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SHORT COMMUNICATION

MICROBIOLOGICAL QUALITY AND SAFETY OF CUSTARD CAKES FROM ADDIS ABABA

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ABSTRACT: A total of 80 samples of custard cakes from ten different pastries in Addis Ababa were examined for pH, moisture content, holding temperatures, bacteriological profile, and antimicrobial sensitivity of *Salmonella* isolates. The mean pH values of custard cakes from ten different pastry houses ranged from 5.4 to 6.6 and the mean moisture content ranged from 44% to 29.9%. About 96.9% of the samples were stored within a temperature range of 15-25^{o} C. About 76% of the custard cakes had counts higher than 8 log cfu/g. *Bacillus* spp. (51.8%) dominated the microflora. Staphylococci and members of Enterobacteriaceae constituted 3.5% and 13.3 % of the microflora, respectively. Five pastry samples yielded *Salmonella* and the dominant resistance pattern of the isolates was Pen/Amp/Amo. This study indicated the need to train pastry producers and handlers in basic principles of hygiene. Keeping custard cake at ambient temperatures must also be avoided.

Key words/phrases: Custard cake, Microorganisms, Pastries, Safety.

INTRODUCTION

Bakery products are important staple foods in most countries and cultures (Guynot *et al*., 2003). Bakery products also include pastry products which offer an excellent environment for bacterial growth owing to their favorable pH, water activity, nutrient availability and storage conditions (Uhitil *et al*., 2004).

Custard is a range of preparations based on milk and eggs, thickened with heat. Most commonly, custard refers to a dessert or dessert sauce. As a dessert, it is made from a combination of milk or cream, egg yolks, sugar, and vanilla. Sometimes flour, corn starch, or gelatin are also added (Longrée et. al., 1966).

Many fillings of pastry products can support the growth of food-borne pathogens, especially if they contain egg or dairy products. Custard products are potential health hazard, as they are prepared from milk and eggs, which

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are possible sources of pathogens due to contamination. *Bacillus cereus* and *Staphylococcus aureus* from custard cake have been implicated in food poisoning outbreaks (Smith *et al*., 2004). Food poisoning outbreaks associated with bakery products, thus, have major public health significance (Bhatia and Zahoor, 2007).

Food-borne outbreaks associated with custard-filled baked goods are attributed primarily to contamination by food handlers followed by inadequate refrigeration during manufacture and storage (Stewart *et al*., 2003). As indicated in Stewart *et al*. (2003), 26-38.5% of food handlers are positive for the carriage of *S. aureus.*

According to Ethiopian Health and Nutrition Research Institute, the food safety control system in Ethiopia is little developed and is not able to support the production, supply and distribution of safe food to the local community (EHNRI, 2003). The system does not encourage the application of Good Manufacturing Practices (GMP) and other recent principles, like Hazard Analysis and Critical Control Point (HACCP), by producers or distributors.

Custard cake products in Ethiopia are usually called cream cakes possibly due to the creamy nature of the custard. Though there are large numbers of custard cake consumers among the urban community in Ethiopia, the safety and quality of these products have not been assessed. Therefore, the purpose of this study was to evaluate the microbial quality and safety of custard cakes as made available to consumers in parts of Addis Ababa.

MATERIALS AND METHODS

A total of 80 custard cake samples were purchased from ten different pastries in Addis Ababa, Ethiopia between November, 2007 and June, 2008. All samples were immediately brought to the Microbiology laboratory, Science Faculty, for microbiological analysis. The holding temperatures of each sample were measured at the point of collection by aseptically inserting a sensing bulb of a laboratory thermometer until the temperature of the custard cake was stabilized.

For microbiological analysis, 25 g of sample was aseptically removed and homogenized in 225 ml sterile 0.1 % bacteriological peptone (Oxoid) for ten minutes. Serial ten-fold dilutions were also prepared for plating.

Aerobic mesophilic bacteria were counted using Plate Count (PC) Agar (Oxoid) plates incubated at 30^{o}C for 72 hours. For Enterobacteriaceae, Violet Red Bile Glucose Agar (Oxoid) was used and plates were incubated

at 30°C for 24 hours. All purple colonies were counted as members of Enterobacteriaceae. Coliforms were counted on Violet Red Bile Agar (Oxoid) and plates were incubated at 30°C for 24 hours. Red to pink colonies, surrounded by precipitated bile, were counted as coliforms. For staphylococci, Mannitol Salt Agar (Oxoid) was surface plated and incubated at 30°C for 36 hours. Bacterial spores were counted after heating the sample for ten minutes in water bath (80°C) and spread-plating 0.1 ml of appropriate dilutions on the pre dried surface of PC plates. Colonies were counted after incubation at 30 to 32° C for 24 hours. Counts of yeasts were determined on Chloramphenicol Bromophenol Blue Agar plates incubated at 25-28°C for three to five days. Chloramphenicol-Bromophenol blue agar consisted of (g/l distilled water) yeasts extract (Oxoid) 5.0, glucose 20, chloramphenicol 0.1, Bromophenol-blue 0.01, agar 15, pH 6.0–6.4. Smooth (non-hairy) colonies without extension at periphery (margin) were counted as yeasts. Microbial counts were transformed to $\log_{10}$ cfu/g.

For flora analysis about 10 to 15 colonies were picked randomly from countable PC plates and inoculated into tubes containing 5 ml Nutrient Broth No 2 (Oxoid). These were incubated at 30-32°C over-night. Cultures were purified by repeated plating and were characterized to the genus level using the following tests: cell morphology, KOH test (Gregersen, 1978), oxidation fermentation (O/F) test (Hugh and Leifson, 1953), catalase test and cytochrome oxidase test (Kovacs, 1956).

For isolation of *Salmonella* spp. pastry samples (25 g) were homogenized in 225 ml buffered peptone water using a Stomacher lab blender (Seward 400) and incubated at 37°C for 24 h. From this, 1ml of culture was transferred into separate tubes each containing 10 ml of Selenite broth, Selenite Cystein Broth, Tetrathionate broth, Mannitol Selenite broth (all from Oxoid). Selenite Cystein and Mannitol Selenite broths were incubated at 37°C for 24 hours and Tetrathionate broth was incubated at 43°C for 48 hours in water bath. After secondary enrichment, culture from each enrichment broth was separately streaked on plates of MacConkey Agar, Salmonella-Shigella (SS) Agar and Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid). Characteristic colonies from each selective medium were picked, purified and tested biochemically on Triple Sugar Iron Agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Urea Agar (Oxoid), Simmons Citrate Agar (Oxoid) and SIM Medium (Oxoid). The ability of Salmonella to ferment mannitol, glucose or sucrose was assessed using a fermentation broth containing the corresponding sugars. Fermentation tubes contained inverted Durham tubes to detect gas production.

The pH of each custard cake samples was determined after blending 10 g custard cake sample with 90 ml distilled water. The pH value of the homogenate was measured using a digital pH-meter. The moisture content of the custard cake was determined by allowing a given weight of samples to dry to constant weight at 35^{o}C.

Antimicrobial susceptibility of *Salmonella* isolates was tested on Mueller-Hinton agar plates following the standardized disk diffusion technique (Jorgenson *et al*. 1999) with Oxoid drug discs: Ampicillin (Amp), (10iu); Chloramphenicol (Chl), (30µg); Gentamycin (Gen), (10µg); Tetracycline (Tet) , (30µg); Ciproflaxin (Cip), (5µg); Ceftriaxone (Cef), (30µg); Penicillin G (Pen), (10ug); Streptomycin (Str), (10µg); Kanamycin (Kan),((30µg); Polymaxin B (Pol),(100iu); Amoxicillin, (Amo), (2 µg).The reference strains, *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922), sensitive to all the drugs used in this study, were routinely tested. Interpretation of readings as sensitive, intermediate or resistant was made according to a chart. Intermediate readings were few and therefore considered as sensitive for the purpose of assessing the data.

To see if there was significant variation in counts within samples of each pastries, sample coefficient of variation (CV) was calculated. Values greater than 10% were considered as significant.

RESULTS

All pastries had mean AMB counts of over 8 log cfu/g and the mean counts of coliforms and members of enterobacteriacea was >6 log cfu/g in all cases. Mean counts of staphylococci was >5 log cfu/g (Table 1). When comparing the microbial load within custard cakes obtained from the same pastry, there was no significant variation in aerobic mesophilic counts (CV<10%), although counts of the other bacterial groups varied significantly between one sample and another obtained from the same pastry in all cases (CV>10%). About 79% of all custard cake samples had aerobic mesophilic counts between 6 and 9 log cfu/g, while about 21% had counts >log 9 cfu/g. Over 90% had coliform and enterobacteriacea counts of 6-9 log cfu/g (data not shown).

The mean pH values of custard cakes ranged between 5.4 and 6.6 and mean moisture content was in the range of 30% to 44%. Variations in pH and moisture content within samples obtained from same pastry were significant (CV>10 %) (Table 2).

Table 1. Mean counts of various microbial groups in custard cakes from different pastry shops (A-J).

Microbial groups	Mean log cfu/g + SD									
	A	B	C	D	E	F	G	H	I	J
Aerobic mesophilic	9.02±0.74	8.58±0.79	8.64±0.50	8.37±0.50	8.48±0.55	8.52±0.14	8.57±0.98	8.33±0.58	8.51±0.66	8.27±0.76
Enterobacteriaceae	6.93±0.74	7.30±0.79	7.87±0.74	6.98±0.78	7.31±0.62	7.75±0.88	7.34±0.67	7.40±0.48	7.11±0.89	7.45±1.15
Coliforms	7.42±0.73	6.74±0.71	7.41±1.01	6.71±0.56	7.01±0.82	7.36±0.91	7.03±0.49	7.08±0.49	6.75±0.85	7.08±0.92
Staphylococci	5.38±1.32	5.49±0.98	5.57±1.21	4.43±0.84	5.34±1.05	5.58±0.99	5.28±0.73	5.03±0.54	5.24±0.78	5.47±0.70
Yeast and Mold	5.53±0.52	5.03±0.82	4.96±0.70	5.48±0.77	5.19±0.49	7.24±0.74	4.92±0.86	5.40±0.91	5.10±0.99	5.51±0.98
Aerobic Spores	2.92±0.66	3.14±0.76	3.25±0.46	3.28±0.52	3.66±1.12	3.44±0.75	3.61±0.67	3.12±0.70	3.85±0.90	3.19±0.84

SD, Standard deviation

Table 2. Holding temperatures, mean moisture content (%) and pH values of custard cakes.

Sample source	Holding Temperatures				Moisture content (%)	pH
	< 15ºC	15-20ºC	21-25ºC	> 25ºC	Mean±S.D	Mean±S.D
A	-	83.3	16.7	-	31.22±4.18	5.69±1.10
B	-	50.0	50.0	-	35.86±7.75	6.35±1.44
C	-	33.3	66.7	-	35.36±7.65	5.92±1.45
D	-	42.9	57.1	-	44.02±11.75	5.76±1.38
E	16.7	33.3	50.0	-	37.36±12.85	6.24±1.07
F	-	33.3	66.7	-	34.74±9.24	6.64±1.53
G	-	57.1	42.9	-	35.99±11.68	5.90±1.40
H	-	28.8	71.2	-	39.18±11.04	6.30±1.49
I	-	42.9	42.9	14.2	33.27±8.98	6.23±1.21
J	-	42.9	57.1	-	29.86±9.24	5.43±1.26

SD, Standard deviation

The various custard cakes were held at different temperatures when they were made available for consumption. About 45% of the samples were within the temperature range of 15-20^{0}C and about 52% were within the temperature range of 21-25^{0}C (Table 2).

A total of eight *Salmonella* isolates were obtained from the custard cakes considered in this study. One isolate was sensitive to all the drugs tested. Resistance was observed only against Pen (8), Chl (2), Kan (1), Amp (6) and Amo (6). Multiple drug resistance (resistance to more than two drugs) was observed in six isolates and the commonest pattern was Pen/Amp/Amo (data not shown).

DISCUSSION

The majority of the investigated food items in this study yielded high microbial load. About 76% of samples in this study had aerobic mesophilic counts of ≥ 8 log cfu/g. According to ICMSF (2001), aerobic mesophilic counts ≥ 7 log cfu/g are considered to be of unsatisfactory quality. Thus, the microbial load of our samples was beyond the acceptable microbiological limits and this indicated poor hygiene or food handling practices. The high pH of our samples (5.4 and 6.6) would also allow growth of the bacterial groups. Smith *et al*. (2004) indicated that any bakery products and their ingredients having a pH of >4.6 would be conducive to the growth of pathogenic bacteria. The high moisture content may also support bacterial proliferation, although our study did not determine the water activity (aw) of custard cakes to assess the availability of moisture to the microflora. As over 50% of the custard cakes were maintained at temperatures over 20^{o}C, most of the mesophilic microbial groups, particularly members of enterobacteriaceae, could multiply luxuriously and compromise the safety of the products. The high variations of coefficient in the various parameters within samples of the same pastry showed that there was no precaution followed in the hygienic preparation of the cakes and most were left to chance contamination.

The high counts of enterobacteriaceae and coliforms among our custard cake samples indicated very unhygienic practices in the preparation and handling of the cakes. As the custard portion is usually not heat-treated, unhygienic handling may result in gross initial contamination and longer holding at room temperature may favor enterobacteriaceae to dominate the flora. The presence of enterobacteriaceae in numbers ≥4 log cfu/g in ready-to-eat foods indicates that an unsatisfactory level of contamination, including of faecal origin, has occurred or there has been under processing

(ICMSF, 2001). The isolation of *Salmonella* from some samples indicated the health hazard associated with the consumption of custard cakes. Low number of coliforms is permitted only at a level of 2 log cfu/g (Jay, 2006). Long holding time at ambient temperature, may help members of enterobacteriaceae to dominate the flora.

The mean count of staphylococci was >5 log cfu/g in nine of the ten pastries. Contamination of ready-to-eat foods with staphylococci is largely a result of contact with skin. Although, according to ICMSF (2001), counts ≥4 log cfu/g are considered as potentially hazardous in foods, enterotoxigenic *Staphylococcus aureus,* if present, may not proliferate to produce enterotoxins as it is not competitive against the high number of background flora found in custard cakes.

Yeasts were also encountered at high levels in this study. They can be problems mainly in intermediate and high moisture bakery products (Smith *et al*, 2004). However, due to short holding period of custard cakes, these groups of microorganisms may have no ecological role other than indicating gross contamination of the cakes.

Despite the ubiquitous nature of *Bacillus* spp., the count of spores was below 4 log cfu/g. According to ICMSF (2001), counts of pathogenic *Bacillus* spp. ≥ 4 log cfu/g are considered potentially hazardous as consumption of foods with this level of contamination may result in food-borne illness.

Salmonella were isolated from few pastries in this study. Costalunga and Tondo (2002), showed that pastry products were among the major food vehicles for outbreaks of *Salmonella* spp. and were responsible for 16.6 % of outbreaks in Rio Grande do Sul state, during the periods of 1997 to 1999. The frequency of isolation of *Salmonella* from custard cake in this study was much lower than that observed in 'leb leb kitfo' (42%) in Addis Ababa (Mezgebu Tegenge and Mogessie Ashenafi,1998) and from chicken carcass and giblet (21.16%) from Debre Zeit and Addis Ababa supermarkets (Bayleyegn Molla, *et al*., 2003). The contamination of custard cakes with *Salmonella* might have arisen from contaminated eggs used for custard preparation and/or *Salmonella* carriers among food handlers. Since custard provides a fertile growth medium, keeping custard cakes for several hours at ambient temperature may support the growth of salmonellae. The number of *Salmonella* isolated in this study was too small to make any reasonable comparison regarding antimicrobial sensitivity with other studies. However, the resistance of most isolates to Pen, Amp and Amo and the appearance of

multiple resistances against the three antibiotics is a point of concern as these drugs are commonly used in Ethiopia to treat salmonellosis.

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