

## Microbiological Quality of Tehena and Development of a Generic HACCP Plan for its Production

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**Abstract:** Tehena, a traditional food in Jordan and neighboring countries, is produced by milling of cleaned, dehulled roasted sesame seeds by two manufacturing methods, the traditional (wet) method and the improved (dry) method. The aerobic plate count (APC) of tehena samples from 14 plants directly after production ranged between  $1 \times 10^2$  cfu g<sup>-1</sup> and  $4 \times 10^4$  cfu g<sup>-1</sup>, with an average of  $5.2 \times 10^3$  cfu g<sup>-1</sup>; the average of APC after two and four months was  $3.4 \times 10^3$  cfu g<sup>-1</sup> and  $1.7 \times 10^3$  cfu g<sup>-1</sup>, respectively. Lactic acid bacteria count (LABC) of tehena samples directly after production ranged between <10 cfu g<sup>-1</sup> and  $5.5 \times 10^4$  cfu g<sup>-1</sup>, with an average of  $4.7 \times 10^3$  cfu g<sup>-1</sup>; the average of LABC after two and four months was  $2.2 \times 10^3$  cfu g<sup>-1</sup>,  $1.5 \times 10^3$  cfu g<sup>-1</sup>, respectively. The coliform count of tehena samples directly after production ranged from <10 cfu g<sup>-1</sup> to  $7.5 \times 10^3$  cfu g<sup>-1</sup>, with an average of  $6 \times 10^2$  cfu g<sup>-1</sup>; the average of coliform count after two and four months was  $3.3 \times 10^2$  cfu g<sup>-1</sup>,  $2.4 \times 10^2$  cfu g<sup>-1</sup>, respectively. *Staphylococcus aureus* count of tehena samples directly after production ranged between <10 cfu g<sup>-1</sup> and  $5 \times 10^2$  cfu g<sup>-1</sup>, with an average of  $7.8 \times 10$  cfu g<sup>-1</sup>; the average of *Staphylococcus aureus* count after two and four months was  $5.4 \times 10$  cfu g<sup>-1</sup> and  $3.5 \times 10$  cfu g<sup>-1</sup>, respectively. Yeasts and molds counts of tehena samples directly after production ranged between <10 cfu g<sup>-1</sup> and  $1 \times 10^2$  cfu g<sup>-1</sup>, with an average of  $2.1 \times 10$  cfu g<sup>-1</sup>; the average count of yeasts and molds after two and four months was 9 cfu g<sup>-1</sup> and 3 cfu g<sup>-1</sup>, respectively. *Salmonella* and *Escherichia coli* were not isolated from any of the examined samples. Generally, microbial counts were higher in samples taken from plants which follow the traditional method than those of improved methods plants and in plants in which hygiene conditions were low. Because of its low  $a_w$  (range 0.12-0.18, average 0.16) low microbial load and absence of pathogens, tehena could be categorized as low risk food. Seven critical control points (CCPs) were identified during the hazard analysis conducted for the tehena HACCP plan. Most of the hazards could be controlled by cleaning sesame seeds from foreign matter and by general measures of quality management and good hygiene practice. Control measures, critical limits, monitoring and corrective action have been specified for the hazards at each of the CCPs.

**Key words:** Tehena • HACCP system • generic HACCP plan • microbiological quality • traditional food • sesame

### INTRODUCTION

*Tehena* (*tehineh* or *tahina*), sesame butter, is among the most important traditional foods in Jordan and other neighboring countries in the Middle East. Tehena is produced in specialized factories by milling of cleaned, dehulled and roasted sesame seeds [1]. It is mainly composed of oil and protein. The proximate analysis of tehena produced in Saudi Arabia was as follows, fat (58.9%), protein (24.7%), crude fiber (2.3%), moisture (<1.0%) and ash (3.0%) [2]. The protein is rich in the

sulfur-containing amino acids methionine, cystine and tryptophan. The oil, sesame oil, is highly stable, with oleic and linoleic acid constitute most of the fatty acids. According to the Jordanian Standard [1], tehena should have the following composition (w/w): fat (as sesame oil), minimum 45%, protein, minimum 19%, moisture, maximum 1.5% and total ash, maximum 3.5%.

Tehena is not usually consumed as such; so the importance of tehena, comes from the fact that it is used, commercially and at household level, to prepare some popular traditional dishes. The processed chickpeas with

tehena, humus (hummus or/ hoummous ) b'tehena (a dip made by blending into a paste boiled chickpeas, tehena, lemon juice and garlic), mottabal al-bathinjan (a dip made by the blending of roasted eggplant, lemon juice, garlic and tehena) and tomatoes and tehena salad are examples to such foods as well as some meat sauces [3, 4]. In addition tehena is a major ingredient of halawa (halva, halvah or chalwa), a low moisture confection produced commercially from tehena and a mix of sugar, citric acid and the root extract of *Saponaria officinalis*. Tehena makes 50% of halawa's composition [5, 6].

Tehena seems to have a long shelf life when kept at ambient temperature, because of its low moisture content (<1.5%) [7], which does not permit microbial growth. Although microbial growth in tehena is not possible, viable microorganisms are usually present in this product. In a study of the microbiological quality of tehena produced in Saudi Arabia, Ayaz, Sawaya and Al-Sogair [2] examined 50 tehena samples collected from ten processing plants. Aerobic plate count ranged from 20 to 170000 cfu g<sup>-1</sup>. The counts of coliforms, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and yeast and mold count ranged from <10 to 300, <10 to 400, <10 to 250, <10 to 100, <10 to 120 and <10 to 50 cfu g<sup>-1</sup>, respectively. Two out of ten tehena plants (20%) were positive to *Salmonella*; these plants were old and the workers were not following good manufacturing practice.

Hazard Analysis and Critical Control Point (HACCP) system has evolved as the system of choice to ensure food safety. HACCP system, which is logical, practical and preventive in nature, identifies, evaluates and controls hazards that are significant for food safety; it has the advantage of being implemented at all stages of food chain [8]. Hence, the joint FAO/WHO Food Standards Programme's Codex Alimentarius Commission has adopted the HACCP system and promoted its application. The guidelines for the application of HACCP systems, as described by the Codex Alimentarius Commission [8], have been accepted internationally as a reference for HACCP application.

HACCP system is characterized by being applied individually to a food production process. This makes it possible to develop a generic HACCP system model for the process which could be implemented to the production of the chosen food at different locations. The idea of developing generic HACCP models is that these models, after being adopted by a regulatory or private agency engaged with food safety, could be used as templates for the relevant food sectors. In this way implementation of generic models has the advantage of creating a high level of uniformity among those who apply

the system. Furthermore, generic models could be used as a material for training on HACCP system and as a reference for inspection. Hence the concept of developing generic HACCP System models has been promoted by the Codex Alimentarius Commission [8] and by some governmental agencies responsible for control of food safety, e.g., in the USA and Canada.

This study was undertaken to assess the microbiological quality of tehena produced in Jordan and to develop a generic HACCP plan for the production of tehena.

## MATERIALS AND METHODS

**Sampling:** Fourteen tehena plants in Amman, Jordan were visited in this study. Three samples of the same batch were taken from each tehena plant directly after filling. The weight of the samples was either 450 or 900 g. One tehena container was examined at the day of sampling; the examination was repeated after two months and four months of sampling on one of the remaining containers, respectively. All samples were kept at room temperature (25 ± 2°C).

**Water activity and pH:** Water activity ( $a_w$ ) of tehena samples was measured using the  $a_w$ -equipment Thermo Constanter Novasina (model TH 200, Germany). A pH-meter, Hanna Instruments pH-meter (model HI 8416, UK), was used to determine the pH value of tehena samples by immersing the electrode in part of the sample diluted 1:1 with distilled water.

### Microbiological examination:

**Sample preparation:** Sample container was wiped from outside with 70% ethanol. Tehena was thoroughly mixed by sterilized glass rod and 25 g of the sample were transferred into a sterile screw capped 500 mL conical flask and diluted with 225 mL of sterilized peptone water (0.1%) and shaken vigorously for 3 min [9]. Flasks were left at room temperature for 30 min then reshaken for another 3 min.

**Aerobic plate count (APC):** APC was determined using pour plate technique as described in Food and Drug Administration's Bacteriological Analytical Manual using plate count agar (Himedia) [10]. Duplicate plate were incubated at 35 ± 1°C for 48 h.

**Lactic acid bacteria (LAB) count and confirmation:** Lab count was determined by pour plate technique using

MRS agar (Himedia) [11]. Duplicate plates were incubated at  $35 \pm 1^\circ\text{C}$  for 48 h. Representative colonies from MRS plates were maintained and confirmed as LAB by direct microscopic examination, Gram stain and catalase test [12].

**Yeast and mold count:** Yeast and mold counts were determined by applying pour plate technique and using plate count agar (Himedia) which contained  $100 \text{ mg L}^{-1}$  each of chloramphenicol and chlorotetracycline - HCl. Duplicate plates were used for each count. The plates were incubated at  $25^\circ\text{C}$  for 5 days [13].

**Coliform count:** Coliform count was determined by the pour plate technique with overlay using violet red bile agar (Himedia) [14]. Duplicate plates were incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. Completed test made by streaking material from typical colonies onto plates of eosine methylene blue agar. Confirmation of the presumptive coliform test was done by transferring typical coliform colonies into 2% brilliant green lactose bile broth (Oxoid) and incubating for 48 h at  $37 \pm 1^\circ\text{C}$ . Gas production indicates a positive confirmed test.

**Escherichia coli count:** Representative colonies from those which gave positive confirmed coliform test were transferred onto nutrient agar (Oxoid) slants and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. These isolates were identified using IMViC tests [15].

**Staphylococcus aureus count:** Spreading technique on Baird-Parker agar (Oxoid) with egg yolk was used [16]. Duplicate plates were incubated at  $37 \pm 1^\circ\text{C}$  for 48 h. Typical colonies were selected, transferred onto slants of tryptic soy agar (Oxoid) and incubated at  $37 \pm 1^\circ\text{C}$  for 24 h. Isolates were confirmed as *S. aureus* by Gram stain, coagulase test and DNase test.

**Statistical analysis:** Data were analyzed using Randomized Complete Block Design produced by statistical system. Differences among means of treatments were tested using LSD test. Level of significance were at ( $p \leq 0.05$ ).

**Development of a generic tehena HACCP plan:** Guidelines for HACCP plan development described by the Codex Alimentarius Commission [8] were followed. This included the assembling of the HACCP team (process authorities and the authors), description of tehena and its distribution, identification of the intended use and the consumers, developing and verifying the flow diagram of tehena production and application of HACCP

principles one through seven in the tehena production process.

## RESULTS AND DISCUSSION

**Water activity and pH of tehena:** Water activity ( $a_w$ ) of tehena samples ranged from 0.12 to 0.18, with an average of 0.16. This very low  $a_w$  is due to the fact that tehena is practically free of water [7, 17].  $a_w$  of tehena does not permit the growth of any type of food borne microorganisms, since when  $a_w < 0.60$  microbial growth is not possible [18]. Although very low  $a_w$  stops the microbial growth, a good part of the microbial flora of the food is usually viable and when conditions change favorably regarding  $a_w$ , growth of these microorganisms may take place. Such conditions could be seen in foods in which tehena is a major component and in which  $a_w$  is high enough to permit microbial growth such as humus [3], mottable- al- bathinjan, tomato and tehena salad [19].

The pH of tehena samples ranged from 5.8 to 6.0, with an average of 5.9. Such near-neutrality pH permits the growth of a wide range of foodborne microorganisms [18]. It is generally agreed upon that bacterial growth become slower when the  $\text{pH} < 5$  [18]. So when tehena is used in preparation of other foods, pH of tehena would not be a limiting factor regarding microbial growth. Furthermore, because of its high protein content tehena may contribute to the buffering capacity of the food in which it is incorporated.

### Microbiology of Tehena:

**Microbial flora of tehena:** Table 1 shows ranges and averages of aerobic plate count (APC) and of the counts of lactic acid bacteria (LAB), coliforms, *S. aureus* and yeasts and molds directly after filling and after 2 and 4 months of filling.

In nine of the samples (64%), APC was  $= 1 \times 10^3 \text{ cfu g}^{-1}$ . A significant differences at ( $p \leq 0.05$ ) in the average APC was noticed only in tehena samples tested directly after filling and after four months of storage. Differences in the decrease in APC could be seen between the samples with prolonged storage period. Generally, the average decrease in count was nearly 67% after four months of storage.

Noticable differences could be also observed between LAB count of different tehena plants. In eight of the samples (57%), LAB count was  $< 1 \times 10^2 \text{ cfu g}^{-1}$ . No significant differences at ( $p \leq 0.05$ ) could be noticed between the average of LAB counts after filling and after two and four months of storage.

Table 1: Microbial content (cfu g<sup>-1</sup>) of tehena samples directly after filling and after 2 and 4 months of filling

Microorganisms	Directly after filling		After 2 months		After 4 months	
	Range	Average	Range	Average	Range	Average
Aerobic plate count	1×10 <sup>2</sup> -4×10 <sup>4</sup>	5.2×10 <sup>3</sup>	1×10 <sup>2</sup> -2.9×10 <sup>4</sup>	3.4×10 <sup>3</sup>	1×10 <sup>2</sup> -1.3×10 <sup>4</sup>	1.7×10 <sup>3</sup>
Lactic acid bacteria count	<10-5.5×10 <sup>4</sup>	4.7×10 <sup>3</sup>	<10-2.5×10 <sup>4</sup>	2.2×10 <sup>3</sup>	<10-1.6×10 <sup>4</sup>	1.5×10 <sup>3</sup>
Coliform count	<10-7.5×10 <sup>3</sup>	6.0×10 <sup>2</sup>	<10-4×10 <sup>3</sup>	3.3×10 <sup>2</sup>	<10-3×10 <sup>2</sup>	2.4×10 <sup>2</sup>
<i>Staphylococcus aureus</i> count	<10-5×10 <sup>2</sup>	7.8×10	<10-4×10 <sup>2</sup>	5.4×10	<10-3×10 <sup>2</sup>	3.5×10
Yeast and molds count	<10-1×10 <sup>2</sup>	2.1 ×10	<10-4×10	9	<10-2×10	3

\* Cfu g<sup>-1</sup> = colony forming unit/g

Coliform counts were generally low in tehena directly after processing (< 10 cfu g<sup>-1</sup>). In 11 of the samples (79%), coliform counts directly after filling were <1 × 10<sup>2</sup> cfu g<sup>-1</sup>. A significant differences in average coliform count was noticed only in tehena samples tested directly after filling and after four months of storage.

In all tehena samples *S. aureus* count was < 1 × 10<sup>3</sup> cfu g<sup>-1</sup>; in 4 samples the count was <10 cfu g<sup>-1</sup>. A significant differences in average *S. aureus* count was noticed only in tehena samples tested directly after filling and after four months of storage.

Yeast and mold count was < 1 × 10<sup>2</sup> cfu g<sup>-1</sup> in ten of the samples (71%). A significant differences in average yeast and mold was noticed only in tehena samples tested directly after filling and after four months of storage.

Averages of APC and averages of the counts of LAB, coliforms and *S. aureus* were higher in plants which follow the traditional method of processing (wet method) than in the plants follow the improved method of production (dry method). Average yeast and mold count was slightly less in plants which follow the traditional method of processing (wet method) than in the plants which follow the improved method of production (dry method).

Aerobic plate count and counts of coliforms, *S. aureus*, yeasts and molds found in this study are comparable to those found by Ayaz, Sawaya and Al-Sogair [2] in samples of tehena produced in Saudi Arabia.

**Isolation of salmonella:** Tehena samples examined in this study were found not to contain *Salmonella*, but tehena samples produced under poor hygienic conditions in Saudi Arabia, were found to contain *Salmonella* [2].

**Microbiological quality and safety of tehena:** Lactic acid bacteria and the aerobic mesophilic spores formed the major part the microbial flora of tehena in 4 different tehena samples. Coliforms were predominant

in 3 other samples. In the rest of the samples the flora was a mix of these bacterial groups. Accordingly the microbial flora of tehena, which is bacterial rather than fungal, from different plants is not homogenous and differences could be expected between the different plants. Method of production and level of hygiene and sanitation have a major effect on the numbers and the types of the microorganism. The traditional tehena plants with relatively less hygiene conditions had the highest bacterial counts.

It is believed that water, especially when recycled, could form the main source of contamination by microorganisms, since it is the only place in the whole process of tehena production where microbial growth is possible. In the traditional tehena plants, where filling is semi-manual, filling could be considered as another important source of microbiological contamination. Temperature and/ or time of roasting seem not to be enough to completely destroy the microorganisms present on the sesame seeds.

Solberg *et al.* [20] considered in their program for microbiological safety for foodservice facilities ready-to-eat foods as acceptable when APC and the counts of coliform, *E. coli*, *S. aureus* and *Salmonella* were <10<sup>5</sup>, <10<sup>2</sup>, <3, <20 and <3 cfu g<sup>-1</sup>, respectively. All tehena samples meets these requirements. Since non of the tehena samples proved to contain *Salmonella*, *E. coli* or *S. aureus* in high numbers and since microbial growth in tehena is not possible, tehena could be categorized as low risk food. Being a food of plant origin contributes to the microbial safety of tehena.

A good part of the microbial flora of tehena remain viable for relatively long time, but unable to grow in thena because of its low a<sub>w</sub>. These microorganisms could grow when water content is raised to suitable levels, as when tehena is used in the production of humus and salads. This state is very similar to that of the microbial flora milk powder, which become able to grow after milk reconstitution.

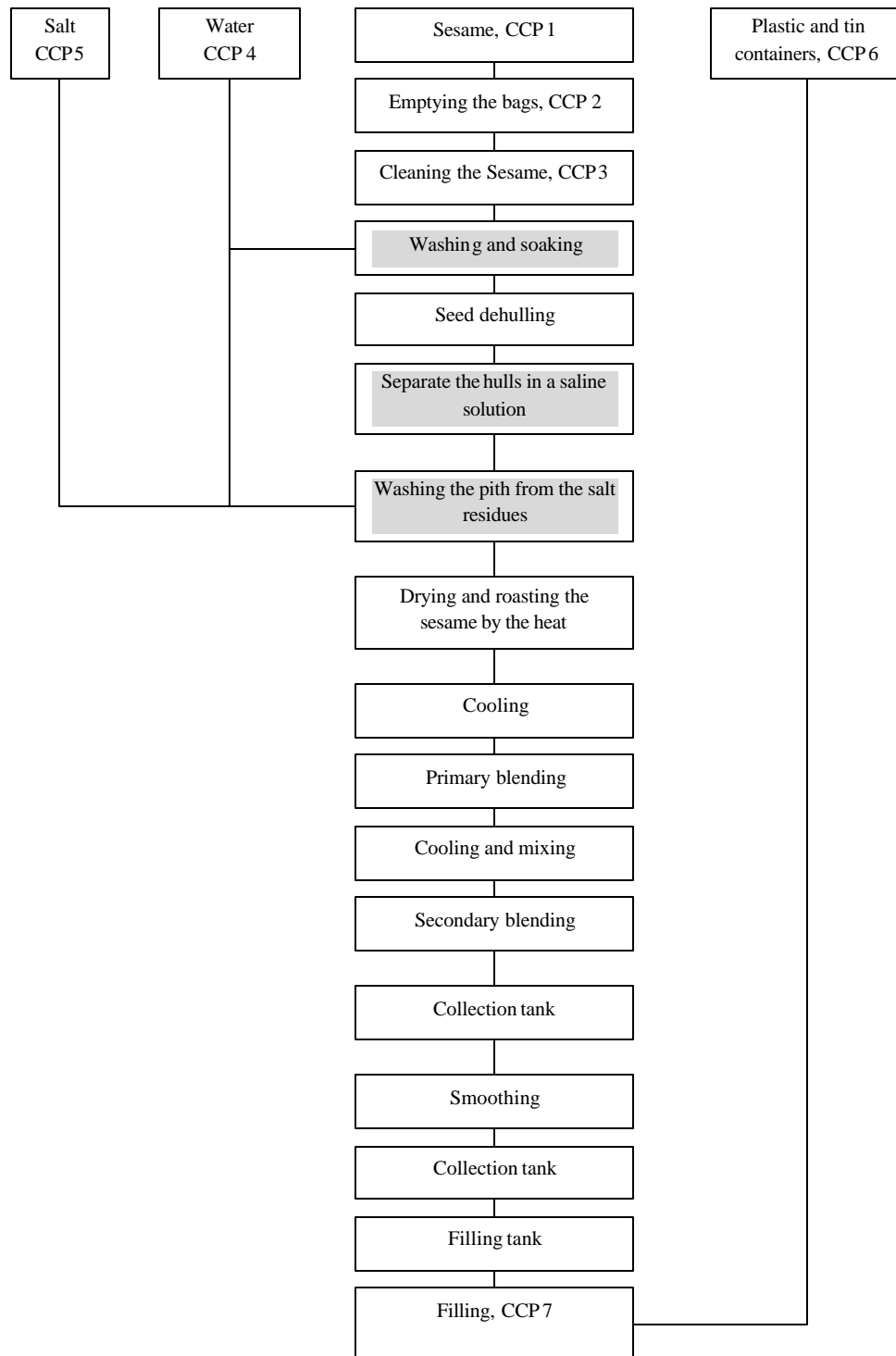


Fig. 1: Process flow diagram for tehena production by the traditional and improved method. Shaded steps are confined to the traditional method

Table 2: HACCP chart for tehana production

Step	Hazard	Control measure	CCP	Critical limit	Monitoring		
					Test	Frequency	Corrective action
Sesame	Biological: insects and toxins of molds	Compliance to legal requirements	2	Sesame legal safety requirements	Check compliance to legal requirements	Each batch	Do not use sesame that do not comply to requirements
	Chemical: pesticide residues	Buy from approved supplier					
Emptying the bags	Biological: insects and toxins of molds	Visual testing	2	Existing of insects and mold growth	Visual inspection	When emptying the bags	Do not use sesame with mold or pest infestation
Cleaning the sesame	Physical: foreign matter	Removal of foreign matter sieves	3	Absence of foreign matter	Check the efficiency of cleaning equipment (sieves, air screen cleaners, density separators, destoners, magnets and aspirator)	Every day	Do not use defect cleaning equipment
Water	Biological: disease-causing microorganisms	Use a potable water supply Assure the adequacy of tanks and the internal net	4	Coliforms not detectable in 100-mL samples	Coliform count	Every month	Do not use water which do not comply to requirements
Salt	Chemical and physical: impurities and foreign matter	Compliance to legal requirements Buy from approved supplier	5	Absence of foreign matter	Visual inspection	Each batch	Do not use salt that do not comply to requirements
Plastic and tin containers	Chemical: toxic chemicals migrating from containers	Using food grade containers	6	Use of food grade containers	Assuring that containers are of food grade.	Daily	Do not use containers that do not comply to requirements
	Biological: disease-causing microorganisms	Keeping the containers in hygienic conditions till use GMP		Proper implementation of GMP	Checking the containers before being used. Check implementation of GMP		
Filling	Biological: insects and disease-causing microorganisms	GMP (pest control and personal hygiene)	7	Proper implementation of GMP	Check implementation of GMP	Daily	Implementation of GMP

### Tehena generic HACCP plan:

**Tehena production and flow diagram:** The process of tehana production is relatively simple, since sesame is the only raw material used and since the steps are straight forward physical and mechanical ones. In Jordan tehana is manufactured in specialized plants most of which are localized in Amman. Some of tehana plants are equipped with improved machines, whereas others are still using the traditional method of manufacturing. However, there are basic steps of production, which are followed in all plants (Fig. 1). The shaded steps in the figure are those still in use in the traditional method of manufacturing, or so-called wet method, which are not used in the the improved dry method. Ten out of the fourteen tehana plant visited in this study were applying the traditional method (wet method) and three of these plants had been using big millstone during milling.

In the traditional method there is a use of large amounts of water. Because of the relatively high cost of water, some of the plants re-use the water for cleaning and soaking. The problem is aggravated by the use of a brine to separate the hulls, since in order to eliminate the salty after-taste in the seeds, large amounts of water may be

needed. These difficulties are not met with in the improved method, in which less amounts of water are used and brine is not used for the separation of the hulls. Furthermore, hulls obtained by the improved method could be used as animal feed, because unlike the hulls of the traditional method, they do not contain residual salt.

The use of the big millstones has a drawback since this part of the process of manufacture is not covered, allowing contamination of the product at this step. This is not the case in the improved method where milling is done in a closed cabinet because the disks used are small.

**HACCP chart:** Hazard analysis (HACCP principle 1) conducted for the HACCP plan of tehana production identified a number of hazards (Table 2) which need to be controlled in order to have a safe product. The steps at which these hazards had been identified were determined (HACCP principle 2) and designated as critical control points (CCPs). Control measures (HACCP principle 1), critical limits (HACCP principle 3), monitoring (HACCP principle 4) and corrective action (HACCP principle 5) were specified for the hazards at the CCPs.

Hazards at most of the CCPs could be controlled by general measures of quality management and good

manufacturing practice (GMP). This confirms the importance of use of sesame which complies to the legal and processing requirements (CCP 1), especially regarding pests and mold growth and the proper implementation of efficient personal hygiene, pest control and cleaning and disinfection (CCPs 2, 7).

Control of the quality of water (CCP 4), salt (CCP 5) and packaging materials (CCP 6) are of paramount importance to ensure the safety of tehena. Cleaning of sesame and removal of foreign matter (CCP3) is the specific CCP in tehena production. Here different combinations of sieves, air screen cleaners, density separators, destoners, magnets and aspirators are used. Validation of the efficiency of cleaning equipment used and monitoring and maintaining them in good condition would ensure having sesame free of foreign matter.

Reviewing of the HACCP plan, internal and third party audits and inspection of the system, especially regarding monitoring of the CCPs and implementation of GMP at short intervals could be a good means to verify proper development, implementation and maintenance of the system (HACCP principle 6). The tailored HACCP plan along with the records necessary for the implementation should be documented in a controlled manner (HACCP principle 7).

Like other generic HACCP models [19], this generic tehena HACCP model is not developed for direct application in all tehena plants. The model need to be used as a starting point for further customization to reflect the particular process and environment. It must be adapted to reflect the specific conditions in each plant.

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