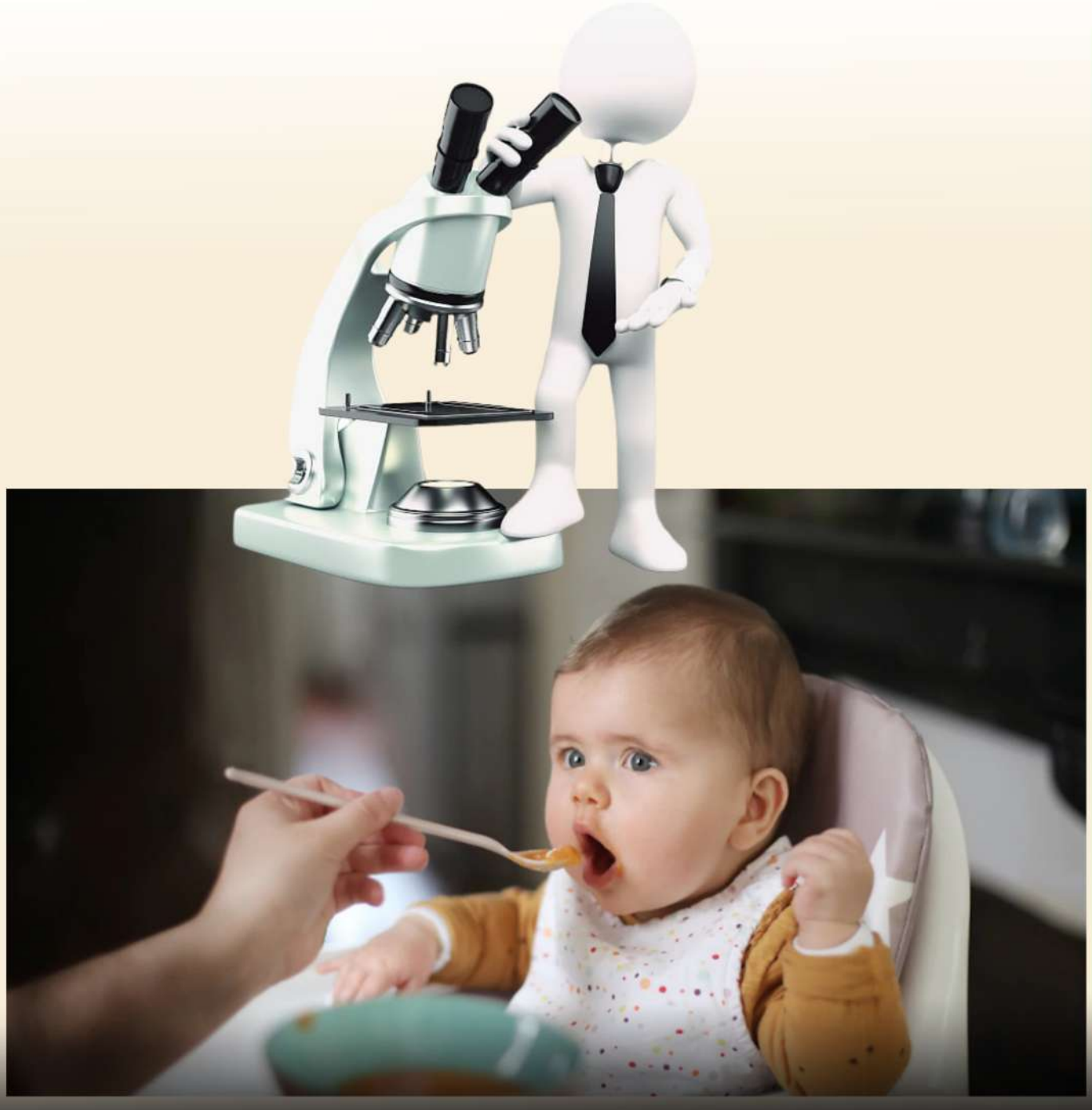
Microbiological Studies on Baby Foods

Dr. Alaaeldin Ali



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By

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B.Sc. (Agric. Microbiol.), Fac. Agric., Ain Shams Univ., Egypt, 1995
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THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences

(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2009

SUPERVISION SHEET

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Department: Agricultural Microbiology **Approval: 18/3/ 2009**

ABSTRACT

Fourty two samples of different infant food products were collected from retail markets and pharmacies around Cairo, Giza and 10th Ramadan cities during 2004 and 2005. These samples were examined for incidence of different types of microorganisms to evaluate their quality. Neither of the tested samples harbored any of the pathogenic bacteria that might be found in such products. Neither of the tested samples had any detectable levels of the aflatoxins.

The colony counts in seven samples *i.e*. Riri with vegetables, Cerelac, Baby king special formula, Babysan 2, S-26 gold, Grebe fruit dessert and Baby calm herbal were considered to be unacceptable according to the Egyptian Standards**.** Yeasts & molds were found in riri with vegetables, cerelac, Grebe mixed vegetables and baby calm herbal samples**.** Coliforms could be detected in the cerelac and baby calm herbal samples.

Preparation of baby calm herbals in hot water (100 °C) reduced the aerobic populations to the acceptable level recommended by the WHO *i.e*. lower than 10^4 cfu/g.

Electron-beam treatment of baby foods such as baby calm at doses below 5 kGy may be required to achieve commertial "sterility" (*i.e*. a total aerobic plate count of <10/g), without affecting the quality change such as color and flavor.

A decrease in the aerobic counts of most microorganisms found in herbal samples was observed at an ethylene oxide dose of 250 mg/l. At higher doses, the effect of the gas fumigation was sterile.

Of 11 ports located on the production line at an infant food packags-manufacturing plant, seven of them contained *Staph. aureus* in counts reached 60 cfu/ swab. Coliforms and *E. coli* could be detected from two ports. Microbial samples collected from workers' hands at the plant under investigation presented considerable bacterial contamination. Of ten employees examined, five of them had *Staph aureus* on their hands. Coliforms could be detected on 2 cases. The microbial load in the air at the time of examination was estimated to 130 cfu/ plate/15 minutes exposure. Examination of three types of infant food packages showed that two of them were within the proposed criteria, since the aerobic counts were either undetectable or only 23 cfu/ 100 cm^2. However, one package, had aerobic counts of 221 cfu/ 100 cm^2 representing about two times higher than the proposed criteria.

Experiments on survival and growth of *E. coli* strain and an isolate of *Staph. aureus* in rice cereal reconstituted with pasteurized milk held at 5 ˚C showed that the counts either (*E. coli*) declined and were undetectable along the experimental duration *i.e*. up to 50 h of inoculation or (*Staph. aureus*) had no changes during the 72 h storage. Cells of *E. coli* grew rabidly at 25 ˚C exceeding 9 $\log_{10}$ cfu/ml of slurry within 26 h. Population of the *Staph. aureus* increased substantially at 25 ˚C within the 48 h inoculation which was >4 logs higher than the initial (0 h) population.

Key words: Infant foods, microbial contamination, *E. coli*, *Staphylococcus*, coliforms, aflatoxins, irradiation, ethylene oxide.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my father, my mother and my wife for their patience and help.

ACKNOWLEDGEMENT

Praise and thanks are to **ALLAH**, the most merciful for assisting and directing me to the right way.

A worm of gratitude is devoted to the sole of **Dr. MOAWAD K. ZAHRA**, Late Professor of Agricultural Microbiology, Fac. Agric., Cairo Univ., for taking the responsibility of supervising this work. His valuable and sincere follow-up have helped me throughout the whole experience, and I will always be proud to be his student. May God let his sole rest in peace.

I would like to express my deep thanks and utmost gratitude to **Dr. REFAE I. REFAE**, Professor of Agricultural Microbiology, Fac. Agric., Cairo Univ., for proposing the research program and plan of study, for his sincere supervision and for giving every possible help and advice throughout this study and in preparing and writing the manuscript. His virtual wise gaudiness and encouragement made my task in this research a joyful experience.

My gratitude is due to **Dr. WALEED D. SALEH**, Lecturer of Agricultural Microbiology, Fac. Agric., Cairo Univ., for his keen supervision, valuable advice, encouragement, and guidance offered through the implementation and discussion of the thesis.

Thanks to all staff members of Agricultural Microbiology Department, Fac. Agric., Cairo Univ., for their sincere help and cooperation.

Thanks to **Dr. MOHAMED NABIL** (El Rashidy Company, 6th October City), Dr. **MOHAMED NASR** (Atom Energy Organization, Nasr City) and **Mr. AHMED SHEHTA** (Cadbury Egypt Company, 10th of Ramadan City) for their kind cooperation respecting the analysis of the samples.

CONTENTS

INTRODUCTION

Infant formulas are liquids or reconstituted powders fed to infants and young children. They serve as substitutes for human milk. Infant formulas have a special role to play in the diets of infants because they are often the only source of nutrients for infants. For this reason, the composition of commercial formulas is carefully controlled and an Egyptian standard requires that these products meet very strict standards. Prepared infant formulas are primarily water and milk whey powder. Among other ingredients, it may include sweeteners, such as lactose, corn syrup or other sugars and fats, such as coconut and soybean oils. Vitamin and mineral supplements are typically universal additives. A few brands contain mono-and diglycerides, emulsifiers that keep the liquid from separating.

Herbs commonly harbor a large number of bacteria and fungi including potential spoilage organisms (Farkas, 1988). The most common bacteria are the aerobic spore-formers, such as *Bacillus* spp., *Clostridium* spp. and *Salmonella* species (McKee, 1995). A major concern of food processors is to assure that the microbial load of ingredients and processing aids does not contribute to spoilage of food and does not diminish its microbial safety. Spices and dried vegetables or herbal teas may not be suitable substrates for the growth or long survival of bacterial pathogens such as salmonellae or other non spore-forming pathogenic bacteria, nevertheless, occasional *Salmonella* contamination is a reality (Bruchmann, 1995).

Many studies have demonstrated an association between infant feeding practice and infant health. The majority of these works indicated that infant morbidity and mortality are influenced by the mode of infant feeding practice. Contamination and growth of bacterial pathogens such as *B. cereus* in infant food are common and increasing. In the study carried out by Becker *et al*. (1994), 261 samples from 17 countries were examined. The authors found that 70 % of the infant formulas in Germany were positive for *B. cereus*, at a level up to 600 cfu/g. In Egypt, El-Prince and Korashy (2003) conducted an research in Assiut to secure information regarding the sanitary conditions as well as existing pathogens in 90 random baby food samples and some dried milk-based infant foods currently available at the retail level. The authors could isolate *B. cereus* and *Staph. aureus* from the tested samples.

Today, three major methods are in use for decontamination of herbs, namely gas (ethylene oxide and propylene oxide), irradiation and steam (Eiss, 2001). However, ethylene oxide gas has been banned in the European Union and other countries (Loaharnu, 1998). Steam degrades the light-weight leafy herbs, and ground products are difficult and sometimes impossible to handle in the steam system (Eiss, 2001). Radiation decontamination of herbs with doses ranging from 3 to 10 kGy proved to be a viable alternative to fumigation or steam. Introduction of irradiation for microorganisms is accepted by 40 countries over the world. This method can irradiate microorganisms safely and efficiently with no residue in the products and without

reduction of the important substances of treated herbs (Biological Sciences Division, 1999).

The present study deals with microbiological and chemical survey for common types of infant food products in Egyptian retail markets and pharmacies. The efficiency of different treatments, *i.e*. irradiation, fumigation and boiled water to reduce the microbial load and to ensure the hygienic quality was evaluated as well. To measure plant hygiene and product quality during manufacturing of infant-food packages, the bacteriological conditions of the equipment, hands of employees, packages and air inside the plant were also checked.

REVIEW OF LITERATURE

Many studies have demonstrated an association between infant feeding practice and infant health. The majority of these works indicated that infant morbidity and mortality are influenced by the mode of infant feeding practice.

Kovar *et al*. (1984) reported that artificial feeding of infants is a method which for success relies upon maintenance of high degree of the home. In developed countries with good nutrition medical care, bottle-feeding is less risky than in the non developing countries. Since the standard of personal hygiene and public sanitation was low in many communities of developing nations, contamination of infant feeds with pathogenic microorganisms may be an important cause of infectious diarrhea. Bacteriological survey of feeds and feeding bottles from Africa and other countries have shown gross contamination of feeding utensils and feeds. Table (1) shows the bacterial contamination at infant formula recalls during 1990-1994.

Table 1. Bacterial contamination in infant formulas recalls 1990-1994*.

Year	Problem	Product	Class **
1994	*Klebsiella* and *Pseudomonas* contamination	Nursoy concentrate (Wyeth Labs)	II
1993	*Salmonella* contamination	Soyalac (Nutrica Inc.)	I
1990	Bacterial contamination	I-Soyalac concentrate formula (Loma Linda)	I

* After Naomi (2001).

**Class I: May cause serious health consequences.

Class II: May cause medically reversible health conditions.

1. Microbial contamination of infant foods

Barrell and Rowland (1979) reported that it is a common practice in rural areas of Gambia to prepare infant foods in quantities which are sufficient to meet the needs of the day rather than one meal. These are then stored at ambient temperatures for periods up to 12 hours to be fed to the child on demand. The total viable counts and levels of *Bacillus cereus, Clostridium welchii, Staphylococcus aureus* and *Escherichia coli* were determined in 294 infant food samples from naught to eight hours after preparation. The presence of *Salmonella* was determined in 10 g samples of food. In the first hour after preparation, the proportion of foods dangerously contaminated was high during the rainy season; significantly more than during the dry season. Foods not consumed fresh were very often hazardous and almost always so after 8 hours. This problem may be a causal factor in weanling diarrhea which also shows marked seasonal variation in prevalence.

Enterococci were isolated from samples of raw and pasteurized milk, cheese, butter, kulfi (a frozen product similar to ice cream but made from milk concentrated in an open container), kulfi mix, ice cream, sweetened condensed milk, dried skim milk and infant foods. Of the 728 enterococci isolated from 208 samples, 216 produced DNAase and 31 of these produced thermonuclease (TNase). Highest incidence of TNase-positive enterococci occurred in dried skim milk, infant food and sweetened condensed milk. Staphylococcal TNase was detected in 30 samples (including 7 of dried skim milk, 6 of cows' raw milk and 4 of Cheddar cheese); these samples also contained

staphylococcal enterotoxins. Enterococcal TNase activity was found in 7 samples (2 of kulfi and 1 each of raw milk, sweet cheese, Cheddar cheese, ice cream and sweetened condensed milk). These results indicate that TNase activity from enterococci can give false-positive results when TNase activity is used to screen foods for the presence of staphylococcal enterotoxin (Batish *et al.*, 1984).

As reported by Kubaeva (1984), the establishment of microbiological standards for infant foods is discussed in the light of experiments which showed that most cells of *Staphylococcus aureus*, enteropathogenic *Escherichia coli*, enterococci and *Bacillus cereus* survived in model media at the pH range of 4.5-6.5 characteristic of the alimentary tract of young infants, and actively multiplied when a dried milk feed was added to these media. Standards in the USSR for foods for children in the 1st year of life are currently based on the count of mesophilic aerobes and facultative anaerobes and absence or present lactose-fermenting coliforms, *E. coli*, *Staph. aureus* and *Salmonella*.

Batish *et al*. (1986) characterized enterococcal isolates recovered from 16 market samples of infant foods and from other sources and subjected them to enterocin typing with 18 indicator strains. Among 150 enterococci isolated from infant foods, 114 (76%) were able to be typed by the indicator strains. Although 24 enterocin patterns were observed, the most prevalent types were X-9, 224, and 65-603. Occurrence of pattern X-9 either singly or in combination with many other types was most frequent. Many of the enterocin patterns were also observed in 35 enterococcal isolates recovered from samples of dairy plant water supply and hand washings of personnel working in

a dairy plant that manufactured infant food; this suggests the possibility of these as sources of contamination. Enterocin typing of enterococci could prove useful in epidemiological studies.

Alvarez *et al*. (1986) microbiologically analyzed a total of 40 samples of 15 different infant formulas available in Santa Cruz, Tenerife. Thirty seven samples had counts of <1000 aerobic mesophilic organisms/g and 38 contained <1000 aerobic thermophilic organisms/g. No sample contained >1000 spore formers/g. Counts were below the permissible maximum specified by Spanish legislation. Coliforms, staphylococci or *Bacillus cereus* were not detected. High quality of all products tested was recorded.

Fifty-three samples (23 baby-food samples bought at supermarkets, 15 soups and 15 samples of milk or milk substitute infant formulas from feeding bottles collected at day-care children's institutions) were examined. The following tests were made: presence or absence of *Salmonella*, *Yersinia enterocolitica* and verification of the most probable number (MPN) of coliforms, counts of mesophilic and thermophilic bacteria, yeasts and molds, sulphite reducing anaerobic bacteria, *Staphylococcus aureus* and *Bacillus cereus*. All samples were negative for *Salmonella*, *Staphylococcus aureus*, *Y. enterocolitica* and anaerobic organisms, There were *B. cereus* and deteriorative microorganisms present in varying proportions in the 3 types of foods. Coliforms and faecal coliforms were present in samples from feeding bottles (Cullen *et al.*, 1986).

Muytjens *et al*. (1988) noticed that members of the family Enterobacteriaceae were cultured from 52.5% of 141 milk substitute

infant formulas obtained in 35 countries. The concentration was 1 cfu/g in all products. Species isolated most frequently were *Enterobacter agglomerans, Enterobacter cloacae, Enterobacter sakazakii* and *Klebsiella pneumoniae*. If infections due to these organisms occur, it can be useful to include a check of hygienic precautions taken during preparation and storage of the formula. Dried milks without members of Enterobacteriaceae might offer extra protection to the newborn if some multiplication does occur in the formula.

Ibanez *et al*. (1992) reported that five of 46 samples of cereal-based infant formulas exceeded the maximum permitted count of various microbial groups. Two samples containing fruit and yoghurt had mesophilic aerobic counts of $3x10^5$ and $8.6x10^5$ cfu /g, identified as *Streptococcus faecium*. Maximum permitted coliform count of 0.01 cfu /g was exceeded in 3 samples, which comprised a milk and cereal preparation containing 300 cfu/g of *Enterobacter cloacae* and *Enterobacter agglomerans* /g, a cereal and cocoa product containing 300 *Citrobacter freundii/*g, and a milk, cereal and honey product, which contained 200 *E. cloacae*/g and also contained 400 *Bacillus cereus*/g. No sample exceeded the maximum permitted yeast and mold level of 300/g and no sample contained *Escherichia coli* in 1 g, *Salmonella* spp., in 30 g or *Yersinia* spp.

Rowan *et al.* (1997) collected twenty four pasteurized infant foods, prepared in a Glasgow hospital, which were examined microbiologically. All produced a satisfactory total aerobic mesophilic count of 1.0×10^4 cfu/g (mean 63 cfu/g) within 1 h of preparation.

Bacillus cereus was detected in 2 infant foods immediately after preparation and one of them had a *B. cereus* count of l.4 x 10^3 cfu/g exceeding the recommended safety limit of 1.0 x 10^3 cfu/g. Subsequent storage for 14 h at 25°C caused the growth of *B. cereus* in further 8 foods, the majority of which exceeded the safety limit of 10^3 cfu/g. The microbiological quality of each infant food depended on the type and number of organisms initially present, and on the temperature and duration of storage. Incubation of foods at 10°C for 14 h did not alter the microbiological quality. While *B. licheniformis* and *B. subtilis* were the predominant organisms isolated within 8 h of incubation (45.8 and 20.8% of foods, respectively), additional storage resulted in the emergence of *B. cereus* I (25%) and II (20.8%) as dominant *Bacillus* spp., The addition of glucose polymers and other supplements to infant foods did not affect the type and number of organisms present. Diarrhoeal enterotoxin was detected in 3 of the 5 formulations which supported the growth of *B. cereus* II via the BCET-RPLA system (*B. cereus* enterotoxin reverse phase latex agglutination test). Although the infant foods were of similar microbiological quality, the majority of *Bacillus* spp. isolated has been previously implicated in food borne illnesses and/or opportunist infections.

In Ethiopia, gastro-enteritis has been a major disease problem among infants and young children. Studies from Ethiopia, South Africa and from other countries on paediatrics diarrhoea indicate that enterotoxigenic coliform such as *Klebsiella, Enterobacter*, and *Citrobacter* are putative casual organisms in addition to known enteric pathogens. Although gastro-enteritis is a major cause of morbidity and

mortality in infants and young children, bacteriological studies of infant food and feeding utensils and its influence on the health of bottle-fed babies in Ethiopia are very scarce. The study did not attempt to isolate pathogens which have special isolation requirements such as *Campylobacter jejuni, Yersinia enterocolitica,* or diarhoeagenic coli other than enteropathogenic *E. coli* (Zeleke, 1998).

In a study by Ergun and Ergun (l999), a total of 100 samples, in original packages, (50 local products and 50 imported products) of food formula for infants were examined for total living bacterial count (*Escherichia coli, Staphylococcus aureus, Salmonella,* yeast and mold). It was found that 12% of local products samples contained germs more than $1.1x10^4$, the number of the same bacterial population in imported formulas did not exceed $l.0xl0^4$, which is accepted as a limit value in the regulations for food products.

Wide variations in total bacterial counts, coliforms, yeast and molds, as well as *Bacillus cereus* and staphylococci counts were found in infant food samples, The differences in counts were significant ($P<0.01$) between different brands. Resuscitation method with pre-incubation of samples in tryptone soya broth for a period of 1 h yielded higher ($P<0.01$) counts than by existing standard methods of enumeration. It was concluded that resuscitation has improved the recovery of sub- lethally injured or stressed cells of all types of microbes that may invariably be found in infant foods (Jyothlrmayi *et al.,* 1999).

El-Prince and Korashy (2003) collected ninety random samples of dried milk-based foods (30 samples each) comprising four brands of

infant's milk powder (for babies after birth, A), three brands of milk-cereal based weaning foods (for 4 months old and above, B) and dried milk powder (for one year old and up, C) from different shops and pharmacies in Assiut City, Egypt, during 2001-2002. The samples were examined for incidence of different types of microorganisms. The obtained results revealed that the average counts of APC, anaerobes, *B. cereus* and yeasts and molds in type A samples were 9.5 x 10^3, 4, 21 and 3.6 x $l0^3$/g, respectively. However, coliforms, psychrotrophs, *Staph. aureus* and thermodurics were not detected. In case of type B, 11 (36.66%), 2 (6.66%) and 13 (43.33%) of the examined samples contained aerobic bacteria, anaerobes and yeasts and molds, respectively, while no *B. cereus*, coliforms, psychrotrophs, *Staph. aureus* and thermodurics were isolated. Moreover, it was noticed that the average values of agar plate count, *B. cereus*, psychrotrophs, thermodurics and yeasts and molds in samples of type C were 6.95 x 10^6, 6.0 x $l0^2$, 9.5 x 10, 1.3 x 10^2 and 3 x 10^3/g, respectively. On the contrary, anaerobes, coliforms and *Staph. aureus* were not detected. The public health and economic significance as well as suggestive measures for improving the microbiological quality of these products were discussed.

Factors affecting the ability of *Escherichia coli* O157:H7 to survive in foods with a_w less than required for growth have not been fully defined. This study was undertaken to determine the ability of *E. coli* O157:H7 to survive in a commercial dry infant rice cereal as affected by a_w (0.35$\pm$0.04, 0.52$\pm$0.03 and 0.73$\pm$0.03), pH (4.0 and 6.8), and temperature (5, 25, 35 and 45ºC), and in nine other reduced a_w

foods. Death of *E. coli* O157:H7 in cereal was enhanced with increased temperature and decreased pH during a 16- to 24-week storage period. Survival was enhanced at pH 6.8 compared to pH 4.0 in cereal at a_w 0.34± 0.04 during initial storage at 5 and 25ºC. The effect of temperature (8, 15, 21 and 30ºC) on survival and growth of acid-adapted cells of *E. coli* O157:H7 inoculated into cereal reconstituted with milk or apple juice at two inoculum levels (8.2–12.3 cfu /ml and 82–123 cfu /ml of slurry) was also studied. Growth occurred in cereal reconstituted with milk at all test temperatures and in cereal reconstituted with apple juice at 15, 21 and 30ºC. Populations increased by > $\log_{10}$ cfu /ml within 3–6 h at 21 and 30ºC. Acid-adapted and unadapted cells had similar growth patterns. The effects of temperature and acid adaptation on survival of *E. coli* O157:H7 in nine commercial foods and food ingredients with pH 4.07–6.49 and a_w 0.17–0.82 were determined. The pathogen survived in these foods for various lengths of time, depending on the storage temperature, with an order of survival of 5ºC > 21ºC > 37ºC. Survival appeared to be enhanced in foods with higher pH, and acid-adapted cells retained higher viability than unadapted cells in only two of the nine test foods. Of particular importance is the ability of *E. coli* O157:H7 to survive well in dry foods with a wide range in a_w and pH, particularly at refrigeration temperature (Yun-Hee *et al*., 1998)

2. Infant botulism

Appuswamy and Ranganathan (1981) collected 547 samples of milk and milk products procured from various markets and dairies in

India and analyzed them for clostridia. Among 310 positive samples, incidence of clostridia was highest in processed cheese (66.4% of 140 samples), followed by infant foods (65.5% of 29 samples), pasteurized milk (59% of 132 samples), raw milk (49.5% of 210 samples) and dried milk (44.4% of 38 samples). Average clostridia spore counts in milk and milk products were: raw milk, 1500/100 ml; pasteurized milk, 300/100 ml: processed cheese, blown cans l000/g and normal cans 500/g; dried milks, 100/g; and infant foods, 150/g.

Guilfoyle and Yager (1983) tested a total of 236 samples of infant foods, including 10 honeys, milk products and canned food, purchased in the New York City area. None contained *Cl. botulinum* spores. The recovery method was validated using foods to which 4 spores /ml had been added, and honey with 40 spores/ml.

Hauschild *et al*. (1988) analyzed a total of 150 honey, 43 syrup and 40 dry cereal samples for *Cl*. *botulinum* spores each in triplicate quantities of 25 g. The foods were sampled randomly, except for 2 lots of honey which were potentially associated with illness. Botulinal spores were detected in a sample of honey associated with infant botulism and in a single sample of rice cereal.

A total of 152 samples of honey were purchased from apiaries and markets in Taiwan, and 50 samples of infant food, including powdered milk, wheat, rice and commercial mixed cereals, as well as juice, were bought from supermarkets in Taipei city from 1988 to April 1989. The samples were investigated for the presence of *Clostridium botulinum* spores. Honey samples were prepared by dialysis to obtain the bacterial spores, whereas infant foods were inoculated directly into

cooked meat medium. Isolated colonies were identified by the Vitek automatic microbial identification system. The supernatants of cooked meat medium enrichment were assessed by typing botulinal toxins via the laboratory animal toxicity test. The results showed that two samples of honey contained the spores of *Cl. botulinum* type B, but there were none in samples of infant foods. The specific responses of clinical signs in mice, after being administered the supernatant of suspected cultural broth, were described (Du *et al.*, 1991).

Luba Vangelova (1995) pointed that infant botulism differs from food-borne botulism in that the toxin itself is not ingested. Instead, *Cl. botulinum* spores swallowed by the infant germinate and produce the toxin in the favorable environment of the baby's large intestine.

Because the spores are nearly everywhere in the environment, children and adults regularly ingest them, yet very rarely suffer ill effects, In a few cases, adults who have had intestinal surgery or whose intestinal tracts have otherwise been altered have contracted the disease the way infants do. This has led researchers to conclude that infants' as-yet "incompletely-developed intestinal flora" may be to blame, says Arnon, one of the co-discoverers of infant botulism in 1976 (Luba Vangelova, 1995).

Infant botulism is serious, but rare and not usually fatal. From 1976 through the end of 1993, 1206 infant botulism cases were confirmed in the United States. About 75 to 100 cases were reported annually. About half of them were in California (presumed to be due to the prevalence of *Cl. botulinum* spores in the state, its high number of births, and the pediatric community's familiarity with the disease

which results in more correct botulism diagnoses). All of the infant cases involve babies less than 1 year old; the disease is most common in the second month of life (Luba Vangelova, 1995).

Infants' immature intestinal tracts offer a "window of vulnerability, and if a baby has the bad luck to swallow a botulism spore during that period, the spore has an opportunity to germinate. The spores travel with microscopic dust particles, so the researchers have concluded that most affected infants have simply inhaled the spores. "They mix with saliva, they're swallowed, and that's how they reach the intestine. Unfortunately, there is no way to prevent the disease in such cases (Luba Vangelova, 1995).

Parents and other caregivers can prevent babies acquiring infant botulism from honey. California researchers have isolated *Cl. botulinum* spores from about 10 percent of store-bought honey samples. Although less than 5 percent of infant botulism patients contract the disease from honey, health officials and pediatricians agree that honey should not be fed to infants under 1 year of age and that is perfectly safe for older children and adults (Luba Vangelova, 1995).

The first sign that an infant has botulism is usually constipation, although this isn't always apparent to parents. Often the baby isn't brought to a doctor until parents notice other symptoms, such as lethargy and poor feeding as the paralysis begins to affect the baby's gag reflex and swallowing ability (Luba Vangelova, 1995).

3. Contamination by *Bacillus cereus* in infant foods

levels in the range 10 to $l0^3$/g) in 38, 193, 17 and 3 of 9465, 1573, 1611 and 377 samples of the infant foods Sunar, Sunarka Feminar and Relakton, respectively, all produced at a factory in Hradec Kralove, Czechoslovakia it was also detected in 2 of 15 samples of cereals used in Sunarka.

A total of 102 samples, comprising 10 brands of infant milk foods and 3 brands of cereal weaning foods were analyzed microbiologically. About 80% of the samples were well within the limits of total viable count standards as prescribed by the Indian Standard Institution (ISI). *Bacillus cereus* was counted in 28% of the samples. Twenty two% of the samples were positive for coliforms and of these only 4.4% were within ISI standards. Staphylococci were also isolated from 57.8% of the samples. No *Salmonella* or clostridia were isolated from any of the samples (Anand and Singh, 1988a).

Becker *et al*. (1994) found that dried milk products and infant food are known to be frequently contaminated with *Bacillus cereus*. Sources of the organism and its behavior in the product and in equipment during processing are discussed with regard to the incidence of *B. cereus* in infant foods. Two hundred and one samples distributed in 17 countries were collected and examined for *B. cereus* content. Fifty four% of the samples were contaminated with *B. cereus* at levels ranging from 0.3 to 600 organisms/g.

Counts >10/g were found in only 27 samples (10%). Most of the positive samples (44%) contained 0.3 to 10/g. Four samples (15%) contained >100 organisms/g reaching a maximum level of 600/g.

When classified into different types of products, about 50% of the infant formulas based on milk, the follow-on formulas and the weaning foods were contaminated with *B. cereus* as well as 69% of those based on soya protein and 92% of the special dietetic foods. Compared with earlier studies from 1982/83, the percentage of contaminated samples from Germany increased from 31 to 70% in the case of infant formulas, from 28 to 55% in the case of follow-on formulas, and from 40 to100% in the case special dietetic foods. The percentage of weaning foods contaminated with *B. cereus* remained unchanged. It should be stressed, however, that the numbers of *B. cereus* were almost the same in both studies with the highest count in 1982/83 being 460 and in 1992 being 600/g. Samples naturally contaminated with counts of about 100g were reconstituted and incubated at 27°C. Levels of 105/g were reached after 7-9 h. Toxigenicity of *B. cereus* in dried milk products and infant foods as well as food poisoning outbreaks attributed to *B. cereus* were also reviewed.

Ranad *et al*. (2006) isolated cereulide producing *Bacillus cereus* from randomly chosen commercial infant foods. The cereulide production in infant food formulas was investigated.

When the reconstituted foods were inoculated with > 10^5 cfu ml^{-1} of cereulide producing *B. cereus*, 2 to 200 μg of cereulide per 100 ml of food accumulated during 24 h of non-refrigerated storage. The amount of cereulide measured in the foods by the accurate chemical assay (LC-MS) matched with that found by sperm micro assay, proving the cereulide was the sole heat stable toxin in the foods and present in its toxic form. The infant formulas containing both cereal

and dairy ingredients were the most supportive for cereulide production. Cereulide accumulation was affected by the infant food composition as well as by the handling of the food. Diluting the reconstituted food with water resulted in increased toxin production expressed as μg per volume. More cereulide was accumulated when the food was incubated stationary compared with moderate shaking. The amount of cereulide accumulated within 24 h at room temperature per 100 ml of cereal and dairy or in rice–nondairy reconstituted infant formulas, inoculated with $\geq 10^5$ cfu ml^{-1} of *B. cereus* strain F4810/72, was higher or similar to the amounts reported for foods implicated in emetic type of food poisonings. Thus, mishandling and temperature abuse of infant foods may cause food poisoning when emetic *B. cereus* is present.

4. Contamination by *Salmonella* in infant foods

Rowe *et al.* (1987) carried out a case control study on Dec. 17-19, 1985, set up as a result of increased widespread occurrence of infants infected with *Salmonella ealing*. They identified a positive relationship with infant consumption of a particular manufacturer's brand of spray-dried milk (product A), which was subsequently withdrawn from the market (Dec. 20, 1985). Microbiological investigation of the factory revealed the source of *S. ealing* to be spray-dried milk behind a hole in the inner skin of the spray drier. Examination of production and quality control records revealed dried milk from certain production runs that had high bacterial viable counts had been blended with powder of low bacterial counts from other production runs before dispatch of 267

sealed milk packets analyzed, *S. ealing* was detected in only 4 which were identified with specific suspect production runs. The factory plant was closed down due to unsuccessful decontamination measures and recommendations were forwarded to limit the transport of raw milk or whey (potential sources of salmonellae) on the factory site.

A glucose/glucose oxidase activated lactoperoxidase system (LPS) delayed the onset of exponential growth of *Salmonella typhimurium* and *Escherichia coli* in infant formulas. Addition of urea peroxidase with the LPS reduced the initial population size, prevented growth of *S. typhimurium*, and extended the lag period before the onset of exponential growth of *E. coli* (Earnshaw *et al.*, 1990). Gelosa (1994) reported a case of food poisoning in 1990 in Italy, in which an unweaned baby was affected. The same serotype of *Salmonella bovis morbificans* was isolated from a stool sample and from the powdered infant milk formula consumed by the child. A description given of the process by which the powdered milk had been produced; it appeared that contamination via an operative carrying the bacterium could have occurred during packing, the only post-pasteurization process performed manually. It is recommended that the principles of HACCP be applied to the infant formula manufacturing process.

Rushdy *et al*. (1998) reported 8 cases of *Salmonella senftenberg* infection in infants identified during January - July 1995 in the UK, 5 of them were indistinguishable *S. senftenberg* strains. A case-control study showed an association between illness and consumption of one brand of baby cereal (P=0.03). The cereal manufacturer reported isolating *S. senftenberg* in June l994 from an undistributed cereal

batch. Outbreak strains and the cereal strain were all plasmid-free in contrast to other human isolates of *S. senftenberg* in the same period. Changes in the production process were implemented to prevent further contamination.

Salmonella has caused food borne illness through consumption of a variety of foods, including dry cereal and infant foods. In particular, reconstituted infant cereals may support growth of recovering or cross-contaminating *Salmonella*, especially if consumption is delayed and the meal is temperature abused. The study assessed survival/growth of *Salmonella* inoculated (four strain mixture) in rice, oatmeal, and mixed rice-oatmeal wheat infant cereal, hydrated (0.5/3.0, w/v) with apple juice, pasteurized milk (2% fat) or water. The inoculated products were stored at 4°C, 15°C, or 25°C for 0, 8 and 24 h to simulate advance home preparation and abuse. Samples were analyzed by plating on tryptic soy agar with 0.6% yeast extract (TSAYE) and on XLT4 agar. There was no growth, but also no death of *Salmonella* in any hydrated cereal stored at 4°C for 24 h. However, at 15°C *Salmonella* increased in cereals hydrated with water or milk from 1.6-2.3 log cfu ml-1 at time 0-4.1 to 5.4 cfu ml-1 in 24 h, while at 25°C the pathogen reached 7.1-7.9 cfu ml-1 24 h. However, in cereals hydrated with apple juice, growth of *Salmonella* was restricted (1.5-2.9 cfu ml-1) after 24 h at 25°C, while weak or no growth occurred at 15°C. When hydrated with apple juice, rice cereal was less supportive of *Salmonella* growth compared to oatmeal and mixed cereals at 15°C and 25°C. Changes in *Salmonella* populations at 8 h of storage had similar patterns; with average increases being smaller compared with those at 24 h. Changes

in bacterial populations on TSAYE followed trends similar to those on XLT4. These results indicate that hydrated infant cereal should be consumed immediately after preparation or held at 4°C for less than 8 h (Abu Shelaibi *et al.*, 2003).

5. Contamination by *Staphylococcus aureus* in infant foods

Kirilenko *et al*. (1983) tested dried milk mixtures for infant feeding that bad been kept for 6-44 months under conditions of' uncontrolled relative humidity (RH) and temperature for *Bacillus cereus* and *Staphylococcus aureus*. *B. cereus* counts/g ranged from 75 to 650 in dried milk mixtures with rice, wheat or oat flour, and from 100 to 450 in Krepysh dried milk mixtures with rice, oat or buckwheat extract *B. cereus* count/g in the dried milk used for these products was only 5 but it was higher in other components, especially dried buckwheat extract (400). *Staph. aureus* was found at concentration of 5/g in dried milk, 50/g in Zdorov' e mixtures with rice or wheat flour, and 10-75/g in Krepysh mixtures with rice or buckwheat extract. It was not found in any of the products containing oat components. Of 44 *B. cereus* strains tested for toxigenicity, 7 (mostly from the Krepysh products) were enterotoxigenic and of 23 *Staph. aureus* strains tested, 3 formed enterotoxin B but none produced enterotoxins A and C.

Mathur and Reddy (1983) analyzed the total bacterial count, and counts of *Escherichia coli* and *Staphylococcus aureus*. They were significantly higher in 8 samples of milk formulas fed to infants from low income group parents than in 10 similar samples fed to infants from high income group parents. Similar, but less pronounced

differences were found between the counts in 14 and 10 samples of weaning foods. Samples of diet were from children under 2 years old in Hyderabad, Andhra Pradesh, India, 20 from families of high income and 22 from poor slum families, In 8 samples of milk feed from the poor families the mean total bacterial count was 353 x 10^3/ml including *Escherichia coli* 220 and *Staphylococcus aureus* 66; in 10 samples from the high-income families the corresponding counts were 8, none and none. In 14 samples of weaning foods from the poor families the counts were 42, 17 and 8 x 10^3/g and in 10 from the high-income families 27, 11 and none. Contamination in hand washings was about the same for high-income as for poor families. In the poor families, milk was often diluted with contaminated water. Water was from a communal tap and utensils were cleaned with ash or mud and water. High-income families had tap water in every house and they sterilized the utensils. Most Indian mothers use their hands to feed the infant, but those of high income generally used a cup or spoon. Findings accounted for the high incidence of diarrhea in such communities, though the incidence was not measured in the present study. The importance of personal hygiene was dear. In was fortunate that prolonged breast feeding was still the practice in rural areas.

Becker *et al*. (1984) collected 206 samples of commercially available infant food and their ingredients were examined for *B. cereus* and coagulase-positive staphylococci as well as for the presence of Salmonellae. *B. cereus* was isolated from 64 samples with numbers between 3 and 460/g (only 2 samples > l00/g). Two samples contained

coagulase-positive staphylococci (4/g each), and no sample contained Salmonellae.

Batish *et al*. (1991) studied the heat resistance of *Staphylococcus aureus* 234 and *S. aureus* NT in phosphate-buffered saline, cow and buffalo milk, reconstituted infant food, and cream. The regression curves of surviving cells were not logarithmic, Among the 3 recovery media, soya bean casein digest (SCD) medium gave maximum numbers of survivors (90 and 260 cfu/ml) in cow and buffalo milk. at 62.5°C after 120 min compared with Baird Parker's Agar (BPA) and Brain Heart Infusion (BHI) Agar, for which the corresponding values were 0,70 and 50,180 cfu/ml, respectively. Similarly, higher F-values (>120 min) were recorded at 62.5°e in cow and buffalo milk when SCD was the recovery medium. The corresponding values on BPA were 120 and >120 min. *S. aureus* NT appeared to be more susceptible to heat-treatment as the F-values in both cow and buffalo milk on SCD medium were only 20 min each. Of the 4 heating media tried, buffalo milk offered maximum protection as revealed by high F-values. The statistical analysis (B-values) also supported these findings. There were significant variations ($P<0.01$) in the heat resistance of *Staph. aureus* 234 between the different heating media, recovery media, time intervals and their interactions. In cream *Staph. aureus* 234 was not completely destroyed at 7l^{o}C even after 120 min.

6. Contamination by *Enterobacter sakazakii* in infant foods

Chantal *et al*. (2003) according to a statement from the company, the US Food and Drug Administration has identified

Enterobacter sakazakii as an emerging foodborne pathogen that can cause sepsis, meningitis, or necrotizing enterocolitis in newborn infants, particularly premature infants or other infants with weakened immune systems.

From the FDA reports over the last several years, investigations of several outbreaks of *Enterobacter sakazakii* infection occurring in neonatal intensive care units worldwide suggest that the outbreak could be associated with milk-based powdered infant formulas. Since powdered formulas are not sterile products, they could contain opportunistic bacteria, such as *Enterobacter sakazakii*. Infant formulas are pasteurized during manufacturing and *E. sakazakii* does not survive such heat treatment. Nevertheless, *E. sakazakii* has been isolated from such infant formulas and it is thought that the pathogen originates from the factory environment, possibly from heat sensitive micronutrients added after pasteurization or from bottle preparation. Even low levels of contamination by *E. sakazakii* in powdered infant formula were considered to be a significant risk factor given the potential for multiplication during the preparation and holding time under certain conditions prior to consumption of reconstituted formula.

7. Occurrence of microbial toxins in infant and baby foods

De Simon *et al*. (1983) analyzed a total of 50 samples of milk/cereal preparations for infants, representing 2 brands. The mycoflora was evaluated, using Czapek-Dox, malt extract and Sabouraud agar media. *Penicillium* spp. predominated, followed by yeasts, *Cladosporium, Aspergillus* and *Monilia* spp. A few *Mucor*,

Scopulariopsis, *Alternaria* and *Phoma* spp. also occurred. Studies on toxigenicity of *P. frequentans*, *P. cyclopium*, *P. decumbens* and *P. expansum* and *Asp. niger*, *Asp. foetidus* and *Asp. clavatus* isolated from these products showed none of these species to form aflatoxin. They have found that most cereal based baby foods, usually the first solid meals given to infants in Canada, regularly contain multiple mycotoxins, potentially harmful molds more often associated with sick building syndrome.

Batish *et al*. (1986) pointed among the different media tested for optimal production of enterotoxin by *Streptococcus faecium* IF-100, trypticase soya broth was the best for maximal enterotoxin production at 37°C after 8 h incubation in agitated culture. The optimum pH was 8.0. Addition of 1% casamino acid enhanced enterotoxin production whereas 1% yeast extract had no appreciable effect. Glucose at 0.5% and acridine orange at 10-20 µg inhibited enterotoxin production. A supplemented medium for maximum enterotoxin production was described.

Anand and Singh (1988b) showed that of 102 infant foods from Karnal, India (10 milk and 3 cereal weaning food brands), 2 milk samples, from different brands, contained 0.7 and 0.34 µg pre-formed *Staphylococcus aureus* enterotoxin type B per 20 g product. Food poisoning can result from ingestion of <1 µg enterotoxin/100 g food. There was no evidence that presence of pre-formed thermostable deoxyribonuclease indicated presence of enterotoxin.

Finoli and Rondinini (1989) tested 26 liquid and dried milk infant formulas for moisture, DM, ash, trace elements, lipopolysaccharide,

aflatoxin M_1 and microbial flora. Microbial counts were lower than the Italian legal limit except for 2 dried samples. The lipopolysaccharide content (an indirect index of contamination by Gram-negative bacteria) indicated poor raw-material quality. Only 3 dried Dink samples contained aflatoxin M_1, the concentrated of which was 13 parts per thousand in reconstituted milk.

Czerwlecki (1994) described a method to estimate ochratoxin A in infant and children wheat-based cereal foods. The mycotoxin was extracted with methanol and phosphoric acid. The extract was clarified with Carrez solutions and defatted with n-hexane. Ochratoxin A was re-extracted with chloroform and estimated by reversed phase-high performance liquid chromatography with fluorimetric detection. The minimum detectable concentration of ochratoxin A was 2 µg /kg and the average recovery was 83%. Aflatoxin M_1 was estimated using commercial immunokits in 376 samples of raw milk from farms in the catchments area of a new factory producing milk-based baby food. 87.8% of samples contained no measurable aflatoxin M_1 (limit of detection, 0.025 µg/liter); 2 samples had an aflatoxin M_1 content of >0.1 µg /liter, which represents the aflatoxin M_1 tolerance limit in the Czech Republic (Fukal and Brezina, 1991).

A psychrotrophic toxin-producing strain of *Aeromonas hydrophila* grew well in a range of food slurries (scallop, prawn, fish, chicken liver pate, liverwurst, chicken luncheon slice, and commercial baby food preparations) held at refrigeration temperatures. In most foods, excluding the baby food preparations, exotoxins were produced at levels comparable with production in bacteriological broth without

apparent food spoilage (all but prawn and fish). Addition of ultra-heat treated milk to toxin-containing broth culture supernatants markedly decreased or removed haemolytic and cytotoxic activities, explaining low levels of toxins found in milk in a previous study. Baby food preparations did not inactivate exotoxins under similar conditions suggesting production of toxins rather than their inactivation was inhibited (Kirov and Brodribb, 1993).

Rowan and Anderson (1997) collected 100 reconstituted milk-based infant formulas (IMF) representative of 10 leading brands available in many European Economic Community countries which were examined for *B. cereus* and for the presence of diarrhea enterotoxin. Sixty three reconstituted IMF supported growth of *B. cereus* after 14 h at 25°C. In 4 IMF, which contained maltodextrin, enterotoxin was detected. Reconstituted IMF (and basal synthetic media) supplemented with 0.1% maltodextrin supported growth of *B. cereus* and diarrhea toxin production when incubated for 14 h or more at 25°C.

Schollenberger *et al*. (1998) randomly collected a total of 237 commercially available samples of cereal-based foods including bread and related products, noodles, breakfast cereals, baby and infant foods, rice and other foods in southwest Germany during the first six months of 1998. The trichothecenes deoxynivalenol [vomitoxin] (DON), 3-and 15-acetyl-deoxynivalenol (3-,15ADON), nivalenol (NIV), fusarenon-X (FUS-X), T -2 toxin (T -2) and HT-2 toxin (HT -2) were determined by gas chromatography/mass spectrometry following clean-up by a two stage solid-phase extraction. Detection limits ranged between 2 and 12

µg /kg. Based on all samples, the incidence of DON, HT -2, T-2, 3-ADON, 15-ADON and NIV was at 71, 18, 4, 4, 4 and 2%, respectively the mean contents in positive samples were at 103, 16, 14, 17, 24 and 109 µg /kg, respectively. Fus-X was not detected in any sample. A lower ($P<0.05$) DON content was found in baby and infant foods as well as in cookies and cakes compared with bread. Overall, based on the incidence and level of all 6 toxins, the degree of contamination was lowest in baby and infant foods. Foods produced from either white or whole grain flour did not differ ($P>0.05$) with regard to the incidence and level of DON. In foods produced from cereals of organic production both the incidence and median content of DON was lower compared with conventional production. Zearalenone, alpha and beta zearalenol were determined by high performance liquid chromatography in 20 selected samples, mostly baby and infant foods. These toxins were not present in excess of the detection limit in any sample.

Three hundred and sixty three samples of cereal-based infant foods were collected from retail marketplaces in Canada over 3 years (1997-1999). The samples included oat, barley, soya, and rice based infant cereals, mixed-grain infant cereals, teething biscuits, creamed maize, and soya-based formulas. Samples were analyzed for targeted mycotoxins (deoxynivalenol, nivalenol, HT-2 toxin, zearalenone, ochratoxin A, fumonisins B_1 and B_2, and 5 ergot alkaloids). Soya-based cereals (which usually contain maize) exhibited the highest incidences of deoxynivalenol (100%), zearalenone (46%) and fumonisins (75%). Overall, deoxynivalenol was the most frequently

detected mycotoxin; it was detected in 63% of samples analysed. Survey results demonstrated the regular occurrence of multiple mycotoxins in cereal-based infant foods (Lombaert *et al.*, 2003).

a. Occurrence of aflatoxins in infant foods raw materials

Toxin-producing organisms could be isolated from natural source and screened for the production of aflatoxins on synthetic media.

Davis *et al.* (1966) studied the production of aflatoxins B_1 and G_1 in nutrient solution consisting of 20% sucrose and 2% yeast extract. Scott *et al.* (1967) reported that *Asp. ostianus* grown for *7* days in mycological broth containing 0.5% yeast extract, produced aflatoxins B_1 and G_1. Scott *et al*. (1970) examined the fungi isolated from food for their ability to produce mycotoxins by growing each isolate on yeast extract sucrose medium (2% yeast extract and 15% sucrose). Cultures were incubated for *7* days at 25°C. They found that fungal isolates can produce 18 mycotoxins.

Burzynska (1971) isolated 124 strains of *Aspergillus, Penicillium, Mucor, Trichothecium* and *Cladosporium* spp. from *74* samples of foods imported into Poland (peanut, walnut, hazelnuts, cocoa beans, cocoa, figs, rice, rye and same seeds). *A. flavus* was the only strain has the capability to produce aflatoxins.

Vanaspati (hydrogenated oil), ghee and both raw and refined mustard, rapeseed, ground nut, and sesame oils were analyzed for aflatoxin content (Sengupata and Roy, 1983). Aflatoxin B_1 was found in raw groundnut oil (0.01-0.0.35 ppm) and in raw sesame oil

(0.02- 0.15 ppm).

Aflatoxin-contaminated corn and cottonseed meal in dairy rations have resulted in aflatoxin M_1 contaminated milks and milk produced, including nonfat dry milk, cheese, and yoghurt. Natural occurrence of mycotoxins in cheeses was found to be a result of mold growth on the cheeses (Northolt *et al.*, 1980).

Scott (1989) extensively reviewed the effects of food processing on selected mycotoxins. He showed that a number of factors, including the process, the food, moistures content, additives, and manner of contamination could affect the stability of mycotoxin during food processing.

Also different mycotoxins have widely different degrees of stability in foods under processing conditions. For example, aflatoxins tend to be stable to moderately stable in most food processes, but are unstable in processes employing alkaline conditions or oxidizing steps.

Mycotoxins can contaminate crops before harvesting, in transport, and in storage. Thus, raw or processed foods and feeds may become contaminated. With the exception of the aflatoxins, the frequency of contamination by mycotoxins is unknown. The aflatoxins are frequently detected in a variety of feeds and foods produced in Untied States as well as in imported commodities and products. Contamination of milk, eggs, and meat can result from animal consumption of mycotoxin-contaminated feed. Aflatoxins, ochratoxin, and some trichothecenes have been given considerable attention, because they are either carcinogenic or of economic concern in animal

health (Marmon and Johnson, 1985).

Rusul and Marth (1988) stated that growth and aflatoxin production by toxigenic aspergilli are partially or completely inhibited by the undissociated form of citric, benzoic, acetic, propionic and sorbic acids.

A collaborative study was carried out by Park *et al.* (1988) for the determination of aflatoxins B_1, B_2, G_1, and G_2 in laboratories of the United States, Canada, South Africa, and Switzerland. Twenty-one artificially contaminated raw peanuts, peanut butter, and corn samples containing varying amounts of aflatoxins B_1, B_2, G_1, and G_2 were distributed to the participating laboratories. For corn, relative standard deviations for repeatability (RSDr) for aflatoxin B_1 ranged from 27.2 to 28.3% for contamination levels from 5 through 50 µg/g. For raw peanuts and peanut butter, RSDr values for aflatoxin B_1 were 35.0 to 41.2% and 11.2 to 19.1%, respectively, for contamination levels from 5 through 25 µg/g. RSDr values for aflatoxins B_2, G_I, and G_2 were similar. Relative standard deviations for reproducibility for aflatoxin B, ranged from 15.8 to 38.4%, 24.4 to 33.4%, and 43.9 to 54.0% for corn, peanut butter, and raw peanuts, respectively.

Faraj *et al.* (1991) stated that highest levels of aflatoxin were produced by *Asp. parasiticus* at 25°C and 0.98 a_w. At 0.90 a_w, toxin production was consistently low at all temperatures.

Fernandez *et al.* (1991) found that minimum a_w, for aflatoxin B_1 (AB_1) production in soybeans infected with *Asp. parasiticus* URRL 2999 was 0.865. At this a_w level, AB_1 production occurred at a temperature higher than 20°C. At lower a_w (0.801 to

0.750), no AB_I production was found at any temperature, *i.e.* 15 to 37°C. Optimum a_w for AB_1 production was 0.99, except for beans held at 37°C where an a_w of 0.9 was optimal.

Doncheva and Diskova (1992) analyzed 6 batches of cocoa beans and found that all batches contained B1aflatoxin (AFB) at a concentration of 5-25 µg/kg. During processing of cocoa beans into cocoa butter, AFB decreased in all batches with each processing step (cocoa grinding, cocoa mass and cocoa cake stages) and was no longer detectable in the cocoa butter. It is believed that the recommended limit of 5 µg/kg for AFB in raw cocoa beans will prevent AFB contamination of the final cocoa product.

Asevedo *et al.* (1993) found that the best conditions for the production of aflatoxins in samples of maize grains, inoculated with toxigenic *Asp. flavus*, were 25°C and 84 and/or 98% relative humidity.

Samples of common Egyptian foods (17 nuts and seeds, 10 spices, 31 herbs and medicinal plants, 12 dried vegetables, and 28 cereal grains) were collected by Selim *et al* (1996) from markets in Cairo and Giza. The highest prevalence of aflatoxin B_1 was in nuts and seeds (82%), followed by spices (40%), herbs and medicinal plants (29%), dried vegetables (25%), and cereal grains (21%). The highest mean concentration of aflatoxin B_1 was in herb and medicinal plants (49 ppb), followed by cereals (36 ppb), spices (25 ppb), nuts and seeds (24 ppb), and dried vegetables (20 ppb). Among nuts and seeds, the prevalence of aflatoxin B1 was highest (100%) in watermelon seeds, in shell peanuts, and

unshelled peanuts. The lowest prevalence and concentrations were in hommos (garbanzo beans). The highest concentrations of aflatoxin B_I were detected in foods that had no potential for field contamination but required drying during processing and storage, such as pomegranate peel, watermelon seeds, molokhia.

The aflatoxin distribution function in individual insect-damaged almonds was determined by Schatzki and Ong (2000) and found to be the sum of two distributions. Substantially all almonds exhibited a positive aflatoxin level between 0.02 and 0.3 ng/g. The precise form of this distribution depending on the lot studied. In addition, 1/1000 of the nuts showed contamination between 60 and $6x10^5$ ng/g; independent of the lot. The latter distribution showed a smooth decrease with log concentration in this range, with no evidence of a minimum. No distribution data between 0.3 and 60 ng/g could be obtained. The distribution below 0.3ng/g was assigned to contamination during post-harvest storage. The distribution above 60ng/g was tentatively assigned to navel orange worm damage occurring when insects enter the kernel during split hulls late in the growing season.

The occurrence of aflatoxin M_1 (AFM_1) in pasteurized milk and dairy products was investigated by Kim *et al.* (2000). Among a total of 180 samples collected in Seoul, Korea, the incidence of AFM_I in pasteurized milk, infant formula, powdered milk and yoghurt was 76, 85, 75, and 83%, respectively, with a mean concentration of 18, 46, 200, and 29 ng/g, respectively.

b. Permissible levels of aflatoxins in foodstuffs

In 1967, it was recommended that level of aflatoxins in food and agricultural commodities must not be exceeded 30 µg/ kg (FAO/WHO /UNICEF 1967). This level decreased to 20 µg/ kg (Duggen and FDA, 1970). The Codex Committee in 1990 decreased this level to only 10 gµ/ kg (Codex Alimentary Commission, 1990).

Recently, the maximum permitted levels of aflatoxins in foodstuffs and dairy products were listed by FAO/FND (1995) in the following Table:

Table 2. The maximum permitted levels of aflatoxins in foodstuffs and dairy products listed by FAO (1995).

Commodity	Aflatoxins	Level (ng/kg)
Peanut products, oil seed products	B_1, B_2, G_1, G_2	10000
Cereal products	B_1	5000
Maize	B_1, B_2, G_1, G_2	20000
Starch and its derivatives	B_1, B_2, G_1, G_2	0
Milk and dairy products	M_1, M_2, G_1, G_2	0

8. Effect of irradiation on contaminated microorganisms in the herbals infant products

In the U.S., over 65 million pounds of spices, herbs and dry ingredients are irradiated each year. Since irradiation is a clearly preferable sanitation method, its use has been allowed by CODEX, and by most countries worldwide. Most countries set maximum dose regulatory limits, and these higher limits allow for higher levels of microbial kill. Storage further enhances the sanitation effect because

injured cells are unable to repair and die off over time (Marcotte, 1993).

Yun-Hee *et al.* (1998) showed that inactivation of *Enterobacter sakazakii, Bacillus cereus, and Salmonella typhimurium* were evaluated in powdered weaning food using electron-beam irradiation. *E. sakazakii, B. cereus,* and *S. typhimurium* were eliminated by irradiation at 16, 8, and 8 kGy, respectively. The D10-vlaues of *E. sakazakii, B. cereus,* and *S. typhimurium* inoculated on powdered weaning food were 4.83, 1.22, and 0.98 kGy, respectively. The results suggest that electron-beam irradiation should inhibit the growth of pathogenic bacteria on baby food without impairing qualities.

Farkas (1998) pointed that decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process. Irradiation is particularly valuable as an end product decontamination procedure. Radiation treatment at doses of 2–7 kGy-depending on condition of irradiation and the food-can effectively eliminate potentially pathogenic nonsporeforming bacteria including both long-time recognized pathogens such as *Salmonella* and *Staphylococcus aureus* as well as emerging or ‘‘new’’ pathogens such as *Campylobacter, Listeria monocytogenes* or *Escherichia coli* O157:H7 from suspected food products without affecting sensory, nutritional and technical qualities. Candidates of radiation decontamination are mainly poultry and red meat, egg products, and fishery products. It is a unique feature of radiation decontamination that it can also be performed when the food is in a frozen state. With today’s demand for high-quality convenience foods, irradiation in

combination with other processes holds a promise for enhancing the safety of many minimally processed foods. Radiation decontamination of dry ingredients, herbs and enzyme preparations with doses of 3–10 kGy proved to be a viable alternative to fumigation with microbicidal gases.

Timprasert *et al***. (**2005**)** carried out an investigation on the effects of irradiation on chemical changes and contaminated microorganisms in turmeric and creat powder. Tumeric powder was bought from a farmer group at Thakhun, Surathani and creat powder was brought from a group of farmers at Kampeangsan, Nakornpathom. These powdered herbs were irradiated with gamma rays at 5, 10, 15 and 20 kGy. The results showed that there were no chemical changes in both turmeric and the creat. There was a significant reduction in colonies per gram of total bacteria, yeast & molds, enterobacteria and *E. coli* in both herbs. There has been no chemical change in the percentage of moisture, ash, non-soluble ash, water soluble substance or curcuminiod. Exceptional percentage of water soluble substance concentration in creat was not significantly reduced compared to nonirradiated treatment (control).

Irradiation treatment with ionizing radiation is an effective means for spice sterilization (Goto *et al*., 1971; Vajdi and Pereira, 1973; Gottschalk, 1977; Eiss, 1984). Irradiation processing of spices with ^{60}Co radiation on the commercial scale has already come true in the USA and a few European countries (Food Irradiation Newsletter, 1984). Consumers of irradiation spices have sense of security thanks to little ratio of spices in meals. So irradiation spices are easily accepted

by the public and approved by governments in quite a few countries.

Farkas (1998) showed that a major concern of food processors is to assure that the microbial load of ingredients and processing aids does not contribute to spoilage of food and does not diminish its microbial safety. Spices and dried vegetables or herbal teas may not be suitable substrates for the growth or long survival of salmonellae or other nonsporeforming pathogenic bacteria, nevertheless, occasional *Salmonella* contamination is a reality (Bockemühl and Wohlers, 1984; Bruchmann, 1995). The microbiological quality of the so-called instant soups which need not be boiled before consumption is of particular importance. If the reconstituted product is held warm, particularly between 30–50^{o}C, eventual pathogens may grow to levels that will cause illness. Radiation decontamination of spices and many other dry food ingredients is a viable alternative to less effective, or toxicologically suspicious other decontamination processes, and it has a great application potential both in developing and the industrialized countries (Farkas, 1988). In addition to strict hygiene in preparation, radiation decontamination of spices, herbs, enzyme preparations and other dry ingredients with doses of 3–10 kGy proved to be a reliable method for improving microbiological safety of such products (Farkas, 1988). The effect of irradiation on the microbial counts of black pepper, one of the most highly contaminated spices, is shown in Table (3).

Table 3. Effect of ionizing radiation on the microbial quality of black pepper*.

Organisms	Log $_{10}$ cfu/g					
	0 kGy	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
Aerobic mesophilic colony count	8.0	6.2	5.2	3.9	2.1	< 1.8
Aerobic mesophilic spore count						
a. Surviving 1 min at 80ºC	7.7	6.5	4.7	3.0	1.8	< 1.8
b. Surviving 20 min at 100ºC	6.0	2.9	0.2	-	-	-
Anaerobic mesophilic spore count						
a. Surviving 1 min at 80ºC	7.5	6.1	3.1	< 1.8	< 1.8	< 1.8
b. Surviving 20 min at 100ºC	5.9	< 2.8	< 1.8	< 1.8	< 1.8	< 1.8
Enterobacteriaceae	4.7	2.8	1.7	1.1	< -0.5	-
Lance field D streptococci	4.9	1.7	0.4	< -0.5	-	-
Molds	4.6	< 1.8	-	-	-	-

*After Soedarman *et al.* (1984).

The use of irradiation instead of ethylene oxide to ensure hygienic quality of spices and dry vegetable seasonings has increased in the last 10 years especially because of the banning of ethylene oxide in the community.

Herbs commonly harbor a large number of bacteria and fungi including potential spoilage organisms (Farkas, 1988). In general, roots, berries, and herbs carry a greater microbiological load than the bark and seed items. Seeds of herbs can have microbial populations as great as millions per gram. The most common bacteria are the spore-formers, such as *Bacillus* spp., *Clostridium* spp. and *Salmonella* species (Pafumi, 1986; Kneif and Berger, 1993; McKee, 1995). Today, three major methods are in use for decontamination of herbs, namely

gas (ethylene oxide and propylene oxide), irradiation and steam (Eiss, 2001). However, ethylene oxide gas has been banned in the European Union and other countries (Loaharnu, 1998). Steam degrades the light-weight leafy herbs, and ground products are difficult and sometimes impossible to handle in the steam system (Ehrenberg and Hussain, 1981; Kligerman *et al*., 1983; Pfeiffer and Dunkelberg, 1980; OSHA, 1984; Eiss, 2001).

Radiation decontamination of herbs with doses ranging from 3 to 10 kGy proved to be a viable alternative to fumigation or steam. The use of irradiation instead of ethylene oxide to ensure hygienic quality of herbs has increased in the last 10 years, especially because of the banning of ethylene oxide (Farkas and Andrassy, 1985; Farkas, 1988; Farkas, 1998; IAEA, 1992; Ito *et al*., 1999).

According to standards established by the WHO (1985), most untreated herbs, harvested and handled under hygienic conditions and tested by appropriate methods of sampling and examination, should contain not more than 1×10^4 bacteria per gram. There is no information available on microbial loads, the quantity of extract and its characteristics for irradiated aniseed, especially when high irradiation doses (up to 20 kGy) are employed.

Louise *et al*. (2006) reported that inactivation of microorganisms by electron-beam irradiation comes from the inhibition of DNA repair mechanism by increased energy demand of homeostasis on the cell. Some species of *Enterobacter* such as *E. sakazakii*, however, were reported to be the most resistant to irradiation. Population of *E. sakazakii* treated with the electron beam at 2 and 8 kGy were reduced

to 5.49 and 4.47 log cfu/g, respectively, compared to 6.29 log cfu/g for the non-irradiated powdered weaning food sample (Hong *et al*., 2008).

9. Effect of ethylene oxide fumigation on contaminated microorganisms in the herbals infant products

Sun drying or ethylene oxide gas treatments are common practices for microbial decontamination in herbs. These practices can kill any microorganisms to some extent and are rather complicated processes. Moreover, with chemical treatment, residue may result in environmental damage and is toxic to the customers.

Introduction of irradiation microorganisms is accepted by 40 countries over the world. This method can irradiate contaminating microorganisms safely and efficiently with no residue in the products as well as no reduction of the important substance of treated herbs (Biological Sciences Division, 1999). Therefore, it is important to evaluate the optimum dose of irradiation.

Further information is now available on some of the reaction products resulting from ethylene oxide fumigation of foodstuffs. The number of known reaction products has increased considerably since 1965 (Lindgren *et al*., 1968). Of these reaction products the ethylene oxide-amino acid adducts are reported to be non-toxic (Lehman, 1965). There is no toxicological information on the reaction products with carbohydrates, vitamins, etc.

Ethylene chlorohydrin has been shown to result from fumigation of foods with ethylene oxide due to interaction with natural chlorides present in the crop. In flour fumigated with ethylene oxide, a level of 260 ppm ethylene chlorohydrin has been detected. Fumigation of

spices also produced ethylene chlorohydrin (Wesley *et al*., 1965). A consideration of the available toxicological data on ethylene chlorohydrin is therefore pertinent to the assessment of ethylene oxide (Anonymous, 1966).

MATERIALS AND METHODS

1. Samples

Twenty seven samples of different infant food products were collected form retail markets and pharmacies Cairo, Giza and 10th Ramadan cities during 2004 and 2005, to study the incidence of different pathogenic bacteria and other microbiological groups. Samples of the infant food products are listed and sorted in Tables (4, 5, 6 and 7) according to the company and ingredients.

Table 4. Label data of the cereal- based infant products.

No.	Product	Company	Infant age	Ingredients
1	Riri with vegetables	Riri	6 months	Rice flour – Skimmed milk powder – Vegetable powders (Peas, Carrots, Potatoes, Tomatoes) – Vitamins – Minerals – Casein
2	Riri with Honey	Riri	5 months	Rice flour – Skimmed milk powder – Honey – Casein– Vitamins – Minerals
3	Riri Flakes	Riri	3 months	Rice flour – Vanillin – Vitamins – Minerals
4	Riri Chocolate	Riri	9 months	Rice flour – Skimmed milk powder – Non fat cocoa – Casein– Vitamins – Minerals
5	Riri Banana	Riri	5 months	Rice flour – Skimmed milk powder – Banana powder – Casein– Vitamins – Minerals
6	Cerelac	Nestle	6 months	Wheat Flour – Skimmed milk– Sucrose – Palm oil – Caramelized Sugar – Corn oil – Vitamins – Minerals – Vanillin

Continued

Table 4. Continued

7	Cerelac with fruits	Nestle	6 months	Wheat Flour – Skimmed milk powder – Sucrose – Palm oil – Dehydrated fruits (Banana, Guava, Mango, Apple) – Corn oil – Vitamins – Minerals – Vanillin
8	Cerelac with chocolate	Nestle	9 months	Wheat Flour – Skimmed milk powder – Sucrose – Palm oil –Sun flower oil – Vitamins – Minerals – Vanillin – Cocoa powder
9	Wheat	Nestle	6 months	Wheat Flour – Sucrose – Vitamins – Minerals – Vanillin
10	Rice	Nestle	6 months	Rice Flour – Sucrose – Vitamins – Minerals – Vanillin
11	Fruity cram	Jotis	6 months	Wheat Flour – Skimmed milk powder – Full cream milk – Sucrose – Dehydrated fruits (Banana, Orange, Pear, Apple) – Vitamins – Minerals
12	Baby King Special formula	Pharama Net Egypt	6 months	Lactose – Carob powder – Chick pea powder – Maltodextrtine – Starch – Vegetable fat – Reduced Skimmed milk powder – Rice Flour – Banana powder – Apple powder – Vitamins – Minerals

The ingredients are listed as indicated by the manufacturer on the package.

Table 5. Label data of the milk- based infant products.

No.	Product	Company	Infant age	Ingredients
1	Nan 1 Infant milk	Nestle	From birth	Deminerlaized cow's milk whey fraction– Palm olein – Low erucic rape seed oil– Coconut oil– Corn oil–Milk whey protein fraction– Soy lecithin– Vitamins – Minerals
2	Bebelac 2	Nutricia Cuijk B.V	6 months	Milk whey– Lactose– Palm olein– Rape seed oil– Coconut oil– Sun flower oil– Glucose- Dietary fiber – Beta carotene – Prebiotics – Vanilline – Soy lecithin – Vitamins – Minerals
3	Bebelac 3	Nutricia Cuijk B.V	6 months	Milk whey – Lactose – Palm olein – Rape seed oil – Coconut oil – Sun flower oil – Glucose – Dietary fiber – Beta carotene – Prebiotics – Vanillin – Soy lecithin – Vitamins – Minerals
4	Promil Gold	Wyeth Nutritionals	6 months	Ethyl vanillin – Milk whey – Lactose – Palm olein – Soy seed oil – Coconut oil – Sun flower oil – Lactose – Soy lecithin – AA (of Martierella alpinea origin) – DHA (of crypthecodinium cohnii origin) – Vitamins – Minerals
5	Babysan 2	Lacto misr	6 months	Milk whey, lactose, palm olein, soy oil, starch, maltodextrine, vitamins and minerals
6	S-26 Gold	Wyeth Nutritionals	From birth	Milk whey–Lactose – Palm olein – Soy seed oil –Coconut oil– Sun flower oil–Lactose– Soy lecithin –AA – DHA (of crypthecodinium cohnii origin) – Vitamins – Minerals.

Continued

Table 5. Continued

7	Biomil 1	Egyco Pharm	From birth	Milk whey – Palm olein – Coconut oil – Rape seed oil – Sun flower oil – Lactose – Vitamins – Minerals

The ingredients are listed as indicated by the manufacturer on the package.

Table 6. Label data of the vegetable and fruit-based infant products.

No.	Product	Company	Infant age	Ingredients
1	Gerber Peas	Gerber	6 months	Water – Green peas
2	Gerber Mixed Vegetables	Gerber	6 months	Pine apple juice – Orange juice – Lemon juice – Sugar – Starch – Water – Vitamin C
3	Gerber Fruit dessert	Gerber	6 months	Carrot – Parsley root – Celery – Corn starch – Rape seed oil – Water
4	Gerber Apple	Gerber	6 months	Apple – Vitamin C

The ingredients are listed as indicated by the manufacturer on the package.

Table 7. Label data of the herbal-based infant products.

No.	Product	Company	Infant age	Ingredients
1	Herbal drink	Milupa	From birth	Balm, fennel – Chamomile – Peppermint – Anise – Maltodextrine – Dextrose
2	Herbal drink	Riri	4 months	Glucose – Sucrose – Extract of (chamomile, palm – Thyme – Anise – peppermint)
3	Baby calm	Sekem	From birth	Caraway – Anise, Liquorices – Chamomile
4	Baby drink	Pharco	From birth	Extract of (Chamomile – Liquorices – Thyme – Anise – Peppermint), Glucose – Sucrose

The ingredients are listed as indicated by the manufacturer on the package.

2. Experimental approaches

The first part of the present study is concerned with accomplishment of microbiological and chemical survey for common types of infant food products in Egyptian retail markets and pharmacies. To realize this objective, the twenty seven samples were divided to four groups according to their type. Twenty five grams of each homogenized sample were placed in 225 ml of peptone water (Difco, 1989), thoroughly mixed and serial dilutions were prepared. Appropriate dilutions from each of the prepared samples were inoculated into different nutrient and selective media. The samples were tested for the presence and estimation of hazardous microorganisms causing poisoning, *i.e. Salmonella*, *Listeria* sp., *Bacillus cereus*, *Staphylococcus aureus*, *Shigella* and *Escherichia coli*; microorganisms causing spoilage (proteolytic and lipolytic bacteria), total aerobic viable counts, yeasts and molds; total coliforms and *Clostridium* sp.

In addition, chemical analysis included the determination of protein, fat, water activity (a_w) and aflatoxins.

The second part of the present work was to investigate the effect of hot water and paper filtration, gamma irradiation and ethylene oxide on the microbial load of the herbals infant products.

3. Microbiological determinations

Appropriate dilutions prepared from each sample were used

for inoculating different nutrient and selective media. The microbial determinations applied were as follows:

a. Total aerobic viable counts

Aerobic counts were estimated on trypticase soy agar medium (APHA, 1978) using the pouring plate technique. Suitable plates were counted after incubation at 37°C for 48 hours.

b. Coliform counts

Coliform and faecal coliform counts were estimated on MacConkey agar (Difco, 1989) using the pouring plate technique. Suitable plates were counted after 24 hours at 37°C and 44.5°C for total coliform and faecal coliform counts, respectively.

c. Yeast and mold counts

Yeast and mold counts were estimated on Sabouraud dextrose agar (Oxoid, 1998). Inoculated plates were incubated at 25°C for 5-7 days.

d. Pathogenic and indicator bacteria

1. *Staphylococcus aureus*

The numbers of *Staph. aureus* were estimated on Baird Parker agar medium **(**Baird-Parker and Devenport, 1965**).** The plates were incubated at 37 °C for 48 hours. On this medium, *Staphylococcus* colonies show features including characteristic

zones which are formed as a result of lipolysis and proteolysis of egg-yolk while the reduction of tellurite to tellurium produces a black color of *Staphylococcus* colonies. The egg-yolk reaction and tellurite reduction are usually found to occur together with a positive coagulase reaction and can thus serve as an index for the latter (Difco, 1989**).**

Coagulase production test

Cultures showing anaerobic fermentation of mannitol and glucose were examined for coagulase production using tube test technique according to Oxoid (1998). To 1:5 saline dilution of citrated rabbit plasma, a large inoculum of *Staphylococcus* grown in broth culture was added. The tubes were incubated at 37°C and examined after half an hour and at 30 minutes intervals up to 3 hours. Clotting usually occurring within 1 hour indicates the presence of coagulase positive staphylococci.

2. *Salmonella* detection

a. *Salmonella* detection by selective media

Twenty-five g of each sample were added to 225 ml of peptone water as a pre-enrichment medium and incubated at 37°C for 24 hours. Twenty five ml from the pre-enrichment culture were added to 225 ml of tetrathionate broth (Oxoid, 1998) as an enrichment medium with incubation at 37°C for 24 hours. After incubation, the culture was streaked on brilliant green agar plates and examined after 18-25 hours (Khan and McCaskey, 1973). On this medium, presumptive *Salmonella* appears as pink colonies surrounded by bright red medium. If the first streaking after 24 hours gave a negative

result, the cultures were further streaked on the same medium and incubated for further 24 hours. Plates were examined and all the suspected *Salmonella* colonies were picked up and streaked on MacConkey agar plates for purification. Non-lactose fermenting colonies were accurately picked up, streaked onto Difco triple sugar iron agar slants and incubated at 37°C for 18-24 hours. Cultures showing alkaline slants with acid butts, and H_2S production were suggested as *Salmonella.*

b. *Salmonella* detection By PCR technique

For pre-enrichment, twenty- five grams of each sample were added to sterile lactose broth. The mixture was incubated at 35ºC for 24hours. Re-growth was conducted by transferring 0.5 ml of the pre-enriched sample into 2.5 ml brain heart infusion broth. Cultures were incubated for 3h at 37ºC. Tow hundred micrometers of the re-grown culture was mixed with 200µl of lyses (0.5% *N*-lauryl sarcosine, 50 mM Tris–HCl, 25 mM EDTA, pH 8.0), vortexed for 1 min and centrifuged at 15,000 rpm for 5 min using Biofuge 15 (Heraeus, Germany). The pellet was resuspended in 200 µl of lysis buffer containing glycogen to a final concentration of 0.03 µg/ml and 4 µl of proteinase K (2 mg/ml) was then added to the suspension. After incubation at 37 °C for 1 h, 300 µl of NaI solution (6 M NaI in 50 mM Tris–HCl, 25 mM EDTA, pH 8.0) and 500 µl of isopropanol was added to the suspension and then centrifuged at 15,000 rpm for 5 min using Biofuge 15 (Heraeus). The pellet was washed in 35% (v/v) isopropanol, dried for a short time and then

resuspended in 50 µl sterile distilled water for PCR.

Polymerase Chain Reaction was conducted according to Jones *et al.* (1993). For this purpose, LHNS-531 (5′-TACCAAAGCTAAACGCGCAGCT-3) and RHNS-682 (5′-TGATCAGGAAATCTTCCAGTTGC-3) were used as sense and antisense primers to identify a 125 pb portion of hns gene coding for a DNA binding protein conserved in all *Salmonella* spp.

Five micrometers of DNA extract was amplified in a 50 µl PCR mixture consisting of 1× PCR buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin), 0.5 µM of each primer, 200 µM of each of the four dNTPs, 2.5 U of Taq polymerase (Bangalore Genie, India). The cycling conditions were as follows. After an initial denaturation for 3 min at 94 °C, first five cycles consisted of denaturation at 94 °C for 1 min, primer annealing at 60 °C for 1 min and primer extension at 72 °C for 1 min. This was followed by 30 cycles consisting of template denaturation at 94 °C for 0.5 min, primer annealing at 60 °C for 0.5 min, and primer extension at 72 °C for 0.5 min. The post-amplification of the flush ends was performed at 72 °C for 5 min. The reactions were performed in a PTC-100 thermal cycler (M.J. Research, USA). In all the cases, the ‘hot start’ method was followed as originally suggested. The products of PCR were separated on a 2% agarose gel, stained with ethidium bromide (0.5 µg/ml) and photographed using gel documentation system (Hero Lab., Germany).

3. *Listeria* spp.

Twenty-five grams from each sample were blended with 225 ml of *Listeria* enrichment broth UVM1 (Oxoid, 1998) as a primary enrichment broth. (ICMSF, 1996). The samples in the primary enrichment broth UVM1 were incubated at 30°C for 24 h, after that 0.1 ml of the inoculated UVMl, was transferred to 10 ml of *Listeria* enrichment broth UVM2 (Biolife, 1991) and incubated at 30°C for 24 h (McClain and Lee, 1988).

Isolation:

A loopful from UVM2 was streaked onto Palcam agar supplemented with *Listeria* palcam supplement (Biolife, 1991) and incubated at 30°C for 48 h (Vannetten *et al*., 1989). Five typical colonies (dew drops-like dark brown or black colonies with brown halo) were picked up, streaked onto trypticase soy agar supplemented with 0.6% yeast extract and incubated at 30°C for 24 h. Pure separate colonies were inoculated into tubes of trypticase soy broth supplemented with 0.6% yeast extract and incubated at 37°C for 24 h. *Listeria* produces browngreen coloured colonies on trypticase soy agar with a black halo (Curtis *et al.*, 1989**).**

4. *Bacillus cereus*

The numbers of *B. cereus* were estimated on *Bacillus cereus* Palcam agar medium according to Mossel *et al*. (1967). The melted medium was cooled to 50^{o} C and for each volume (470 ml), 5 ml of polymyxin B sulfate (an antibiotic) were added to the medium (100 µl/ml of melted medium). The antibiotic was dissolved in 5 ml of

distilled water before the addition to the medium. Egg yolk emulsion was also aseptically added in a ratio of 25 ml / 470 ml of the melted medium. Medium plus the additives were mixed and poured into inoculated Petri dishes. Sample dilutions were subjected for pasteurization at 80ºC for 15 min before dispensing the dilution into plates. Plates were then incubated at 37ºC for 24 to 48 h and *B. cereus* colonies were characterized as 5 mm in diameter, turquoise blue in color, surrounded by distinct opaque zone of egg yolk precipitation with the same color.

5. *Shigella*

The samples were mixed with sterile peptone water in a ratio of 1:10 and incubated at 37°C for 24 h. One ml of the pre-enriched broth was transferred to a sterile test tube containing 9 ml of Gram-negative broth (Oxide 1989) as an enrichment medium. The tubes were incubated for 16-18h at 37°C, after which a loopful from each enriched broth was streaked on the surface of both Xylose-Lysine Desoxycholate (XLD) agar and *Salmonella-Shigella* (S.S) agar. The plates were incubated invertedly at 37°C for 24h. On XLD agar, *Shigella* colonies are red and on S.S. agar, Shigella gives translucent small colonies (Ellis *et al.,* 1976).

6. *Escherichia coli*

Twenty five g of the sample were transferred to a sterile flask, mixed well with 225 ml of MacConkey broth and incubated at 37°C for 24h, after which a loopful from the incubated homogenate was streaked on Eosin Methylene Blue (EMB) agar plates which were

incubated for 24h at 37°C. Medium size colonies with green metallic sheen were suspected to be *E. coli*. The suspected colonies were transferred to the surface of slant agar for purification after which biochemical identification was done (Collins *et al.*, 1998).

Biochemical confirmatory tests for *E. coli* isolates

a. Gram staining

E. coli are Gram negative non-spore forming short rods.

b. Indole production

To 48 hours old culture incubated at 37°C in 1% peptone water, an amount of 0.5 ml of Kovac's reagent was trickled down on the side of the tube. Development of rosy color indicates a positive reaction.

c. Methyl red test

To an amount of 5 ml of 48 hours of peptone water 1% culture incubated at 37°C, 5 drops of methyl red reagent were added. Red color indicates a positive reaction, while the negative one gives yellow color.

d. Vogus-Proskauer test (V.P.)

To 5 ml of 48 hours of peptone water 1% culture incubated at 37 °C, 3 ml alcoholic solution of alphanaphthol and 1 ml of 40% KOH were added. The mixture was thoroughly sharked, if bright pink coloration is developed after 15 minutes, this indicates a positive result, while a yellow color indicates a negative one.

e. Citrate utilization test

Microbes were inoculated onto citrate agar slants and incubated for 24-48h. Positive reactions gave slants with blue color; indicating the ability of the organism to utilize citrate as the sole carbon source. Retaining the medium green color indicated negative results.

7. *Enterobacter* spp.

Enterobacter spp. counts were estimated on Violet Red Bile Glucose agar (VRBGA) (Oxoid, 1998) using the pouring plate technique. Inoculated plates were incubated at 30^{o}C for 2 days (Pridmore and Silley, 1998).

8. *Clostridium* spp.

Differential reinforced clostridia medium (DRCM) (Gibbs and Frame, 1965) was used for detection of *Clostridium*. Before use, sterile solution of anhydrous sodium sulfite and ferric citrate was added to the heated medium to give a final concentration of 0.04% and 0.07%, respectively. Medium was inoculated with dilutions pasteurized at 80^{o}C for 15 minutes. 1ml of each dilution was inoculated into each of 2 tubes of DRCM and incubated at 30^{o}C for upto 3-5 days.

e. Microbial groups responsible for spoilage

1. Proteolytic bacteria

Proteolytic bacteria were estimated on milk agar (James and Natalie, 1987) using the plate method technique with incubation at 30°C for 24 hours. Colonies which were surrounded by clear zones were estimated as proteolytic bacteria.

2. Lipolytic bacteria

Lipolytic bacteria were estimated on butter-fat agar (James and Natalie, 1987). Plate method technique was used with incubation at 30°C for 1-2 days. Colonies of lipolytic bacteria were detected by flooding the plates with saturated aqueous copper sulfate for 10 min, the plates were subjected to running water for few minutes. Colonies with a turquoise blue zone beneath or around the colony were estimated as lipolytic bacteria.

4. Chemical determinations

All foodstuff samples under investigation were subjected to chemical determinations including the following parameters:

a. Protein content

Crude protein in various foodstuffs under investigation was determined by the automated method using Kjel-foss automatic (Model 16210) as described in AOAC (2002). For sample digestion, 3 Kjel tablets were placed in its special flask and simultaneous addition of 10 ml 30-35% H_2O_2 and 12-15 ml of 96-98% H_2SO_4 was done. One gram of sample was added, digested for 3 minutes and then re-digested for additional 3 minutes. The flask was cooled and 140 ml H_2O was added, after which NaOH solution was introduced in excess. Released NH_3 was steam distilled quantitatively into a 200 ml beaker containing 50 ml mixed indicator solution (1.0 g methyl red and 0.25 g methylene blue in 1.0 L ethyl alcohol) and titrated with diluted

H_2SO_4 Crude protein was automatically calculated. Calibration of the instrument was carried out using aliquots of $(NH_4)_2SO_4$.

b. Fat content

Fat content was determined according to the method described in AOAC (2002). A known weight of the dried samples was extracted by petroleum ether in a Soxhlet apparatus for 4 hours. The solvent was evaporated under reduced pressure and the total fat content was accurately weighed.

c. Water activity (a_w)

Water activity was estimated in the samples using hygroscopic rotronic instrument (model AD-250, Rotronic Instrument Corp., USA).

d. Aflatoxins

Levels of aflatoxins (B_1, B_2, G_1 and G_2) were determined according to the method described by Vidyasagar *et al*. (1997) using the immunoaffinity chromatography and fluorometric techniques.

1. Extraction

a. Fifty gram sample, 5 g NaCl and 100 ml of 80% methanol were mixed in a 250 ml beaker.
b. Mixing using a blender was carried out for one minute.
c. Filtration was carried out using Whatman No. 1 filter paper.
d. The diluted solution was filtered using glass microfiber paper.

2. Purification

a. Ten ml of filtrate were passed throughout the column.
b. The first and second washing were done by 10 ml of distilled water for each.

3. Estimation

a. One ml of 100% methanol (HPLC grade) was added throughout the column.
b. The solute was collected in a measuring cell, and measured by a fluorometer (VICA series – 4 fluorometer model VICAM V1.0).

5. Effect of different treatments on contaminating microorganisms in infant food component from herbals

a. Effect of irradiation on contaminated microorganisms

Nineteen samples of herbs infant food products were collected form retail markets and pharmacies around Cairo, Giza and 10th Ramadan cities during 2004 and 2005. Ten grams of seeds were transferred into a polyethylene bag, and every 24 bags were packed in a carton box. Packed seeds were treated with 0, 1, 2, 3, 4, 5 and 10 kGy of gamma rays using a ^{60}Co source with a dose rate of 719 Gy/h. The irradiation was carried out at the Atomic Energy Agency, Nasr City.

b. Effect of ethylene oxide on contaminating microorganisms

Sterilization / fumigation with ethylene oxide was performed in vacuum or gas-tight chambers designed for use with ethylene oxide. The following is a list of ranges for the critical variables which should be in proper relationship for ethylene oxide to be an effective

sterilizing/ fumigating agent.

Temperature: 30 °C

Pre-vaccum*:* typically 0.86kg/cm2 (25 inches of mercury). Vacuums and/or inert gas purges should be compatible with the products and packages to be sterilized/fumigated, and such that explosive atmospheres are never present in the chamber.

Moisture*:* relative humidity of 66 %

Gas concentration*:* 250 mg/l to 1500 mg/l (mg/l means milligrams of ethylene oxide per liter of chamber volume) restricted 3.00 kg.100 m^3

Exposure time*:* 8 hours

The fumigation was carried out at the National Papering Medicine Co., 10^{th} Ramadan City.

c. Effect of hot water on microbial contamination of infant food components

1. Infant food components from herbals

Infant food preparations packed in filter paper packets (baby calm; a total of 15 packets from different batches; 1.5 g each) were placed in 100 ml boiling tap water. The preparation was allowed (about 15 min) to cool to the room temperature and used to inoculate different culture media for bacterial enumeration.

2. Infant food components based on vegetables or cerelac

Twenty five g of either Riri with vegetables or cerelac samples were placed in 225 ml of boiling tap water. Immediately and at 15 min intervals up to 60 min, the microbiological analysis was investigated.

The aim of this experiment was to evaluate the survival of the different microbial groups during the first hour of infant food preparation using boiling water.

6. Hazard analysis during manufacturing of infant food packaging materials

For testing plant hygiene and product quality during manufacturing of infant food packages, the following microbiological measurements were made: (i) Sanitation tests, *i.e*. environmental swabs from hands of workers and machines (equipment) to measure the bacteriological condition of both. (ii) Microbiological quality of the air inside the plant. (iii) The bacteriological quality of the packages.

a. Environmental swabs

The swab contact method was applied. In this method, a sterile swab is dipped in a sterile phosphate buffer solution and then used to "wash" the surface. This lifts the bacteria onto the swab. The swab is rubbed over a selected area, rolling back and forth and criss-cross to thoroughly cover the few square inches involved. The swab is dipped back into the sterile solution several times during the cleaning so that the bacteria are rinsed off into the tube. The final step is to break off the tip of the swab and place it in the solution. The tube is shaken hard to rinse all the bacteria out of the swab and into the solution. The solution is subsequently diluted and poured onto the culture media plates.

In the present study, moistened sterile cotton swabs were rubbed over the hands (*ca.* 30 cm^2) of different workers or different test surfaces of the machines used to manufacture the infant food packages. The following

microbiological determinations were made: total coliforms adopting MPN technique and MacConkey broth medium, *E. coli* using EMB agar and *Staph. aureus* using Vogel-Johnson agar. The surface of both EMB and Vogel-Johnson media was streaked with the swabs.

b. Microbiological quality of the air inside the plant

The culture settling plate technique (Sveum *et al*., 1992) was applied to determine the air quality. In this method, open Petri dishes containing 20 ml of culture media (nutrient agar or MacConkey agar) were distributed around the production lines and exposed for about 15 minutes. The Petri dishes were closed and incubated fat 35 ˚C/48 h for aerobic plate count and 37 ˚C/48 h for total coliform. Results were expressed as CFU plate^{-1} 15 minutes^{-1} exposure. The culture settling plate is currently classified by the APHA as method D, which used to be considered standard.

c. Assessment of the hygiene of packaging materials

Sterile cotton swabs were rubbed over a specified area, *i.e.* about 100 cm^2 of the interior surface of the packing material and used to enumerate the total aerobic counts (nutrient agar), total coliforms (MacConkey broth and MPN technique), *E. coli* (EMB agar) and *Staph. aureus* (Vogel-Johnson agar).

7. Survival and growth of both *E. coli* or *Staph. aureus* in reconstituted cereal hydrated with milk

a. Bacterial cultures and preparation of inoculum

The inoculua used in the present study were an isolate of

Staphylococcus aureus isolated, from the swabs taken from the workers hands employing at the plant under investigation, and a strain of *E. coli* ATCC 25922. Stock cultures of both bacteria, maintained on nutrient agar slants at 5 ˚C, were transferred to 10 ml of nutrient broth and incubated at 35 ˚C for 24 h. After incubation, 2 ml aliquots of the broth culture of each organism were mixed into sterilized 15 ml tubes. The cultures were diluted serially as appropriate with 0.1 % buffered peptone water (Difco, 1989). Populations of inocula were determined by serially diluting the cell suspension in sterile 0.1 % BPW and surface plating (0.1 ml), in duplicate, on nutrient agar (for enumeration of *Staphylococcus aureus*) or VRBDA (for enumeration of *E. coli*) plates. Colonies were counted after incubation at 35 ˚C for 24 h.

b. Cereal preparation and inoculation

One kind of the commercially available infant cereal (rice) and pasteurized milk (2 % fat) were purchased from a local supermarket. Cereal was a brand-named dried product in flake form manufactured by extrusion processing. Refrigerated pasteurized milk was aseptically placed in a pre-sterilized flask and kept at room temperature (21 °C) for 1 h to maintain constant temperatures of the ingredients at cereal hydration. One hundred ml of milk was added to each cereal sample (10 g) based on the manufacturer's recommendation. Reconstitution resulted in cereal slurry. For storage at 5 °C, the slurry was inoculated with 1 ml of inoculum per 100 ml to yield 10^3 cfu ml^{-1}, while reconstituted cereal for storage at 20 °C was inoculated with 1 ml of inoculum per 100 ml to yield 10^2 cfu ml^{-1}.

c. Microbiological analyses of the reconstituted cereal

After inoculation, the samples were mixed will for 10 min and stored at 4 °C or 20 °C. The reconstituted cereal samples were analyzed microbiologically at 0, 4, 8, 12, 24 and 48 h of storage. Serial decimal dilutions were made in BPW and plated in duplicate on either Vogel-Johnson agar or VRBDA plates to determine *Staph. aureus* and *E. coli* populations, respectively. Colonies on agar plates were counted after 48 h of incubation at 30 °C.

8. Media

a. Trypticase soy agar medium (APHA, 1978)

Component	g/l
Peptone	17.0
Yeast extract	6.0
Dipotassium phosphate	2.5
Sodium chloride	5.0
Glucose	2.5
Agar	18.0

pH 7.0±0.2

b. MacConkey agar (Difco, 1989)

Component	g/l
Peptone	17.0
Protease	3.0
Lactose	10.0
Bile salts no.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

pH 7.1±0.2

c. Baird Parker agar (Baird Parker and Davenport, 1965)

Component	g/l
Peptone	10.0

Meat extract	5.0
Yeast extract	1.0
Sodium pyruvate	10.0
Glycine	12.0
Lithium chloride	5.0
Agar	15.0

pH 6.8±0.2

Five milliliters of egg yolk emulsion and 0.0105g of potassium telluride are to be added.

d. Buffered peptone water (1%) (Difco, 1989)

Component	g/l
Peptone	10.0
Sodium chloride	5.0
Distilled water	1000ml

pH 7.0±0.2

e. Tetrathionate broth base (Difco, 1989)

Component	g/l
Lab-Lemco' powder	0.9
Peptone	4.5
Yeast extract	1.8
Sodium chloride	4.5
Calcium carbonate	25.0
Sodium thiosulphate	40.7

pH 8.0±0.2

f. Brilliant green agar (Khan and McCaskey, 1973)

Component	g/l
Proteose peptone	10.0
Yeast extract	3.0
Lactose	10.0
Saccharose	10.0
Sodium chloride	5.0
Agar	20.0
Brilliant green	0.0125
Phenol red	0.08

pH 6.9±0.2

g. Triple sugar iron agar (Difco, 1989)

Component	g/l
Beef extract	3.0
Yeast extract	3.0
Peptone	1.50
Proteose peptone	5.0
Sodium chloride	5.0
Lactose	10.0
Dextrose	1.0
Ferrous thiosulphate	0.3
Phenol red	0.024
Agar	12.0

pH 7.4 $\pm$ 0.2

h. *Listeria* enrichment broth medium (Oxoid, 1998)

Component	g/l
Triptic soy broth	30.0
Yeast extract	6.0
Cycloheximide	0.05
Nalidixic acid	0.04

pH 7.3±0.2

i. Palcam agar base (Oxoid, 1998)

1. Basal medium

Component	g/l
Columbia blood agar base	39.0
Yeast extract	3.0
Glucose	0.5
Aesculin	0.8
Ferric ammonium citrate	0.13
Mannitol	10.0
Phenol red	0.08

pH 7.2±0.2

2. Palcam selective supplement

Component	mg/vial
Polymyxin B	5
Aciflavine	2.5
Ceftazidime	10

j. Thioglycolate broth medium (Oxoid, 1998)

Component	g/l
Lab-lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Glucose	5.0
Sodium chloride	5.0
Sodium thioglycolate	1.1
Agar	1.0
Methylene blue	0.002

pH 7.2±0.2

k. *Bacillus cereus* base agar medium: (Mossl *et al.*, 1967)

Component	g/l
Peptone	1.0
Mannitol	10.0
Sodium chloride	2.0
Magnesium sulphate	0.1
Disodium phosphate	2.5
Potassium phosphate	0.25
Sodium pyruvate	10.0
Bromothymol blue	0.12
Agar	15.0

pH 7.2±0.2

l. GN (Gram-Negative) Broth (Oxoid, 1998)

Component	g/l
Pancreatic digest of casein	10.0
Peptic digest of animal tissue	10.0
Dextrose	1.0
D-Mannitol	2.0
Sodium citrate	5.0
Sodium desoxycholate	0.5
Dipotassium phosphate	4.0
Monopotassium phosphate	1.5
Sodium chloride	5.0

pH 7.2±0.2

m. Xylose Lysine Desoxycholate (XLD) agar medium (Taylor, 1965)

Component	g/l
Xylose	3.5
L-Lysine	5.0
Sodium desoxycholate	2.5
Sodium chloride	5.0
Sodium thiosulphate	6.8
Lactose	7.5
Sucrose	7.5
Ferric ammonium citrate	2.5
Phenol red	0.025
Yeast extract	3.0

pH 7.4±0.2

n. *Salmonella Shigella* (S.S) agar (Difco, 1989)

Component	g/l
Protease peptone	5.0
Lactose	10.0
Bile salt no.3	8.5
Sodium citrate	8.5
Sodium thiosulphate	8.5
Ferric citrate	1.0
Agar	13.5
Brilliant green	0.33
Neutral red	0.025
Beef extract	5.0

pH 7.0±0.2

o. MacConkey broth (Oxoid, 1998)

Component	g/l
Peptone	20.0
Lactose	10.0
Bile salts no.3	5.0
Sodium chloride	5.0
Neutral red	0.075

pH 7.4±0.2

p. Eosin methylene blue (EMB) agar (CM 69/70) (Oxoid, 1998)

Component	g/l
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0

pH 6.8±0.2

q. Milk agar (James and Natalie, 1987)

Component	g/l
Yeast extract	3.0
Peptone	5.0
Agar	15.0
Skim milk	30.0 ml

pH 7.2 ± 0.1

r. Butter-fat agar (James and Natalie, (1987)

Component	g/l
Yeast extract	3.0
Peptone	5.0
Agar	15.0
Butter fat	50.0

pH 7.2 ± 0.1

s. Sabouraud dextrose agar (Oxoid, 1998)

Component	g/l
Mycological peptone	10.0
Glucose	40.0
Agar	15.0

pH 5.6±0.2

t. Vogel-Johnson agar (Oxoid, 1998)

Component	g/l
Tryptone	10.0
Yeast extract	5.0
Mannitol	10.0

K_2HPO_4	5.0
Lithium chloride	5.0
Glycine	10.0
Phenol red	0.025
Agar	16.0

p H 7.1 ± 0.2

u. Phosphate saline solution (ISO 8261/2001)

Component	g/l
KH_2PO_4	42.5

p H 7.2 ± 0.2

One ml of this stock solution was added to 10^3 ml of water for use as diluents.

v. Violet Red Bile Glucose agar (VRBGA) (Oxoid, 1998)

Component	g/l
Peptone	7.0
Yeast extract	3.0
Glucose	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0

pH 7.4±0.2

w. Differential Reinforced Clostridial Medium (DRCM) (Gibbs and Frame, 1965)

Component	g/l
Yeast extract	1.5
Beef extract	10.0
Peptone	10.0
Soluble starch	1.0
Sodium chloride	5.0
Hydrated sodium acetate	3.0
L- cysteine	0.5
Agar	0.5

pH 6.8±0.2

RESULTS AND DISCUSSION

To throw light on microbial contamination in infant food products, samples from retail markets and pharmacies, collected during a period extended from 2004 to 2005, were subjected to microbiological and chemical analysis. The presence of aflatoxins B_1, B_2, G_1 and G_2 was studied in the products. Effect of hot water & filtration, effect of irradiation and ethylene oxide fumigation on contaminated microorganisms in the herbals infant products was investigated as well.

1. Investigation of microbial contamination and chemical analysis

a. Microbial contamination and chemical analysis of cereal–based infant formulas

The obtained results showed that neither of the tested samples harbored any of the pathogenic bacteria that might be found in such products, *i.e. E. coli*, *Salmonella*, *Shigella*, *Staph. aureus*, *Bacillus cereus*, *Clostridium* sp., *Listeria* sp. or *Enterococcus*. The more sensitive method for detection of *Salmonella* using the PCR technique could not confirm the presence of the genus *Salmonella*. These results may reflect good manufacturing practices of the products under examination. However, other studies found that contamination and growth of bacterial pathogens such as *B. cereus* in infant food are common and increasing. For example, in the large study (261 samples from 17 countries) carried out in Germany by Becker *et al.* (1994), it was found that in 1982 and 1992, 31% and 70% of the infant formulas

respectively, were positive for *B. cereus*, at levels up to 600 cfu/g. The Maximum allowed levels for *B. cereus* in dried infant food have been set in several countries, *e.g*. Finland and Sweden, to be 10^3 and 10^4 cfu g^{-1}, respectively.

Data in Tables (8-a & 8-b) show that the colony counts in three samples, *i.e*. Riri with vegetables, Cerelac and Baby King special formula (nos. 1, 6 and 12, respectively) were considered to be unacceptable according to the Egyptian Standards (2005, 2006 and 2007a, b). The counts according to these standards are as follows: total aerobic counts not more than 100 cfu/g in infant foods which are prepared by boiling for at least 3 minute, yeasts & molds count not more than 10 cfu/g in infant foods which are prepared by boiling for at least 3 minute and free from yeasts & molds in infant foods which are not prepared by boiling, it must be free of pathogenic microorganisms and their toxins, free from coliform group and *E. coli*.

The total mesophilic microbial counts detected in the three above mentioned samples of the present study were 135, 115 and 115 cfu/g, respectively. Therefore, these formulas might be considered unsafe. Yeasts & molds were found only in Riri with vegetables (no. 1) and Cerelac (no. 6) samples, reaching 60 and 35 cfu/g. Coliforms could be detected only in the Cerelac sample (no. 6) and their counts did not exceed 20 cfu/g (Table, 8-a).

The counts reported in the present study were rather low compared to those observed by some investigators such as Ibanez *et al*. (1992) who reported that 5 of 46 samples of cereal–based infant

formula exceeded the maximum permitted count of mesophilic aerobic counts ($3 \times 10^5 - 8.6 \times 10^5$ cfu / g), coliform (> 0.01 cfu / g),

Table 8-a. Microbial contamination and chemical analysis of cereal based infant formulas.*

Analysis	Sample No.					
	1	**2**	**3**	**4**	**5**	**6**
	Microbiological counts (cfu/g)					
Total aerobic counts	135	65	10	65	55	115
Yeasts &molds	60	0	0	0	0	35
Coliforms	0	0	0	0	0	20
Salmonella	0	0	0	0	0	0
Shigella	0	0	0	0	0	0
E. coli	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	0
***Clostridium* sp.**	0	0	0	0	0	0
***Listeria* sp.**	0	0	0	0	0	0
***Enterococcus* sp.**	0	0	0	0	0	0
Lipolytic bacteria	75	0	0	0	5	25
Proteolytic bacteria	110	20	15	0	20	55
	Chemical analysis					
Water activity (aw)	0.146	0.299	0.149	0.209	0.326	0.179
Aflatoxin (ppb)	0	0	0	0	0	0
Protein (g/100g)	15.9	16.0	7.5	15.0	16.0	15.0
Fat (g/100g)	9.6	0.5	0.5	1.5	0.5	9.0

* For the label data, see Table (4).

Enterobacter sp. (3×10^2 cfu/g), *Citrobacter* (3×10^2 cfu/g), *E. cloacae* (200 cfu/g) and *B. cereus* (4×10^2 cfu/g). However, the counts of yeast & mold did not exceed the maximum permitted (3×10^2 cfu/g). Neither *E. coli* nor *Salmonella* could be detected.

The same trend was noticed by Anand and Singh (1988a), in India where 102 samples, comprising 10 brands of infant milk foods and 3 brands of cereal weaning foods were analyzed microbiologically. About 80 % of the samples were well within the limits of total viable

count standards as prescribed by the Indian Standard Institution (ISI). *Bacillus cereus* was counted in 28 % of the samples. Twenty two % of the samples were positive for coliforms and of these only 4.4 % were

Table 8-b. Microbial contamination and chemical analysis of cereal based infant formulas.*

Analysis	Sample No.					
	7	**8**	**9**	**10**	**11**	**12**
	Microbiological counts(cfu/g)					
Total aerobic counts	15	10	40	50	50	115
Yeasts & molds	0	0	0	0	0	0
Coliforms	0	0	0	0	0	0
Salmonella	0	0	0	0	0	0
Shigella	0	0	0	0	0	0
E. coli	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	0
***Clostridium* sp.**	0	0	0	0	0	0
***Listeria* sp.**	0	0	0	0	0	0
***Enterococcus* sp.**	0	0	0	0	0	0
Lipolytic bacteria	5	0	10	0	0	0
Proteolytic bacteria	0	0	0	0	35	30
	Chemical analysis					
Water activity (aw)	0.221	0.265	0.203	0.184	0.104	0.378
Aflatoxin (ppb)	0	0	0	0	0	0
Protein (g/100g)	15.0	15.0	8.5	6.2	15.9	18.5
Fat (g/100g)	9.0	9.0	0.9	0.6	9.6	8.4

* For the label data, see Table (4).

within the ISI standards. Staphylococci were also isolated from 57.8 % of the samples. No salmonellae or clostridia were isolated from any of the samples.

For all examined samples of infant food component from cereals in the present study, the proteolytic bacteria group was detected only in 58 % of the samples, *i.e.* 7 samples with a mean of 40.7 cfu/g (Tables, 8-a & 8-b). Lipolytic bacterial counts ranged from 5 to 75 cfu/g with a

mean of 24 cfu/g and were detected only in 5 samples representing 41.7% of the tested samples. The average of water activity was found to be 0.2219, while the fat content ranged from 0.5 to 9.6 % and the protein content ranged from 6.2 to18.5 %. Neither of the tested samples had any detectable levels of the aflatoxins (Tables, 8-a & 8-b).

The presence of lipolytic and proteolytic bacteria raises the issue of possibility of spoilage in such foods. This points out the significance of holding the water activity value at low levels to avoid spoilage. The recorded water activities in this study (0.104-0.378) were in all cases below the critical threshold of 0.7 (Mossel and Ingram, 1955; Van Arsdel *et al.*, 1973 and Cullen *et al.*, 1986). Thus, such foods may be exposed to spoilage if good care of proper closure is not taken.

b. Microbial contamination and chemical analysis of the milk-based infant formulas

The obtained data presented in Table (9) show that the mesophilic colony counts in two samples, *i.e*. Babysan 2 and S-26 gold (nos. 5 & 6) were considered to be unacceptable according to the Egyptian Standards (2005, 2006 and 2007a,b). The determined total aerobic counts were 130 and 125 cfu/g, respectively. Lipolytic bacteria ranged form 10 to 65 cfu/g, while proteolytic bacteria ranged form 10 to 15 cfu/g. The mean water activity of the infant food components from milk was 0.267 with a fat content value ranging from 20 to 28.1. Protein content values ranged from 9.5 to 16. None of the samples harbored any of the tested pathogenic bacteria.

Cullen *et al.* (1986) found similar results when studied baby food in Brazil. They examined 53 samples (23 baby-food samples bought at

supermarkets, 15 soups and 15 samples of milk or milk substitute infant formulae from feeding bottles collected at day-care children's institutions). Similar to this work all samples were negative for *Salmonella*, *Staphylococcus aureus*, *Y. enterocolitica* and anaerobic organisms, There were *B. cereus* and deteriorative microorganisms present in varying proportions in the 3 types of foods. Coliforms and faecal coliforms were present in samples from feeding bottles.

Table 9. Microbial contamination and chemical analysis of milk-based infant food formulas.*

Analysis	**Sample No.**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
	Microbiological analysis (cfu/g)						
Total aerobic counts	20	25	50	45	130	125	80
Yeasts &molds	0	0	0	0	0	0	0
Coliforms	0	0	0	0	0	0	0
Salmonella	0	0	0	0	0	0	0
Shigella	0	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	0	0
***Clostridium* sp.**	0	0	0	0	0	0	0
***Listeria* sp.**	0	0	0	0	0	0	0
***Enterococcus* sp.**	0	0	0	0	0	0	0
Lipolytic bacteria	10	0	0	15	35	30	65
Proteolytic bacteria	0	0	0	10	10	10	15
	Chemical analysis						
Water activity (aw)	0.223	0.246	0.250	0.327	0.266	0.232	0.327
Aflatoxin (ppb)	0	0	0	0	0	0	0
Protein (g/100g)	9.5	15.2	15.2	16.0	14.6	12.0	12.6
Fat (g/100g)	27.7	20.1	20.4	20.0	20.6	28.0	28.1

* For the label data, see Table (5).

On the contrary, El-Prince and Korashy (2003) in Assiut- Egypt collected 90 random samples of dried milk-based foods during 2001-2002. Their results revealed that the average counts of aerobic plate

counts, anaerobes, *B. cereus* and yeasts & molds in samples for babies after birth were 9.5 x 10^3, 4, 21 and 3.6 x $l0^3$/g, respectively. However, coliforms, psychrophiles, *Staph*. *aureus* and thermodurics were not detected. In samples for 4 months old and above, 11 (36.66%), 2 (6.66%) and 13 (43.33%) of the examined samples contained aerobic bacteria, anaerobes and yeasts and molds, respectively, while no *B. cereus*, coliforms, psychrophiles, *Staph. aureus* and thermodurics were isolated. Moreover, it was noticed that the average values of agar plate count, *B. cereus*, psychrotrophes, thermodurics and yeasts and molds in samples of type C were 6.5 x 10^4, 6.0 x $l0^2$, 9.5 x 10, 1.3 x 10^2 and 3 x 10^3/g, respectively. Anaerobes, coliforms and *Staph*. *aureus* were not detected. The contradiction between their work and the present one may be attributed to the difference in the brands tested.

c. Microbial contamination and chemical analysis of vegetable and fruit-based infant food formulas

Results in Table (10) show that only one sample, *i.e*. Gerber fruit dessert (no. 3) was shown to be unacceptable according to microbiological standards of the Egypt (2005, 2006 and 2007a,b). The total aerobic mesophilic count in this sample was 675 cfu/g. Yeasts & molds could be detected only in one sample, *i.e.* Gerber mixed vegetables (sample no. 2) in low numbers hardly exceeding 10 cfu/g. For all examined infant food components from vegetables and fruits, lipolytic bacteria were detected in all samples and ranged form 5 to 150 cfu/g, while proteolytic bacteria ranged form 15 to 125 cfu/g. The mean water activity of infant food component from vegetables was 0.3837

with a fat content value ranging from 0.1 to 1.8 % and protein content value of 0.4 to 4.0 % (Table, 10).

Table 10. Microbial contamination and chemical analysis of vegetable and fruit-based infant food formulas.*

Analysis	Sample No.			
	1	**2**	**3**	**4**
	Microbiological analysis (cfu/g)			
Total aerobic counts	60	20	675	15
Yeasts &molds	0	10	0	0
Coliforms	0	0	0	0
Salmonella	0	0	0	0
Shigella	0	0	0	0
E. coli	0	0	0	0
Staph. aureus	0	0	0	0
Bacillus cereus	0	0	0	0
***Clostridium* sp.**	0	0	0	0
***Listeria* sp.**	0	0	0	0
***Enterococcus* sp.**	0	0	0	0
Lipolytic bacteria	110	5	150	15
Proteolytic bacteria	125	15	20	25
	Chemical analysis			
Water activity (aw)	0.371	0.419	0.342	0.403
Aflatoxin (ppb)	0	0	0	0
Protein (g/100g)	4.0	1.1	0.5	0.4
Fat (g/100g)	0.2	1.8	0.1	0.4

For the label data, see Table (6)

d. Microbial contamination and chemical analysis of herbal-based infant food formulas

Data in Table (11) showed that only one sample, *i.e*. Baby Calm herbal (sample no. 3) was not acceptable according to the Egyptian Standards (2005, 2006 and 2007a, b). The sample had a total aerobic mesophilic count of 10^3 cfu/g. Compared to the other analyzed herbal

samples, the baby calm sample had detectable numbers of yeasts & molds (20 cfu/g), coliforms (50 cfu/g), proteolytic bacteria (75 cfu/g) and lipolytic bacteria (50 cfu/g). The sample was positive for *Clostridium* sp. Other samples (nos. 1, 2 & 4) met the Egyptian Standards (2005, 2006 and 2007a, b). The mean water activity of the infant food component from milk was 0.297. No fat or protein could be detected, except marginal amounts measured only in sample no.1 (Table, 11). Figure (1) shows the distribution of the unacceptable samples respecting the aerobic plate counts, yeasts & molds and coliforms.

Table 11. Microbial contamination and chemical analysis of herbal-based infant food formulas.*

Analysis	**Sample No.**			
	1	**2**	**3**	**4**
	Microbiological analysis (cfu/g)			
Total aerobic counts	0	20	$1x10^3$	10
Yeasts &molds	0	0	20	0
Coliforms	0	0	50	0
Salmonella	0	0	0	0
Shigella	0	0	0	0
E. coli	0	0	0	0
Staph. aureus	0	0	0	0
Bacillus cereus	0	0	0	0
***Clostridium* sp.**	0	0	+ve	0
***Listeria* sp.**	0	0	0	0
***Enterococcus* sp.**	0	0	0	0
Lipolytic bacteria	0	0	50	0
Proteolytic bacteria	0	0	75	10
	Chemical analysis			
Water activity (aw)	0.325	0.296	0.258	0.310
Aflatoxin (ppb)	0	0	0	0
Protein (g/100g)	0.1	0	0	0
Fat (g/100g)	0.1	0	0	0

* For the label data, see Table (7).

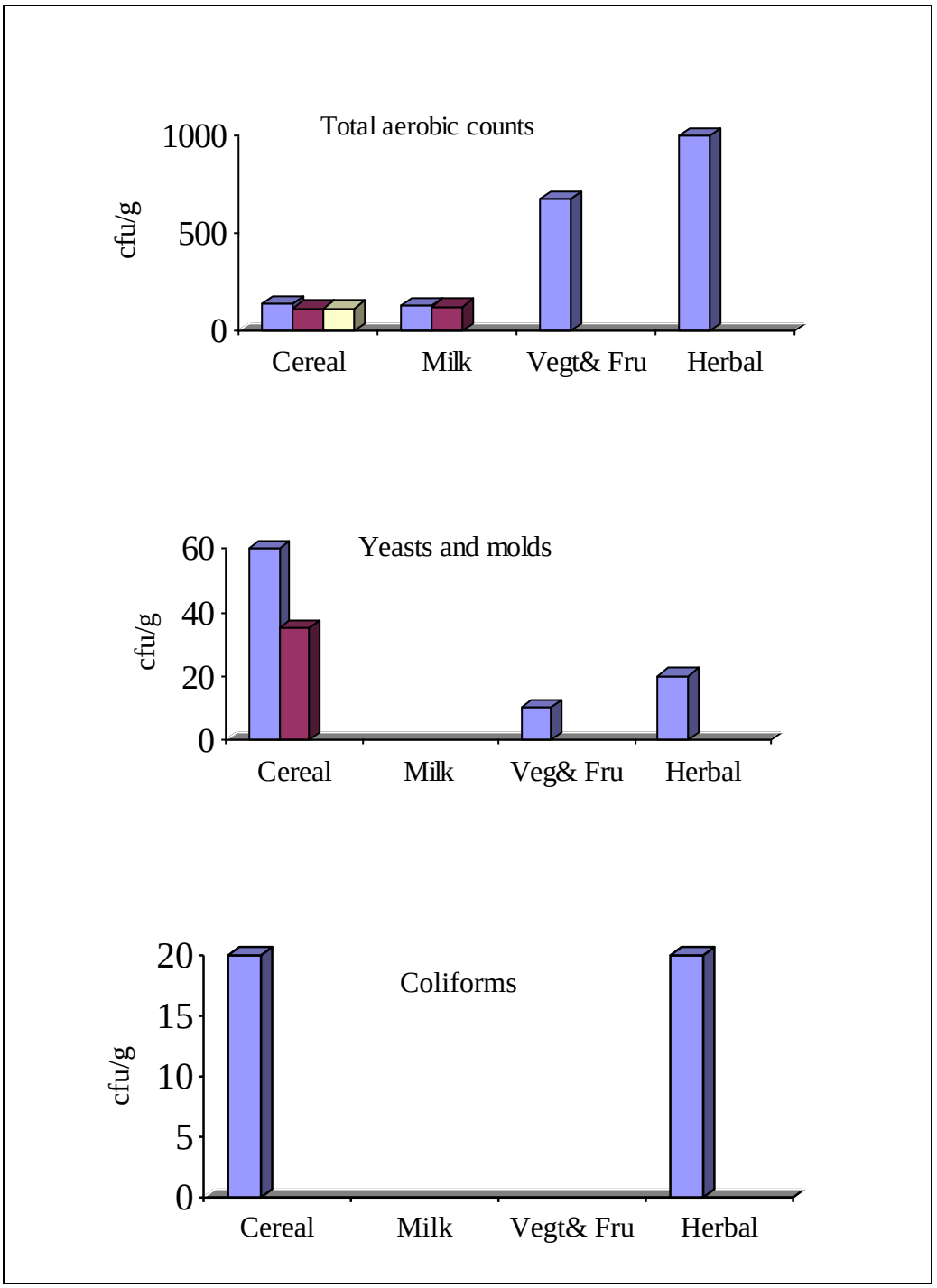

Fig. 1. Unacceptable samples (cereals, milk, vegetables & fruits and herbals)

e. Microbial contamination of different samples of Baby Calm herbals

Since the Baby Calm sample (no. 3; Table, 11) had the highest numbers of the microorganisms, 15 samples belonging to Baby Calm were chosen to investigate whether all these samples contain such high numbers of microorganisms. The obtained results show that all tested samples were free from the pathogenic bacteria that might be found in such products such as *Salmonella*, *Shigella*, *Staph. aureus* and *Bacillus ceurus* (Tables 12a & 12b). However, *E. coli* could be detected in two samples (nos. 1 & 2). It is obvious that considerable levels of microbial contamination were recovered in most of the surveyed samples. For all examined samples, the total aerobic mesophilic counts ranged from 1.1 x 10^2 – 1.0 x 10^5 cfu/g. However, *Clostridium* sp. was detected only in 7 samples, representing about 47 % of the tested samples (Fig., 2). Yeast & mold counts ranged from undetectable counts to 100 cfu/g. Coliforms were found in all tested samples and ranged from 15 to 4.2 x 10^3 cfu/g.

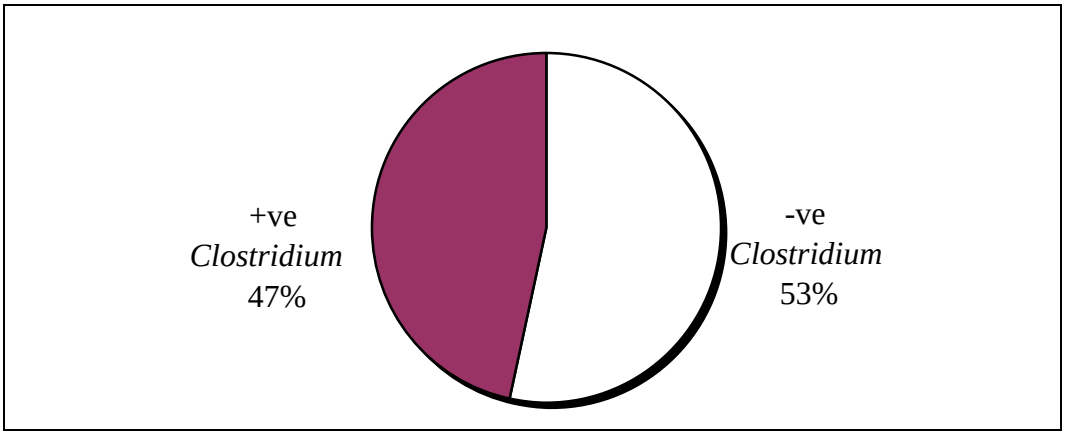

Fig. 2. Distribution of *Clostridium* - positive and negative samples (Baby Calm).

Table 12 – a. Microbial contamination (cfu/g) of Baby Calm herbals.*

Analysis	Sample No.						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Total aerobic counts	3.5x 10^4	6.0x 10^4	1.0x 10^3	7.0x10^3	1.0x 10^4	1.0x 10^5	2.0x 10^3
Yeasts &molds	30	20	0	0	0	0	0
Coliforms	2.1 x 10^3	1.7 x 10^3	35	3.1 x 10^2	15	3.2 x 10^3	3.5 x 10^2
E. coli	+ve	+ve	0	0	0	0	0
Salmonella	0	0	0	0	0	0	0
Shigella	0	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	0	0
***Clostridium* sp.**	+ve	+ve	0	0	+ve	+ve	0

* Samples belonging to Baby Calm herbals shown in Table (7).

Table 12 – b. Microbial contamination (cfu/g) of Baby Calm herbals.*

Analysis	Sample No.							
	8	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Total aerobic counts	9.5x 10^4	7.5x 10^4	2.0x 10^2	3.5x10^2	1.5x 10^2	1.1x 10^2	1.0x 10^3	1.3 x 10^3
Yeasts &molds	0	0	1.0 x 10^2	1.0 x 10^2	1.0 x 10^2	0	0	0
Coliforms	4.2 x 10^3	1.9 x 10^3	10	3.0 x 10^3	70	95	2.1x 10^3	1.7 x 10^3
E. coli	0	0	0	0	0	0	0	0
Salmonella	0	0	0	0	0	0	0	0
Shigella	0	0	0	0	0	0	0	0
Staph. aureus	0	0	0	0	0	0	0	0
Bacillus cereus	0	0	0	0	0	0	0	0
***Clostridium* sp.**	0	0	+ve	+ve	0	0	+ve	0

* Samples belonging to Baby Calm herbals shown in Table (7).

It was interesting to note that, in spite of the procedures adopted, except 2 Baby Calm samples (nos. 1 & 2) [Table, 12a], no *E. coli* strains were recovered from all infant food samples (a total of 42) analyzed which suggests that there was no recent fecal contamination of the formulas. The absence of the bacterial pathogens in the most of analyzed samples might support this finding.

These results agree with those obtained by Farkas (1998) who reported that herbals commonly harbor a large number of bacteria and fungi including potential spoilage organisms.

The present results agree with those reported by McKee, 1995; Kneif and Berger, 1993 and Pafumi, 1986, who noted that the most common bacteria are the spore-formers. According to standards established by the WHO (1985), most herbs, harvested and handled under hygienic conditions and tested by appropriate methods of sampling and examination, should contain not more than 1×10^4 bacteria per gram. Of the 15 samples examined in the present study, 5 samples contained aerobic counts higher than 10^4 cfu/g (Tables, 12-a & 12-b) and consequently did not meet the above mentioned standard. According to the Egyptian Standards (2005, 2006 and 2007a, b), 14 samples in the present study are shown to be microbiologically unacceptable (Tables, 12-a & 12-b). Figure (3) summarizes the distribution of the unacceptable baby calm samples.

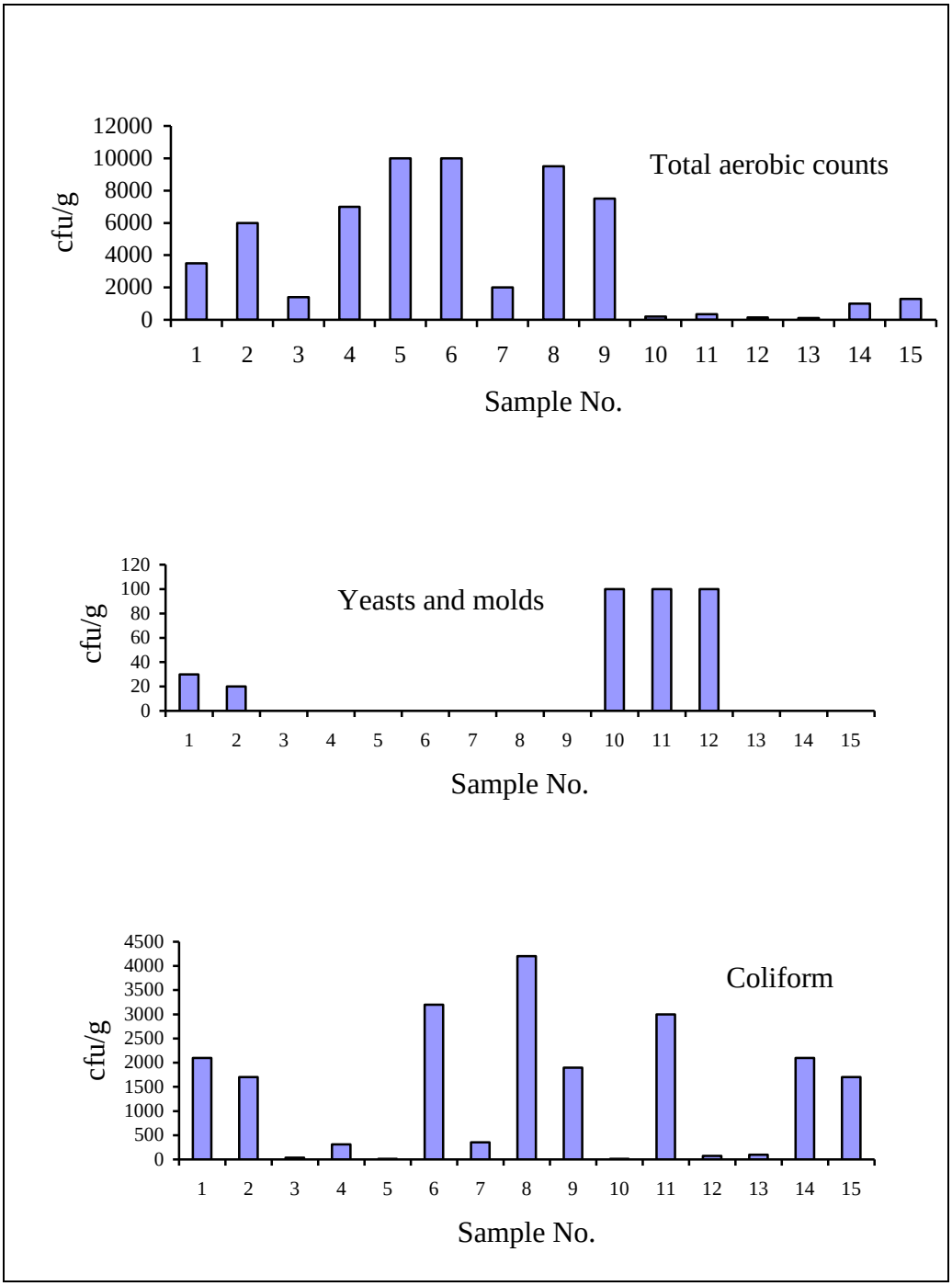

Fig. 3. Unacceptable samples (Baby Calm).

2. Survival of microorganisms in infant food formulas after preparation in pre-boiled (about 100 ˚C) water

a. Survival in vegetable-based infant food formulas

Preparation of a vegetable-based sample in pre-boiled water resulted in a relative reduction of the counts along the one hour period (Table, 13). The total aerobic counts decreased by about 60 %, suggesting that the non-spore forming cells might represent the majority of the existed microflora. This was evident when the counts of yeasts and molds were determined, where their counts decreased by about 50 %.

Table 13. Survival of microorganisms in Riri with vegetables after preparation in pre-boiled (100ºC) tap water (counts determined as cfu/g).

Analysis	Time (min)				
	0	**15**	**30**	**45**	**60**
Total aerobic counts	135	40	50	50	80
Yeasts &molds	60	30	50	30	30
Coliforms	0	0	0	0	0
Salmonella	0	0	0	0	0
Shigella	0	0	0	0	0
E. coli	0	0	0	0	0
Staph. aureus	0	0	0	0	0
Bacillus cereus	0	0	0	0	0
***Clostridium* sp.**	0	0	0	0	0

b. Survival in cereal-based infant food formulas

Contrary to the vegetable-based sample, the recovered total aerobic counts from the cereal-based formula survived without any considerable reduction (Table, 14). However, the initial low numbers of yeasts & molds (35 cfu/g) and coliforms (20 cfu/g) disappeared rapidly, since no detectable numbers could be observed after preparation.

Table 14. Survival of microorganisms in cereal based infant food after preparation in pre-boiled (100°C) tap water (counts determined as cfu/g).

Analysis	Time (min)				
	0	**15**	**30**	**45**	**60**
Total aerobic counts	115	90	90	120	200
Yeasts &molds	35	0	0	0	0
Coliforms	20	0	0	0	0
Salmonella	0	0	0	0	0
Shigella	0	0	0	0	0
E. coli	0	0	0	0	0
Staph. aureus	0	0	0	0	0
Bacillus cereus	0	0	0	0	0
***Clostridium* sp.**	0	0	0	0	0

c. Survival in herbal-based infant food formulas

Since the Baby Calm herbal formula was shown to be microbiologically unacceptable (Tables, 11 and 12a & 12b), it was interesting to investigate whether the observed relatively high numbers of microflora could be reduced to the acceptable levels by means of preparation in hot water. For this purpose, 15 samples belonging to Baby Calm herbals formula were tested. Each infant herbal packet was placed in boiling water for about 5 min. This resulted in an obvious reduction of the microbial counts (Figure, 4 and Tables, 15-a & 15-b). A reduction of 0.14 – 2.62 $\log_{10}$ cycle cfu/g of the total mesophilic counts was achieved in 11 samples of the 15 samples tested. Expectedly, the coliform group either completely disappeared or highly decreased in all tested samples. *E. coli* detected in two samples could not be recovered after preparation. Unexpectedly, the anaerobic spore-forming bacteria detected in 7 samples could not be recovered from these samples after preparation in the hot water.

The results of this experiment indicate that the preparation of such infant formula types in hot water for a short time is sufficient either to kill the indicator microorganisms or to reduce other microflora to a high extent. According to standards established by the WHO (1985), the heat treatment in the present study reduced the aerobic populations of Baby Calm herbals to an acceptable level *i.e*. lower than 10^4 cfu/g in the 15 tested samples (Tables, 15a & 15b). Consequently, following up the company precautions is highly significant to maintain good hygienic quality of the product.

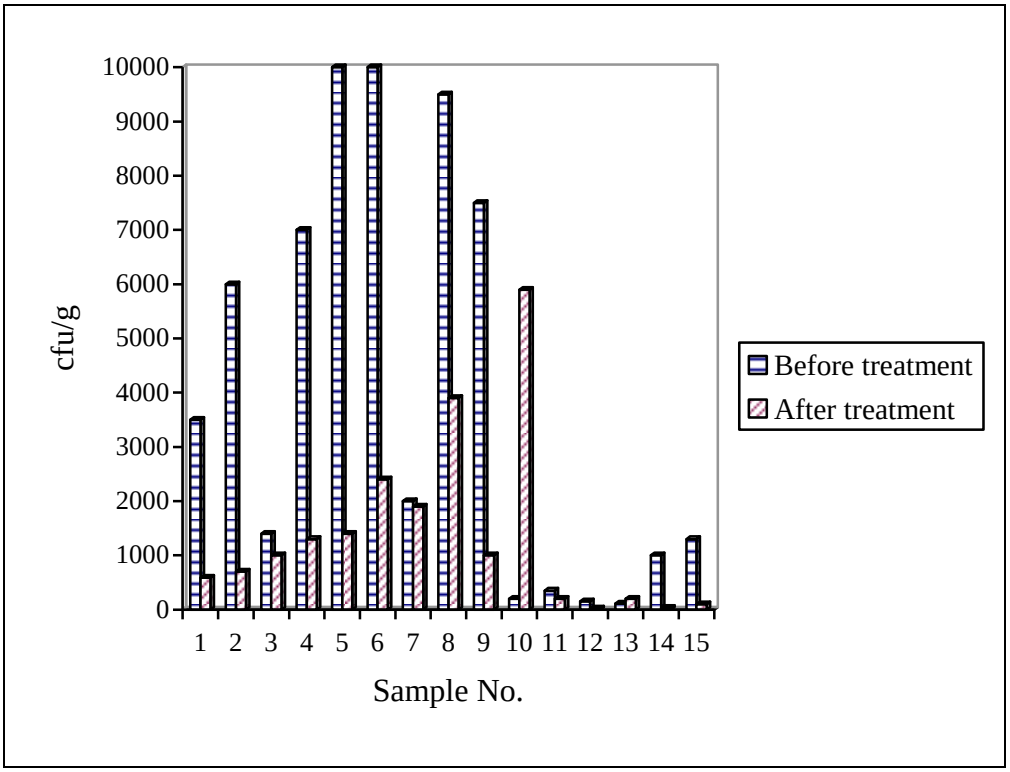

Fig. 4. Effect of pre-boiled water (100°C) on aerobic plate counts in Baby Calm herbals.

Table 15-a. Effect of pre-boiled water (100ºC) on microbial contamination (cfu/g) in Baby Calm herbals (mode of use as company precaution).

Analysis	Sample No. *							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Total aerobic counts	5.9×10^3 (3.5×10^4)	7×10^2 (6.0×10^4)	1.4×10^3 (1.0×10^3)	1.3×10^3 (7.0×10^3)	1.4×10^3 (1.0×10^4)	2.4×10^3 (1.0×10^5)	1.9×10^3 (2.0×10^3)	3.9×10^3 (9.5×10^4)
Yeasts &molds	100 (30)	100 (20)	100 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Coliforms	0 (2.1×10^3)	0 (1.7×10^3)	0 (35)	0 (3.1×10^2)	0 (15)	0 (3.2×10^3)	0 (3.5×10^2)	0 (4.2×10^3)
E. coli*	0 (+ve)	0 (+ve)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Salmonella	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Shigella	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Staph. aureus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bacillus cereus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
***Clostridium* sp.**	0 (+ve)	0 (+ve)	0 (0)	0 (0)	0 (+ve)	0 (+ve)	0 (0)	0 (0)

* Values between brackets are counts before placement of the sample in pre-boiled water.

* Determined by Eijkman test.

Table 15-b. Effect of pre-boiled water (100ºC) on microbial contamination (cfu/g) in Baby Calm herbals (mode of use as company precaution).

Analysis	Sample No. *						
	9	**10**	**11**	**12**	**13**	**14**	**15**
Total aerobic counts	1.0×10^3 (7.5×10^4)	5.9×10^3 (2.0×10^2)	2.0×10^2 (3.5×10^2)	70 (1.5×10^2)	2.0×10^2 (1.1×10^2)	35 (1.0×10^3)	1.0×10^2 (1.3×10^3)
Yeasts &molds	0 (0)	1.0×10^2 (1.0×10^2)	0 (1.0×10^2)	0 (1.0×10^2)	0 (0)	0 (0)	0 (0)
Coliforms	0 (1.9×10^3)	0 (10)	5 (3.0×10^3)	35 (70)	40 (95)	35 (2.1×10^3)	5 (1.7×10^3)
E. coli*	0 (0)	(0) 0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Salmonella	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Shigella	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Staph. aureus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Bacillus cereus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
***Clostridium* sp.**	0 (0)	0 (+ve)	0 (+ve)	0 (0)	0 (0)	0 (+ve)	0 (0)

* Values between brackets are counts before placement of the sample in pre-boiled water.

* Determined by Eijkman test.

3. Effect of ionizing irradiation on microbial contamination in herbal-based infant formulas

The effectiveness of irradiation to control the microbiological contamination in 3 Baby Calm herbal samples was evaluated. This type of samples was chosen, since the Baby Calm herbal was characterized with obvious high numbers of microorganism (Tables, 11 and 12a & 12b). The experiment was carried out to follow the microbiological changes during exposure of the samples to different doses of irradiation. For this purpose, packages of samples were irradiated at doses of 1, 2, 3, 4, 5 and 10 kGy. As shown in Tables (12-a & 12-b), the initial numbers of the total aerobic counts decreased from 5.7 $x10^3$ cfu/g before irradiation reaching their lowest level of 18 cfu/g after treatment by a dose of up to 4 kGy, representing a reduction percentage of about 99.7 %. On the other hand, higher doses of 5-10 kGy might result in an immediate sterilization of the samples, since no microorganisms could be detected in any of the tested samples. Irradiation, at the energy levels commonly used (4 kGy), effectively kills bacteria, molds and yeasts. Similar findings were reported by Marcotte (1993) who showed that a dose of 5 -10 kGy results in an immediate 2-3 log cycle reduction of bacteria

These results are similar to that of Farkas (1998) who demonstrated that radiation decontamination of dry ingredients, herbs and enzyme preparations with doses of 3–10 kGy proved to be a viable alternative to fumigation with microbicidal gases.

The present results agree with those reported by Rodriguez *et al.* (2006) who showed that electron-beam irradiation destroyed 99.9 % of the major food pathogenic bacteria. In the present study, irradiation decreased the microbial populations of both coliforms and

Enterobacter with increasing irradiation dose (Figure, 5 and Tables, 16-a, 16-b & 16-c). Both populations were eliminated at 4 kGy or higher. On the opposite, the results obtained Hong *et al*. (2008) showed that some species of *Enterobacter* such as *E. sakazakii*, however, were reported to be the most resistant to irradiation.

The present study is one of few who dealt with food supplement irradiation and its effects on pathogenic bacteria. Osaili *et al*. (2007) have reported that *E. sakazakii* in dehydrated infant formula needs gamma irradiation of 5.13 kGy to obtain 3 log reduction while, Sarrias *et al*. (2003) also have reported that *B. cereus* in raw rice was eliminated by irradiation at 7.5 kGy. According to the results of the present study, electron-beam treatment of baby foods such as Baby Calm at doses below 5 kGy appears to achieve microbial decontamination, without affecting the quality change such as color and flavor.

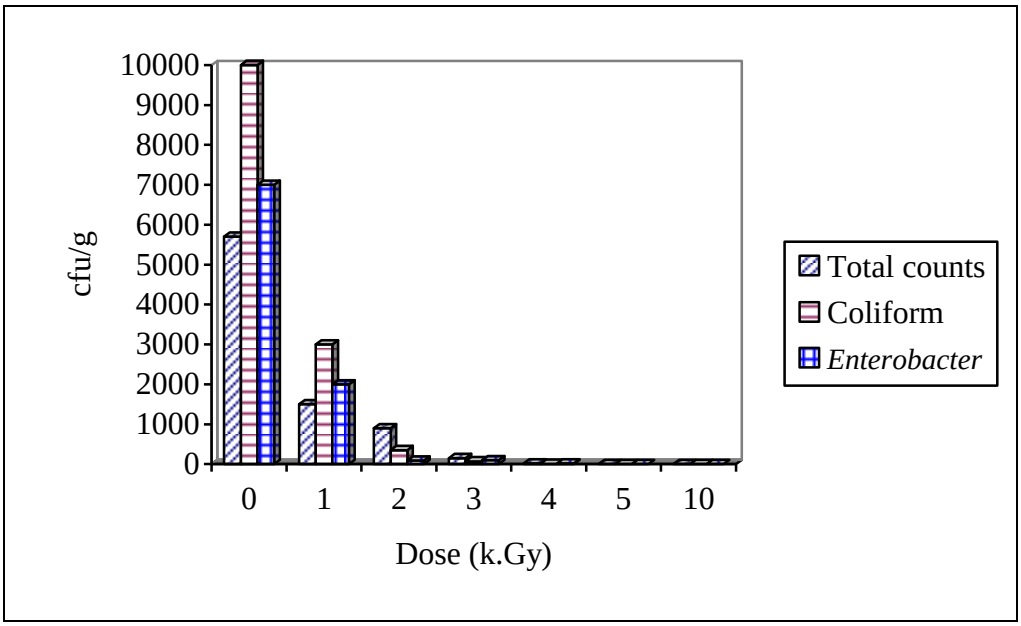

Fig. 5. Effect of irradiation on microbial contamination in baby calm herbals.

Table 16- a. Effect of irradiation on microbial load in Baby Calm herbals (sample no. 1).

Test / Dose (k.Gy)	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7.0×10^3
1	15×10^3	0	3×10^3	2.0×10^3
2	9×10^2	0	3.5×10^2	90
3	150	0	70	100
4	20	0	5	20
5	0	0	0	0
10	0	0	0	0

Table 16- b. Effect of irradiation on microbial load in Baby Calm herbals (sample no. 2).

Test / Dose (k.Gy)	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7.0×10^3
1	6.3×10^3	0	7.0×10^3	2.1×10^3
2	9.0×10^2	0	9.0×10^2	3.0×10^2
3	70	0	65	60
4	25	0	10	5
5	0	0	0	0
10	0	0	0	0

Table 16- c. Effect of irradiation on microbial load in Baby Calm herbals (sample no. 3).

Test / Dose (k.Gy)	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7×10^3
1	1.3×10^3	0	5.5×10^3	3.0×10^3
2	9.0×10^2	0	7.0×10^2	3.7×10^2
3	35	0	30	30
4	10	0	15	0
5	0	0	0	0
10	0	0	0	0

4. Effect of fumigation with ethylene oxide on microbial contamination of herbal-based infant formulas

The effectiveness of fumigation with ethylene oxide to control the microbiological contamination in 3 Baby Calm herbal samples was evaluated. This type of samples was chosen, since Baby Calm was characterized with obvious high numbers of microorganism (Tables, 11 and 12-a & 12-b). The experiment was carried out to follow the microbiological changes during exposure of the samples to different doses of ethylene oxide.

Data recorded in Tables (17-a, 17-b & 17-c) and Figure (6) show a decrease in the counts of most microorganisms found in herbal samples at a gas dose of 250 mg/l. At higher doses, the effect of ethylene oxide fumigation was sterile, since no microorganisms could be detected. From these results it is obvious that the ethylene oxide had a very effective killing action against contaminating microorganisms.

Although ethylene oxide is commonly used to sanitize spices with varying degrees of success, it is also banned in many countries such as Japan, some of EEC and the United Kingdom because it reacts with organic spice components. However, the use of ethylene oxide in some countries such as the United States and Canada is under review (residues levels of 50 and 1500 ppm are currently allowed, respectively). The instability and flammability of ethylene oxide requires it to be mixed with another gases. Now, ethylene oxide is stabilized with much less effective CO_2 and delivered with steam.

Table 17- a. Effect of fumigation with ethylene oxide on microbial load in Baby Calm herbals (sample no. 1).

Test / Dose (mg/l)*	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7×10^3
250	60	0	15	0
500	0	0	0	0
750	0	0	0	0
1000	0	0	0	0
1500	0	0	0	0

* mg ethylene oxide per liter of chamber volume (see materials and methods).

Table 17- b. Effect of fumigation with ethylene oxide on microbial load in Baby Calm herbals (sample no. 2).

Test / Dose (mg/l)*	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7×10^3
250	35	0	0	0
500	0	0	0	0
750	0	0	0	0
1000	0	0	0	0
1500	0	0	0	0

* mg ethylene oxide per liter of chamber volume (see materials and methods).

Table 17- c. Effect of fumigation with ethylene oxide on microbial load in Baby Calm herbals (sample no. 3).

Test / Dose (mg/l)*	Total aerobic counts	*E. coli*	Total coliforms	*Enterobacter* sp.
0	5.7×10^3	0	1.0×10^4	7×10^3
250	10	0	5	0
500	0	0	0	0
750	0	0	0	0
1000	0	0	0	0
1500	0	0	0	0

* mg ethylene oxide per liter of chamber volume (see materials and methods).

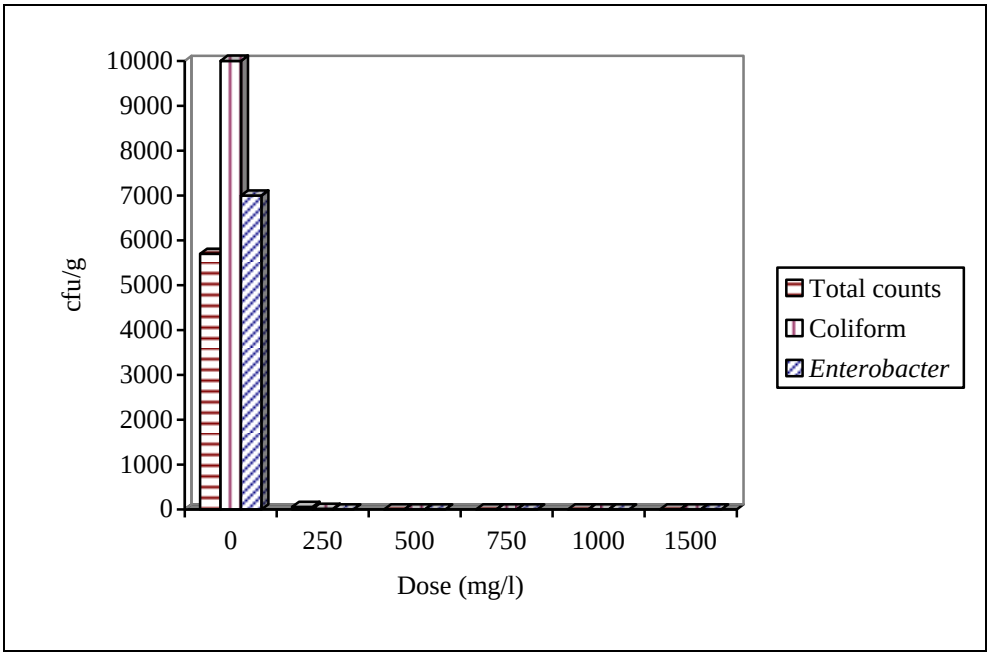

Fig. 6. Effect of fumigation with ethylene oxide on microbial contamination in baby calm herbals

Oppositely (Marcotte, 1993) noted that ethylene oxide is far less effective than irradiation. Ethylene oxide is less effective than irradiation. He noted that fumigation has to be repeated to reach the suitable microbial levels. That study regarded that to the addition of CO_2 at 80 % level for stabilization and using steam for delivery. In addition the anther noted that the dense packing of the herbs prevents the penetration of ethylene oxide.

5. Hazard analysis during manufacturing of infant food packaging materials

The majority of studies on pathogens in foods are devoted to their presence in raw materials or in their growth and behavior in the finished products. Since food packaging materials constitute a part of

the food chain affecting the quality of the final product, an investigation in the present study was performed to evaluate the possible microbial contamination of the infant food packages during its manufacturing.

A survey performed by the WHO (1985) in Europe indicated that almost 25 % of the food-borne outbreaks could be traced back to recontamination. The most important factors contributing to the presence of pathogens in prepared foods were insufficient hygiene (1.6 %), cross-contamination (3.6 %), processing or storage in inadequate rooms (4.2 %), contaminated equipment (5.7 %) and contamination by personnel (9.2 %).

For testing plant hygiene and product quality during manufacturing of the infant food packages, the following microbiological measurements were made: (i) Sanitation tests, *i.e*. environmental swabs from hands of workers and machines (equipment) to measure the bacteriological condition of both. (ii) Microbiological quality of the air inside the plant. (iii) The bacteriological quality of the packages.

a. Contamination from contact surfaces

Recontamination of products through contaminated surfaces has been observed in many cases and is a major issue. Unclean, insufficiently or inadequately cleaned pieces of equipment have been identified as the source of the pathogens, *e.g*. transfer of *Salmonella* ealing into infant formulas from the environment of processing, lines and equipment (Rowe *et al*., 1987). Containers used for holding or transporting unprocessed raw materials have subsequently been used

for processed products without any cleaning (Llewllvn *et al*., 1998). This certainly represents a major deviation from good hygienic practices. Recontamination has also occurred as the consequence of ineffective or inadequate cleaning and disinfection. The poor hygienic design of equipment is then often the cause of these problems. The correct hygienic design and proper maintenance of equipment are crucial to avoid recontamination through, for example, dripping condensation water or accumulating residues, cracks or micro-holes in heat exchangers or double walled equipment, errors in the design or installation of the equipment allowing contact between unprocessed and processed product (Lecos, 1986).

1. Production line (equipment)

Of 11 points located on the production line, seven contained *Staph. aureus* in counts reached 60 cfu/ swab (Table, 18). Coliforms could be detected from only two points, while *E. coil* was found on only two points. The cleaning and sanitizing of equipment must be performed in a manner that prevents contamination of food, food-contact surfaces, and food packaging materials. The first step in cleaning equipment is to remove excess soil.

2. Hands of workers (employees)

The hands of the workers who handled the packages during their manufacturing and transport were microbiologically evaluated. Swabs taken from workers' hands presented considerable bacterial contamination. Of ten employees examined, five (50 %) had *Staph.*

Table 18. Microbiological examination of the swab samples taken from different parts of the production line during manufacturing of infant food packages.

Place	*Staph. aureus* (cfu/ swab)	Total coliforms (MPN/ swab)	*E. coli* (cfu/swab)
Feeder	6	0	5
Withdrawal role	0	0	0
Glue cylinder	1	0	0
Withdrawal column	5	0	0
Role belt	1	0	0
Knife	1	0	0
Suction	0	0	0
Net belt 1	5	140	6
Bag Withdrawal column	0	0	0
Cutting Knives	0	0	0
Net belt 2	60	0.9	0

aureus on their hands (Table, 19). Coliforms could be detected only on two cases, while *E. coli* could not be isolated. The presence of *Staph. aureus* and coliforms on the workers' hands indicated that the employees needed to improve personal hygiene practices. Educational programs should be established to continually reinforce food-safety principles. In some cases, high numbers of *Staph. aureus* (up to 2.0 x 10^4 cfu/hand) were detected on the workers' hands(Almeida *et al*., 1999).

Bacteria grow very well on skin, and hands are always contaminated with these bacteria. Improper hand washing or lack of hand washing is a major cause of many food poisonings. The predominance of *Staph. aureus* over *E. coli* on the worker's hands in the present study might be explained by the fact that when human

Table 19. Microbiological examination of the swab samples taken from the hands of workers employing at the plant under investigation.

Worker No.	*Staph. aureus* (cfu/ swab)	Total coliforms (MPN/ swab)	*E. coli* (cfu/swab)
1	9	0	0
2	20	0	0
3	60	0	0
4	20	0	0
5	0	0	0
6	7	0.9	0
7	0	140	0
8	0	0	0
9	0	0	0
10	0	0	0

fingertips are exposed to Gram negative *E. coli*, the bacteria are killed by a brief incubation (Gläser *et al.*, 2005) In contrast, the Gram-positive bacteria *Staph. aureus* remained viable after the same skin exposure. The authors found that the keratinocytes in the skin secrete an 11-kDa protein, which is called psoriasin, which has potent bactericidal activity against *E. coli*. This protein was present not only in extracts of the epidermis but, more importantly, at the skin surface. A study to determine carriage of *E. coli* O157:H7 by persons living on dairy farms revealed elevated antibody titers against surface antigen of *E. coli* O157:H7 (Doyle, *et al*., 1997).

b. Microbiological quality of the air inside the plant

Although equipment and employees are of the most common sources of pathogens which might be introduced to infant foods, other sources such as dust in the air that comes in contact with the food or food preparation surfaces must be considered possible microbial

sources. For this reason, the air inside the plant was examined. The microbial load in the air at the time of examination was estimated to 130 cfu per plate and 15 minutes of exposure. Counts of coliform did not exceed 5 cfu, while *E. coli* could not be detected. Several factors could be contributing to air contamination at the manufacturing plant. Among them are plant localization, ventilation system and manufacturing practices. The absence of *E. coli* and the low numbers of coliforms in the present study suggest that these microorganisms do not survive well in the air.

c. Microbiological contamination of infant food packages

Microbiological contamination of packaging materials is most likely to occur during construction, transport, storage and usage of packaging materials since the harsh conditions during processing render the materials either sterile or near-sterile (Dallyn and Shorten, 1988). Studies in which the microbial load of packaging materials is determined are limited (Kneifel and Kaser, 1994). Most work has been focused on aseptic packages and packages made from paper and board (Narciso and Parrish, 1997). In general, the microbiological contamination levels of packages made from conventional and biobased materials are relatively low and negligible, will bellow the standard of 1 organism/ cm^2 or 250 cfu/ gram paper and board homogenate proposed by the US Department of Health, Education and Welfare (Dallyn and Shorten, 1988). The microbiological condition of packaging materials should in keeping with that of the products packed in them. A total bacterial count of not more than 10 /100 cm^2 or 10 /100

ml capacity, and a coliform count of 0/100ml or 100 cm^2 are proposed (Lück and Gavron, 1990).

Examination of three types of infant food packages in the present study (Table, 20) showed that two of them, *i.e.* royal hibiscus and royal caraway were within the above mentioned criteria, since the aerobic counts were either undetectable or only 23 cfu/ 100 cm^2, respectively. However, one package, *i.e*. royal camomile had aerobic counts of 221 cfu/ 100 cm^2 representing about two times higher than the proposed criteria. *Staph. aureus* could be detected only on the surface of the last packaging type. Neither of the examined packages contained detectable counts of *E. coli*. Precautions are usually taken to avoid contamination during storage and usage or measures are taken to reduce the microbial load.

Table 20. Microbiological contamination of packages manufactured for packing of infant foods.

Package	Total viable counts*	*Staph. aureus***	*E. coli***
Royal hibiscus	0	0	0
Royal caraway	23	0	0
Royal chamomile	221	1	0

* Count as cfu/100 cm^2 of the interior surface of each package.
** Counts as cfu/swab.

6. Growth and survival of either *E. coli* ATCC 25922 or *Staph. aureus* in reconstituted cereal

E. coli and *Staph. aureus* were selected as inoculants since both bacteria could be isolated from either workers hands, infant food packages or production line at the plant under investigation (Tables, 18-20). Even *E. coli* was found in two Baby Calm herbal samples

(Table, 12a). *Staph. aureus* and pathogenic *E. coli* have been associated with several outbreaks of foodborne disease. Pathogenic *E. coli* is known to cause several diseases, especially in young children (Doyle *et al*., 1997). Monitoring the survival and growth characteristics of both bacteria would provide valuable information useful in predicting their behavior in food systems. It is possible for dry infant cereals to contain dormant pathogenic cells such as *Salmonella*, *E. coli* and *Staph. aureus*. Such cells may recover and grow when cereal is reconstituted, if temperature and time permit. Also, cross-contamination of reconstituted cereal from raw foods and contaminated utensils may occur in the kitchen (Humphery, 2001). Overall, few studies have examined the survival and growth of bacterial pathogens in reconstituted cereal preparations. Additional research is needed to examine the behavior of foodborne pathogens in reconstituted infant cereals and provide recommendations to consumers. Research must consider type of cereal, hydration liquid, and the time and temperature of storage, as well as the bacterial species, as primary factors that affect the behavior of pathogens in infant cereals.

a. Growth and survival of *E. coli* ATCC 25922

Survival and growth characteristics of *E. coli* ATCC 25922 in rice cereal reconstituted with pasteurized milk held on 5 and 25 ˚C at different intervals were determined (Table, 21 and Figure, 7). Initial population was determined by plating cells on VRBDA and calculating $\log_{10}$ cfu/ml of reconstituted cereal slurry. At 5 ˚C, the counts of *E. coli* cells slightly increased after 4 h of inoculation, then declined to about

30 % after 20 h of inoculation and were undetectable along the experimental duration, *i.e*. up to 50 h of inoculation. On the other hand, the *E. coli* cells grew rabidly at 25 ˚C exceeding 9 $\log_{10}$ cfu/ml of slurry within 26 h. This population resulted in a sour taste and different aroma. Deng *et al*. (1998) reported populations of *E. coli* O157:H7 exceeding 10^8 cfu/ml of cereal reconstituted with milk resulted in a remarkable change in aroma. In the present study, the lag phase of growth in the reconstituted cereal could not be observed. The population increased by about 1 $\log_{10}$ cfu/ml of slurry within 4 h at 25˚C. Meanwhile, abundant (near 10 logs) growth of *E. coli* occurred in the cereal reconstituted with pasteurized milk kept at 25 ˚C for 26 h (Table, 21), indicating that infant cereals support growth of bacterial pathogens following reconstitution with a hydrated liquid that has little or no antimicrobial effect.

Table 21. Population of *E. coli* ATCC 25922 recovered from reconstituted rice cereal inoculated with *E. coli* cells.*

Time (h)	Population ($\log_{10}$ cfu/ml)	
	5ºC	**20ºC**
0	0.90	0.30
4	0.93	1.11
8	0.64	2.05
20	0.30	7.10
26	<0	9.94
30	<0	8.95
50	<0	8.34

* Original culture, $1.5x10^8$ cfu/ml.

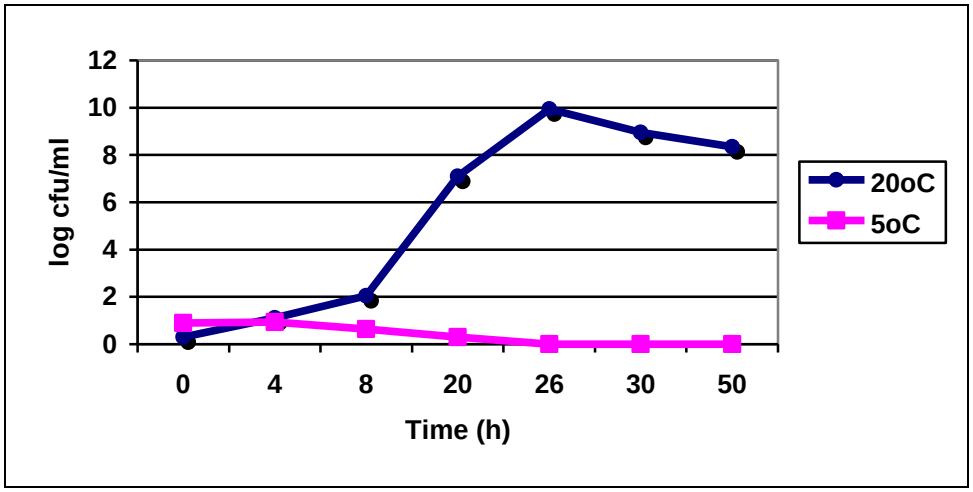

Fig. 7. Growth and survival of *E. coli* in reconstituted rice cereal at either 20 or 5 °C.

b. Growth and survival of *Staph. aureus*

Survival and growth characteristics of *Staph. aureus*, isolated from hands of employee during manufacturing of infant food packages, in rice cereal reconstituted with pasteurized milk held at 5 and 25 ˚C were determined at different intervals (Table, 22 and Figure, 8). *Staph. aureus* population on Vogel- Johnson agar showed no changes during the 72 h storage at 5 ˚C, indicating survival of the pathogen without reproduction. However, at 25 ˚C the population of the organism

Table 22. Population of *Staph. ouraus* recovered from reconstituted rice cereal inoculated with *Staph. aureus* cells.

Time (h)	Survival of population*	
	5ºC	20ºC
0	0	0
12	0	2.0
24	0	3.0
48	0	4.2
72	0	3.12

* Number of $\log_{10}$ cycles cfu/ml increased (=$\log_{10}$ cfu/ml at 0 h subtracted from $\log_{10}$ cfu/ml at any given time).

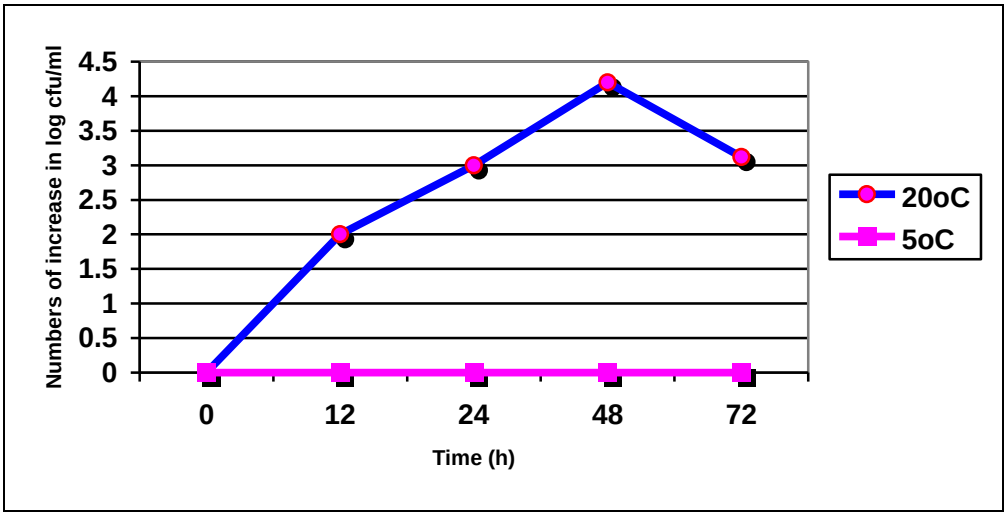

Fig. 8. Growth and survival of *Staph. aureus* in reconstituted rice cereal at either 20 or 5 °C.

increased substantially within the first 48 h of inoculation. This population was >4 logs higher than the initial (0 h) population. This result indicates the crucial influence of temperature on growth of the pathogen in infant cereals when reconstituted with a hydration liquid such as pasteurized milk.

The results of the last 2 experiments are in general agreement with previous data on the behavior of *B. cereus* and *E. coli* O157:H7 in rice infant cereals reconstituted with milk (Deng *et al*., 1998 and Jaquette and Beuchat, 1998). There were no major increases in the populations in the reconstituted rice cereal stored at 5 ˚C for 72 h. These findings suggest that adequate refrigeration would prevent growth of potential *E. coli* and *Staph. aureus* contaminants in reconstituted infant cereals. Abusive condition of temperature (25 ˚C), however, stimulated growth of both organisms during storage. In summary, both organisms showed high potential for survival and

growth in reconstituted infant cereals stored at 5 and 20 ˚C for up to 72 h. Growth of the organisms in cereal hydrated with milk increased as temperature and time of storage increased. Thus, small number of the organisms could grow to more dangerous levels at 25 ˚C over a period of time. The behavior of such pathogens in cereals hydrated with other liquids, such as orange juice, carrot juice, or other kinds of juices, needs to be investigated. Based on the results of this study, it is recommended that hydrated infant cereals are consumed immediately after preparation or held at less than 5 ˚C to be consumed in less than 4 h to reduce potential risks associated with pathogens. Additional studies are needed to evaluate survival and potential growth of psychrophilic pathogens in such products.

CONCLUSIONS

1. Examination of the infant food formulas reflected good manufacturing practices of these products, since no pathogenic bacteria could be detected in any of the tested samples. However, precautions should be considered since the indicator bacterium *E. coli* was detected in a few portions of the samples.
2. The presence of lipolytic and proteolytic bacteria in all samples raises the issue of possibility of spoilage in such foods. However, the recorded water activity values in all samples were below the critical threshold. Consequently, such foods may be exposed to spoilage if good care of proper closure is not taken.
3. Preparation of dehydrated infant foods in boiled water for a short time (5–15 minutes) is sufficient either to kill the indicator organisms or to reduce other microflora to a high extent. Consequently, following up the company precautions is highly significant to maintain good hygienic quality of the product.
4. Treatment of baby foods such as Baby Calm at doses below 5 kGy appears to achieve microbial decontamination, without affecting the quality change. The present study is one of few who dealt with food supplement irradiation and its effects on pathogenic bacteria.
5. Ethylene oxide had a very effective killing effect against contaminated microorganisms in the herbal-based samples. A gas dose of 250 mg/l is sufficient either to completely kill or to reduce the counts to a high extent.

6. Recontamination of infant food packages through worker's hands and contaminated surfaces has been observed. The presence of *Staph. aureus* and coliforms indicates that employees needed to improve personal hygiene practices
7. Abusive temperature (25 °C) stimulated the growth of either food poisoning (*Staph. aureus*) or indicator bacteria (*E. coli*) in the hydrated infant cereals. Consequently, these preparations must be consumed immediately after preparation or held at less than 5 °C and consumed within not more than 4 h.

SUMMARY

The present study deals with the accomplishment of microbiological and chemical survey for common types of infant food products in Egyptian retail markets and pharmacies. The efficiency of different treatments, *i.e*. irradiation, fumigation and boiled water to reduce the microbial load and hence ensure the hygienic quality of the tested samples was evaluated as well. To measure plant hygiene and product quality during manufacturing of infant-food packages, the bacteriological conditions of the equipments, hands of employees, packages and air inside the plant under investigation were checked. The growth and survival of health-related bacteria, *i.e*. *Staphylococcus aureus* and *E. coli* commonly found in such products was evaluated as well.

The obtained results could be summarized as follows:

1. Investigation of microbial contamination and chemical analysis

a. Microbial contamination and chemical analysis of cereal–based infant formulas

Neither of the tested samples harbored any of the pathogenic bacteria that might be found in such products, *i.e. E. coli, Salmonella, Shigella, Staph. aureus*, *Bacillus ceurus*, *Clostridium* sp., *Lisreria* sp. or *Enterococcus*. The colony counts in three samples, *i.e*. Riri with vegetables, cerelac and baby king special formula were considered to be unacceptable according to the Egyptian standards. The total

mesophilic microbial counts detected in the above mentioned three samples of the present study were 135, 115 and 115 cfu/g, respectively. Therefore, these formulas might considered to be unsafe. Yeasts & molds were found only in Riri with vegetables and cerelac samples, reaching 60 and 35 cfu/g. Coliforms could be detected only in the cerelac sample and their counts did not exceed 20 cfu/g.

For all examined samples of infant food component from cereals, the proteolytic bacteria group was detected only in 58 % of the samples, *i.e*. 7 samples with a mean of 40.7 cfu/g. Lipolytic bacterial counts ranged from 5 to 75 cfu/g with a mean of 24 cfu/g and were detected only in 5 samples representing 41.7% of the tested samples. The average of water activity was found to be 0.2219, while the fat content ranged from 0.5 to 9.6 % and the protein content ranged from 6.2 to18.5 %. Neither of the tested samples had any detectable levels of the aflatoxins.

b. Microbial contamination and chemical analysis of the milk-based infant formulas

The mesophilic colony counts in two samples, *i.e*. Babysan 2 and S-26 gold were considered to be unacceptable. The determined total aerobic counts were 130 and 125 cfu/g, respectively. Lipolytic bacteria ranged form 10 to 65 cfu/g, while proteolytic bacteria ranged form 10 to 15 cfu/g. The mean water activity of the infant food components from milk was 0.267 with a fat content value ranging from 20 to 28.1. Protein content values ranged from 9.5 to 16.

c. Microbial contamination and chemical analysis of vegetable and fruit-based infant food formulas

Only one sample, *i.e*. Grebe fruit dessert was shown to be unacceptable according to microbiological standards of the Egyptian. The total aerobic mesophilic count in this sample was 675 cfu/g. Yeasts & molds could be detected only in one sample, *i.e.* Grebe mixed vegetables in low numbers hardly exceeding 10 cfu/g. For all examined infant food components from vegetables and fruits, lipolytic bacteria were detected in all samples and ranged form 5 to 150 cfu/g, while proteolytic bacteria ranged form 15 to 125 cfu/g. The mean water activity of infant food component from vegetables was 0.3837 with a fat content value ranging from 0.1 to 1.8 % and protein content value of 0.4 to 4.0 %.

d. Microbial contamination and chemical analysis of herbal-based infant food formulas

Only one sample, *i.e*. baby calm herbal was not acceptable according to the Egyptian standards. The sample had a total aerobic mesophilic count of 10^3 cfu/g. Compared to the other analyzed herbal samples, the bay calm sample had detectable numbers of yeasts & molds (20 cfu/g), coliforms (50 cfu/g), proteolytic bacteria (75 cfu/g) and lipolytic bacteria (50 cfu/g). The sample was positive for *Clostridium* sp. Other samples met the Egyptian standards. The mean water activity of the infant food component from milk was 0.297. No fat or protein could be detected, except marginal amounts measured in one sample.

e. Microbial contamination of different samples of baby calm herbals

All tested samples were free from the pathogenic bacteria that might be found in such products such as *Salmonella, Shigella, Staph. aureus* and *Bacillus ceurus*. However, *E. coli* could be detected in two samples. It is obvious that considerable levels of microbial contamination were recovered in most of the surveyed samples. For all examined samples, the total aerobic mesophilic counts ranged from 1.1 x 10^2 – 1.0 x 10^5 cfu/g. However, *Clostridium* sp. was detected only in 7 samples, representing 46.6 % of the tested samples. Yeast & mold counts ranged from undetectable counts to 100 cfu/g. Coliforms were found in all tested samples and ranged from 15 to 4.2 x 10^3 cfu/g.

2. Survival of microorganisms in infant food formulas after preparation in pre-boiled (100 ˚C) water

a. Survival in vegetable-based infant food formulas

Preparation of a vegetable-based sample in pre-boiled water resulted in a relative reduction of the counts along the one hour period. The total aerobic counts decreased by about 60 %, suggesting that the non-spore forming cells might represent the majority of the existed microflora. This was evident when the counts of yeasts and molds were determined, where their counts decreased by about 50 %.

b. Survival in cereal-based infant food formulas

Contrary to the vegetable-based sample, the recovered total aerobic counts from the cereal-based formula survived without any considerable reduction. However, the initial low numbers of yeasts &

molds (35 cfu/g) and coliforms (20 cfu/g) disappeared rapidly, since no detectable numbers could be observed after preparation.

c. Survival in herbal-based infant food formulas

Since the baby calm herbal formula was shown to be microbiologically unacceptable, it was interesting to investigate whether the observed relative high numbers of microflora could be reduced to the acceptable levels by means of preparation in hot water. Each infant herbal packet was placed in pre-boiled water (about 100 ˚C) for about 5 min. This resulted in an obvious reduction of the microbial counts. A reduction of 0.14 – 2.62 $\log_{10}$ cycle cfu/g of the total mesophilic counts was achieved in 11 samples of the 15 samples tested. Expectedly, the coliform group either completely disappeared or highly decreased in all tested samples. *E. coli* detected in two samples could not be recovered after preparation. Unexpectedly, the anaerobic spore-forming bacteria detected in 7 samples could not be recovered from these samples after preparation in the hot water. The results of this experiment indicate that the preparation of such infant formula types in hot water for a short time is sufficient either to kill the indicator microorganisms or to reduce other microflora to a high extent.

3. Effect of ionizing irradiation on microbial contamination in herbal-based infant formulas

The effectiveness of irradiation to control the microbiological contamination in 3 baby calm herbal samples was evaluated. This type of samples was chosen, since the baby calm herbal was characterized with obvious high numbers of microorganism. The initial numbers of

the total aerobic counts decreased from 5.7 x10^3 cfu/g before irradiation reaching their lowest level of 18 cfu/g after treatment by a dose of up to 4 kGy, representing a reduction percentage of about 99.7 %. On the other hand, higher doses of 5-10 kGy might result in an immediate sterilization of the samples, since no microorganisms could be detected in any of the tested samples. Irradiation, at the energy levels commonly used, effectively kills bacteria, molds and yeasts.

4. Effect of fumigation with ethylene oxide on microbial contamination of herbal-based infant formulas

The effectiveness of fumigation with ethylene oxide to control the microbiological contamination in 3 baby calm herbal samples was evaluated. This type of samples was chosen, since the baby calm herbal was characterized with obvious high numbers of microorganism. A decrease in the counts of most microorganisms found in herbal samples was observed at a gas dose of 250 mg/l. At higher doses, the effect of ethylene oxide fumigation was sterile, since no microorganisms could be detected. From these results it is obvious that the ethylene oxide had a very effective killing effect against contaminated microorganisms.

5. Hazard analysis during manufacturing of infant food packaging materials

a. Contamination from contact surfaces

1. Production line (equipment)

Of 11 ports located on the production line, seven of them contained *Staph. aureus* in counts reached 60 cfu/ swab. Coliforms

could be detected from only two ports, while *E. coli* was found on only two ports.

2. Hands of workers (employees)

Microbial samples collected from workers' hands presented considerable bacterial contamination. Of ten employees examined, five (50 %) of them had *Staph. aureus* on their hands. Coliforms could be detected only on two cases, while *E. coli* could not be isolated. The presence of *Staph. aureus* and coliforms on the workers' hands indicated that the employees needed to improve personal hygiene practices.

b. Microbiological quality of the air inside the plant

The microbial load in the air at the time of examination was estimated to 130 cfu per plate and 15 minutes exposure. Counts of coliform did not exceed 5 cfu, while *E. coli* could not be detected.

c. Microbiological contamination of infant food packages

Examination of three types of infant food packages showed that two of them, *i.e.* royal hibiscus and royal caraway were within the above mentioned criteria, since the aerobic counts were either undetectable or only 23 cfu/ 100 cm^2, respectively. However, one package, *i.e.* royal camomile had aerobic counts of 221 cfu/ 100 cm^2 representing about two times higher than the proposed criteria. *Staph. aureus* could be detected only on the surface of the last packaging type. Neither of the examined packages contained detectable counts of *E.*

coli. Precautions are usually taken to avoid contamination during storage and usage or measures are taken to reduce the microbial load.

6. Growth and survival of either *E. coli* ATCC 25922 or *Staph. aureus* in reconstituted cereal

a. Growth and survival of *E. coli* ATCC 25922

Survival and growth characteristics of *E. coli* ATCC 25922 in rice cereal reconstituted with pasteurized milk held at 5 and 25 ˚C were determined. At 5 ˚C, the counts of *E. coli* cells slightly increased after 4 h of inoculation, then declined to about 30 % after 20 h of inoculation and were undetectable along the experimental duration *i.e*. up to 50 h of inoculation. On the other hand, the *E. coli* cells grew rabidly at 25 ˚C exceeding 9 $\log_{10}$ cfu/ml of slurry within 26 h.

b. Growth and survival of *Staph. aureus*

Survival and growth characteristics of *Staph. aureus*, isolated from hands of employee during manufacturing of infant food packages, in rice cereal reconstituted with pasteurized milk held at 5 and 25 ˚C were determined. *Staph. aureus* population showed no changes during the 72 h storage at 5 ˚C, indicating survival of the pathogen without growth. However, at 25 ˚C the population of the organism increased substantially within the 48 h inoculation. This population was >4 logs higher than the initial (0 h) population. This result indicated that temperature abuse conditions stimulated growth of the pathogen in infant cereals when reconstituted with a hydration liquid such as pasteurized milk.

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الملخص العربي
دراسات ميكروبيولوجية على أغذية الأطفال

يعتبر لبن الأم هو الغذاء الأول والمثالي في حياة الطفل حيث أن هذا الغذاء يتناسب تماماً مع احتياجات الطفل الفسيولوجية ومتطلباته الحيوية, وقد يتعذر إرضاع الطفل طبيعياً كما يحدث في بعض الحالات مثل مرض الأم بأمراض معدية أو في حالة نقص الإفراز اللبني نقصاً شديداً أو في حالة وفاة الأم أو غير ذلك من الأسباب التي تعجز معها الأم عن إرضاع طفلها. ففي هذه الحالات يلزم تغذية الرضيع باستعمال ألبان خارجية معدلة في تركيبها بحيث تماثل لبن الأم.

وقد تستخدم في مراحل عمرية معينة أغذية تكميلية أثناء فترة الفطام أو كغذاء مكمل للبن الأم وهذه الأغذية توفر العناصر الغذائية سواء التي لا توجد أو توجد بكميات غير كافية في أغذية الرضع الأساسية.

ولقد تم تقسيم أغذية الأطفال في هذه الدراسة إلى أربع أقسام:

1. أغذية أطفال مصنعة من الألبان.
2. أغذية أطفال مصنعة أساساً من الحبوب.
3. أغذية أطفال مصنعة أساساً من الخضروات والفواكه.
4. أغذية أطفال مصنعة من الأعشاب.

ومن المعلوم أن أطفال والحوامل وكبار السن والمرضي هم أقل الأشخاص مناعة للتلوث بالميكروبات المرضية لذا كان من الضرورة القصوى أخذ كل الاحتياطات اللازمة أثناء إعداد الأغذية الخاصة بالفئات السابقة. ولذا عنيت الهيئة المصرية للتوحيد القياسي وضبط الجودة بوضع مواصفة قياسية خاصة لأغذية الأطفال برقم 2072 لسنة 1991 وكذلك هيئة التقييس لدول مجلس التعاون الخليج برقم 677 لسنة 1997 وكذلك منظمة Codex العالمية برقم **CAC/RCP 21**.

وتهدف هذه الدراسة إلى:

1. عمل دراسة أولية علي كل أنوع أغذية الأطفال المتداولة في السوق المصري سواء محلية الصنع أو المستوردة والتي تشمل كذلك الفئات العمرية المختلفة (منذ الولادة – أربعة

شهور – ستة أشهر – تسعة أشهر) وذلك بدراسة أهم المجموعات الميكروبية وكذلك الميكروبات المرضية وكذلك عمل التحاليل الكيمائية والفيزيقية للمواد المؤثرة على النمو الميكروبي مثل معامل النشاط المائي ونسب البروتين والدهون والسموم الفطرية في المواد الغذائية ومقارنة النتائج بالمواصفات القياسية المصرية لأغذية الأطفال.

2. مقارنة تأثير المعاملة بالإشعاع والتبخير بغاز أكسيد الإثيلين وكذلك استخدام الماء المغلي علي الحمل الميكروبي للأغذية التكميلية المصنعة أساساً من الأعشاب.
3. دراسة مقدرة بعض الميكروبات الممرضة علي التأقلم والنمو في بعض أغذية الأطفال.
4. دراسة بعض العوامل المؤدية للتلوث الميكروبي للأغذية التكميلية المصنعة أساساً من الأعشاب مثل مواد التعبئة والتغليف وأيدي العمال.

ويمكن تلخيص النتائج المتحصل عليها فيما يلي:

1. دراسة التلوث الميكروبي وأهم الخصائص الكيميائية لأغذية الأطفال

أ. أغذية الأطفال المكونة أساساً من الحبوب

لوحظ أن كل العينات المختبرة خالية تماماً الميكروبات المرضية أو دلائل التلوث مثل *E. coli, Salmonella, Shigella, Staph. aureus, Bacillus cereus, Clostridium* sp., *Listeria* sp. or *Enterococcus*. بينما يوجد ثلاث عينات فقط غير مطابقة للمواصفات القياسية المصرية من حيث العدد الكلي للبكتريا وهم ريري بالخضروات وسيريلاك و بيبي كينج التركيبة الخاصة وكانت الأعداد على الترتيب هي 135, 115, 115 مستعمرة/جم. كذلك وجدت الفطريات في عينتين هما ريري بالخضروات وسيريلاك بأعداد 60 , 35 مستعمرة/جم, بينما المجموعة القولونية وجدت في عينة واحدة كذلك وهي سيريلاك بأعداد 20 مستعمرة/جم.

في كل العينات المختبرة وجدت البكتريا المحللة للبروتين في 58% فقط من العينات بمتوسط 40.7 مستعمرة/جم, بينما البكتريا المحللة للدهون وجدت في 41.7% من العينات بمتوسط 24 مستعمرة/جم.

متوسط معامل النشاط المائي للعينات المختبرة 0.2219 بينما تتراوح قيم نسبة الدهون بين 0.5–9.6% ونسبة البروتين بين 6.2–18.5%, مع ملاحظة أن كل العينات المختبرة لم يكتشف بها أي آثار للسموم الفطرية.

ب. أغذية الأطفال المكونة أساساً من الألبان

تشير النتائج إلي وجود عينتين فقط غير مطابقة للمواصفات القياسية المصرية من ناحية العدد الكلي للبكتريا وذلك في عينتي بيبي زان2 و س 26 جولد حيث كانت الأعداد المقدرة هي 130 , 125 مستعمرة/جم علي الترتيب. بينما البكتريا المحللة للدهون كانت تتراوح ما بين 10–65 مستعمرة/جم, وبالنسبة للبكتريا المحللة للبروتين كانت تتراوح بين 10–15 مستعمرة/جم. وكان متوسط قيمة معدل النشاط المائي 0.267 ونسبة الدهون تتراوح بين 20–28.1 بينما نسبة البروتين تتراوح بين 9.5–16.

ج. أغذية الأطفال المكونة أساساً من الخضروات والفواكه

أظهرت النتائج وجود عينة واحدة فقط خارج المواصفات القياسية المصرية من حيث العدد الكلي للبكتريا وهي جربر فواكه حيث كان العدد 675 مستعمرة/جم, بينما وجدت في عينة واحدة كذلك فطريات وهي جربر خليط خضروات حيث كان العدد 10 مستعمرة/جم, وفي كل العينات المختبرة كان معدل البكتريا المحللة للدهون يتراوح بين 5–150 مستعمرة/جم, بينما متوسط معامل النشاط المائي 0.3837 مع نسبة دهون تتراوح بين 0.1–1.8% ونسبة بروتين تتراوح بين 0.4–4%.

د. أغذية الأطفال المكونة أساساً من الأعشاب

فقط عينة واحدة من ضمن العينات المختبرة كانت غير مطابقة للمواصفات القياسية المصرية وهي عينة بيبي كالم حيث كان العدد الكلي للبكتريا 1000مستعمرة/جم, بينما كانت أعداد الفطريات 20 مستعمرة/جم وأعداد المجموعة القولونية 50 مستعمرة/جم وكانت من ضمن العينات الذي أظهرت نتيجة إيجابية لوجود بكتريا *Clostridium* sp. بينما كانت أعداد البكتريا المحللة للبروتين 75 مستعمرة/جم وأخيراً كانت أعداد البكتريا المحللة للدهون 50مستعمرة/جم, واللافت للنظر أن باقي العينات المختبرة كانت كلها مطابقة للمواصفات القياسية المصرية.

هـ. التلوث الميكروبي لعينات من منتج بيبي كالم

كل العينات التي تم تحليها خالية من الميكروبات المرضية مثل *Salmonella, Shigella, Staph. aureus, Bacillus cereus* بينما تم إكتشاف *E. coli* في عينتين فقط, ومن الجدير بالذكر أن كل العينات المختبرة كانت غير مطابقة للمواصفات القياسية المصرية من حيث العد الكلي للبكتريا والذي تراوح بين 1.1×10^{2} – 1.0×10^{5} مستعمرة/جم بينما وجد *Clostridium* sp. في سبع عينات فقط والتي تشكل 46.6% من

إجمالي العينات المختبرة. تراوحت أعداد الفطريات في العينات المختبرة 0–100 مستعمرة/جم, بينما بكتريا المجموعة القولونية والتي وجدة في كل العينات المختبرة تراوحت بين 15–4.2x 10^3 مستعمرة/جم.

2. تأثير الماء المغلي أثناء تجهيز أغذية الأطفال علي الميكروبات الملوثة

أ. بالنسبة للأغذية المكونة أساساً من الخضروات

وجد أن المعاملة أدت إلى اختزال العدد الكلي للبكتريا إلي حوالي 60%, بينما اختزل عدد الفطريات والخمائر بنسبة 50% تقريباً.

ب. بالنسبة للأغذية المكونة أساساً من الحبوب

علي النقيض من الأغذية المكونة أساساً من الخضروات لم يظهر أي تأثير للمعاملة بالماء المغلي علي الأغذية المكونة أساساً من الحبوب, بينما ظهرت بسرعة أعداد من الفطريات وصلت إلي 35 مستعمرة/جم وكذلك بكتريا المجموعة القولونية والتي كان تعدادها 20مستعمرة/جم.

ج. بالنسبة للأغذية المكونة أساساً من الأعشاب

من النتائج المتحصل عليها والتي أكدت أن منتج بيبي كالم كان الوحيد الغير مطابق للمواصفات القياسية المصرية كان من الضروري إيجاد المعاملة التي من شأنها خفض الحمل الميكروبي بالعينة, وبالفعل عند معاملة البكوات (العينة داخل فلتر الورق) بالماء المغلي لمدة 5 دقائق أدي هذا إلي اختزال العدد الكلي للبكتريا من 2.62 إلي 0.14 لو$_{10}$ مستعمرة/جم وذلك في 11 عينة مختبرة من أصل 15عينة, وكذلك تم حدوث اختزال كبيير لعدد بكتريا القولون, بينما ظهرت *E. coli* في عينتين ولم تتأثر البكتريا اللاهوائية المتجرثمة بالمعاملة علي الإطلاق.

3. تأثير المعاملة بالإشعاع علي الميكروبات الملوثة لأغذية الأطفال المكونة من الأعشاب

وجد أن معاملة عينات من منتج بيبي كالم بجرعة من الإشعاع حوالي 4 كيلو جراي أدي إلي اختزال العدد الكلي للبكتريا من 10x5.7 ^{3} إلي 18مستعمرة/جم بينما الجرعات 5–10 كيلو جراي كان لها تأثير معقم للعينات.

4. تأثير المعاملة بأكسيد الإثيلين علي الميكروبات الملوثة لأغذية الأطفال المكونة من الأعشاب

من خلال التجارب التي أجريت لتبخير العينات بجرعات مختلفة بغاز أكسيد الإثيلين 250–1500 ملجم/لتر وجد أن المعاملة بتركيز 250ملجم/لتر أدي إلي اختزال عدد الميكروبات الملوثة بينما الجرعات الأعلى فلها تأثير معقم علي العينات.

5. تحليل المخاطر الحادثة أثناء تصنيع مواد التعبئة والتغليف الخاصة بأغذية الأطفال

أ. التلوث الحادث نتيجة الأسطح الملامسة

1. خطوط الإنتاج

من خلال التجارب التي أجريت علي 11 جزء مختلف من خط الإنتاج تبين تلوث الخطوط بميكروبات *Staph. aureus* والتي وصلت إلي 60 مستعمرة/مسحة بينما وجد كل من بكتريا القولون و *E. coli* في مكانين فقط.

2. أيدي العمال

الجدير بالذكر أن 50% من العمال الذين أجري عليهم الاختبارات (11 عامل) كانت أيدهم ملوثة بالميكروبات العنقودية *Staph. aureus* بينما بكتريا القولون وجدت في حالتين فقط, ولم يكتشف وجود تلوث ببكتريا *E. coli*.

ب. الحمل الميكروبي للهواء داخل خطوط الإنتاج

الحمل الميكروبي للهواء داخل خطوط الإنتاج كان 130 مستعمرة/طبق خلال 15 دقيقة من التعرض, بينما لم تتعدي أعداد بكتريا القولون عن 5 خلايا/طبق, ولم يكتشف وجود أى تلوث ببكتريا *E. coli*.

ج. التلوث الميكروبي لمواد التعبئة والتغليف الخاصة بأغذية الأطفال

تم دراسة التلوث الميكروبي لثلاث أنواع مختلفة من مواد التعبئة والتغليف الخاصة بأغذية الأطفال وأشارت النتائج المتحصل عليها إلي عدم وجود أي تلوث ببكتريا *E. coli* بينما ظهرت *Staph. aureus* في عينة واحدة فقط وهي علبة رويال كاموميل والتي كان العدد الكلي للبكتريا بها 221مستعمرة/100سم^2 بينما لم يزيد العدد الكلي للبكتريا في العينات الأخرى عن 23 مستعمرة/100سم^2.

6. تأقلم ونمو بعض الميكروبات الممرضة في أغذية الأطفال

أ. تاقلم ونمو *E. coli* ATCC 25922

بدارسة تأقلم ونمو *E. coli* ATCC 25922 في عينة رقائق الأرز المضاف له لبن مبستر علي درجتي حرارة 5 و 25^{o}مئوية تبين أن أعداد الميكروب ببطء تزيد بعد 4

ساعات من التحضين علي 5° مئوية ثم يقل العدد بنسبة 30% بعد 20 ساعة من التحضين ثم تختفي تماماً بعد 50 ساعة من التحضين, ومن ناحية أخري تنمو *E. coli* بسرعة عند التحضين علي25°مئوية حيث تصل خلال 26 ساعة إلي 9 لو10 مستعمرة/مل.

ب. تأقلم ونمو *Staph. aureus*

بدارسة تأقلم ونمو الميكروب الذي تم عزله من أيدي العمال علي عينة رقائق الأرز المضاف لها لبن مبستر علي درجتي حرارة 5 و 25°مئوية تبين عدم حدوث تغير لأعداد الميكروب خلال 72 ساعة من التحضين علي 5°مئوية, ومن ناحية أخري عند التحضين علي 25°مئوية لمدة 48 ساعة حدث نمو كبير للبكتريا وصل إلي أكثر من 4لو10 من العدد الأولي عند بدء التجربة.

اسم الطالب: علاء الدين محمد صادق علي **الدرجة:** الدكتوراه

عنوان الرسالة: دراسات ميكروبيولوجية على أغذية الأطفال

المشرفون : الأستاذ الدكتور : رفاعي إبراهيم رفاعي **الدكتور :** وليد ضياء الدين صالح
قسم: الميكروبيولوجيا الزراعية
تاريخ منح الدرجة: 18 /2009/3

المستخلص العربي

في هذه الدراسة تم تجميع 42 عينة ممثلة لأغذية الأطفال من السوق المحلي والصيدليات من مدن القاهرة والجيزة والعاشر من رمضان خلال الفترة 2004–2005 والتي تم اختبارها من الناحية الميكروبيولوجية لتقييم جودتها. وتبين من النتائج أن كل العينات المختبرة خالية من الميكروبات المرضية وكذلك لم يكتشف بها أي آثار من السموم الفطرية.

تبين أنه في 7 عينات فقط وهي ريري بالخضروات و سيريلاك و بيبي كينج ذو التركيبة الخاصة و بيبي زان2 و س 26 جولد و جربر خليط فواكه و بيبي كالم أعشاب غير مطابقة للمواصفات القياسية المصرية من حيث العدد الكلي للبكتريا, ولم يكتشف الفطريات والخمائر إلا في 4 عينات فقط وهم ريري بالخضروات و سيريلاك و جربر خليط من الخضروات و بيبي كالم أعشاب, بينما بكتريا القولون وجدت فقط في عينتين هما سيريلاك و بيبي كالم أعشاب.

بمعاملة بيبي كالم أعشاب بالماء المغلي لمدة 5 دقائق أدي هذا لاختزال العدد للبكتريا الهوائية إلي الحد المسموح به حسب منظمة الصحة العالمية أقل من10^4 مستعمرة/جم, وبدراسة تأثير المعاملة بالإشعاع علي العينة وجد أن جرعة 5 كيلو جراي تعتبر كافية لتعقيم العينة حيث كان العدد الكلي للبكتريا بعد المعاملة أقل من 10 مستعمرة/جم وبدون التأثير علي اللون أو النكهة, بينما أدت المعاملة بأكسيد الإيثيلين بجرعة 250 ملجم/لتر إلي اختزال التلوث الميكروبي بشكل واضح ولوحظ أن الجرعات الأعلى كانت ذات تأثير معقم.

من ضمن 11 مكان في مصنع أغذية الأطفال وجد 7 أماكن ملوثة بميكروب *Staph. aureus* بمعدل 60 مستعمرة/مسحة بينما وجدت بكتريا القولون و *E. coli* في مكانين فقط, وبدراسة التلوث الميكروبي لأيدي العمال تبين تلوث أيدي 5 عمال من ضمن 10 بميكروب *Staph. aureus*, بينما وجدت بكتريا القولون في حالتين فقط, وكان الحمل الميكروبي في الهواء المحيط بمنطقة التصنيع 130مستعمرة/طبق/15 دقيقة تعريض. و بدراسة التلوث الميكروبي لثلاث أنواع مختلفة من مواد التعبئة والتغليف الخاصة بأغذية الأطفال وأشارت النتائج المتحصل عليها إلي عدم وجود أي تلوث ببكتريا *E. coli* بينما ظهرت *Staph. aureus* في عينة واحدة فقط وهي علبة رويال كاموميل والتي كان العدد الكلي للبكتريا بها 221مستعمرة/100سم^2 .

بدراسة نمو وتأقلم سلالات من *Staph. aureus* و *E. coli* علي رقائق الأرز مضاف إليه لبن مستر علي درجات حرارة 5°م أثبت النتائج إختزال أعداد *E. coli* وأختفائها تماماً بعد 50ساعة بينما لم تتغير أعداد *Staph. aureus* بعد 72 ساعة. بينما عند تحضين *E. coli* علي 25°م حدث نمو سريع لـ *E. coli* وصل لـ$\log_{10}$ 9 بعد 26 ساعة بينما وصل نمو *Staph. aureus* علي 25°م أكبر من $\log_{10}$ 4 بعد 48 ساعة.

الكلمات الدالة: أغذية الأطفال, التلوث الغذائي, المعاملة بالإشعاع, أكسيد الإيثيلين, ميكروبات القولون, البكتريا العنقودية

دراسات ميكروبيولوجية على أغذية الأطفال

رسالة دكتوراه الفلسفة
في العلوم الزراعية
(ميكروبيولوجيا زراعية)

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للحصول على

درجة دكتوراه الفلسفة

في

العلوم الزراعية
(ميكروبيولوجيا زراعية)

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كليـــــة الزراعـــة
جـامعــة القــاهرة
مصـــــــر

2009

نبذة عن المؤلف

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