



United States Agency for International Development  
Peanut Collaborative Research Support Program

**USA ♦ PHILIPPINES**

● **MONOGRAPH SERIES** ●

**No. 3**



# **CONTROL OF AFLATOXIN IN RAW PEANUTS THROUGH PROPER MANUAL SORTING**

Department of Food Science and Technology  
University of Georgia  
Griffin, Georgia  
USA



Food Development Center  
National Food Authority  
Taguig, Metro Manila  
Philippines



Department of Food Science and Nutrition  
University of the Philippines  
Diliman, Quezon City  
Philippines



Department of Agricultural Chemistry and Food Science  
Leyte State College  
Leyte  
Philippines



---

**July 2002**

**United States Agency for International Development**  
**Peanut Collaborative Research Support Program**  
**Project 04**  
**(USA and Philippines)**  
**MONOGRAPH SERIES**  
**No. 3**

Department of Food Science and Technology  
University of Georgia  
1109 Experiment St.  
Griffin, Georgia 30223-1797  
USA

Food Development Center  
National Food Authority  
Department of Agriculture  
FTI Complex, Taguig 1632  
Metro Manila, Philippines

Department of Food Science and Nutrition  
College of Home Economics  
University of the Philippines  
Diliman 1101  
Quezon City, Philippines

Department of Food Science and Technology  
Leyte State College 6521-A  
Leyte, Philippines

# **CONTROL OF AFLATOXIN IN RAW PEANUTS THROUGH PROPER MANUAL SORTING**

Flor Crisanta F. Galvez<sup>1</sup>  
Ma. Leonora D. L. Francisco<sup>2</sup>  
Alicia O. Lustre<sup>3</sup>  
and  
Anna V. A. Resurreccion<sup>4</sup>

<sup>1</sup>Dean, College of Home Economics, UP Diliman 1101

<sup>2</sup>Assistant Professor, College of Home Economics, UP Diliman 1101

<sup>3</sup>Director, Food Development Center 1632

<sup>4</sup>Professor, University of Georgia, Griffin, Georgia 30223-1797

**JULY 2002**



## ACKNOWLEDGMENT

The authors acknowledge the Peanut-Collaborative Research Support Program (P-CRSP) of the United States Agency for International Development (USAID) for providing research funds through Grant No. LAG-G-00-96-00013-00.

We would also like to thank Dr. Tim Williams, Program Director of Peanut-CRSP for his support of our research activities; Dr. Anna V. A. Resurreccion, for her leadership as principal investigator of this Peanut-CRSP project; Dr. Manjeet Chinnan and Dr. Larry Beuchat, co-investigators of Peanut-CRSP, for their contributions to the research and training activities.

Our gratitude is extended to Marigold Commodities Corporation and especially to Mr. Kim Lapuz, for collaborating with us in this project, and for unselfishly agreeing to have the Peanut-CRSP team in the Philippines transfer the sorting technology to other interested peanut manufacturing companies even before the date of termination of the secrecy agreement, recognizing the importance of providing the Filipino consumers with aflatoxin-free peanut products.

We are indebted to all the staff of the Department of Agriculture (DA) National Food Authority (NFA) Food Development Center (FDC) who, in one way or another, contributed to the efficient conduct of this important study.

The support of Ms. Marina Escaño, Division Chief, FDC Support Services, for budgetary matters and reporting of expenses is appreciated. We likewise recognize the assistance of Mr. Alberto Cariso, Division Chief, Industry Liaison Services Division, FDC, for coordinating the initial visits to Marigold Commodities Corporation, leading to their collaboration as industrial partners.

We appreciate the assistance of Dr. Marlene Bulgarelli, for the cover design of the monograph series and the production of this monograph, and Ms. Blanca J. Villarino, for technical assistance.



## TABLE OF CONTENT

### CONTROL OF AFLATOXIN IN RAW PEANUTS THROUGH PROPER MANUAL SORTING

	<b>Page</b>
I. Acknowledgement	3
II. Abstract	7
III. Introduction	8
A. Peanuts	8
B. Molds in Peanuts	8
C. Mycotoxins	8
D. Aflatoxins	9
E. The Fungus	10
F. Natural Occurrence in Foods	10
G. Factors Affecting Production of Aflatoxin	10
H. Control of Aflatoxin Production	11
I. Methods for Aflatoxin Detection	11
J. Aflatoxin Detoxification	12
K. Health Hazards	13
IV. Objectives	14
V. Methods	14
A. Establishment of Collaboration	14
B. Experimental Design	15
C. Development of the Sorting Process	15
D. Pilot Scale Trials	15
E. Verification of the Efficiency of the Sorting Process	15
F. Commercial Scale Trials	16
G. Development of Guidelines for Collaborator's Workers	16
VI. Results	16
A. Development of the Sorting Process	16
B. Pilot Scale Trials	19
C. Verification of the Efficiency of the Sorting Process	20
D. Commercial Scale Trials	22
E. Development of Guidelines for Collaborator's Workers	22
VII. Impact	23
VIII. Conclusion	24
IX. References	25

## X. Appendices

Appendix A	Developed Guidelines on the Manual Sorting of Aflatoxin-Contaminated Peanut Kernels	27
Appendix B	Calendar of Events for Collaboration with Marigold Commodities Inc. on the Adoption of Sorting Technology	37
Appendix C	Impact Monitoring of Sorting Technology for Aflatoxin Control in Kare-kare Mix	41

## LIST OF TABLES

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
Table 1	Aflatoxin content of dry blanched peanut samples obtained during the development of the sorting process	19
Table 2	Aflatoxin content of dry blanched peanut samples obtained during pilot scale trials of the developed sorting process	20
Table 3	Aflatoxin content of dry blanched peanut samples during verification trials of the developed sorting process	21
Table 4	Aflatoxin content of dry blanched peanut samples obtained during the commercial scale trials of the developed sorting process at the collaborator's facilities	22

## LIST OF FIGURES

<b>Fig No.</b>	<b>Title</b>	<b>Page</b>
Fig. 1	Flow diagram of the developed manual sorting procedure for raw peanut.	17
Fig. 2	Examples of discolored and damaged kernels sorted out from the raw materials.	18



## ABSTRACT

A manual sorting procedure to eliminate aflatoxin contamination in raw peanuts was developed. Pilot scale and verification trials using the peanut roaster at the Department of Agriculture (DA) National Food Authority (NFA) Food Development Center (FDC) were done. Blanching 20 Kg of peanuts at approximately 140°C for 25 min using the FDC peanut roaster facilitated sorting of aflatoxin-contaminated kernels after de-skinning. This rendered the dry blanched sound sorted peanuts aflatoxin-free (no aflatoxin detected), starting with raw materials with aflatoxin that was extremely high (300 ppb). Verification trials confirmed that the sorting process employed in this study was efficient in detecting and separating contaminated peanut kernels from raw materials, whether contamination was high or low. The sorting procedure was adapted to the system used at the collaborator's facilities. The blanching time that was found to facilitate proper de-skinning and subsequent sorting of the aflatoxin-contaminated peanut kernels ranged from 45 to 55 min using 50 Kg of raw peanuts. The blanching time was determined to be longer than the established blanching time since the collaborator does not preheat the roaster prior to blanching. All de-skinned sound sorted raw materials were found either not to contain aflatoxin or to contain extremely low levels.

# INTRODUCTION

## **Peanuts**

Peanut (*Arachis hypogaea* L.) is a popular food item because of its good taste, pleasing aroma and flavor, and even-dry texture (Woodroof, 1983). It has high protein and energy values and is suitable for producing other food products. It is one of the major components of products being manufactured by the food processing industry. It is, however, more popularly consumed in the Philippines as nuts, either boiled or roasted in or out of the shell.

Peanut is known locally in the Philippines as *mani*. In other parts of the world, it is known as groundnut, earthnut, pistache de terre, grober, monkey nut, Manila nut, ground beans, and pindar (Arthur, 1953). It is an annual herb that grows best in well-drained, loose friable sandy loams and is noted for the production of underground fruits called pods.

## **Molds in Peanuts**

Molds may grow on peanuts before it is used in the processing of products. This may happen from harvesting the peanuts up to shelling before use in the process. These molds produce mycotoxins, specifically, aflatoxin. Aflatoxin can cause illness if it is not removed from peanuts that are being processed. The peanut products with high levels of “aflatoxin” are not permitted by the Bureau of Food and Drug (BFAD) of the Philippines and US Food and Drug Administration (USFDA) in America to be sold in the market to protect the consumers.

In trying to control aflatoxin contamination in peanut products, an aflatoxin management program must be well established in the company (Beuchat, 2000). The regulatory limits governing specific products must be known and an efficient monitoring program must be established which should include a good sampling plan, a listing of permitted uses of aflatoxin-contaminated peanuts, and a proper designation of the use for violative products. The sampling plan should specify the methods that are intended to be used for sample collection, sample preparation, and sample analysis. There is likewise a need to ensure that Good Manufacturing Practices (GMP) are strictly followed and a good quality control system is in place in the company. The final product must be evaluated or analyzed for aflatoxin content to know the efficiency of the methods employed and whether the objectives were met or not.

## **Mycotoxins**

Mycotoxins are substances produced as secondary metabolites by fungi, especially molds. They probably have affected mankind since the beginning of organized crop production. For example, Miller (1991) conjectured that the severe depopulation of Western Europe in the thirteenth century was caused by the replacement of rye with wheat, an important source of *Fusarium* mycotoxins.

Mycotoxins occur in a wide variety of foods and feeds and have been implicated (Coker, 1979) in a range of human and animal diseases. Exposure to mycotoxins can produce both acute and chronic effects ranging from death to effects upon the central nervous, cardiovascular and pulmonary systems, and upon the alimentary tract. Mycotoxins may be carcinogenic, mutagenic, teratogenic and immunosuppressive; the latter being their most important effect.

Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and domestic and international trade. This is a particular problem in developing countries, where the food staples are susceptible to contamination.

Some of the mycotoxins demonstrated to occur in foods include the aflatoxins, *Alternaria* toxins, citrinin, ochratoxins, patulin, penicillic acid, sterigmatocystin, and zearalenone (Jay, 1992).

Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus*, and are the most widely studied of all mycotoxins. *Alternaria* toxins are produced by *Alternaria* spp. (*A. citri*, *A. alternata*, *A. solani*, and *A. tenuissima*) in apples, tomatoes, blueberries, and others.

Citrinin is produced by *Penicillium citrinum*, *P. viridicatum*, and other fungi and has been recovered from polished rice, moldy bread, country-cured hams, wheat, oats, rye, and other similar products.

Ochratoxins are produced by a large number of storage fungi, including *A. ochraceus*, *A. alliaceus*, *A. ostianus*, *A. mellus* and other species of aspergilli. The penicillia that produce ochratoxins include *P. viridicatum*, *P. cyclospium*, *P. variable*, and others. This mycotoxin has been found in corn, dried beans, soybeans, oats, barley, citrus fruits, Brazil nuts, moldy tobacco, country-cured hams, peanuts, coffee beans, and other similar products.

Patulin, which includes clavacin and expansin, is produced by a large number of penicillia, including *P. Claviforme*, *P. expansum*, *P. patulum*, by some aspergilli (*A. clavatus*, *A. terreus* and others), and by *Byssochlamys nivea* and *B. fulva*, and is found in moldy bread, sausage, fruits (including bananas, pears, pineapples, grapes and peaches), apple juice, cider, and other similar products.

Penicillic acid has biological properties similar to patulin. It is produced by many penicillia like *P. puberulum* and *P. cyclospium*, as well as members of the *A. ochraceus* group. It has been found in corn, beans, and other field crops.

Sterigmatocystin is structurally and biologically related to the aflatoxins, and like the latter, they cause hepatocarcinogenic activity in animals. It is produced by *Aspergillus versicolor*, *A. nidulans*, *A. rugulosus*, and others, and has been found in wheat, oats, Dutch cheese, and coffee beans.

## **Aflatoxins**

Aflatoxins are secondary metabolites produced by particular strains of *Aspergillus flavus*, *A. vesicolor*, and *A. indulans*. The term "aflatoxin" was coined in the early 1960s when the death of thousands of turkeys ('Turkey X' disease), ducklings and other domestic animals was attributed to the presence of *A. flavus* toxins in groundnut meal imported from South America (Austwick, 1978). The chronic effects of low dietary levels (parts per billion) of aflatoxin on livestock are also well documented (Coker, 1979).

The aflatoxin-producing molds occur throughout the world, in-sub-tropical and tropical climates. The aflatoxins may be produced, both before and after harvest, especially on oilseeds, edible nuts and cereals (Coker, 1979).

The first aflatoxins were B1, B2, G1 and G2. They were classified according to fluorescence and Rf values. The blue-fluorescence spot observed under ultra-violet (UV) light with Rf values of 0.4 and 0.36 were designated aflatoxin B1 and B2. On the other hand, those that fluoresced green with slightly lower Rf values of 0.34 and 0.31 were characterized as aflatoxin G1 and G2, respectively.

The molecular formula for aflatoxin B1 and G1 were deduced to be  $C_{17}H_{12}O_6$  and  $C_{17}H_{12}O_7$ , respectively. Aflatoxin B2 and G2 were known to be dihydroderivatives of B1 and G1 with the molecular formula of  $C_{17}H_{12}O_6$  and  $C_{17}H_{12}O_7$ , respectively.

Aflatoxin B1 is a human carcinogen (IARC, 1993) and is one of the most potent hepatocarcinogens known, and is also acutely toxic at high levels of contamination. Immunosuppressive effects are known in animals, and reported in humans; the aflatoxins could play a significant role in the aetiology of human disease in some developing countries.

## **The Fungus**

*A. flavus* and *A. parasiticus* are widely distributed in soil and air and are easily carried from one place to another due to the extreme buoyancy of its reproductive structure known as conidial spores. The fungal spores enter through natural openings or sites caused by insects and mechanical injury. These then germinate if the environmental condition is suitable for the development of the mold. However, only approximately 60% of 1400 isolates of the *A. flavus* group were toxin producers.

## **Natural Occurrence In Foods**

Foods high in carbohydrates are most susceptible to aflatoxin contamination. Agricultural commodities implicated with aflatoxin are peanuts or groundnuts, barley, beans, cotton seed, rice, wheat, copra, cassava and peas. Meat and dairy products are possible sources of the mycotoxin if the animal was fed with feeds contaminated with aflatoxin. Cured meats like ham, sausage and bacon are also found to be vulnerable to aflatoxin contamination.

## **Factors Affecting Production Of Aflatoxin**

### Aeration

Biosynthesis of aflatoxin is an aerobic process, i.e., aeration favors aflatoxin production. Depletion of oxygen to 0.1% in the atmosphere greatly lowers the production of aflatoxin. Increasing the concentration of  $CO_2$  likewise adversely affects the production of the toxin.

### Relative Humidity and Moisture

The moisture content of the food substrate is the most critical factor affecting fungal growth and aflatoxin formation. Maximum production of aflatoxin in peanuts can be attained with moisture content of 25% and RH of 85 % (Woodroof, 1983)

### Temperature

*A. flavus* and *A. parasiticus* has been classified as mesophilic organisms. However, the minimum and maximum temperature for their growth is affected by other factors like moisture, oxygen concentration and nutrients available. The minimum temperature for growth of the fungus is 6-8°C, optimum at 36-38°C and maximum at 44-46°C. Temperature also influences the type of aflatoxin produced.

### Microbial Interaction

Association of *A. flavus* with other organisms promotes microbial competition for the available substrate. This eventually restricts the formation of aflatoxin in substrates. Among the

1,000 microorganisms screened by Ciegler *et al.* (1966) for the ability to degrade aflatoxin, only one bacterium, *Flavobacterium aurantiacum* NRRL B-184, was found to have the ability to irreversibly remove aflatoxin from the solution.

### Pod and Seed Damage

Physical and biological damages to the peanut shell and seed cause decay that enhance production of the toxin that destroys the exposed tissues. The openings present in an infested testa, a protective covering of a peanut kernel, provide an easy entrance for the fungi *A. flavus*. Also, it renders the nut rancid by freeing the oil through the cracked/damaged part.

### Other Factors

Light, pH and maturity of the pod also affect the development of aflatoxin. Aflatoxins are sensitive to light while acidic medium was found to enhance the formation of the toxin. It was also found that the mature pods are easily penetrated by fungus compared to young fruits.

## **Control Of Aflatoxin Production**

### Pre-Harvest

Soil preparation is necessary for planting peanut in order to reduce the incidence of aflatoxin contamination. Crop rotation is advisable because it replenishes organic matter in the soil thereby improving its fertility and enhances the growth of microorganisms that have inhibiting effects on *A. flavus* (Pattee and Young, 1982). Also, planting time should be planned such that the harvesting time will coincide with dry season to avoid aflatoxin contamination (Flach, 1987).

### Harvesting

During harvesting, mechanical damage to peanuts must be avoided because it enhances susceptibility to contamination. Moreover, only mature peanuts should be harvested since fungal infection is more likely to occur in shriveled and cracked kernels (Grybauskas *et al.*, 1992). Removal of soil from pods is also another factor to consider since *Aspergillus* is indigenous to soil that might increase the risk of molding.

### Storage Condition

During the subsequent process after harvesting, moisture content and temperature are the most critical factors for controlling aflatoxin. Prior to storage, peanuts must be dried to certain moisture content where it will be at equilibrium with the relative humidity of the warehouse to prevent invasion of aflatoxin to peanuts. When stored in the silos, rewetting must be avoided since it increases by 10-fold the production of aflatoxin (Pattee and Young, 1982). In Indonesia, it was found that the contamination of peanuts usually happens at the retailer stage, and it can be explained by the fact that retailers keep the nuts in uncovered containers, and they may keep the nuts for quite sometime (Flach, 1987). Peanuts should be stored at temperatures low enough to suppress sporulation and inhibit mold growth. This may be achieved by using refrigerated temperature or below 5°C.

## **Methods For Aflatoxin Detection**

There are numerous methods being used at present to detect presence of aflatoxin. The indirect method includes the Visible *A. flavus* (VAT) Method where it is used to segregate aflatoxin-

suspect loads from non-suspect loads at farm level (Domer, 1990). At the processing level, separation of contaminated peanuts is done by hand sorting and density segregation (Gnanasekharan *et al.*, 1992). Hand sorting is the most effective method, however, it is time-consuming making it impractical for commercial use. Color sorting on the other hand, is relatively rapid and may be done by machine but loss of uncontaminated peanut is probable or some contaminated kernels with aflatoxin may still be left in the sorted kernels. Density segregation is a potential alternative for separation of contaminated peanuts. Studies were done to find efficient methods that would utilize density segregation without liquid absorption of peanuts.

Direct aflatoxin quantification methods include thin layer chromatography (TLC), minicolumn chromatography, gas chromatography, high-performance thin layer chromatography, immunoassay: Enzyme Linked Immunosorbent Assay (ELISA), Fluorometer (FTM) and differential pulse chromatography (Domer, 1990).

## **Aflatoxin Detoxification**

### Physical Method

Physical approaches to aflatoxin destruction generally involve treating with heat, UV light, or ionizing radiation, none of which is entirely effective. Heat treatment is a useful reduction method for aflatoxin as long as it does not affect the nutritional value of the food. The temperature that is usually required to effect destruction of aflatoxin is in the vicinity of 270°C (Beuchat, 2000). The effect of heat treatment has an average reduction effect of 43-83% depending on the level of aflatoxin in raw peanuts and roasting conditions. However, it is not considered to be a satisfactory detoxification method because problem of oxidative degradation arises. Ionizing radiation showed no reduction on the toxicity of the meal. Some studies reported that susceptibility of products to the storage fungi may even be increased by irradiation. Contradicting reports also showed that the molds on the kernel surfaces were effectively eliminated by a dose greater than 5.0 Kgy (Chiou *et al.*, 1990).

### Chemical Inactivation

Chemical degradation of aflatoxins is usually carried out by the addition of chlorinating (sodium hypochlorite, gaseous chlorine), oxidizing (hydrogen peroxide, ozone, sodium bisulfite) or hydrolytic agents (acids, alkalis, ammonia). Consideration in choosing chemicals to use must include its ability to maintain the nutritive value of the food and must not produce toxic residues. Of the methods mentioned above, ammoniation is the most widely accepted. Although effective, ammoniation can require expensive equipment and may result in losses in nutritional quality of the treated feed (Samarajeewa *et al.*, 1990). Chemicals like acids, bases and oxidizing agents are among the chemical inactivators. Oxidizing agents inactivate aflatoxin B1, G1 and M1 which have terminal double bond in the hydrofuran ring that is sensitive to reactive forms of oxygen. Acids are used due to its ability to destroy the biological activity of aflatoxin B1 and G1. Inorganic and organic bases are used to destroy and remove aflatoxin contaminated agricultural products. Although they were able to destroy aflatoxin, the protein efficiency ratio (PER) of the treated food products were lowered and some of the chemicals produce residues that are toxic.

### Biological Degradation

Many bacteria, yeasts, molds, actinomycetes and algae can remove or degrade aflatoxin in foods and feeds (Marth and Doyle, 1979). The most widely reported, however, is *Flavobacterium aurantiacum* NRRL B-184 (Ellis *et al.*, 1991). Ciegler *et al.* (1966) demonstrated the ability of *F. aurantiacum* to remove aflatoxin B1 from milk, corn oil, peanut butter, corn, soybeans and peanuts. Hao and Bracket (1988) likewise detoxified peanut milk using this organism. However, the

mechanism by which this organism detoxifies aflatoxin remains unknown. Line and Brackett (1995) studied the effects of cell populations (viable and heat-inactivated), culture age, and transfer history on aflatoxin removal by *F. aurantiacum* NRRL B-184 in a test system. It was found that 72-hr cultures removed more toxin from solution than 24-hr cultures. Likewise, populations of  $10^{10}$  cells removed aflatoxin at a faster rate than  $10^9$  cells did. However, populations of  $\leq 1 \times 10^9$  cfu/ml heat-inactivated cells were unable to remove aflatoxin B1 from phosphate buffer. Transferring cultures in tryptic soy broth every 3 days for over 8 months had no apparent effect on the ability of the organism to remove measurable amounts of aflatoxin B1 solution.

### Physical Separation Of Contaminated Kernels

At present, electronic color sorting and handpicking are widely used to separate aflatoxin-contaminated kernels from sound kernels. Electronic color sorting, however, is only 72% efficient in removing aflatoxin-contaminated kernels (Dickens and Whitaker, 1975), while handpicking, on the other hand, although more selective, is deemed impractical in the United States. Density-based separation schemes are theoretically feasible but loss of peanuts is high (Gnanasekharan and Chinnan, 1989) and efficiency of separation is highly variable. A water-flotation method has been patented based on the observation that contaminated kernels are usually less dense than sound kernels (Henderson *et al.*, 1989). This procedure has not gained wide commercial acceptance due to an additional drying step after the flotation treatment.

Takeuchi *et al.* (1970) developed a hydrogen peroxide blanching process for peanuts based on the principle that catalase will react with hydrogen peroxide to yield water and oxygen. It has been shown that aflatoxin-contaminated peanuts floated more rapidly than sound kernels when submerged in hydrogen peroxide solution. Clavero *et al.* (1992) demonstrated that *A. parasiticus* produces catalase when grown in peanut milk. It was hypothesized that catalase produced by *A. parasiticus* will react with hydrogen peroxide and promote the formation of oxygen bubbles on the surface of the kernels. The higher the catalase activity, i.e., the more severely infected the peanuts, the more rapid would be the evolution of oxygen, thus causing mold-infected kernels which may contain aflatoxin to float.

### **Health Hazards**

Presence of aflatoxin at low amounts is believed to pose a risk to human health due to its extreme toxicity. Furthermore, since they are considered “unavoidable contaminants” of major plant commodities, it is considered as a consumer food-safety issue and inadvertently caused economic losses to producers and to food handlers and processors (Domer, 1990).

In response to the disturbing effect of aflatoxin in agriculture and public health, the USFDA set a maximum allowable level of total aflatoxin at 20 ppb. In the United Kingdom, the allowable limit for aflatoxin was set at 4 ppb (MAFF, 1996). Commodities for human and animal consumption must have aflatoxin levels below these numbers. In Asia, countries such as China, India, Malaysia, Thailand and the Philippines have established national maximum levels for aflatoxin which ranges from 20-30 ppb for commodities for human consumption (Flach, 1987).

Aflatoxin B1 was found to be the most lethal among the types of aflatoxin. Susceptibility to toxin is influenced by dosage, duration, sex and age of animals or humans. Studies showed that male rats are more susceptible to aflatoxin B1, while old animals are more resistant to aflatoxin compared to young ones.

Aflatoxin has been reported to cause liver damage and aflatoxicosis both on domestic animals and humans (Chenault, 1996; Anonymous, 1996). The only documented health effect that could be

expected from low-level exposure to aflatoxin would be an increased prevalence of liver cancer years to decades after exposure (Anonymous, 1996). However, the actual or true rate of liver cancer associated with aflatoxin is not known (Wagstaff, 1993). For aflatoxicosis, on the other hand, initial manifestations are loss of weight and lack of appetite, other symptoms include vomiting, abdominal pain, pulmonary edema, gastrointestinal hemorrhage, convulsions, coma which may eventually lead to death (Anonymous, 1996). Hepatotoxicity resulted to pale, firm and fibrotic liver. Teratogenic effect was also observed on hamsters. Degree of severity was influenced by the period of gestation upon which the toxin was administered.

Epidemiologic studies implicated aflatoxin in an outbreak of liver cancer in India involving consumption of contaminated maize in 1974 wherein symptoms of the disease included brief period of fever associated with vomiting and anorexia, followed by jaundice and some, death. Findings showed that aflatoxin poses a potential hazard to humans. Moreover, there is strong evidence that aflatoxin is the leading cause of liver cancer in Africa and China (Chenault, 1996).

## **OBJECTIVES**

One of the problems of the peanut industry in the Philippines is aflatoxin contamination of raw peanuts. Peanut products exported from the Philippines have suffered detention problems at the ports of countries where they are shipped to because of high levels of aflatoxin. In U.S. and U.K., the enforced maximum levels of aflatoxin in foods are 20 ppb (Flach, 1987) and 4 ppb (MAFF, 1996), respectively. Consumer demand and the world export market for commodities susceptible to aflatoxin contamination, however, are pushing towards zero tolerance. There is no other method that peanut product manufacturers in the Philippines can use to reduce aflatoxin levels in peanuts except for manual sorting of raw peanuts to separate kernels that are not fit for processing.

This study was undertaken to develop a technology for manual sorting of peanut kernels to eliminate aflatoxin contamination. Specific objectives were to: 1) determine methods for manual sorting of peanuts, 2) evaluate and verify the effects of manual sorting in laboratory and pilot scale levels, 3) introduce the developed manual sorting to the collaborator, and 4) develop guidelines and procedures that would be used by the collaborating company to ensure separation of aflatoxin-contaminated peanuts.

## **METHODS**

### **Establishment of Collaboration**

A consultation meeting was set up with one peanut product manufacturing company that expressed interest in a possible collaboration on the technical needs of the company. Discussions with the owner and top management revealed possible collaboration on control of aflatoxin through proper sorting techniques. According to the owner, the problem of the company was that they were unable to incorporate peanuts in their exported Kare-kare Mix because of high levels of aflatoxin. He indicated that if aflatoxin would be eliminated from the peanuts that they were using, the company would incorporate peanuts in their exported products, specifically the Kare-kare Mix. An agreement on the collaboration was drafted, discussed and signed by the representative from the collaborating company,



by Dr. Alicia O. Lustre as P-CRSP Principal Investigator in the Philippines and by Dr. Flor Crisanta F. Galvez as P-CRSP Co-Principal Investigator who conducted the research. The collaborator supplied the raw materials used in the study.

### **Development of the Sorting Process**

Studies on the sorting process were conducted in the laboratory at the Food Development Center (FDC). A prototype roaster was used to test the applicability of the blanching procedure suggested by Woodroof (1983). The FDC roaster had nine gas-fired burners each of which has its own control knob. The roaster was pre-heated to 140°C by setting all burners to maximum temperature. When the roaster attained the temperature of 140°C, burner nos. 2,6, 7,8 and 9 were adjusted to minimum settings that would maintain the desired temperature. All other burners were turned off. Five-Kg shelled peanuts (large seed variety from Vietnam) were weighed, sorted (sorting 1) fed to the roaster and dry-blanching at 140°C for 25 minutes. This was conducted in two replications. After dry-blanching, the peanuts were removed from the roaster, cooled with the use of an electric fan de-skinned and sorted (sorting 2) manually for discolored and damaged kernels. Percent yield of peanuts was calculated by subtracting the weights of discolored and damaged nuts and skin from the weight (5 Kg) of the sample. Two hundred grams of representative samples for both dry-blanching, de-skinned unsorted peanuts and de-skinned sound sorted peanuts and all kernels sorted as discolored and damaged were analyzed aflatoxin using AOAC Official Methods # 49.2.09, 970.45 – BF Method and # 49.2.08, 968.22 - CB Method. The aflatoxin value for the total weight (5 Kg) of the raw peanuts was derived using the formula:

$$\text{Total aflatoxin value (ppb)} = \frac{\text{aflatoxin content (ppb)} \times \text{wt of discolored and damaged peanuts(g)}}{\text{wt of starting raw peanuts (g)}}$$

### **Pilot Scale Trials**

Twenty Kg of peanuts were weighed, fed into the pre-heated roaster, dry blanching for 25 min at 140°C and sorted using the sorting procedure developed previously. Two trials were conducted. Percent yield of peanuts was calculated using the same procedure used previously. One thousand grams of representative samples for both dry-blanching unsorted and sound sorted peanuts, and all kernels sorted as discolored and damaged were analyzed for aflatoxin using the method applied previously. Two separate batches of 20 Kg peanuts were analyzed to serve as the sample before sorting. The aflatoxin value for the total weight (20 Kg) of the starting raw material was computed using the equation used previously.

### **Verification of the Efficiency of the Sorting Process**

The verification studies were conducted at FDC. Eleven 20-Kg samples of raw peanuts of the same variety used previously were obtained from different sources and markets to cover a wide range of expected aflatoxin levels in raw peanuts in the market. Twenty Kg of peanuts were weighed, fed into the pre-heated roaster, dry-blanching for 25 min at 140°C and sorted using the sorting procedure developed previously. Percent yield of peanuts was calculated using the same procedure used previously. One thousand grams of representative samples for both dry-blanching unsorted and sound sorted peanuts, and all kernels sorted as discolored and damaged were analyzed for aflatoxin using the method applied previously. The aflatoxin value for the total weight (20 Kg) of the raw peanuts was calculated.

## **Commercial Scale Trials**

Trials were conducted at the collaborator's plant using the procedure employed by the company during normal manufacturing operations such as flame setting and no preheating of roaster. The roaster used did not have temperature controllers. Fifty Kg raw peanuts were dry blanched at 140°C for 45 min. Because the roaster was not preheated, after 25 min of dry blanching, it was found that the skins of the peanuts were still intact. Blanching time was extended to 45 min until the skins can be easily removed from the peanut kernels using the de-skinner of the company. The blanched peanuts were transferred to the de-skinning room but the initial de-skinning process resulted to broken peanuts, so the machine was adjusted. Skins were separated from the de-skinned kernels with the use of electric fan. Manual sorting of aflatoxin-contaminated kernels were conducted and samples were submitted for analysis as described previously.

Two succeeding trials were conducted to verify the procedure in blanching peanuts purchased by the company from different suppliers in terms of ease of de-skinning the peanuts after blanching. In some cases when there was difficulty in de-skinning peanuts blanched for 45 min, blanching time was extended to 55 min. It was observed that the maturity of the peanuts was different in these cases so heating was extended to loosen the skin. After blanching, the peanuts were subjected to the same procedures as previously described.

## **Development of Guidelines for Collaborator's Workers**

The steps to be followed and the guidelines to be employed at the collaborator's facilities were developed in a way that these were easily understood by workers and would explain to them the importance of strictly following these guidelines.

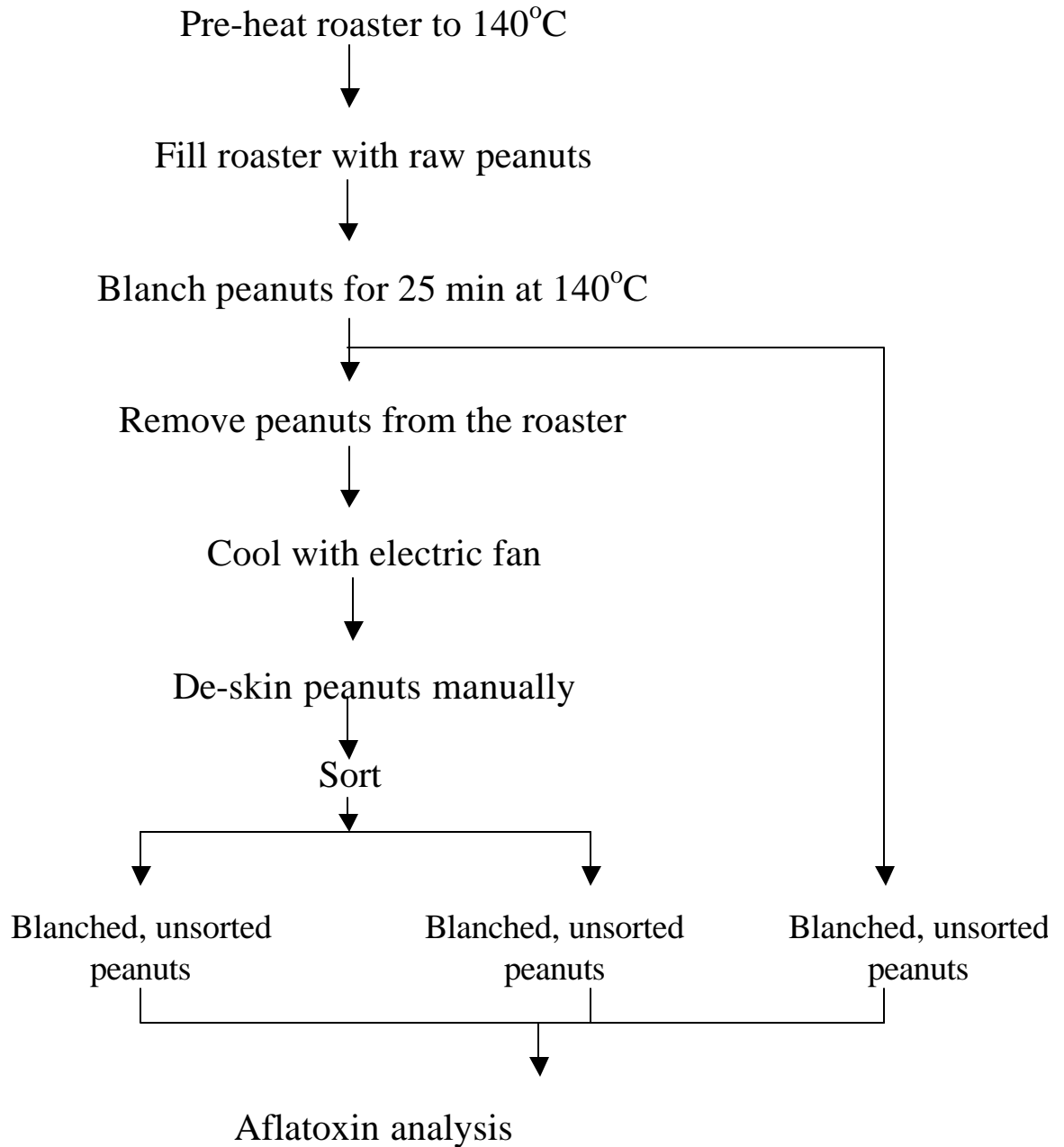
# **RESULTS**

## **Development of the Sorting Process**

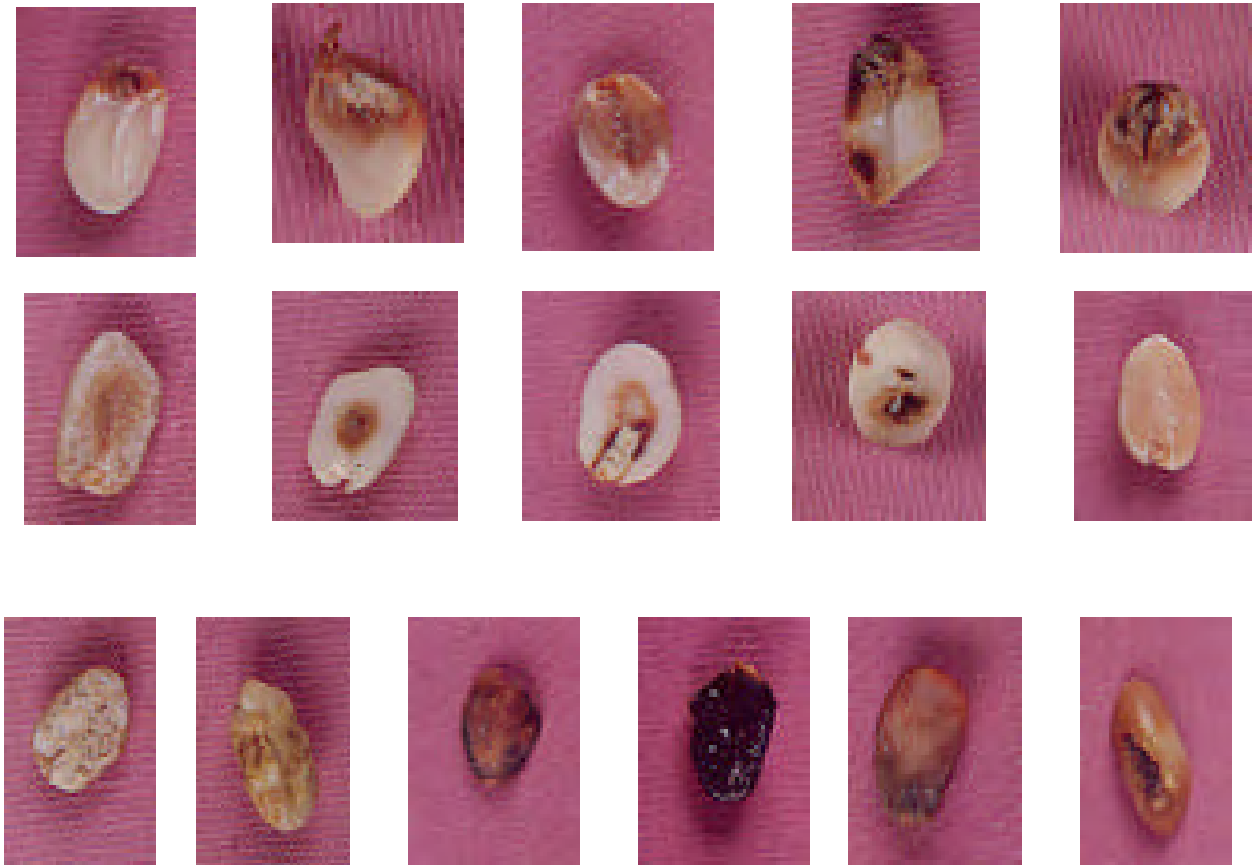
The flow diagram of the developed process for sorting is presented in Fig. 1. Dry blanching of raw peanuts facilitated sorting for discolored and damaged peanuts since they appeared more moldy, shriveled and discolored compared to unblanched peanuts. Examples of discolored and damaged peanut kernels that were unfit for processing are shown in Fig. 2.

Manual sorting of 5-Kg raw peanuts resulted to an average of 4.36 Kg (87.2%) de-skinned peanuts, which is 87.2% (Table 1). The skins, unrecovered peanut kernels from the roaster and wastes during the transfer of peanuts from blanching to sorting accounted for the 12.8% loss. From the de-skinned peanuts an average of 98.7% was sorted as sound kernels and 1.3% as discolored and damaged peanuts.

Results of the analysis for aflatoxin content of samples collected during the development of the sorting process are shown in Table 1. Although aflatoxin was not detected in the dry-blanched de-skinned unsorted and sound sorted peanuts, the sorted de-skinned discolored kernels had aflatoxin ranging from 95 to 114 ppb, equivalent to 1.25 ppb in the starting raw materials (5 Kg). The calculated aflatoxin content (1.25) of the raw materials was low compared to the 15 ppb level allowed in the Philippines. These results implied that even though aflatoxin analyses of the de-skinned unsorted and sound sorted peanuts revealed negative aflatoxin content, the starting raw material is in fact contaminated with aflatoxin. These results indicated that if sorting was not applied to raw peanuts the



**Fig. 1. Flow diagram of the developed manual sorting procedure for raw peanut (Aflatoxin analysis used AOAC Official Methods, # 49.2.09,970.45-BF Method and # 49.2.08,968.22-CB Method).**



**Fig. 2. Examples of discolored and damaged kernels sorted out from the raw materials.**

**Table 1. Aflatoxin content of dry blanched peanut samples obtained during the development of the sorting process<sup>1</sup>**

Sample	Trial	Total Weight of Peanut Samples (Kg)	Weight of Sample Submitted for Analysis (g)	Aflatoxin Content (ppb)
Dry blanched, de-skinned, unsorted peanuts	1	4.34	200.00	None detected
	2	4.37	200.00	None detected
Dry blanched, de-skinned sound sorted peanuts	1	4.30	200.00	None detected
	2	4.30	200.00	None detected
Dry blanched, de-skinned discolored and damaged sorted peanuts from raw materials	1	0.04	44.10	95
	2	0.07	72.98	114

<sup>1</sup>using 5-Kg raw materials

resulting product would be contaminated with aflatoxin. These results also demonstrated the efficiency of the sorting process to eliminate aflatoxin contamination from peanuts even at low level of contamination. These results may be accounted to the ineffective sampling procedure employed for analysis of the peanut samples. The problem of accurate determination of aflatoxin content in a large quantity of raw peanuts maybe due to the large variability associated with the sampling procedure (Whitaker *et al.* 1974; 1976; 1979). The sampling procedure for granular products generally consists of three steps : (1) a sample is taken from the lot, (2) the sample is comminuted to reduce particle size and a subsample is removed from the comminuted sample for analysis, and (3) the aflatoxin is extracted and quantified. Studies by the same researchers on peanuts and cottonseed indicated that sampling variability is the largest source of errors in aflatoxin analysis, especially for small sample sizes. Sampling error is large because aflatoxin is found only in a small percentage (<0.1%) of the kernels in the lot (Whitaker and Wisner, 1969), but the concentration in a single kernel maybe extremely high. Cucullu *et al.* (1966, 1977) reported aflatoxin concentrations  $>1 \times 10^6$  ng/g (ppb) for individual peanut kernels and  $5 \times 10^6$  ng/g for cottonseed. Shotwell *et al.* (1974) reported finding  $> 4 \times 10^5$  ng/g of aflatoxin in a corn kernel.

### Pilot Scale Trials

Manual sorting of 20-Kg raw peanuts resulted to an average of 18 Kg (90%) de-skinned peanuts (Table 2). The skins, unrecovered peanut kernels from the roaster and wastes during the transfer of peanuts from blanching to sorting accounted for the 10% loss. From the de-skinned peanuts an average of 97.8% was sorted as sound kernels and 2.2 % as discolored and damaged peanuts.

Results of aflatoxin analysis on samples obtained during the pilot scale trials at FDC are shown in Table 2. Actual analysis of the starting raw material showed that the sample did not contain aflatoxin. However, the de-skinned unsorted peanuts were found to contain 300 ppb, which is high

compared to the maximum allowable limit of 15 ppb in the Philippines. Likewise, the de-skinned discolored and damaged sorted peanuts had high aflatoxin (611 ppb in 500 g to 16,000 in 280-g samples), equivalent to a calculated 15 and 224 ppb of aflatoxin in the starting raw materials. These results again demonstrated the importance of the sorting process in raw peanuts. If sorting was not applied in the raw materials the final product would be contaminated with aflatoxin, even when analysis of the starting raw materials shows otherwise. The results again showed the problem of large variability associated with the sampling procedure.

**Table 2. Aflatoxin content of dry blanched peanut samples obtained during pilot scale trials of the developed sorting process<sup>1</sup>**

Sample	Trial	Total Weight of Peanut Samples (Kg)	Weight of Sample Submitted for Analysis (g)	Aflatoxin Content (ppb)
Dry blanched, de-skinned, unsorted peanuts	1	18.18	1000	300
	2	17.80	1000	300
Dry blanched, de-skinned sound sorted peanuts	1	17.90	500	None detected
	2	17.30	500	None detected
Dry blanched, de-skinned discolored and damaged sorted peanuts from raw materials	1	0.28	280	16,000
	2	0.50	500	611

<sup>1</sup>using 20-Kg raw materials

### Verification of the Efficiency of the Sorting Process

Manual sorting of 20-Kg raw peanuts resulted to an average of 17.2 Kg (86%) de-skinned peanuts. The skins, unrecovered peanut kernels from the roaster and wastes during the transfer of peanuts from blanching to sorting accounted for the 14% loss. From the de-skinned peanuts an average of 96.4% was sorted as sound kernels and 3.6% as discolored and damaged peanuts.

Table 3 shows the results of the aflatoxin analysis performed on samples collected during the verification of the efficiency of the sorting process using 20 Kg of starting raw materials obtained from different sources. Aflatoxin was detected in only two of the 11 samples of de-skinned raw peanuts. One sample of dry blanched de-skinned and sound sorted peanut exhibited an aflatoxin content of 5 ppb, which is low compared to the set limit of 15 ppb in the Philippines. The de-skinned discolored and damaged peanuts sorted from raw materials exhibited aflatoxin content ranging from 9 ppb in 100-g sample to 2791 ppb in 750-g sample. These values are equivalent to 0.045 ppb and 104.70 ppb aflatoxin, respectively, in the 20- Kg starting raw materials. These results again showed the significance of sorting peanuts as verifications tests confirmed that raw peanuts are contaminated with aflatoxin even though analysis showed otherwise. These results also indicated that the sorting process employed was able to separate the aflatoxin-contaminated kernels from the raw materials that

**Table 3. Aflatoxin content of dry blanched peanut samples obtained during the verification trials of the developed sorting process<sup>1</sup>**

Sample	Dry blanched, De-skinned Unsorted Peanuts			Dry blanched, De-skinned Sound Sorted Peanuts			Dry blanched, De-skinned Damaged and Aflatoxin-Contaminated Sorted Peanuts from Raw Materials			Aflatoxin Content of 20-Kg Starting Raw Materials (ppb)
	Total Wt of Peanut Sample (Kg)	Wt of Sample Submitted for Analysis (Kg)	Aflatoxin Content (ppb)	Total Wt of Peanut Sample (Kg)	Weight of Sample Submitted for Analysis (Kg)	Aflatoxin Content (ppb)	Total Wt of Peanut Sample (Kg)	Wt of Sample Submitted for Analysis (Kg)	Aflatoxin Content (ppb)	
1	16.70	1.0	0	16.60	1.0	0	0.10	0.10	9	0.04
2	18.32	1.0	0	18.20	1.0	0	0.12	0.12	15	0.09
3	17.60	1.0	0	16.80	1.0	0	0.80	0.80	10	0.40
4	16.80	1.0	0	16.00	1.0	0	0.80	0.80	40	1.60
5	16.90	1.0	0	16.10	1.0	0	0.80	0.80	80	3.20
6	17.70	1.0	0	17.20	1.0	0	0.50	0.50	744	18.60
7	17.70	1.0	0	16.90	1.0	0	0.80	0.80	600	24.00
8	15.95	1.0	10	15.20	1.0	0	0.75	0.75	1017	38.10
9	17.20	1.0	400	16.30	1.0	5	0.90	0.90	1200	54.00
10	17.35	1.0	0	16.70	1.0	0	0.65	0.65	2837	92.20
11	17.15	1.0	0	16.40	1.0	0	0.75	0.75	2791	104.70

<sup>1</sup>using 20-Kg raw materials

have high (104.7 ppb) and low (0.045 ppb) aflatoxin content. The difficulty in sampling for aflatoxin analysis was again demonstrated here, as previously discussed.

### Commercial Scale Trials

Manual sorting of 50-Kg raw peanuts resulted to a mean weight of 47 Kg (94%) de-skinned peanuts (Table 4). The skins, unrecovered peanut kernels from the roaster and wastes during the transfer of peanuts from blanching to sorting accounted for the 6% loss. From the de-skinned peanuts an average of 97.9% was sorted as sound kernels and 2.1% as discolored and damaged peanuts. Table 4 shows the aflatoxin content of dry-blanching peanut samples obtained from commercial scale trials of the sorting process. Although samples of dry blanching de-skinned unsorted and sound sorted peanuts did not exhibit aflatoxin, the 200-ppb mean aflatoxin value of the de-skinned discolored and damaged peanuts showed that the raw material is contaminated with aflatoxin. This value is equivalent to 2 ppb in the 50-Kg starting raw materials. The results in the commercial scale trial demonstrated the current situation that happens in the peanut manufacturing industries in the Philippines. Without proper sorting of peanuts, the peanut products commercially marketed in the Philippines are possibly contaminated with aflatoxin.

### Development of Guidelines for Collaborator’s Workers

The developed guidelines for the collaborator are shown in Appendix A. The developed guidelines were introduced to the collaborator and the employees were trained on the sorting procedure. The sorting procedure would be employed by the collaborator in the peanuts they use in their products. The company presently uses an average of 2 MT of peanuts every month. This was expected to increase by 20% every year. They have identified facilities that can be used for this step in the process and would hire people specifically for this. The developed guidelines were written both in Filipino and English and were made into posters that displayed inside the sorting room of the collaborating company.

**Table 4. Aflatoxin content of the dry blanching peanut samples obtained during the commercial scale trials of the developed sorting process at the collaborator’s facilities<sup>1</sup>**

Sample	Trial	Total Weight of Peanut Samples (Kg)	Weight of Sample Submitted for Analysis (g)	Aflatoxin Content (ppb)
Dry blanching, de-skinned, unsorted peanuts	1	47.1	1000	None detected
	2	46.9	1000	None detected
Dry blanching, de-skinned sound sorted peanuts	1	46.0	500	None detected
	2	46.0	500	None detected
Dry blanching, de-skinned discolored and damaged sorted peanuts from raw materials	1	1.1	500	200
	2	0.9	500	200

<sup>1</sup>using 50-Kg raw materials



## IMPACT

The additional step of sorting resulted in an expected change in the procedure being followed on the roasting of the raw peanuts at the collaborator's facilities. The company now has to follow a 2-stage roasting process, the first stage of which is the blanching process to facilitate sorting of the aflatoxin-contaminated peanut kernels. The company is willing to do this because of the advantages that the process offers. Appendix C discusses the detail of the study of the impact of the sorting technology as applied by the collaborator in their peanut products.

1. The blanching stage was observed to have resulted to the loss of the undesirable aroma (which was thought to be rancidity) in the roasted peanuts of the collaborating company that was thought to develop after only a few hours after roasting before the sorting technology was adopted. This is considered by the collaborator to be an improvement in the quality of their product.
2. The shelf life of the products that contain peanuts was improved from six months to two years.
3. The collaborating company increased their production volume from the use of 2 MT to 4 MT of raw materials
4. Five (5) additional laborers were hired by the company specifically for the sorting process.
5. The collaborating company would be sorting for aflatoxin-contaminated kernel for all the peanuts that they use in their products. They never sorted for aflatoxin-contaminated kernels in the past. They only sorted manually for kernels that were not fit for processing and for weevil-infected nuts. All peanuts incorporated in the company's products would now be aflatoxin-free.
6. The demand for the company's product has gone up because the company decided to expand its market and the company is now even refusing orders from clients.
7. The collaborating company is now again exporting their Kare-kare Mix with peanuts and is again trying to get a USFDA approval for this product in the U.S.A. after five shipments of aflatoxin-free products. It has exported a total of 1,122.08 Kg of Kare-kare Mix to the U.S.A.
8. The collaborating company would be promoting their Kare-kare Mix, through multi-media advertising, in the local market as "aflatoxin-free". This would make people, as well as other manufacturers of peanut products, aware that this is possible and would make them conscious of aflatoxin contamination. Eventually, all peanut product manufacturers would adopt the technology and this would ensure all peanut products in the local market to be aflatoxin-free.
9. The collaborator has likewise extended their peanut product line, with the addition of peanut sauce.
10. The efficiency of the sorting process for the elimination of aflatoxin from raw peanuts was verified resulting in more confidence in the transfer of the technology to the peanut industry.

## CONCLUSION

Results confirmed that the sorting process employed in this study was efficient in detecting and separating contaminated peanut kernels from raw materials, whether said contamination was high or low. There can now be greater confidence in transferring the technology of the sorting process to the peanut industry. This is expected to provide the Filipino consumers, both the local and foreign, with aflatoxin-free peanut products.

## REFERENCES

- Anonymous. 1996. Biological warfare agents. [Online], Available: <http://www.inform.umd.edu>
- Arthur, J. S. 1953. *Advances in Protein Chemistry*. Vol. 8. p. 393. Academic Press, Inc., New York:
- AOAC. 1995. *Official Methods of Analysis*. 5<sup>th</sup> ed. p.1-10. Association of Official Analytical Chemist. Washington, D.C.
- Austwick, P.K.C. 1978. Mycotoxicoses in poultry. In: *Mycotoxic fungi, mycotoxins, mycotoxicose: an encyclopedia handbook. Volume 2: mycotoxicoses of domestic and laboratory animals, poultry, and aquatic invertebrates and vertebrates*. Wyllie, T.D.; Morehouse, L.G. (Eds.) p. 279-301. Marcel Dekker, Inc., New York.
- Beuchat, L.R. 2000. Peanut microbiology: a focus on aflatoxin. A paper presented at the *Training-Workshop on Control of Aflatoxin in Raw Peanuts Through Proper Sorting*. July 31 to August 1, 2000. Food Development Center, FTI Complex, Taguig, Metro Manila.
- Bungay, A.A. 1993. Health hazards of aflatoxin. A paper presented at the *21<sup>st</sup> Conference of the Veterinary Practitioner's Association of the Philippines (VPAP)*. July 16, 1993. Philippine Village Hotel, Nayong Pilipino, Pasay City.
- Chenault, E. A. 1996. Aflatoxin research shows promise. [Online], Available: <http://www.agnews.amu.edu>.
- Ciegler, A., Lillehoj, B., Peterson, R.E., and Hall, H.H. 1966. Microbial detoxification of aflatoxin. *Appl. Microbiol.* 14:934-939.
- Chiou, R.Y.-Y., Lin, C.M., and Shyu, S.L. 1990. Property characterization of peanut kernels subjected to gamma irradiation and its effect on the outgrowth and aflatoxin production by *Aspergillus parasiticus*. *J. of Food Sci.* 55: 210-213.
- Clavero, M.R.S., Hung, Y.C, Beuchat, L.R and Nakayama, T. 1992. Catalase content in peanut milk as affected by growth by *Aspergillus parasiticus*. *J. Food Prot.* 56:55-57.
- Clavero, Ma.R.S., Hung, Y.C, Beuchat, L.R., and Nakayama, T. 1993. Separation of aflatoxin-contaminated kernels from sound kernels by hydrogen peroxide treatment. *J. Food Prot.* 56:130-133, 146.

- Coker, R.D. 1979. Aflatoxin: past, present and future. *Tropical Sci.* 21:143-162.
- Dickens, J.W. and Whitaker, T.B. 1975. Efficacy of electronic color sorting and handpicking to remove aflatoxin contaminated kernels from commercial lots of shelled peanuts. *Peanut Sci.* 2:45-50.
- Diener, V.L., Pettit, R.E. and Cole, R.J. 1982. Aflatoxins and other mycotoxins in peanut. In: *Peanut Science and Technology*. Pattee, H.E. and C.T. Young (Eds). American Peanut Research and Education Society Inc. Youkum, Texas, U.S.A.
- Domer, J.W. 1990. Methods of analysis and confirmation of aflatoxins in commodities. In: "A Perspective of Aflatoxin in Field Crops and Animal Food Products in the US: A Symposium". [Online], Available: <http://www.inform.imd.edu>.
- Ellis, W.O., Smith, J.P., and Simpson, B.K. 1991. Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. *Crit. Rev. Food Sci. Nutrition* 30:403-439.
- Flach, M. 1987. The prevention and control of mycotoxins in Thailand. In: Joint FAO/WHO/UNEP Second International Conference In Mycotoxins. [Online], Available: <http://www.fao.org/inpho/vlibrary/x0036E19.htm>.
- Gnanasekharan, V. and Chinnan, M.S. 1989. Density characteristics of aflatoxin contaminated peanuts. *Am. Soc. Agric. Engr. Tech. Paper No.* 89-6510. St. Joseph, MI 49085.
- Gnanasekharan, V., Chinnan, M.S., and Donner, J.W. 1992. Methods for characterization of kernel density and aflatoxin levels of individual peanuts. *Peanut Sci.* 19,24-28.
- Grybauskas, A.P., Thomson, P.R., and Cassel, E.U. 1992. Aflatoxins. [Online], Available: <http://www.inform.umd.edu>
- Hao, D.Y-Y. and Brackett, R.E. 1988. Removal of aflatoxin B1 from peanut milk inoculated with *Flavobacterium aurantiacum*. *J. Food Sci.* 53:1384-1386.
- Henderson, J.C., Kreutcher, S.H., Schmidt, A.A., and Hagen, W.R. 1989. Flotation separation of aflatoxin contaminated grain or nuts. U.S. Patent No. 4.795.651.
- Jay, J.M. 1992. *Modern Food Microbiology* 4<sup>th</sup> Ed. Chapman and Hall, International Thomson Publishing, New York
- Hui, Y.D. 1992. Aflatoxin. *Encyclopedia of Food Science and Technology*. p. 6-10, Wiley and sons, Inc, New York
- IARC 1993. Aflatoxins. In: IARC monographs on the evaluation of carcinogenic risks to humans, Lyon, France; International Agency for Research on Cancer, 56, pp. 245-395.
- Lillehoj, E.B., Ciegler, A., and Hall, H.H. 1967. Aflatoxin B1 uptake by *Flavobacterium aurantiacum* and resulting toxic effects. *J. Bacteriol.* 93:464-471.
- Line, J.E. and Brackett, R.E. 1995. Factors affecting aflatoxin B1 removal by *Flavobacterium aurantiacum*. *J. Food Prot.* 58(1):91-94.

- Line, J.E., Brauhel, R.E. and Wilkinson, R.E. 1994. Evidence of degradation of Aflatoxin B1 by *Flavobacterium aurantiacum*. J. of Food Prot. 5: 788-791.
- MAFF.1996. MAFF-UK Aflatoxin contamination of peanut butter and peanuts (February 1995) *In: Food Surveillance Sheets*[Online], Available: <http://www.archive.food/infsheet/1996/no78/78afla.htm>
- Marth, E.H. and Doyle, M.P. 1979. Update on molds: degradation of aflatoxin. Food Technol. 1(1):81-87.
- Miller, J.D. 1991. Significance of grain mycotoxins for health and nutrition. *In: Fungi and Mycotoxins in Stored Products* Champ, B.R., Highley, E., Hocking, A.D., Pitt, J. I. (Eds.) ACIAR Proceedings Series 36, 125-135.
- Pattee, H.E. and Young, C.T 1982. *Peanut Science and Technology*. p. 569-599, American Peanut Research and Education Society, Inc., Texas, U.S.A.
- Samarajeewa, U., Sen, A.C., Cohen, M.D. and Wei, C.I. 1990. Detoxification of aflatoxins in foods and feeds by physical and chemical methods. J. Food Prot. 53:489-501
- Takeuchi, Y., Kawabata, A. and Mazumoto, Y. 1970. New skinning process. Peanut J. Nut World. 50:12.
- Wagstaff, J. 1993. Epidemiologic studies of the association of aflatoxin exposure and human liver cancer. . *In: "A Perspective of Aflatoxin in Field Crops and Animal Food Products in the US: A Symposium"*. [Online], Available: <http://www.inform.umd.edu>.
- Whitaker, T.B. and Wiser, E.H. 1969. Theoretical investigation into the accuracy of sampling shelled peanuts for aflatoxin. J. Am. Oil Chem. Soc. 46:377.
- Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1974. Variability of aflatoxin test results. J. Am. Oil Chem. Soc. 49:590.
- Whitaker, T.B., Dickens, J.W., and Monroe, R.J. 1979. Variability associated with testing corn for aflatoxin. J. Am. Oil Chem. Soc. 56:789.
- Whitaker, T.B., Whitten, M.E., and Monroe, R.J. 1976. Variability associated with testing cottonseed for aflatoxin. J. Am. Oil Chem. Soc. 53:502.
- Woodroof, J.P. 1983. *Peanut: Production, Processing, Products*, 3<sup>rd</sup> Ed. AVI Publishing Co., Inc., Westport, Connecticut.

**APPENDIX A**

**DEVELOPED GUIDELINES  
ON THE  
MANUAL SORTING  
OF AFLATOXIN-CONTAMINATED  
PEANUT KERNELS**



**MGA GABAY sa  
PAGPILI at PAGHIHIWALAY ng  
MGA BUTONG MAY AFLATOXIN  
MULA sa MANI na GINAGAMIT sa MGA  
PRODUKTO ng MARIGOLD COMMODITIES  
CORPORATION (MCC)**

*(Guidelines on Sorting Aflatoxin-contaminated Kernels  
from the Raw Peanuts Used for Products of Marigold Commodities  
Corporation)*





## Paunang Salita

Ang mani ay isang popular na pagkain dahil sa kanais-nais na lasa nito. Ito ay isang pangunahing sangkap sa mga produktong ginagawa sa kumpanyang ito, tulad ng *Kare Kare Mix*.

Lingid sa kaalaman ng nakakarami sa atin, ang mani ay maaaring tubuan ng amag habang ito ay hindi pa isinasama sa pagproseso ng mga produkto. Ito ay maaaring mangyari mula sa lupa na pinagtataniman hanggang sa bodega na pinagtataguan ng mani bago gamitin sa nasabing pagproseso ng mga produkto.

Ang mga amag na ito ang nagiging sanhi at siyang pinagmumulan ng tinatawag na "aflatoxin". Ang "aflatoxin" ay maaaring makapagdulot ng sakit kung hindi ito maaagapang tanggalin sa mani na ginagamit sa pagproseso ng mga produkto. Dagdag pa rito, ang mga produkto na may mataas na lebel ng "aflatoxin" ay hindi pinapayagan ng mga ahensiyang katulad ng Bureau of Food and Drugs (BFAD) sa Pilipinas at ng US Food and Drug Administration (USFDA) sa Amerika, na maipagbili sa mga tindahan. Ito ay upang mapangalagaan ang kalusugan ng mga mamimili.

Lubos na tataas ang kalidad o "quality" ng mga produkto ng Marigold na sinasamahan ng mani kung ang mga buto ng mani na may "aflatoxin" ay maihihiwalay at hindi makakasama sa pagproseso ng mga nasabing produkto. Sa ganitong pamamaraan, makakaasa tayong lahat, kasama ng mga mamimili, na ligtas at malinis ang produkto ng kumpanyang ito sa pamilihan.

Subali't ang lahat ng ito ay mangyayari lamang kung ang mga pamamaraang gagamitin sa planta ay sumusunod sa mga alituntunin ng kalinisan na nasasaad sa mga probisyon ng GMP (Good Manufacturing Practices).

## FOREWORD

*Peanuts are popular food items because of its good taste. It is one of the major components of products being manufactured by this company, like the Kare Kare Mix.*

*However, molds may grow on peanuts before they are used in the processing of products. This may happen from harvesting the peanuts up to storing before use in the process. These molds produce "aflatoxin". "Afltoxin" can make a person sick if this is not removed from peanuts that are being processed. In addition to this, peanut products with high levels of "aflatoxin" are not permitted by the Bureau of Food and Drug (BFAD) of the Philippines and US Food and Drug Administration (USFDA) in America to be sold in the market. This is done to protect the consumers.*

*Peanut products of this company can be assured of higher quality by sorting aflatoxin-contaminated seeds from the raw peanuts to make sure that peanuts being processed are aflatoxin-free. In this way, the company and all consumers can have safe and clean products from Marigold.*

*Let us not forget, however, that this will only be possible if everyone will adhere to proper and sanitary practices in manufacturing as specified and recommended in GMP (Good Manufacturing Practices) Guidelines.*

- 1        Magtimbang ng 50 kilo ng hilaw na mani. Kailangang eksakto ang timbang ng mani. Lubhang napakahalaga at importante ang pagtimbang dahil ang tagal ng pag-init ng mani sa roaster ay depende sa dami nito.  
*Weigh 50 Kg of raw peanuts. The roasting time that will be applied on the peanuts is dependent on its weight.*
  
- 2        Ilagay sa roaster ang mani.  
*Feed the peanuts into the roaster.*
  
- 3        Isarang mabuti ang roaster. Ito ay kailangan upang hindi matapon ang mga mani habang niluluto.  
*Tightly close the roaster. Closing the roaster properly will prevent the spilling of peanuts during the roasting process.*
  
- 4        Paikutin muna ang roaster.  
*Turn on the roaster rotator first.*  
Buksan at sindihan ang kalan sa pinakamalakas na apoy. Kailangang umiikot muna ang roaster na may mani bago buksan at sindihan ang kalan upang hindi masunog ang mani na nasa ilalim ng roaster kung ito'y hindi umiikot kapag binuksan ang kalan.  
*Turn on the burner to full setting. The roaster should be made to rotate first before turning on the burner so that peanuts at the bottom of the roaster will not get burnt.*
  
- 5        Painitin ang mani ng 45-55 minuto. Kailangang eksaktong 45-55 minuto ang pagpapainit ng mani. Kung mas maiksi ang oras ay mahihirapan sa pagtanggap ng balat. Kung mas mahaba naman ang oras, ay maaaring lumampas sa ninanais na kulay ng mani at hindi na makita ang mga palatandaan ng may "aflatoxin" na buto.  
*Blanch for 45-55 minutes. If less than 45 minutes, de-skinning will be difficult to perform. If blanching is longer than 55 minutes, color development will be more intense and signs of aflatoxin-contamination on peanuts may not be seen.*
  
- 6        Patayin ang kalan subalit huwag ang rotator. Pabayaang umiikot pa rin ang roaster matapos patayin ang kalan. Ito ay upang hindi masunog ang mga mani na nasa gitna ng roaster dahil sa maiipong init dito kung ihihinto ang roaster.  
*Turn off the burner only, not the rotator. This is done so that peanuts in the middle of the roaster will not get burnt.*

- 7 Ilagay ang tray sa ilalim ng roaster.  
*Put the tray below the mouth of the roaster.*
- 8 Ihinto ang pag-ikot ng roaster.  
*Turn off the roaster rotator.*
- 9 Itaob ang roaster para maisalin ang mani sa tray.  
*Tilt the roaster to transfer content to the tray.*
- 10 Palamigin ang mani, hanggang ito ay nahahawakan na ( $\approx 45^{\circ}\text{C}$ ), sa pamamagitan ng dalawang bentilador. Haluin paminan-minan ang mani para mapabilis ang paglamig nito. Ito ay upang hindi magpatuloy ang "pagkaluto" ng mani at hindi sumobra sa kulay na ninanais na makuha at upang mapadali ang pagtanggap ng balat ng mani. Ang balat ng mani ay nakakapit pang mabuti sa buto kapag mainit pa ang mani matapos ang "blanching" dahil mataas pa ang taglay nitong tubig.  
*Cool the peanuts with the aid of 2 electric fans and mix occasionally to facilitate cooling. This is done to stop the "roasting" process and to maintain the desired color of the blanched peanuts. When peanuts are still hot, the skin are not easily removed because its moisture content is still very high. Cooling ends when peanuts can be handled by the workers ( $\approx 45^{\circ}\text{C}$ ).*
- 11 Ilipat ang pinalamig na mani sa malaking timba na may plastic na malinis.  
*Transfer cooled peanuts in a big pail with clean plastic lining.*
- 12 Ilipat sa lugar na tanggalan ng balat.  
*Transfer to a de-skinning room.*
- 13 Tanggalin ang balat sa pamamagitan ng makinang pantanggap ng balat. Kailangang ang nagpapaandar ng makina ay bihasa o eksperto sa paggamit nito dahil kailangang iwasang madurog ang mani. Kapag nadurog ang mani ay hindi na makikita ang mga palatandaan ng kontaminasyon ng "aflatoxin".  
*Remove the skin by using a peanut de-skinner. A trained worker should handle the equipment and he should take extra care not to crush the peanuts while de-skinning. If peanuts are crushed, sorting for aflatoxin-contaminated peanuts will be difficult to perform.*

- 14 Tapatan ng bentilador na may lakas na no. 3 ang mani na napadaan na sa makinang pantanggal ng balat para mahiwalay o liparin ang mga balat.  
*Separate the skins from the de-skinned peanut kernels by passing the peanuts that came from the des\*kinner in front of an electric fan set at no. 3.*
- 15 Ilipat sa maliwanag na kuwarto. Kailangang maliwanag ang ilaw upang makitang mabuti ang mga palatandaan ng kontaminasyon ng "aflatoxin".  
*Transfer to a well-lighted room. Adequate lighting is needed so as to see clearly the aflatoxin.*
- 16 Ilagay ang nabalatang mani sa malaking lamesang patungan.  
*Place the de-skinned peanuts in a big tabletop tray.*
- 17 Tanggalin ang mga mani na sira o hindi angkop sa pagproseso at ang mga may palatandaan ng kontaminasyon ng *aflatoxin* o may amag. Tanggalin din ang balat ng mga butong hindi nabalatan ng makina upang makita ang mga palatandaan ng kontaminasyon.  
*Remove mold-infected and damaged peanuts. Also remove the skin of peanuts that were not properly de-skinned so as to see the aflatoxin-contamination.*
- 18 Ang mga halimbawa ng maning kontaminado ng *aflatoxin* o sira at hindi angkop sa pagproseso ay makikita sa Fig. 2.  
*Examples of aflatoxin-contaminated and damaged peanuts that are not fit for processing are shown in Fig. 2.*
- 19 Ilipat ang mga napiliang mani sa plastic bag na nakapaloob sa malaking timba.  
*Transfer sorted peanuts into a big pail with plastic lining.*
- 20 Itago ang mga napiliang mani hanggang ito ay gamitin.  
*Store sorted peanuts until use.*



**APPENDIX B**

**CALENDAR OF EVENTS ON COLLABORATION WITH  
MARIGOLD COMMODITIES, INC. ON THE ADOPTION OF  
SORTING TECHNOLOGY**





**CALENDAR OF EVENTS ON COLLABORATION WITH MARIGOLD COMMODITIES,  
INC. ON THE ADOPTION OF SORTING TECHNOLOGY**

<b>Date</b>	<b>Activities</b>
November 1997	Plant visit to peanut processors
December 1997	Seminar on optimization
January 1998	Start of collaboration with peanut processors
June 3, 1998	Conducted a Seminar on Technical and Policy Issues
December 1998	Use of technology in production line
April 1999	Trial shipment of aflatoxin-free Kare-kare Mix to the USA
June 1999	Formal turn-over of sorting technology to industry collaborator Signing ceremony First shipment of aflatoxin-free Kare-kare Mix to the USA
January – March 1999 <sup>1</sup> , October 1999	Total production of Kare-kare mix without peanuts - 11,486.24 Kg Total produced for export without peanuts – 264.24 Kg Total produced for the US market – 264.24 Kg
July – December 2000	Total production of Kare-kare mix with peanuts – 29,600.00 Kg Total production of Kare-kare mix with peanuts for export – 5,304.88 Kg Total produced for the US market – 14,640 Kg
January – March 2001 <sup>2</sup>	Total production of Kare-kare mix with peanuts – 14,640 Kg Total produced for export – 1,766.24 Kg Total produced for USA – 1,122.08 Kg

<sup>1</sup>There was sporadic production in 1999 before technology adoption whereas production was continuous from July 2000 to March 2001

<sup>2</sup>Partial data for 2001



## **APPENDIX C**

### **IMPACT MONITORING OF SORTING TECHNOLOGY FOR AFLATOXIN CONTROL IN KARE-KARE MIX**



## **Impact Monitoring of Sorting Technology for Aflatoxin Control in Kare-kare Mix**

R. N. B. Buan, A. O. Lustre, A. R. Cariso, and G. M. Agustin  
Food Development Center, National Food Authority, Department of Agriculture  
Taguig, Metro Manila PHILIPPINES

### **INTRODUCTION**

All projects funded under the Peanut CRSP do not end after the products are launched and adopted by the collaborators. The impact on the industry, the consumer and society as a whole and the potential for sustaining this impact, should be evaluated. This information should be monitored for a length of time adequate to come up with firm conclusions on whether objectives are achieved.

This study was undertaken to monitor perceptible social and economic benefits of adopting the technology of sorting aflatoxin-contaminated peanuts by an industry collaborator in order to eliminate aflatoxin contamination in Kare-kare Mix. The impact was evaluated based on the acceptance/expansion of export shipments for Kare-kare Mix to the U.S. Also, the impact of technology adoption by the industry collaborator on product quality at the market was evaluated. Likewise, other socio-economic benefits that were provided by the transfer of technology were studied.

### **METHODS**

#### **Preparation of a checklist for use in the measurement of the sales performance, production and socio-economic impact.**

A questionnaire was prepared and was used as guide in gathering data on the sales performance of the product, monthly changes in the production volumes, employment opportunities and promotion of women welfare. The questionnaire was provided to the collaborator.

#### **Collection and Verification of Data**

Quarterly interviews with the collaborator were likewise conducted at least once for the period to gather and/or verify information supplied on production volume, sales volume, promotional activities and number of additional hired labor.

#### **Collection and Analysis of Samples**

Samples of Kare-kare Mix were likewise obtained from Metro Manila stores and/or supermarkets on a monthly basis for aflatoxin analysis. Samples collected came from production batches with the highest production for the month, which were supplied by the industry collaborator. Thirteen packs of Kare-kare Mix were either bought in supermarkets and public market or taken directly from the industry collaborator's plant. In addition to the above, six shipments of Kare-kare Mix (with peanuts added) intended for the U.S. market were analyzed prior to shipment to ensure that the product will not encounter any detention due to presence of aflatoxin. Samples were then submitted to the Food Development Center (FDC), Chemistry Section for analysis.

## **RESULTS**

### **Monitoring the Sales Performance, Production and Socio-Economic Benefits**

A total of five quarterly interviews were conducted and in attendance were, Mr. Kim Lapus, General Manager, Mr. Juan Bernad, the Management Trainee for Domestic Sales, Mr. Frank Aguba, Deputy Director for Sales and Marketing, Ms. Oteyza Peñero, R & D Head and Ms. Evangeline Tayag, Production Supervisor.

### **Sales Performance**

Before the introduction of the sorting technology for aflatoxin control, the product sold by the industry collaborator to the U.S. market is a type of Kare-kare Mix without peanuts, to prevent the product from being detained due to aflatoxin contamination. Consumers of the product were required to add peanuts or peanut butter to the sauce during cooking to enhance the peanut flavor, which is a characteristic of the product. After the collaborator adopted the technology, they tried shipping Kare-kare Mix with peanuts to the U.S. initially in small quantities starting June 1999. Since no reports of detention problems were received from its distributor in the United States, the industry collaborator after building confidence started accepting orders. The biggest shipment of the product made to the U.S. was reported in January 2001 at 1,040 Kg (Table 1).

During the monitoring period, the collaborator did not provide any figures of their sales. Only production volumes were provided. Since the collaborator claimed that all products produced for export were based on orders they received, it was then assumed that volume of sales for export are the volume produced for the given period. In an interview conducted on December 6, 2000, Mr. Frank Aguba, Deputy Director for Sales and Marketing, mentioned that the company sometimes had to deny some orders both in the domestic and export market because of the high demand and limited production situation.

Promotional activities were reportedly conducted by the collaborator in the U.S. in November 2000 in the Los Angeles, California area where Filipino population is high. Almost at the same time, an intensified promotion of aflatoxin-free Kare-kare mix with peanuts were conducted in the local market through the "Pistang Mama Sita". The Pistang Mama Sita is a promotional activity where cooking demonstrations and free tasting activities were held in supermarkets and groceries.

### **Production Volume**

The collaborator claimed that they have no production data for the period April 1999 to June 2000 except for the volume shipped to the U.S. market during the period June-October 1999 where they claimed to have produced and exported a total of 246.24 Kg of the product (Table 1). The collaborator however, cannot identify how much was produced and exported on a monthly basis.

A comparison of production for the period of January to March 2001 showed an increase of more than 30.0% from the total production of the same months in 1999. A total of 1,766.24 Kg of Mama Sita's Kare-kare Mix was exported during the first three months of year 2001 comprising 12.1% of total production while the volume produced for the U.S. market made up 7.7% of total production and 63.5% of the total export. All exports to the U.S. market have not been tested by the USFDA.

The fluctuations in the total monthly production levels from July 2000 to March 2001 is not based on sales forecasts but on the availability of the supply of good quality shelled peanuts.

### **Socio-Economic Benefits**

With the adoption of the sorting technology for aflatoxin control, the collaborator reportedly hired five additional male workers to perform the sorting operation. The male workers were preferred because unlike its female counterparts, the male workers can carry the sack-loads of peanuts in the sorting table without any help.

### **Monitoring the Aflatoxin Content in Kare -kare Mix**

For the period March 1999 to January 2001, a total of 21 samples were obtained from the supermarkets and the collaborator's plant. All samples tested negative for aflatoxin content (Table 2). Likewise, samples taken from six shipments to the U.S. tested negative for aflatoxin contamination. According to the industry collaborator, it has not received any reports of detention due to aflatoxin contamination. Above findings are indication of the effectiveness of the sorting technology in controlling aflatoxin in Kare-kare Mix.

### **CONCLUSION**

The technology enabled the collaborator, Marigold Commodities Corp., to export their Kare-kare Mix with peanuts not only to the U.S. but also in Middle East and Hong Kong vis-à-vis zero export of Kare-kare Mix with peanuts before the adoption of the technology. The negative aflatoxin content constantly obtained in samples of products obtained during the monitoring period, built a high degree of confidence on the part of the collaborator, resulting in the development of the "Java" sauce adapting the sorting technology as a major step in its production.

The improved quality of their product i.e. addition of peanuts, have contributed in the increase in sales. The adoption of the technology also enabled the collaborator to develop a related product which will be initially named as "Java Sauce"

**Table 1. Total production volume of Kare -kare Mix (with peanuts added), volume for export and volume produced for the U.S. market<sup>1</sup>**

Production Date (Month)	Total Production (Kg)			Total Production For the Export Market (Kg)			Total Production for the U.S. Market (Kg)		
	1999	2000	2001	1999	2000	2001	1999	2000	2001
January	3,920.00	-	3,120.00	-	-	1,520.00	-	-	1,040.00
February	3,880.00	-	5,760.00	-	-	-	-	-	-
March	3,440.00	-	5,760.00	-	-	246.24	-	-	82.08
April	-	-	-	-	-	-	-	-	-
May	-	-	-	-	-	-	-	-	-
June	-	-	-	-	-	-	-	-	-
July	-	6,240.00	-	-	171.36	-	-	171.36	-
August	-	4,240.00	-	-	492.48	-	-	492.48	-
September	-	4,880.00	-	-	41.04	-	-	41.04	-
October	246.24	3,600.00	-	246.24	1,680.00	-	246.24 <sup>2</sup>	143.64	-
November	-	5,920.00	-	-	1,160.00	-	-	53.35	-
December	-	4,720.00	-	-	1,760.00	-	-	880.00	-
Total	11,486.24	29,600.00	14,640.00	246.24	5,304.88	1,766.24	246.24	1,781.87	1,122.08

<sup>1</sup>Based on monthly report submitted to FDC by Marigold Commodities Corp.

<sup>2</sup>The amount represents all shipments made to the U.S. from June to October 1999.



**Table 2. Aflatoxin content in samples of Kare-kare Mix (with peanuts added) taken from supermarkets in Metro Manila, provinces and Marigold Plant.**

<b>Production Date</b>	<b>Place Bought</b>	<b>Date Analyzed</b>	<b>Aflatoxin content (ppb)</b>	<b>Remarks</b>
Feb. 1999	EDSA Central Public Market	Aug. 31, 1999	None detected	Initial sample before sorting was introduced.
July 1999	Rustan's Supermarket, Mandaluyong	Aug. 31, 1999	None detected	3 months after introduction of the sorting process.
Sept. 1999	SM Megamall	Oct. 26, 1999	None detected	
Oct. 1999	Marigold Plant	Dec. 29, 1999	None detected	1 <sup>st</sup> shipment to the U.S.
Jan. 2000	Marigold Plant	Jan. 9, 2000	None detected	2 <sup>nd</sup> shipment to the U.S.
Jan. 2000	Rustan's Mandaluyong	May 11, 2000	None detected	Shipping to the U.S. & Middle East at 300 cases/month average
Feb. 2000	Marigold Plant	Feb. 28, 2000	None detected	3 <sup>rd</sup> shipment to the U.S.
Feb. 2000	SM Makati City	May 11, 2000	None detected	Experienced no detention problems in the U.S.
March 2000	Rustan's Mandaluyong	May 11, 2000	None detected	
April 2000	Sunshine Mall Taguig	July 13, 2000	None detected	
May 2000	Sunshine Mall Taguig	July 13, 2000	None detected	
June 2000	SM Megamall	Aug. 8, 2000	None detected	
July 2000	Makro, Sucat	Aug. 4, 2000	None detected	
August 2000	Rustan's Mandaluyong	May 22, 2000	None detected	
Sept. 2000	Rustan's Supermarket	Nov. 20, 2000	None detected	
Oct. 2000	Landmark Supermarket, Makati City	Dec. 5, 2000	None detected	
Nov. 2000	Landmark Supermarket, Makati City	Dec. 5, 2000	None detected	
Dec. 2000	Agoo Supermarket, Agoo, La Union	Dec. 12, 2000	None detected	Provincial sample.
	Marigold Plant	Dec. 12, 2000	None detected	Produced for the U.S. market. 4 <sup>th</sup> monitoring batch
Jan. 2001	Marigold Plant	Jan. 18, 2001	None detected	Produced for the U.S. market. 5 <sup>th</sup> monitoring batch.
	Marigold Plant	Jan. 18, 2001	None detected	Produced for the U.S. market. 6 <sup>th</sup> monitoring batch.

..









