Food Control 36 (2014) 88–93

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Microbiological safety in pistachios and pistachio containing products

Marwa Al-Moghazy [a,b], Silvio Boveri [a], Andrea Pulvirenti [a,*]

[a] Dept. of Agricultural and Food Sciences, University of Modena and Reggio Emilia, Reggio Emilia, Italy
[b] Food science and nutrition division, National Research Center, Dokki, Cairo, Egypt

ARTICLE INFO

Article history:
Received 23 March 2013
Received in revised form
16 July 2013
Accepted 23 July 2013

Keywords:
Pistachio
Salmonella spp.
Klebsiella spp.
Aflatoxins

ABSTRACT

Pistachio nuts, including salted pistachios, unsalted pistachios and shelled pistachios which are ready to eat foods, recently appeared as possible sources of infection with foodborne bacteria besides the high levels of aflatoxins. Several types of pistachio and pistachio containing products were evaluated for the presence of *Staphylococcus aureus*, and *Salmonella* spp; also aerobic mesophilic bacteria, coliforms and yeasts and moulds, were enumerated. The analysis was performed in three different sampling times. Aflatoxins levels were determined using a commercial kit. Unsatisfactory levels of mesophilic, coliforms and yeasts and moulds counts were obtained in 55%, 22% and 15% of the samples respectively. Samples contained $\geq 10^5$ CFU/g moulds were tested for aflatoxins. For *Salmonella* detection, conventional methods were used, and all the suspected colonies on two different selective media were confirmed biochemically. Isolates that biochemically identified as *Salmonella* spp. were confirmed using multiplex PCR which included primers for 16S rDNA (bacteria control), invA (for *Salmonella* spp.) and STM4057 (for *Salmonella enterica* subsp. enterica). Due to presence of 16S rDNA bands only or other bands not explained by any of used primers in multiplex PCR patterns, some isolates were sequenced. Sequencing results revealed that those isolates included *Klebsiella pneumonia* and *Salmonella* sp. These results reveled that pistachios and pistachio products can be a possible source of infection with foodborne bacteria and aflatoxins contamination.

1. Introduction

Export of Italian pistachios (*Pistacia vera* L.), is between 1000 and 2000 tons per year (Anonymous, 2000) and it is consists mainly of shelled and peeled pistachios, whereas imports (about 9000 tons) consist mainly of in-shell pistachios, consumed as salted, roasted snacks and as familiar ingredient in confectionery and ice cream industry for domestic markets and, partially, for re-exportation (United Press International, 2009).

Italian pistachio is growing mainly in Sicily region and 80% of its yearly production occurred in Bronte area (Gentile et al., 2007). Fruits harvesting is done manually because the fruits do not become mature at the same time and also because of the nature of soil in this region. After harvesting and de-hulling, the fruits are dried naturally by sun, or under plastic tunnels to accelerate the drying process which takes 4–5 days in which the temperature varied from 40 to 50 °C (Avanzato, Caruso, Marra, Vaccaro, & Vassallo, 2008). Traditional harvest and post-harvest treatments of pistachio usually occur with no or minimum hygiene procedures increase the probability of contamination with variable microorganisms from different environmental sources such as soil, cattle or other animals, insects, reptiles, human sources and others (Marcus and Amling, 1973; Weinzirl, 1927).

The Codex recommended International Code of Hygienic Practice for tree nuts states that these products should be free from pathogenic microorganisms (CAC, 1972). Pistachio fruits and pistachio-containing products were usually tested for its microbiological quality like mycoflora contamination and aflatoxins levels (Walter, Brunschweiler, Leimbacher, & Schneider, 1992) but recently it's appear to be a possible source of pathogenic bacteria as *Salmonella*. On March, 2009 the US Food Drug Administration (FDA) informed CDC (Centre For Disease Control and Prevention) that multiple samples of pistachio nuts and pistachio-containing products collected over several months from a single company were contaminated with several serotypes of *Salmonella* (CDC, 2009a).

Recently, it was confirmed that *Klebsiella* (Umeh & Berkowitz, 2002) can be epidemic bacteria since outbreak that happened to students of the Indian Institute of Technology. About 600 students got pneumonia symptoms after consuming food contaminated with *Klebsiella* as the stole analysis revealed later (Maryland Department of Health and Mental Hygiene, 2011)

As long before, the nuts like pistachio with low a_w, were not considered as possible source pathogenic bacteria, but actually in many cases low a_w can only stop the reproduction of *Salmonella* but not its surviving (Kinsella et al., 2008; Kotzekidou, 1998).

* Corresponding author. Tel.: +39 0522522004.
E-mail address: andrea.pulvirenti@unimore.it (A. Pulvirenti).

0956-7135/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2013.07.030

Pathogenic bacteria (FREC, 2007) such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* cannot regenerate on nuts, but can survive on these products for periods extended more than one year (Beuchat & Scouten, 2002; Blessington, Mitcham, & Harris, 2012; Uesugi, Danyluk, & Harris, 2006). *Salmonella* was detected in other nuts such as row almond (*Prunus amygdalus*) (CEN, 1998; Danyluk et al., 2007; Eglezos, Huang, & Stuttard, 2008). *Salmonella* also was found in 0.8% of 2886 edible nut kernels collected from retail markets in UK (Little, Rawal, de Pinna, & McLauchlin, 2010). Salmonellosis outbreak which toke place in USA in the years 2006–2007 associated with peanut butter (CDC, 2009b; Zink, 2008).

Tree nuts (almonds, walnuts and pistachio) are subjected to infection by a variety of microorganisms that can cause foodborne illness, spoilage or toxic effects on human (aflatoxicosis). Aflatoxins are secondary metabolites of various strains of *Aspergillus flavus* and *Aspergillus parasiticus* (Abdulkadar, Al-Ali, & Al-Jedah, 2000). Due to their hepatocarcinogenic potential, aflatoxins are highly regulated in many countries around the world. European Community fixed 4 ppb as a maximum level of total aflatoxins (Commission of European Community, 1998), while in USA the FDA (Food and Drug Administration) has set a guidance level for tree nuts of 20 ppb (Food and Drug Administration, 1996).

This article focused on testing the microbiological quality and safety of different pistachio nuts and pistachio containing products as well the effect of packaging type and processing by analysing the aerobic mesophilic bacteria, Coliform, Staphylococcus aureus and *Salmonella* spp. Yeasts and moulds counts were evaluated in all samples and aflatoxins levels were determined exclusively in the samples much more contaminated with fungus. Molecular identification was performed for suspected isolated *Salmonella* spp colonies which obtained from classical selective media. PCR methods have been defined to detect pathogens in clinical and food samples (Lampel, Orlandi, & Kornegay, 2000; Whelen & Persing 1996. Therefore, the present paper is a representation of the overview of PCR-based modern identification of pathogens existing in pistachio and pistachio containing products in addition to conventional microbiological and biochemical analyses.

2. Material and methods

2.1. Sampling

The analyses of the samples were conducted in three different sampling times, for a total period of three month. For each time, nine different kinds of pistachio and pistachio containing products were purchased from local market of Bronte in Italy; type, number and description of samples are listed in Table 1.

2.2. Microbiological analysis

Ten grams of each sample were weighted in sterilized stomacher bag and homogenized in a stomacher using sterile physiological saline solution (0.9% NaCl) and serial dilutions were performed. Pour plate method was applied for aerobic mesophilic bacteria count, using PCA (Plate Count Agar, Oxoid). PCA plates were incubated for 48 ± 3 h at 30 °C. Counting of total yeasts and moulds were performed on SDA (Sabouraud Dextrose Agar, Oxoid), all plates were incubated at 25 °C for 5 days. For total coliforms counts, the method described by APHA (1992) was used. Growth of coliforms was detected using VRBA (Violet Red Bile Agar, Oxoid) incubated at 37 °C for 48 h, while confirmation was performed by subculturing of suspected colonies into BGLB (Brilliant Green Lactose Bile Broth, Oxoid) and monitoring gas production at 37 °C for 48 h, and at 44 ± 0.5 °C for 24 h. To detect and count *Staphylococcus aureus*; 0.2 ml of the first dilution was spread onto a plate of Baird Parker Agar (Oxoid) containing 5% of Egg Yolk Tellurite Emulsion (Oxoid). After incubation at 37 °C (±2 °C) for 48 h (±4 h), the plates were observed for typical colony morphology of *S. aureus*. *Salmonella* detection was performed according BS EN 12824 which used to recover all strains of *Salmonella* likely to cause illness. This method provides by homogenizing 25 g of food sample with 225 mL of buffered peptone water and incubation at 37 °C for 18 ± 2 h. Enrichment mix was subcultured into Rappaport-Vassiliadis (RV, Oxoid) and Selenite Cystine (SC) broth tubes and incubated at 42 °C and 37 °C respectively for 20–24 h. Then, subculturing a loopfull of each broth tube into two selective media BGAm (Brilliant Green Agar Modified, Oxoid) and BSA (Bismuth Sulphite Agar, Oxoid) and put in incubator at 37 °C for 20–24 h; BSA plates should be incubated up to 48 h.

For the biochemical confirmation suspicious colonies were transfered from selective media plates to LDX (Lysine Decarboxylase, Oxoid) broth and put in the incubator for 24 h at 37 °C; positive tubes turns to purple colour.

Table 1
List and description of the samples used in this work.

commercial type of pistachio	n° of specimen *per* sampling time	Code	Utilization
Unshelled pistachio	2	US1, US2	Ready to eat (snack)
Shelled pistachio	1	S	Snack and ingredient
Shelled pistachio (vacuum bag)	1	SV	Snack and ingredient
Pistachio flour (vacuum bag)	1	FV	Ingredient for biscuits and cakes
Pistachio salty cream	2	P1, P2	For pasta and salty snack dressing
Pistachio sweet cream	2	N1, N2	For snack

2.3. Aflatoxins levels assay

Aflatoxins levels were determined by using AFLACHECK commercial kit (VICAM), for the detection of aflatoxins in the samples that gave count of yeasts and moulds more than legal limits (TFC, 2008). A protocol for this kit with a 10 ppb cut-off for total aflatoxins was applied

2.4. Physico-chemical analysis

pH of pistachio's different samples were measured using Crison pH meter (microPH 2002). Water activity values (a_w) were assessed with a Novasina apparatus (Novasina Thermoconstanter TH200) at atmospheric temperature.

2.5. DNA isolation and Multiplex PCR

Boiling method was used for DNA isolation of suspected *Salmonella* spp. isolates (Kawasaki et al., 2005). Cells from 24 h old solid cultures of different isolates were resuspended in 100 μl of Tris–EDTA (TE) buffer and then kept in a hot dry path at 98 °C for 10 min. After cooling in ice for 5 min, the samples were centrifuged at 12,500*g* for 5 min and supernatant transferred to new eppendorf tubes, concentration and purity of the extracted DNA was evaluated by UV–vis analysis, NanoDrop®, Spectrophotometer ND-1000. 2 μl of the supernatant was used directly as a template for Multiplex PCR (Germini, Masola, Carnevali, & Marchelli, 2009).

Primers pairs were chosen according to target genes that reported in literature (Table 1); 16S rRNA as an internal control for bacterial amplification, invA to detect *Salmonella* spp., and STM4057 for *Salmonella enterica* subsp. I. All multiplex PCR reactions were performed in a final volume of 25 μl using 2 μl of extracted DNA as template. The final concentrations of the reagents