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Microbiological quality of ready-to-eat foods of Tehran province

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Recontamination of ready-to-eat (RTE) products during post-processing may be the cause of outbreaks of food-borne disease. In this study, a total of 150 RTE samples were obtained for bacteriological examination (*coliforms*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Bacillus cereus*, *Psychrotrophic bacteria* and *Psychrophilic* bacteria). Various types of RTE food products that contained frozen (cooked and semi-cooked) and refrigerated (cooked) poultry meat foods, were purchased randomly periodically in January and March, 2012. 65% of cooked samples and 62% of semi cooked samples contain more than 10^2 CFU/g coliform, while *S. aureus* was more than 10^2 CFU/g in 35 and 40% of samples, respectively. Also 28% of cooked samples and 44% of semi cooked samples contained *E. coli*. 14% of all samples were contaminated by *Salmonella*. The results for enumeration of *B. cereus*, psychrophilic and psychrotrophic microorganisms were: 2/96 ± 0/09 log CFU/g, 5/02 ± 1/77 log CFU/g and 3/05±0/04 log CFU/g, respectively.

Key words: Foodborne pathogens, coliforms, *Escherichia coli*, *Staphylococcus aureus, Salmonella, Bacillus cereus*, psychrotrophic bacteria, psychrophilic bacteria, cooked, semi-cooked.

INTRODUCTION

According to EC Regulation No. 2073/2005, “microbiological criterion is a criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch” and “‘food safety criterion means a criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market”. Ready-to-eat (RTE) foods have become increasingly popular in the last two decades, particularly in metropolitan areas (Peck et al., 2008). In Tehran, Capital of Iran, there has been a marked increase in the sales of RTE food products in recent years. Familiarity taste, low-cost and convenience are some of the appealing factors that make RTE foods popular as food source. The RTE food products provide a source of readily available and nutritious meals for the consumer. However, questions

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have been raised about the safety and microbiological quality of these food products. The incidence of foodborne illness is increasing worldwide (Kaneko et al., 1996; Mead et al., 2009; Nguz, 2007). High counts of *Escherichia coli* and total coliform (TC) in foods usually indicates lack of hygiene in handling and production operations, inadequate storage and post-process contamination (De Sousa et al., 2002). Therefore, *E. coli* and TC enumeration are used as a food-quality parameter. *Bacillus cereus* is frequently isolated from both the natural environment (soil and growing plants) and foods, meat products, raw meat and meat product additives.

Psychrotrophic and psychrophilic bacteria are the main contributors to the spoilage of sea foods at refrigeration temperatures, in addition they are important in ready to eat food with chicken meat origin. *Salmonella* can frequently be isolated from raw foods of animal origin. Environmental contamination can also result in *Salmonella* being present in a wide variety of foods, although generally at lower numbers. Foods that are frequently implicated in Staphylococcal food poisoning include meat and meat products, poultry and egg products. Enterotoxin production of *Staphylococcus aureus* is also a public health concern owed to its ability to grow in environments of high salt concentration such as salami. Such foods can be important vehicles for infection by *Salmonella*, *Listeria monocytogenes* and *E. coli* O157 (Emberland et al., 2006; Swaminathan and Gerner-Smidt, 2007). The aim of this study was to identify and enumerate *Salmonella*, *Bacillus cereus*, psychrophilic and psychrotrophic microorganisms on frozen (cooked and semi-cooked) food for 104 samples and for 46 samples of refrigerated (cooked) poultry meat ready-to-eat food.

MATERIALS AND METHODS

Selection sampling

A total of 150 RTE samples were obtained for bacteriological examination. Various types of RTE food products were obtained from 23 brands that contained frozen (cooked and semi-cooked) and refrigerated (cooked) poultry meat foods. They were purchased randomly, periodically during January and March 2012. All samples were randomly purchased before their best before date, transported to the laboratory in their original package and kept 2 days at -18°C until their analysis.

Microbiological analysis

Twenty-five grams of each sample was added to a culture medium/diluent (1:10; homogenized for 2 min in a Stomacher), in agreement with specific standard methods for *Coliforms* (AFNOR/NF BIO 12/20-12/06), *E. coli* (ISO, 16649-2:2001) and the pathogenic bacteria *Salmonella* (ISO 6579:2002; AFNOR BIO 12/01-04/94 protocol) and *L. monocytogenes* (ISO, 11290-1:2004; AFNOR BIO-12/11-03/04 protocol) and meat foods standard of Iran.

Determination of coliforms

For investigation of coliforms, violet red bile agar (VRBA medium, Merck, Germany) were used after incubation at 30 ± 1°C for 24 h, as recommended by the manufacturer. Those positive tubes, which have formed a gas at the end of incubation period, were planted into the brilliant green bile (2%) broth (BGB), which has again contained a Durham tube and then they underwent the incubation process at 35°C for 48 h. Those tubes, that have formed a gas as a result of incubation process, were evaluated according to the MPN table and their total coliform counts were determined in this way. To defined *E.coli* by MPN method, gas positive BGB tubes were transferred to loop of each suspension and tubes were streaked to eosin methylene blue agar (EMB) and incubated at 37°C for 24 h. Ideally, *E. coli* should not be detected and as such a level of <3 per gram (the limit of the most probable number test) has been given as the satisfactory criteria for this organism.

Identification and numeration of *S. aureus*

Enrichment of 1 g sample in 10 mL cooked meat medium (Difco), streaking a loopful of the 24-h enrichment culture on Baird-Parker agar (BPA, Merck) containing egg yolk and potassium tellurite (Merck), and finally, incubation at 37°C for 48 h was done

Identification of *Salmonella*

For identification of Salmonella spp., 25 g of each food sample was pre-enriched in lactose broth (Merck) at 37°C for 18 h. Then, 1 mL was transferred into 10 mL selenite cysteine broth (Merck) for enrichment, incubated at 37°C for 24 h. Finally, Salmonella Shigella (SS) agar (Merck), bismuth sulfite agar (Merck) was used as selective media, triple sugar iron agar (Merck), lysine iron agar (Merck) as differential media and urease (Merck) as complement media.

Identification and numeration of *B. cereus*

Surface plate method on *B. cereus* selective agar (Merck) were used for identification of typical B. *cereus* colonies and incubated at 37°C for 24 h.

Determination of psychrotrophic microorganisms

69 g of Nutrient Agar powder was suspended in 3 L of distilled water. It was allowed to soak and brought to boil. They were distributed into suitable containers and sterilized in the autoclave at 121°C for 15 min.

Determination of psychrophilic microorganisms

114 g of king agar powder was suspended in 3 L of distilled water. It was allowed to soak and brought to boil. They were distributed into suitable containers and sterilized in the autoclave at 121°C for 15 min.

Statistical analysis

Probability value $p < 0.05$ was defined statistically significant. Data analysis was performed using SPSS 18 (IBM, PASW Statistics 18.0, USA).

Table 1. Mean±standard deviation in *B. cereus*, psychrotrophic, psychrophilic, coliform and *S. aureus* bacteria of cooked semi, cooked, refrigerated, frozen foods and total.

Condition of storage	Cooked	Semi cooked	Refrigerated	Frozen (cooked and semi cooked)	Total
N	65	39	46	104	150
B. cereus	2/98 ± 0/07	3/84 ± 0/05	4/44 ± 1/41	3/21 ± 0/06	2/96 ± 0/09
Psychrotrophic	3/63 ± 0/05	3/37 ± 0/09	3/96 ± 0/07	3/56 ± 0/08	3/05 ±0 /04
Psychrophilic	5/2 ± 0/08	5/73 ± 1/3	4/34 ± 0/04	5/34 ± 0/09	5/02 ± 1/77
Coliform	2/9 ± 0/09	3/15 ± 0/06	5/42 ± 1/53	4/47 ± 1/02	4/02 ± 0/07
E .coli	2/4±0/4	3/2 ±0/9	2/1 ±0/4	1/76±0/7	3/46±0/8
S. aureus	2/17 ± 0/08	2/04 ± 0/07	3/09 ± 0/09	3/42 ± 0/07	3/41 ± 0/09

N: Number of samples.

Table 2. Percentage of *B. cereus*, psychrotrophic, psychrophilic, *Salmonella* and *E. coli* bacteria in cooked, semi cooked, refrigerated and frozen foods.

Condition of storage	Frozen (cooked and semi cooked)		Refrigerated		Cooked		Semi cooked	
B. cereus	u	76/5	u	56/2	s	27/6	u	24/2
Psychrotrophic	u	76/5	u	43/8	u	22/3	u	54/7
Psychrophilic	u	97/3	u	87/5	u	41/5	u	35/8
Salmonella	u	14/7	u	12/5	u	4/3	u	7/4
E. coli	u	47/2	u	49	u	28	u	44
Coliform	u	71/2	u	78	u	65	u	65
S. aureus	s	26	u	33	u	35	u	40

s = Satisfactory, u = unsatisfactory.

RESULTS AND DISCUSSION

The results for enumeration of *B. cereus*, psychrophilic and psychrotrophic microorganisms were: 2/96 ± 0/09, 5/02±1/77 and 3/05±0/04 Log CFU/g, respectively. The fourteen percent of all samples were contaminated by *Salmonella*. Percentage of *Salmonella* contamination in cooked frozen samples were higher than semi-cooked ones, because of cross-contamination and inappropriate usage of time-temperature chain. The contamination percentage of *B. cereus* was higher in semi-cooked samples than cooked samples. Minimum and maximum and mean ± SD (standard deviation) of coliform and *Staphylococcus aureus* in frozen cooked samples are 5 (2/9±0/09) and 4 (2/17 ± 0/08), respectively. The number and mean ± SD of coliform and *S. aureus* in semi cooked samples are 12 (3/15 ± 0/06) and 14 (mean±SD, 2/4±0/7) respectively. 65% of cooked samples and 62% (62% of 39 samples contain more than 10^2 CFU/g coliform) of semi cooked samples contain more than 10^2 CFU/g coliform, while *S. aureus* was in more than 10^2 CFU/g in 35 and 40% of samples, respectively. Also, 28% of cooked samples, 44% of semi cooked samples, 47/2% of frozen samples and 49% refrigerator samples contain *E. coli*. Therefore, the level of contamination of cooked and semi cooked foods by these bacteria is high (Tables 1 and 2).

This study has shown that, *Salmonella*, *B. cereus*, *Coliforms*, *E. coli*, *S. aureus*, psychrophilic and psychrotrophic microorganisms can be isolated from many different ready-to-eat foods. Several investigations regarding the microbiological quality of various ready-to-use food products, such as vegetable salads (Albrecht et al., 1995; Garcı´a-Gimeno et al., 2005; Kaneko et al., 1999; Odumeru et al., 1997) cold and hot meals served by airlines (Hatakka, 1998a, b); cooked rice (Nichols et al., 1999), street-vended foods (King et al., 2000; Kubheka et al., 2001; Mosupye and von Holy, 2013), hot-held foods (Chiou et al., 1996), catering dishes (Alberghini et al., 2000), sliced meat and meat products (Gillespie et al., 2010; Soriano et al., 2000) and shrimp (Hatha et al., 1998; Valdimarsson et al., 2008), have been reported.

The Brazilian Food Sanitation Standard (Brazil. Agencies Nacional de Vigilance Sanitaria, 2001) used for the ‘‘ready-to-eat hot sandwich and finger food and cold sandwich categories were: Fecal coliforms 2 log MPN/g; *B. cereus* 3 log cfu/g (HS) and 3:7 log cfu/g (CS); coagulase positive *Staphylococcus* 3 cfu/g (HS) and 3:7 cfu/g (CS) and the present study found high count of *Salmonella*, *B. cereus*, psychrophilic and psychrotrophic microorganisms among frozen ready-to-eat food for a single sample. In the previously cited research carried out in Latin America, the incidence of *B. cereus* in counts

above the safe level ranged from 1.7 to 8.1% of street food samples, except in one country, where this number reached 32.2% (Almeida et al., 1996).

In South Africa, this frequency was 22% of the 51 street food samples, but the counts were below 1 log cfu/g (Mosupye and von Holy, 2013) and in this study, enumeration of *B. cereus* was 2/96 ± 0/09 Log10 cfu/g. In a study carried out in Zaria, Nigeria on street food contamination, *B. cereus* and *S. aureus* were observed in 26.3 and 15% of the samples, respectively (Umoh and Odoba, 2009). The detection of high levels (>103 cfu per gram) of *B. cereus* could result in an investigation of the food handling controls used by the food business. Levels of ≥104 cfu per gram are considered potentially hazardous as consumption of foods with this level of contamination may result in food borne illness. In the multicenter study of street foods in 13 towns, 41% of sandwich samples did not meet the bacteriological criteria. The proportion of unsuitable samples due to *E. coli* contamination ranged from 4.5 to 70.2%; the prevalence of *B. cereus* was between 0.4 and 3% and from 1.9 to 10.1% for *S. aureus* (Garin et al., 2002). *S. aureus* was found in only one sample (3 log cfu/g) (2.5%), suggesting that recontamination of food by this organism after cooking was not common. At the study area, contact of the consumers with the street foods was not observed, except in the case of industrialized product (chocolates, crackers, candies, etc). In the study carried out in South Africa, *S. aureus* was not detected in any of the street food samples (Mosupye and von Holy, 2013), whereas in Latin American cities, its occurrence ranged from 1.9 to 25.2% of the street food samples (in counts above 10^3 cfu/g) (Almeida et al., 1996).

In the study from Nigeria, none of the samples from mobile food vendors was contaminated with *S. aureus*, whereas those from stationary vendors, without shelter, had the highest frequency of contamination by *S. aureus* (22.9%) and *B. cereus* (32.9%) (Umoh and Odoba, 2009). In this study, 40% of cooked samples and 35% of semi cooked samples contain more than 10^2 cfu/g *S. aureus*. During the years 1986 to 1995, 104 outbreaks caused by *B. cereus* were reported in Taiwan, and this bacterium was noted to be the third most commonly implicated food-borne pathogen in this country (Pan et al., 1997). The increasing prevalence of precooked refrigerated food products could potentially exacerbate the problems associated with *B. cereus* (Choma et al., 2000; Nichols et al., 1999; Kaneko et al., 1996; Hatakka, 1998a, b).

Considering *Salmonella*, the results of Dom et al. (2014) study suggest a generally low prevalence of this microorganism in all analyzed products, with the exception of dried pork sausages. Previous studies of ice cream and cheese reported levels of less than 0.1% or no isolation (EFSA and ECDC, 2012; Ortolani et al., 2010). Salmonella in meat preparations, intended to be eaten without any additional treatment, were reported by Cabedo et al. (2008) with 2% in cooked ham and 11.1% in cured dried pork sausage. In this study, 14% of all samples were contaminated by *Salmonella*. All the tested ready-to-eat products in this study were of unsatisfactory quality according to coliforms (AFNOR/NF BIO 12/20-12/06), *E. coli* (ISO, 16649-2:2001) and the pathogenic bacteria *Salmonella* (ISO 6579:2002; AFNOR BIO 12/01-04/94 protocol) and *L. monocytogenes* (ISO, 11290-1:2004; AFNOR BIO-12/11-03/04 protocol).

Conclusions

This study shows that most ready-to-eat food samples (all types and brands) analyzed presented unsatisfactory microbiological quality according to the Iranian guidelines and they have high risk for consumer. Contaminated food is the usual source of human infections, and poultry products are considered the major infectious route for humans (Mead, 1999; Stern et al., 2001). Moreover, evidence exists that inadequate hygiene practices within food processing plants may result in the contamination of product with pathogens (Metaxopoulos et al., 2003) and therefore pose a subsequent risk in the product's safety. On the other hand, complete elimination of pathogens from raw materials (Eisel et al., 1997) and food processing environment (Tompkin, 2012) is difficult, particularly when many food pathogenic are known to be able to attach on food contact surfaces (Fonnesbech-Vogel et al., 2001; Jessen and Lammert, 2009; Deza et al., 2005).

Conflict of interests

The author(s) did not declare any conflict of interest.

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