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RESEARCH ARTICLE

Effect of Storage Conditions on Bacteriological Growth in Infant Cereals

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ABSTRACT:

A total of 28 samples of infant cereals from 3 brands purchased from many pharmacies and markets in Lattakia, were evaluated in terms of bacteriological quality at opening time (initial point) and within a month at different temperatures. Bacteriological tests included the determination of total plate counts (TPC) and the detection of *Salmonella* spp. and *Enterobacteriaceae* using Salmonella Shigella Agar (SSA) and MacConkey agar (MAC), respectively. In this study, all the samples of infant cereals showed bacterial counts below the permitted level of Syrian Standard for Infant Cereals 2006 of 10^4 CFU/g. None of the tested samples had either *Salmonella* spp. or *Enterobacteriaceae*.

KEYWORDS: infant cereals, microbiological analysis, *Enterobacteriaceae*, *Salmonella* spp., weaning.

1. INTRODUCTION:

Weaning is the process by which the baby gets used to family foods or adult foods and relies less and less on breast milk[1,2]. As the baby grows, breast-milk alone is insufficient to meet the full nutritional and psychological needs of infant especially from 6 months age[3]. Infant cereals provide an essential amount of carbohydrates and proteins as well as, supply source of vitamins, minerals, and bioactive compounds[4]. Infants and young children are considered to be of high-risk individuals, since their immune systems may have not yet been fully developed [5]. It is therefore, important to investigate the bacterial safety of these foods, whereas many women store baby food cans after opening in refrigerator or keep them at room temperature, so microbial load could change and alter the quality of food as well as pathogenic bacteria have been isolated from refrigerators[6,7].

The ability of microorganisms, except viruses, to grow in foods is determined by the food environment as well as the conditions in which the food is stored, involving the intrinsic and extrinsic environment of food.

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Intrinsic factors include nutrients, growth factors, and inhibitors (or antimicrobials), water activity, pH, and oxidation–reduction potential. Extrinsic factors are temperature, relative humidity, and gaseous environment [8,9,10,11].

According to Syrian Standard for infant cereals *Enterobacteriaceae* and *Salmonella* spp. must not be present in each 1g powder, additionally, the aerobic plate count must not exceed 10^4 CFU/g[12]

This study aims to determine the microbiological quality of infant cereal products commercially available in lattakia in terms of the presence of *salmonella* spp. and *Enterobacteriaceae*, the total bacterial counts as well as the effect of temperature and storage time on plate counts[13].

2. MATERIALS AND METHODS:

Materials:

Three commercial brands of infant cereals (A, B and C) were obtained from different markets and pharmacies in Lattakia. Each brand is available on rice and wheat products, some of them were flavored (A: wheat-honey) (B: wheat-honey, wheat-banana, wheat-fruit and rice-vegetables). (C: wheat-honey, wheat-banana and wheat-fruit).

Brand A was fortified with *Bifidobacterium lactis*. This probiotic is considered as functional component used to support babies intestinal flora[14,15].

Storage Conditions:
The samples of the three brands (without flavor) were opened, packed and stored at 2 temperature (4 and 25) ºC for a month while the flavored samples of the three brands were stored under the same condition for just a week (the rest flavors of each company: honey, banana, fruit and vegetables). The presence of *Enterobacteriaceae* and *salmonella* spp. as well as the total bacterial counts were investigated for all products at the moment of opening time and after 3, 7, 10, 14, 21 and 30 days (rice and wheat) and 0, 3, 7 days (flavored products).

Microbiological Tests:
Microbiological tests conducted at opening time included the determination of bacterial plate counts, and detection of *Enterobacteriaceae* and *Salmonella* spp. The TPC (total plate count) were determined Following the pour spread plate method: 9 grams of each sample was added to 100ml of sterile water rehydrated at room temperature. After rehydration, the sample was diluted many times in the water until a 10^{-3} dilution. After that, an aliquot of 1ml of each dilution was spread in the center of sterile petri plate using a sterile pipette, and then cooled nutrient agar (NA) was added into the petri dish and mixed well. After solidification of agar, plates were inverted and incubated at 37ºC for 24-48 h.[16].

To detect *Enterobacteriaceae* in infant cereals, 1ml of rehydrated samples was mixed with MAC. MacConkey agar in petri plate, to incubated for 48 h. at 37°C [17].

In order to detect *Salmonella* spp. 25g of cereal products was suspended in 225ml of sterile water, after that an aliquot of 1ml reconstituted sample was transferred to plate and S.S.A. was added. Finally, the plate was incubated for 24 h. at 37ºC.

3. RESULTS AND DISCUSSION:

3.1. Initial Points:

No bacterial growth was detected in non-flavored rice and wheat products from A Brand, while TPC was 2.12 $\log_{10}$ CFU/g (CFU: colony forming units) in honey flavored products. Fig. 1. shows the TPC in Brand A at opening time.

Most of Brand B samples had positive results on NA except the product with honey flavor. The viability of germs growth was found to be the highest in rice product (2.60 $\log_{10}$ CFU/g), followed by wheat product (2.38 $\log_{10}$ CFU/g), then vegetable, fruit while banana flavored samples had the lowest counts. Fig. 2. shows the TPC in B Brand at opening time.

All products of C Brand had bacterial growth in all products. Fig. 3. shows the TPC in C Brand at opening time.

Salmonella spp. and *Enterobacteriaceae*: were not detected in all samples from all Brands.

Comparing to a study involved 90 samples of powdered infant formula, 61 samples had acceptable aerobic plate counts APC ($<10^4$ CFU/g). It is important to take into account that the added *Bifidobacterium lactis* to infant formula and cereals did not contribute to the high APC levels because of the anaerobic condition required by them[18].

A study in Tanzania on complementary foods to children aged 10-15 months, showed that bacterial numbers were 4.63±0.56 CFU/g which it did not change between rice and porridge samples[19].

Another study was carried out in Yemen on weaning dried foods, showed TPC of 4.83±0.79 $\log_{10}$ CFU/g, while *salmonella* spp., *Entrobacteriacae* and coliforms were not detected[20].

Another study was carried out in Libya has shown the absence of *salmonella* spp. in baby cereals with TPC ranged from <1.0 to 6.43 $\log_{10}$ CFU/g. However, *Enterobacteriaceae* were presented in most samples[21].

A Korean study has investigated the microbiological safety of various foods intended for consumption by infants where APC ranged from 1 $\log_{10}$ CFU ml^{-1} (or g^{-1}) to 7.85 $\log_{10}$ CFU g^{-1}. *Cronobacter* spp. was detected in 6 samples of infant cereal products, while both *salmonella* spp. and *Escherichia coli* were not detected [22].

Potgieter and his collage investigated the microbiological safety of 94 samples of weaning food in the Venda Region of South Africa, results showed the presence of *salmonella* spp. in 5 samples, the presence of *Escherichia* coli in 83 samples, while coliforms ranged between $4.9x10^2$ and $5.8x10^3$ CFU 100 ml^{-1}[23].

Shigella and *Salmonella* spp. were not detected in the weaning food formulations in Nigeria, total bacterial count ranged from 2.48 to 2.57 $\log_{10}$ CFU/g, while coliform counts ranged from 1.67 to 2.15 $\log_{10}$ CFU/g [24].

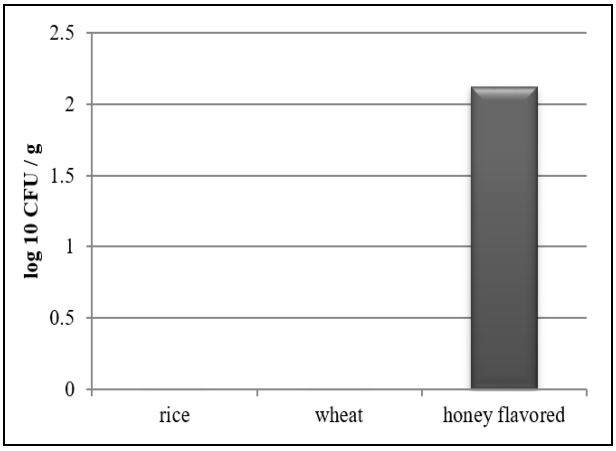

Fig. 1: TPC in Brand A at opening time.

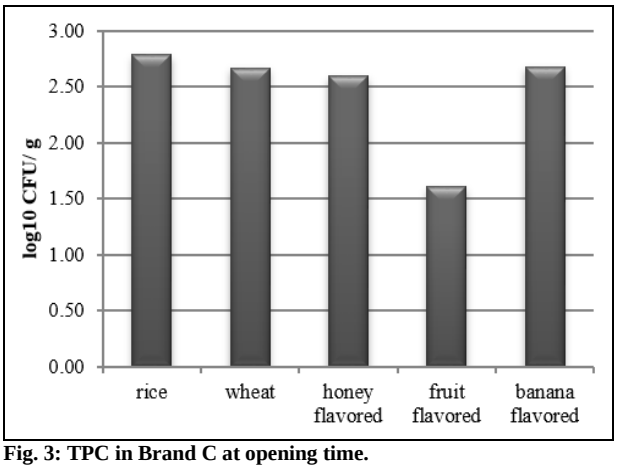

Fig. 3: TPC in Brand C at opening time.

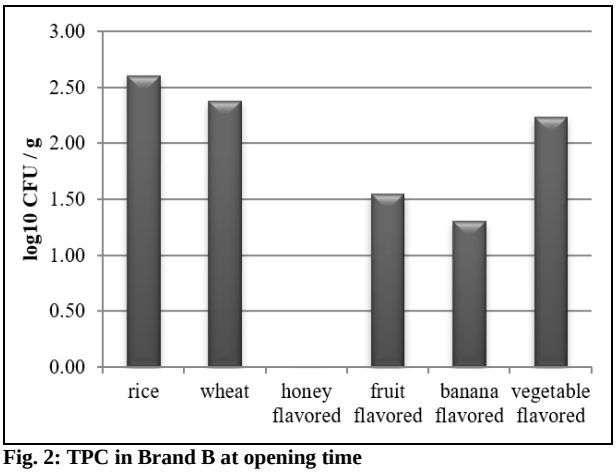

Fig. 2: TPC in Brand B at opening time

3.2. TPC in different storage conditions after opening the package:

Brand A:

Generally, TPC increased in samples stored at room temperature more than those stored in refrigerator. Only one rice batch had bacterial growth in refrigerator higher than at room temperature on the third day of storage. Similar results were obtained after 7 days of storage in the 2 batches of wheat-honey flavor. Increasing the storage time led to increase the bacterial counts regarding 4°C or 25°C. All samples were approved by Syrian Standard during storage. Results are shown in (Table 1).

Table 1: Effect of storage time and temperature on bacterial counts in Brand A.

A Brand	days	0	3	7	10	14	21	30
		Log_{10} CFU/g ± SD						
Rice	A_1	-	1.60±0	2.26±0.02	2.94±0.03	3.08±0.04	3.22±0.02	3.32±0.01
	A_2	-	-	-	2.59±0.03	2.91±0.02	3.23±0.02	3.32±0.02
	A_1 R	-	2.48±0	2.66±0.04	2.78±0.03	2.93±0.04	3.14±0.02	3.20±0.02
	A_2 R	-	-	-	2.18±0.01	2.63±0.03	3.10±0.02	3.19±0.03
Wheat	A1	-	2.20±0	3.11±0.02	3.10±0.03	3.37±0.03	3.40±0.02	3.51±0.02
	A2	-	2.24±0.09	3.27±0.02	3.12±0	3.22±0.04	3.34±0.04	3.53±0.02
	A1 R	-	2.04±0	3.15±0.02	2.59±0.05	2.95±0.01	3.22±0.06	3.45±0.01
	A2 R	-	2.02±0.03	2.94±0.04	2.58±0	3.07±0.03	3.26±0.02	3.43±0.03
Honey f.	A_1	-	2.79±0	3.14±0.02				
	A_2	2.42±0.03	2.83±0.04	3.00±0.02				
	A_1 R	-	2.69±0.03	2.20±0.04				
	A_2 R	2.42±0.04	2.78±0.03	2.33±0.04				

A_1: first batch, A_2: second batch, A_1 R: first batch stored in refrigerator, A_2 R: second batch stored in refrigerator. - = nil, honey f.: honey flavor

Brand B:

Like brand A, TPC increased in samples stored at room temperature more than those stored in refrigerator. Increasing the storage time led to increase the bacterial counts in all samples until the 10th day of storage regarding of 4°C or 25°C.

Anyway, some samples stored in refrigerator showed bacterial growth higher than those stored at room temperature such as the samples of rice products and honey flavored products stored for three and seven days. Even after 30 days of storage all samples were accepted by Syrian Standard. Results are shown in (Table 2).

Brand C:

All samples showed bacterial colonies on N.A. petri plates at opening time.

Generally, TPC increased in the samples stored at room temp. more than those stored in refrigerator. Increasing

the storage time led to increase the bacterial counts in all samples until the 10th day of storage regarding of 4°C or 25°C except wheat, fruit-flavored and banana flavored samples after 7 days of storage in refrigerator. At 10th day, TPC got down in rice and wheat samples except one wheat batch stored at refrigerator. Anyway, some samples stored in refrigerator showed bacterial growth higher than those stored at room temperature, such as one wheat batch product stored from 7 to 30 days. Even after 30 days of storage all samples were approved by Syrian Standard. Results are shown in (Table 3)

Table 2: Effect of storage time and temperature on bacterial counts in Brand B.

B Brand	days	0	3	7	10	14	21	30
		Log_{10} CFU/g ± SD						
Rice	B_1	2.58±0.02	2.74±0.03	3.14±0.04	3.13±0.04	3.20±0.01	3.32±0.02	3.27±0.03
	B_2	2.62±0.03	2.78±0.03	3.09±0.02	3.01±0.03	3.06±0.04	3.37±0.03	3.40±0.03
	B_1 R	2.58±0.02	2.96±0.01	3.09±0.02	3.03±0.03	3.02±0.03	3.23±0.04	3.18±0.01
	B_2 R	2.62±0.03	2.76±0.04	2.92±0.04	3.01±0.01	2.86±0.04	3.07±0.03	3.14±0.01
Wheat	B_1	2.42±0.03	3.12±0.04	2.90±0	2.97±0.04	2.70±0	3.24±0.02	3.26±0.04
	B_2	2.38±0	3.07±0.01	2.94±0.04	2.92±0.01	2.76±0.03	3.18±0.04	3.21±0.01
	B_1 R	2.42±0.03	2.57±0.03	2.65±0	3.26±0.01	2.63±0	3.18±0.01	3.18±0.01
	B_2 R	2.38±0	2.84±0.03	2.70±0.04	3.07±0.04	2.64±0.04	2.8±0.04	3.13±0.01
Honey f.	B_1	-	3.41±0.04	3.45±0.01				
	B_2	-	3.37±0.02	3.40±0.04				
	B_1 R	-	3.34±0	3.24±0.02				
	B_2 R	-	3.29±0.02	3.18±0.04				
Fruit f.	B_1	1.48±0	3.11±0.03	2.44±0.03				
	B_2	1.60±0	3.09±0.03	2.76±0.04				
	B_1 R	1.48±1	3.20±0	2.11±0				
	B_2 R	1.60±1	3.09±0.02	2.06±0.03				
Banana f.	B_1	1.30±0	3.35±0.02	2.00±0				
	B_2	1.30±2	3.2±0.04	2.06±0.03				
	B_1 R	1.30±1	3.23±0.03	1.85±0				
	B_2 R	1.30±3	3.20±0.03	1.90±0				
Vegetable f.	B_1	2.25±0.03	2.54±0.04	1.70±0				
	B_2	2.20±0	2.69±0.03	1.70±0				
	B_1 R	2.25±0.04	2.32±0	2.00±0				
	B_2 R	2.20±1	2.43±0.02	2.04±0				

B1: first batch, B2: second batch, B1 R: first batch stored in refrigerator, B2 R: second batch stored in refrigerator. - = nil, honey f: honey flavored, fruit f: fruit flavored, banana f: banana flavored, vegetable f: vegetable flavored.

Table 3: Effect of storage time and temperature on bacterial counts in brand C.

C Brand	days	0	3	7	10	14	21	30
		Log_{10} CFU/g ± SD						
Rice	C_1	2.57±0.01	2.69±0.04	3.14±0.03	3.09±0.04	3.08±0.03	3.30±0.03	3.53±0.02
	C_2	2.92±0.03	3.01±0.03	3.25±0	2.92±0.01	3.26±0.04	3.32±0.03	3.16±0.04
	C_1 R	2.57±0.01	2.94±0.01	3.04±0.01	2.93±0.04	2.99±0.03	3.20±0.04	3.44±0.01
	C_2 R	2.92±0.03	3.09±0.01	3.16±0.02	2.68±0.03	3.08±0.04	3.26±0.04	3.22±0.04
Wheat	C_1	2.82±0.03	3.41±0.02	2.90±0.04	2.87±0.04	2.72±0	3.34±0.01	3.54±0.01
	C_2	2.42±0.03	3.22±0.01	3.13±0.03	2.53±0.04	2.93±0.05	3.31±0.01	3.10±0.02
	C_1 R	2.82±0.04	3.07±0.02	2.62±0.03	2.75±0.05	2.65±0.03	3.25±0.04	3.45±0.02
	C_2 R	2.42±0.04	3.06±0.03	2.94±0.04	2.70±0	2.91±0.04	3.19±0.04	3.29±0.02
Honey f.	C_1	2.62±0.04	3.43±0.02	3.46±0.02				
	C_2	2.56±0.03	3.39±0.01	3.47±0.01				
	C_1 R	2.62±0.05	3.36±0.01	2.58±0.02				
	C_2 R	2.56±0.03	3.34±0	2.65±0.03				
Fruit f.	C_1	1.60±0	3.35±0.01	2.66±0				
	C_2	1.60±0	3.28±0.03	2.64±0.07				
	C_1 R	1.60±0	3.50±0.03	1.95±0				
	C_2 R	1.60±0	3.21±0.03	1.98±0.03				
Banana f.	C_1	2.68±0.05	3.39±0.01	3.47±0.01				
	C_2	2.68±0	3.28±0.03	3.44±0.01				
	C_1 R	2.68±0.05	3.23±0.04	2.45±0.02				
	C_2 R	2.68±0	3.11±0.03	2.41±0.02				

C1: first batch, C2: second batch, C1 R: first batch stored in refrigerator, C2 R: second batch stored in refrigerator. honey f: honey flavored, fruit f: fruit flavored, banana f: banana flavored.

The results of our study showed the absence of *E.coli*, coliforms and *Salmonella* in all samples, this is in general agreement with a study in Bangladesh on cereal foods prepared from essential materials for young children, whereas Coliform and *Salmonella* were not present in any of test samples[25].

To our knowledge, no previous studies describe the bacterial assessment of dry infant cereals after opening the package and during the storage at different temperatures for a month. However, our previous study investigated the fungal growth during storage in infant cereals after opening[26]. Additionally, some studies investigated the chemical stability certainly lipid and protein oxidation in infant formula during storage the opened package for one month[27]. However, various researches have been reported on microbes in hydrated weaning food and other foods. For example, samples of infant cereal, hydrated with each of apple juice, pasteurized milk and water were stored at temperatures 4°C, 15°C and 25°C for 24h, where results indicated that growth of salmonella was restricted in samples hydrated with apple juice[28].

To understand the bacterial kinetics at different storage temperatures, many studies has been carried out. For example, one study on refrigerated food products (white pudding) were stored for 16 days, at 4°C. results showed that the growth of *Lactococcus lactis* and *Serratia* sp. stopped at day 12, while *Carnobacterium maltaromaticum* reached its maximal growth on 12th day and took apart 90.7% of total counts then it entered its stationary phase[29].

Another study on beef stored at (3-5-7)°C, showed that longer storage at higher temperatures resulted in higher microbial loadings, so an increase in TPC was noticed [30].

Bacterial tests of refrigerated human milk showed that TPC were relatively the same during storage time. TPC were 11.9, 10.7, 12.0, 12.1 and 12.3 CFU ml^{-1} at 0, 12, 24, 36 and 48 hours of storage, respectively[31].

Another study aimed to evaluate the effect of prolonged refrigeration of human milk on microbial profile within 96 h. found that LAB (lactic acid bacteria) and TAB (total aerobic bacterial) counts were around 4.3 and 4.5 $\log_{10}$ CFU mL^{-1}, respectively, without significant change during storage. In some samples, the coagulase-positive Staphylococci contamination decreased around 40% in 96 h, in comparison to 0 h. *Enterobacteriaceae* were detected only in one sample, with count of 2.9 $\log_{10}$ CFU mL^{-1}, and no significant change during storage[32].

4. CONCLUSION:

All samples were approved by Syrian Standard for infant cereals whereas no presence of bacterial pathogens like *Enterobacteriaceae* and *Salmonella* spp., as well as the aerobic plate counts did not reach to 10^4 CFU/g in any sample during a month of storage after opening cans.

This study puts a spotlight on the importance of bacterial assessment of dry infant cereals after opening the package during different storage conditions of temperature and time.

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