

Generic Plan of Food Safety Management System Based on ISO 22000:2005 for Aflatoxin Control in Raw Pistachio Processing Units from Raw Material Reception to Packaging

A. Fallah ^{1*}, M. Bolandi ¹, L. Nouri ¹

¹Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran

Abstract: Pistachio is one of the most important agricultural crops of Iran. It is a nut from *Anacardiaceae* family and its domesticated species is called *Pistacia vera* L. Regarding to pistachio importance and usage and by the expanding of pistachio cultivate, it is necessary to improve agricultural situation and by establishing well equipped processing and packaging units near the farms, it is possible to supply high quality and quantity of products for foreign and domestic markets. In this case, aflatoxin is one of the major problems. It is *Aspergillus* metabolite produced in good humidity and temperature condition. Thus for pistachio safety, usage of effective scientific methods based on preventive action in pistachio production chain from planting to packaging, storing and distribution is recommended. Since quality assurance systems and process control based on ISO 22000:2005 can be a good method for aflatoxin contamination control, in this research, ISO 22000:2005 requirements were implemented in a pistachio processing unit and aflatoxin B1 content was measured by HPLC before and after six stages of processing including washing, immersing pool, wet sorting, drier, temporary storing and dry sorting. After analyzing measured data, the most reducing stage in aflatoxin content was wet sorting and drying and temporary storing had the preventing role in aflatoxin content, so they had no significant effect in aflatoxin reducing. As a whole it is concluded that OPRP and CCP have no difference important in hazard control but they are different from type of control measure aspect. Besides for implementing FSMS effectively, suitable prerequisite programs are necessary.

Keywords: Aflatoxin, CCP, ISO22000, OPRP, Pistachio

INTRODUCTION

Pistachio is a nut from *Anacardiaceae* family and its domesticated species is called *Pistacia vera* L [6, 17]. It has high nutrition value and contains 57.4% fat and 21.7% protein. Its unsaturated fatty acids content is 89.8% that 18.2% of them are PUFA (Poly Unsaturated Fatty Acids). Pistachio is rich in intakeable iron so it is known as a blood maker food.

Based on FAO statistics in 2008, the cultivated area in the world was 609 hectares that 440 hectares of

it belongs to Iran. Iran is the biggest producer and exporter of pistachio in the world [17].

Regarding to pistachio importance and usage and by the expanding of pistachio cultivate, it is necessary to improve agricultural situation and by establishing well equipped processing and packaging units near the farms, it is possible to supply high quality products for foreign and domestic markets. In this case, aflatoxin is one of the major problems. It is *Aspergillus* metabolite produced in good humidity and temperature

*Corresponding author: A. Fallah, Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran E-mail: amif81@gmail.com

condition. *Aspergillus flavus* and *Aspergillus parasiticus* can grow in 10°C to 45°C. Their optimum growth temperature is 25°C to 32°C and aflatoxin is produced in 25 °C to 28°C [16, 19]. Aflatoxins are considered as the potentially carcinogen compounds and consist of several type that their toxicity is B1>G1>B2>G2. Among them aflatoxin B1 is considered to be the most potent carcinogen [5, 7, 17]. In recent years European Union determines the maximum residue limit of aflatoxin B1 as 2ppb [17], but according to Iran national standard no.5925 it is 5ppb.

Thus for pistachio safety, usage of effective scientific methods based on preventive action in pistachio production chain from planting to packaging, storing and distribution is recommended. So quality assurance systems and process control based on ISO 22000:2005 can be a good method for aflatoxin contamination control. ISO 22000:2005 is the requirements of food safety management system that is edited by the ISO/TC34 technical commission of international organization for standardization (ISO) and published in 2005. This standard consists of 8 clauses that its applicable clauses start at clause no.4 [13].

In 2011 Imad Gaaloul and his colleagues assessed the implementation of ISO 22000 in cereal food industry “SMID” in Tunisia and determined the survival of cereal insects in raw material storage (wheat), the presence of foreign bodies in cleaning, contagion and proliferation of mushrooms in moistening, The proliferation of mushrooms and insects in Transfer and storage of finished products (flours end semolina), etc as CCPs [9]. In 2011 Alessandra J. Weyandt and his colleagues surveyed on environmental and food safety management systems, according to ISO 14001 & ISO 22000 in fish processing plants and determined related PRPs, OPRPs and CCPs [1].

MATERIALS AND METHODS

In this research, ISO 22000:2005 requirements

were implemented in a pistachio processing unit that is described briefly as below.

Food safety management system requirements

Food safety management system (FSMS)

Requirements of this clause was divided into 2 sub clause about hazard identification and documentation requirements, that for this reason document and records control procedure were written [13, 15].

Management Responsibility

The basic requirement for establishing and developing of this standard is management commitment. Food safety policy is a document that defines overall intentions of the organization expressed by top management. FSMS planning shall ensure that the integrity of the FSMS is maintained when changes happen in the organization. Organization must ensure that sufficient information on issues concerning pistachios safety is available throughout the pistachios chain by internal and external communication. Also Top management shall establish, implement and maintain procedures to manage potential emergency situations and accidents that can impact pistachios safety and which are relevant to the role of the organization in the pistachios chain. At the end, top management reviewed the organization's food safety management system at planned intervals to ensure its continuing suitability, adequacy and effectiveness [13, 15].

Resource Management

The organization provided adequate resources for the establishment, implementation, maintenance and updating of the food safety management system. The human resources included food safety team and the other personnel carrying out activities having an impact on pistachios safety was competent and have appropriate education, training,

skills and experience. So training courses must be set by the Ministry of Agriculture and other organizations for the farmers and effective persons in GAP (Good Agriculture Practices) chain to improve pistachios safety. The scope of these training courses must be in some extent that personnel be informed with aflatoxin production factors and the relevant preventive actions. The organization shall provide the resources for the establishment and maintenance of the infrastructure and work environment needed to implement the requirements of this International Standard [13, 15].

Planning and Realization of Safe Products

This clause contains ten sub clauses. It defines how to manage and control potential hazards of pistachio chain. First step is implementing PRPs (Prerequisite Programmes). PRPs are basic conditions and activities that are necessary to maintain a hygienic environment throughout the pistachio chain [13]. Some of them are:

- Good placement for garden making (spring coldness, hot & long summer, low relative humidity, raining time, etc)
- Suitable variety selection (low early split percentage)
- Suitable distance between trees
- Pre harvesting factors (suitable feeding, regular irrigating, good recision, pest control, etc)
- Harvesting factors
- Post harvesting handling
- Processing conditions
- Post drying storage conditions
- End processing [3, 4, 14, 18]

The next step is risk assessment that must be done as below:

- Food safety team appointment

At first for full understanding of FSMS and the ability for identification of hazards and CCPs (Critical Control Points) and OPRPs (Operational

Prerequisite Programmes), organization set the multidisciplinary team [13, 15].

- Raw materials and product description and intended use

Food safety team described Pistachio that must conforms to Iran national standard No.15 and No.5925 about mycotoxin acceptable level and its intended use as shown in Table 1.

- Flow diagrams preparation

Flow diagrams, process steps and control measures had be defined and verified by food safety team as shown in Fig 1.

- Hazard analysis and control plans

After above preliminary steps all food safety hazards that are reasonably expected to occur in relation to the aflatoxin production and increasing were identified by food safety team as shown in Table 2.

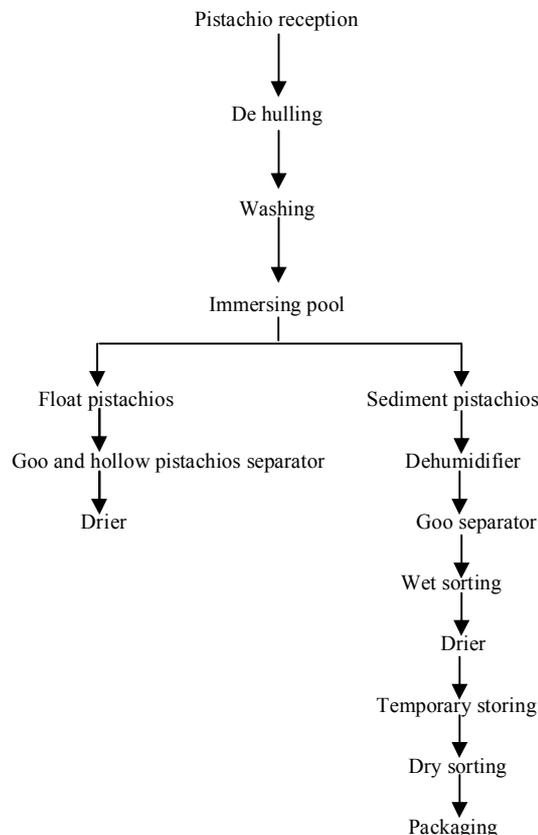


Fig 1. Process flow diagram

Table 1. Product description work sheet

Product name: raw pistachio
Biological, chemical and physical characteristic: Pistachio must have no unmoral odor and taste, rancidity and ever kind of alive pest. The maximum level of extracted peroxide must be one meq.gr/Kg. Maximum water content must be 5% and the water activity must be less than 0.7. Aflatoxin B1 and total aflatoxin respectively must be less than 5ppb and 15ppb or must conform to the customer requirements. Other characteristics must meet the Iran national standard no.15 [11].
Shelf life and storage conditions: In a good condition including relative humidity less than 65%, temperature less than 10°C and away from the sun light, shelf life can be 2 years [10].
Packaging: Raw pistachio can be packed in sacks made from natural or synthetic yarn with high diameter (120gr/m ²), or cartoons or every legally permitted packaging materials that have suitable strength. If packaging is not in vacuum or inert gas filled shape, air conditioning must be considered. Packaging materials must be clean, dry, safe and odorless and keep the product safe during handling and distributing.
Labeling: Labeling must be done according to clause no. 11 in Iran food law and Iran national standard no.2135 and must contain at least name and grade of product, production and expire date, batch number, net weight, production license number, and storage condition.
Distribution methods: It has no particular condition for distribution.
Intended use: This product can be use directly for all range of age or use as the raw material for further processing in other industries.

Table 2. Potentially hazards affecting contamination to aflatoxin

Stage	Hazard	Control measure
Pistachio reception	Production or increasing of aflatoxin because of long time between harvesting and processing	- Suitable GAP - Good time tabled pistachios reception by coordinating with farmers - Processing based on reception batches sequence (FIFO [First In First Out])
De hulling	No hazard detected	-
Washing	Cross contamination because of potentially contaminated water	Supplying hygienic water
Immersing pool	Inadequate separation of contaminated pistachios	- Immersing pool function monitoring
Dehumidifying	No hazard detected	-
Goo separating	No hazard detected	-
Wet sorting	Remaining contaminate suspicious pistachios	- Personnel training - Wet sorting belt monitoring
Drying	Aflatoxin contamination because of bad controlling of drying temperature and time	- drying temperature and time controlling till moisture content reach to less than 6%
Temporary storing	Aflatoxin contamination because of increasing of relative humidity and temperature of stores	Suitable GSP (Good Storage Practices)
Dry sorting	Remaining contaminate suspicious pistachios	- Personnel training - Dry sorting belt monitoring
Packaging	No hazard detected	-

After hazard identification, risk assessment was done by suitable method based on the possible severity of adverse health effects and the likelihood of their occurrence. In this way food safety team used the scaling method from 1 to 4 that 1 was the least and 4 was the most possible severity of adverse health effects or the likelihood of hazard

occurrence. The risk number was calculated by multiplying the severity number in likelihood of occurrence number, so the risk number above 5 considered as the actual hazard that must be controlled by the HACCP plan or OPRP plan. This risk assessment is shown in Table 3.

Table 3. Risk assessment

Stage	Hazard	Severity of hazard	Likelihood of hazard occurrence	Risk	Actual hazard
Pistachio reception	Production or increasing of aflatoxin because of long time between harvesting and processing	3	4	12	√
Washing	Cross contamination because of potentially contaminated water	3	2	6	√
Immersing pool	Inadequate separation of contaminated pistachios	3	3	9	√
Wet sorting	Remaining contaminate suspicious pistachios	3	3	9	√
Drying	Aflatoxin contamination because of bad controlling of drying temperature and time	3	2	6	√
Temporary storing	Aflatoxin contamination because of increasing of relative humidity and temperature of stores	3	2	6	√
Dry sorting	Remaining contaminate suspicious pistachios	3	2	6	√

The next step was selecting suitable control plan which was OPRP plan or HACCP plan. This selection was done regarding to the type of main control measure. If the control measure was as the type as the prerequisite programmes, it considered OPRP plan otherwise it considered HACCP plan. OPRP plan and HACCP plan are the same with a bit different in critical limits of the CCPs in HACCP plan. The mix OPRP plan and HACCP plan is shown in Table 4 [2, 12, 13, 15].

correction and corrective action procedures, and set traceability and withdrawal procedures.

Validation, Verification and Improvement of FSMS

This clause includes five sub clauses that demonstrate planning and performance verification, measuring equipment calibration and the requirements related to auditing, improvement and updating [8, 13, 15].

After planning OPRP plan and HACCP plan the organization defined verification planning,

Table 4. HACCP plan / OPRP plan

No.	Stage	Hazard	OPRP / CCP	Critical limit	Monitoring	Correction / Corrective action
1	Pistachio reception	Production or increasing of aflatoxin because of long time between harvesting and processing	OPRP	-	Recording harvest starting time and processing starting time	- Rejecting input pistachios - Processing according to nonconformity batch
2	Washing	Cross contamination because of potentially contaminated water	OPRP	-	Water safety tests	- Water refinery correction - Processing according to nonconformity batch

Con.Table 4. HACCP plan / OPRP plan						
No.	Stage	Hazard	OPRP / CCP	Critical limit	Monitoring	Correction / Corrective action
3	Immersing pool	Inadequate separation of contaminated pistachios	CP	-	Visual inspection	- Identifying , separation and recycling of nonconformities to Immersing pool - If it is impossible to do as above out the batch from processing - Machine operation correction
4	Wet sorting	Remaining contaminate suspicious pistachios	CP	- Max. 5% Goo - Max. 5% contaminated suspicious pistachios	Sampling and test of each batch	- Resorting - If it is impossible to do as above out the batch from processing - Optimizing sorting stage
5	Drying	Aflatoxin contamination because of bad controlling of drying temperature and time	CCP	- In cabin drier, Stopping time min. 3 hr. & at temp. min. 55°C - In solar drier, Stopping time min. 24 hr. at room temp. - Final moisture content of pistachios is max. 5%	- Controlling and recording drying time and temperature - Moisture content test	Continue drying to 6% or less moisture content
6	Temporary storing	Aflatoxin contamination because of increasing of relative humidity and temperature of stores	OPRP	-	Controlling and recording relative humidity and temperature of stores	Suitable conditioning up to desire relative humidity and temperature of stores
7	Dry sorting	Remaining contaminate suspicious pistachios	CCP	- Max. 1% Goo - Max. 1% Off color pistachios - Max. 5% damaged - Max. 1% bad shape	- Visual inspection - sampling and physical test from each batch	- Resorting - If it is impossible to do as above out the batch from processing - Optimizing sorting stage

After implementation of ISO 22000:2005 requirements, aflatoxin B1 content was measured by HPLC method before and after six stages of processing including washing, immersing pool, wet sorting, drying, temporary storing and dry sorting and the received data were analyzed.

Aflatoxin measurement

Materials and standard solution

The slurry was prepared by extra pure methanol

with concentration $\geq 99.9\%$ and extra pure N-hexane with concentration $\geq 95\%$. Phosphate buffered saline solution (PBS) was prepared by 0.2 gr. KCl 99.0-100.5% mixed with 0.2 gr. Phosphate dihydrogen sodium 98-100.5% and 8 gr. NaCl $\geq 99.5\%$ and 1.16 gr. anhydrous phosphate hydrogen disodium 99.0-100.5% or 2.92 gr. Phosphate hydrogen disodium with 12 molecules H₂O and then 900 cc distilled water was added. This mixture was shaken completely and reached to

PH 7.4 by HCl 0.1N or NaOH 0.1N. After that this solution was reached to volume 1000cc in volumetric flask.

Sampling

Three samples before and three samples after six stages of processing were selected according to Iran national standard no.13534 and sent to laboratory with cool box in 4°C.

Extraction procedure

Aflatoxin extraction was done according to Iran national standard no. 6872 with a little modification. At first the samples were blend with distilled water in ration of 1 to 1.5 until the samples changed to paste form called slurry. Then 50 gr. slurry was mixed with 5 gr. Merck grade NaCl and added by 100 cc N-hexane and 120 cc extra pure methanols. Then this mixture was blend for 3 minutes in blender. After that homogen mixture was filtered through a Falten filter paper. A 20 cc volume of filtrate was mixed with 130 cc four times diluted distilled water (electrical conductivity less than 1 μ s/cm), and then it was filtered through a multi-fold cellulosic filter paper. Finally, 70 cc of extract was purified with [Neogen] immunoaffinity column prior to HPLC analysis.

Immunoaffinity column preparation and sample collection

The column was warmed to laboratory temperature and set up in the instrument. It was washed by 10 cc PBS at a flow rate 1-2 drops/s. 70 cc extract was cleaned up through the column at a flow rate 1-2 drops/s, then aflatoxin was eluted slowly from the column at first by 500 μ lit methanol and then by 1000 μ lit methanol HPLC grade. The eluted samples were collected in small glass vials and were added by 1500 μ lit distilled water HPLC grade.

HPLC quantification

The HPLC system (Knauer, Germany) consists of fluorescence detector (RF/10AXL) ($\lambda=254$ nm), [Uve] derivator (LC Tech, Germany), smart line pump 1000 (V7603 10/2005) that supported by Chrome gate software. The separation was carried out on a C18 column (Capital HPLC) (15cm \times 4.6mm) kept constant at 30°C in an oven. The mobile phase consisted of a mixture of acetonitrile/methanol/water (1:2:4, v/v/v), that all of them were HPLC grade. The flow rate of mobile phase was set on 1 ml/min and then 100 μ lit of sample was injected. The quantification of aflatoxin B1 was performed by measurement of peak area at aflatoxin B1 retention time and comparison with the relevant calibration curve (0.4, 2, 2.8, 3.6 ppb, $r=0.9983$).

Statistical analysis

The statistical analysis was performed with the SAS software 9.1 and the data were analyzed by Duncan test in 5% level of probability.

Method validation

In order to validate the methods, we used the recovery test. So the blank without aflatoxin B1 were contaminated with 10ppb aflatoxin B1 and after preparation as described, we injected it to HPLC system for quantification. The recovery factor of the test was calculated as 91.3%. Besides the limit of detection of this method was 0.01ppb.

RESULTS AND DISCUSSION

As shown in Table 5 aflatoxin B1 was analyzed before and after six stages of processing including washing, immersing pool, wet sorting, drying, temporary storing and dry sorting. By analyzing these data in probability value of 0.05, it was found out washing, drying and temporary storing had no significant effect on aflatoxin reduction and only immersing pool, wet sorting and dry sorting reduced aflatoxin significantly.

Besides by comparison the differences between aflatoxin B1 content in before and after immersing pool, wet sorting and dry sorting we can observe that the maximum aflatoxin B1 decreasing was in immersing pool from scale aspect, but since the initial aflatoxin B1 differed from each stage to another, so the percentage of aflatoxin B1 reduction in each stage was calculated, as it is shown in Table 6. As it is clear, from the aflatoxin B1 reduction percentage aspect, wet sorting had the maximum effect on aflatoxin B1 decreasing.

CONCLUSION

Based on analyzing data maximum effect on aflatoxin B1 decreasing was in wet sorting, because in this stage the mechanism of operation is separation of spotted, immature, pest affected, etc. kernels that have the most concentration of aflatoxin and since this stage is at the beginning of pistachio processing and the aflatoxin B1 concentration is high at this stage, so it has the most reducing effect. Some stages like drying or temporary storing are preventive treatment for aflatoxin increasing, so they had no significant effect on aflatoxin reduction.

Table 5. Aflatoxin B1 content before and after six stages of pistachio processing. The values are shown mean value ± standard deviation

stage	Aflatoxin B1 before treatment (ppb)	Aflatoxin B1 after treatment (ppb)
washing	100.08±4.44	96.34±3.76
immersing pool	97.25±2.35	45.50±4.06
wet sorting	43.13±2.87	3.94±0.70
drier	4.07±0.86	4.61±0.61
temporary storing	4.81±0.98	6.86±1.08
dry sorting	6.92±1.05	1.33±0.42

Table 6. Aflatoxin B1 content reduction in pistachio processing. The values are shown mean value ± standard deviation and by percentage

stage	Aflatoxin B1 content reduction mean	Aflatoxin B1 content reduction percentage (%)
immersing pool	51.76±1.74	53.22
wet sorting	39.19±3.57	90.86
dry sorting	5.60±0.63	80.84

As a whole it is concluded that OPRP and CCP have no difference important in hazard control but they are different from type of control measure aspect. Besides for implementing FSMS effectively, suitable prerequisite programs are necessary. So it is not true to say the maximum hazard control is done in CCP, but CCP is the last point for hazard control and must be concentrated for the most monitoring actions.

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