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# MIROBIAL PROFILE OF SOME EGYPTIAN TRADITIONAL FOODS AS AFFECTED BY STORAGE

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## ABSTRACT

The safety of ready-to-eat foods is an important topic in today's life. Improper handling of ready-to-eat food items may result in foodborne outbreaks. In this study, koshari, vegetable salad and couscous were selected as the target ready-to-eat foods for a microbiological survey. The aim of this study was to evaluate the microbiological quality of koshari, vegetable salad and couscous sold in Zagazig shops in Sharkia Governorate. A total of 39 samples were collected from food stores in winter and summer seasons. They were tested for the presence of pathogenic microorganisms causing food poisoning (*Salmonella* spp., *Shigella* spp., enterococci and staphylococci), spoilage microorganisms (fungi and yeasts), total aerobic viable counts, lactic acid bacteria and coliform groups. The study also aimed to monitor the population changes of the previous microbial groups during the storage of these foods at refrigerator temperature (5 – 7ºC) and abuse conditions of room temperature (22±2 ºC) . By comparing the three tested foods, generally vegetable salad had the highest microbial populations, followed by koshari, then couscous. The numbers of *Salmonella* and *Shigella* were 6.15, 5.47and 4.89 log cfu/g in summer for vegetable salad, koshari and couscous, respectively. Also, the average microbial counts in summer are clearly higher than those in winter. Generally, the microbial numbers kept increasing for several days, then started to decrease. For most microbial groups, the peak of their numbers were at days 5, 3-5 and 6-8 for koshari, vegetable salad and couscous, respectively. By looking at microbial numbers changes in vegetable salad stored in refrigerator, enterococci increased up to 2.26 log cfu/g after 5 days, staphylococci increased up to 0.12 log cfu/g after 1 day, coliforms increased up to 0.77 log cfu/g after 3 days and *Salmonella* and *Shigella* increased up to 0.45 log cfu/g after 2 days. All of these groups started to decrease thereafter. Based on the current results, it is higher recommended to follow routine inspections and training of vendors to improve the microbiological quality of food products.

**Key words:** Storage, traditional food, foodborne pathogens, koshari, vegetable salad, couscous.

## INTRODUCTION

Traditional foods are considered as high-risk foods because no further treatment such as heating is required before eating. Improper handling of traditional foods may cause contaminations and display at improper temperature favours the rapid growth of pathogens and may result in foodborne outbreaks. Therefore, control of bacterial levels is critical to ensure the safety of such ready-to-eat foods. Ready-to-eat (RTE) food is defined as food that can be consumed at the point of sale without further preparation or treatment. It could be raw, partially or fully cooked and hot, chilled or frozen (FEHD, 2007; USFDA, 2009). Ready-to-eat food can be animal food, plant food, fruits and vegetables, or bakery products (USFDA, 2009). Methods of processing, storage, handling and display can affect the levels of microorganisms in ready-to-eat food (Fang *et al.*, 2003 ; Christison *et al.* 2008).

Foodborne disease outbreaks linked with RTE foods have been associated with various

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foodborne pathogens (Gilbreth *et al.,* 2005; Gibbons *et al.,* 2006). The initial microbiological load on RTE food ingredients is important, however, factors such as handling, processing, storage and display may influence the microbiological load of RTE foods at the point of sale (Beuchat and Ryu, 1997; Angelidis *et al.,* 2006).

Human pathogens like *Listeria monocytogenes*, *Salmonella* and *Escherichia coli* O157-H7 may contaminate the product during plant cultivation and processing (Francis *et al.,* 1999; Brandl, 2006; Franz and Van Bruggen, 2008). Contamination with enteric pathogens may occur through various routes, including use of organic waste as fertilizers, contamination of irrigation water with feacal material, direct contamination by livestock and wild animals, hygienic problems in handling and processing (Heaton and Jones, 2007). The significant negative relationship found between produce visual quality and the levels of total aerobic viable count, coliforms and lactic acid bacteria (LAB) confirms the association between spoilage and LAB levels and the statement that RTES shelf-life is largely conditioned by the microbiological and sensory quality levels at the processing factory gate (Sinigaglia *et al.,* 1999). Enterobacteriaceae are useful indicators of hygiene and post processing contamination of heat – processed food. The bacterial counts and coliforms are indicators of sanitation and could signify unhygienic conditions during food handling and preparation. *Escherichia coli* is a significant diarrhoeal causing organisms usually found in localities of poor sanitary conditions Umoh and Odobab (1999). It has been associated with "travelers' diarrhoeal and hemor-rhagic colitis. Therefore, consumption of this food could be associated with diarrhoeal diseases (Hanoshiro *et al.,* 2004).

The purpose of this study is to determine the extent to which koshari, vegetable salad and couscous were contaminated with aerobic bacteria, hygiene indicator bacteria, potential foodborne pathogen, yeast and fungi at the point of sale in Sharkia Governorate. In addition, the effect of different storage temperatures on the presence of aforementioned microorganisms was investigated.

# MATERIALS AND METHODS

## Food Sampling Procedures

In this study, traditional food products were obtained from shops, Sharkia Governorate, Egypt. A total of 39 samples of three traditional food products, *i.e.* koshari; vegetable salad and couscous were randomly colected from June to September in 2012 and December to March in 2013. These samples were transported in an ice box to the laboratory of Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University, Egypt for microbiological analyses.

## Microbiological Analyses

Ten grams of each of koshari, vegetable salad and couscous were separately transferred to a 250 ml Erlynmayar flask containing 90 ml of sterile peptone saline solution (0.1% peptone and 0.85% NaCl) and well mixed, then serial dilutions up to $10^7$ were prepared. One tenth ml of each dilution was spred on the surface of plate count agar medium (P.C. agar, Oxide) then incubated at 30 ±2ºC for 48 hr., for enumeration the total bacterial count (Hausler, 1972). For lactic acid bacteria (LAB) enumeration, MRS agar (De-Man, Rogosa and sharp) were used for counting lactobacilli after incubation at 30ºC for 24– 48 hr., (De-Man *et al.,* 1960). *Enterococcus* was enumerated on Kanamycin Aesculin Azide – agar medium (Difco, 1989), then incubated at 37ºC for 48 hr. Black colonies on Kanamycin Aesculin Azide-agar are typical colonies of enterococci. *Staphylococcus* spp. was enumerated on Baird-parker's medium (Oxide CM 275) (Baird-Parker and Davenport, 1965). The total coliforms were counted according to Harrigen and Mccance-Margart (1976) using MacConkey – agar medium. Plates were incubated at 37ºC for 24 hr. Pink colonies on MacConkey agar are typical colonies of coliform. *Salmonella* and *Shigella* spp. were counted using S.S. agar (Oxide CM 99). All plates were incubated at 37ºC for 24 hr., (Harrigen and Mccance-Margart, 1976). Black and pink colonies on S.S. agar are typical colonies of *Salmonella* spp. and *Shigella* spp., respectively. For yeast enumeration, yeast extract-malt extract agar (YM-agar, Kreger-van Rij, 1984) was used. Colonies were counted after incubation at 28 ± 2ºC for 48 hr. Moulds were enumerated by using Potato – dextrose –

agar (PDA) medium at 28ºC for 72 hr. The samples were tested for the presence of hazard microorganisms causing poisoning and spoilage.

### Storage Effect on Microbial Load

The samples under investigation were kept at refrigerator temperature (5 – 7ºC) and sampled for microbial groups enumeration daily for up to 9 days. Other samples were kept for 36 hours at room temperature (22 ±2ºC) and sampled for enumeration at 0, 12, 24 and 36 hours. Ten grams of each sample were homogenized with 90 ml of sterile peptone saline solution and serial decimal dilutions were prepared. Appropriate dilutions were inoculated onto petri dishes of different nutrients and selective media.

### Statistical Analysis

One-way analysis of variance was used to analysis the data using the General Linear Model procedure by SAS 1998 (Statistical Analysis System). Duncan's multiple range test system (1955) was used to compare the least significant difference (LSD) of treatments at P value of $\leq 0.05$.

## RESULTS AND DISCUSSION

### Microbial Counts of Traditional Food Products in Sharkia Governorate (Zagazig City)

Eight different microbial groups were enumerated in the three traditional food products (koshari, vegetable salad and couscous). Data in Table 1 show the different microbial counts in traditional foods obtained from different restaurants in Zagazig City, Sharkia Governorate in winter and summer. Each number in this table reflects the average of 6 samples enumerations bought from different stores during each season.

The average microbial counts in winter of aerobic bacteria, lactic acid bacteria, enterococci, staphylococci, coliform and *Salmonella* and *Shigella* in koshari samples were 7.04, 3.23, 4.03, 2.65, 4.56 and 4.32 log cfu/g, respectively. Yeasts and fungi did not show any growth in winter. In summer, the average counts for the same eight different microbial groups in koshari samples were 7.68, 6.41, 5.50, 5.07, 6.22, 5.47, 0.43 and 0.78 log cfu/g, respectively. Umoh and Odobab (1999) counted the aerobic bacteria in some Nigerian foods and found that the count in the boiled white rice was 4.14 log cfu/g, while the average count for two other food types (Soup and Moin-moin) was 3.62 log cfu/g. They also showed in their study that while rice accounted for 26.2% of the positive samples for *B. cereus*, six other foods (Fried fish, Tuwo, Soup, Kosai, Kunu and Moin-moin) accounted for 73.8% (an average of 12.3% for each one). The percentage of rice positive samples for *S. aureus* was 16.7%, while the other six foods accounted for 83.8% (an average of 12.3% for each one). This result indicated that koshari is expected to have higher microbial load than other food types, especially since the other major component of Koshari is pasta, which is mainly composed of starch an easily degradable food source for microorganisms. Mensah *et al.* (2002) found that the mean bacterial count in macaroni samples analyzed was 5.48 log cfu/g. This food is often prepared by heating, but gets cold by the time it is served because the sellers are not able to keep the food at a good holding temperature and therefore ambient temperatures provide a suitable condition for the growth of microorganisms. The contamination of this food was not surprising because after cooking the food serving was performed with bare hands.

In Vegetable salad samples, the average microbial counts in winter for aerobic bacteria, lactic acid bacteria, enterococci, staphylococci, coliform and *Salmonella* and *Shigella*, yeasts and fungi were 6.58, 5.89, 4.58, 4.25, 4.40, 4.37, 1.72 and 5.33 log cfu/g, respectively. In summer, the average counts for the same eight different microbial groups in Vegetable salad samples were 8.13, 7.92, 6.77, 6.32, 6.20, 6.15, 2.01 and 5.54 log cfu/g, respectively. Microbiological surveys of retail ready to eat salad (RTES) have considered the occurrence of enteric bacterial pathogens, *Escherichia coli*, coliforms, total aerobic and spoilage bacteria, fungi and yeasts. Most reported counts range between 4 and 8 log cfu/g for total aerobic bacteria and around 6 log cfu/g for coliforms (Wojcik-Stopczynska, 2004; Tournas, 2005; Fröder *et al.*, 2007; Pavan da Silva *et al.*, 2007 ; Pianetti *et al.*, 2008). These numbers are in accordance with the obtained results in the

current study, expect for coliform count in winter (4.40 log cfu/g), which is still in

**Table 1. Average of different microbial enumerations[a] (log cfu/g) for different traditional foods in winter and summer**

| Traditional food | Study season | Aerobic bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|---|
| Koshari | Winter | 7.04 | 3.23 | 4.03 | 2.65 | 4.56 | 4.32 | 0.00 | 0.00 |
| | Summer | 7.68 | 6.41 | 5.50 | 5.07 | 6.22 | 5.47 | 0.43 | 0.78 |
| Vegetable salad | Winter | 6.58 | 5.89 | 4.58 | 4.25 | 4.40 | 4.37 | 1.72 | 5.33 |
| | Summer | 8.13 | 7.92 | 6.77 | 6.32 | 6.20 | 6.15 | 2.01 | 5.54 |
| Couscous | Winter | 5.27 | 2.42 | 1.97 | 3.37 | 1.60 | 1.29 | 0.00 | 0.68 |
| | Summer | 5.61 | 5.34 | 4.48 | 4.10 | 5.28 | 4.89 | 0.93 | 0.91 |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

agreement with Gitahi *et al.* (2012) who found that total coliform count in vegetable salad was 4.48 log cfu/g. Gitahi *et al.* (2012) found that *Staphylococcus aureus* counts were 4.03 log cfu/g in vegetables. Higher occurrence of *Staphylococcus aureus* suggests a contamination which originates from food handling that might have occurred in the street foods during handling, processing or vending. *Staphylococcus aureus*, being a part of the microflora present on/in several parts of the human body, is a good indicator of contamination due to poor personnel hygiene practices (Nester *et al*. 2001).

The average microbial counts in couscous samples for aerobic bacteria, lactic acid bacteria, enterococci, staphylococci, coliform and *Salmonella* and *Shigella* and fungi in winter were 5.27, 2.42, 1.97, 3.37, 1.60, 1.29and 0.68 log cfu/g, respectively. Yeasts showed no growth in winter. In summer, the average counts for the same eight different microbial groups in couscous samples were 5.61, 5.34, 4.48, 4.10, 5.28, 4.89, 0.93 and 0.91 log cfu/g, respectively.

By comparing the three tested foods, generally vegetable salad had the highest microbial populations, followed by koshari then couscous. An obvious reasons for that, is the absence of heat treatment in vegetable salad preparation, which allows for microbial growth, a situation that contradicts with the preparation method of koshari and couscous. Also, the average microbial counts in summer are clearly higher than those in winter for the same food; this is most likely be due to the lower temperature in winter than in summer season.

## Effect of Storage at Refrigerator and Room Temperature on Microbial Load of the Tested Foods

### Effect of refrigerator storage on koshari

Data in Table 2 show the effect of refrigerator storage (5–7ºC) on microbial counts of koshari sample. The population of the total bacterial count showed the lowest rate of increase by only

increasing 24.8% after four days, while yeasts showed the highest increase, reaching 522% after four days. Other microbial groups, *i.e.* lactic

**Table 2. Changes in Koshari microbial popualtion[a] during refrigerator storage for 5 days**

| Microbial count / Storage time (day) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 7.41 c | 6.62 d | 4.96 d | 4.02 d | 6.34 e | 6.27 e | 0.00 d | 0.00 c |
| **1** | 7.60 c | 7.16 c | 6.99 c | 5.87 c | 7.24 d | 7.14 d | 0.00 d | 0.00 c |
| **2** | 8.62 b | 8.58 b | 8.03 b | 6.16 bc | 8.12 c | 8.07 c | 3.70 c | 0.00 c |
| **3** | 9.07 a | 8.97 a | 8.24 b | 6.46 b | 8.52 b | 8.61 b | 5.03 b | 0.00 c |
| **4** | 9.25 a | 9.11 a | 8.82 a | 7.05 a | 8.77 b | 8.88 b | 5.22 ab | 3.30 b |
| **5** | 8.91 ab | 9.11 a | 8.84 a | 7.08 a | 9.64 a | 9.46 a | 5.37 a | 3.70 a |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

acid bacteria, enterococci, staphylococci, coliforms, *Salmonella* and *Shigella* and fungi showed the rate of increase by 37.6, 78.2, 76.1, 52.1, 56.9 and 370 percent, respectively, after five days. Yeasts and fungi showed the lowest numbers (no growth) at zero time, while the total count of bacteria showed the highest numbers (7.41) at zero time. These results indicated that all microbial counts, significantly increased ($p < 0.05$) when stored at refrigerator temperature for 5 days.

Due to the lake of available microbiological studies about Koshari, rice was used as reference in some discussions. A study on the cooked rice stored at refrigerator temperature (5-7ºC) for 24 hours showed no viable bacterial load, while after another 24 hours of storage, the refrigerated rice showed $6.9x10^5$cfu/g (Ali *et al.*, 2008). According to the FAO/WHO expert consultation of microbiological specifications, a maximum of $5x10^4$ cfu/g of mesophilic aerobic bacteria are safe for human consumption. Also, Frazier and Westhoff (1995) mentioned that the values at or above $10^6$ are unacceptable.

### Effect of refrigerator storage on vegetable salad

Data in Table 3 represented the effect of refrigerator storage on microbial counts of Vegetable salad samples. Concerning, aerobic count of bacteria, data show that a significant increase was found after 24 hr., until the 3rd day, then started to decrease thereafter. Aerobic bacteria and coliform counts showed rates of population increase by 56.9 and 23.8%, respectively during the first 3 days of refrigerator storage, then showed a reduction by 29.7 and 42.5%, respectively. Enterococci and yeasts counts showed rates of population increase by 40.2 and 107.03%, respectively during the first 5 days of refrigerator storage, then showed a rate of decrease by 11.6 and 2.87%, respectively afterwards. These results indicate that all microbial counts, significantly increased ($p < 0.05$) when stored at refrigerator temperature for 7 days. Lactic acid bacteria and fungi counts showed rates of increase by 42.5 and 37.7%, respectively during the first 4 days of refrigerator storage, then non-significantly decreased afterwards. Similar results were found by Francis and O'Beirne (2001) who found that the populations of *E. coli* O157:H7 on coleslaw mix (80% shredded cabbage, 20% shredded carrot) stored at 8ºC had increased by approximately 1.5 log cycles by day 5, then declined by 1 to 2 log cycles, depending on the strain. Populations of *E. coli* O157:H7 decreased at 4ºC by 1 to 1.5 log cycles, but viable cells were still detected at the end of the storage period. Abdul-Raouf *et al*. (1993) reported that

populations of *E. coli* O157:H7 numbers on shredded carrots (12ºC) increased during initial days of storage and subsequently declined on extended storage. Arias *et al.* (2001) stated that

**Table 3. Changes in vegetable salad microbial popualtion[a] during refrigerator storage for 7 days**

| Microbial count / Storage time (day) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 5.06 e | 5.81 d | 5.62 c | 3.69 a | 3.23 cd | 3.40 b | 3.41 e | 3.34 c |
| **1** | 6.38 c | 6.33 c | 6.95 b | 3.81 a | 3.56 bc | 3.64 ab | 3.76 d | 3.63 c |
| **2** | 7.12 b | 7.09 b | 7.00 b | 3.30 b | 3.91 ab | 3.85 a | 5.59 c | 4.20 b |
| **3** | 7.94 a | 8.07 a | 7.66 a | 3.08 b | 4.00 a | 2.90 c | 6.41 b | 4.48 ab |
| **4** | 7.78 a | 8.28 a | 7.76 a | 3.00 b | 3.82 ab | 2.80 c | 6.90 a | 4.60 a |
| **5** | 7.31 b | 8.15 a | 7.88 a | 0.00 c | 3.11 d | 2.70 c | 7.06 a | 4.23 b |
| **6** | 7.25 b | 8.13 a | 7.56 a | 0.00 c | 2.95 d | 2.60 c | 6.95 a | 4.20 b |
| **7** | 5.58 d | 8.10 a | 7.00 b | 0.00 c | 2.30 e | 2.30 d | 6.86 a | 4.18 b |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

populations of *E. coli* O157: H7, significantly increased on chopped cabbage after 48 hr., of storage at 6ºC, followed by significant ($p<0.05$) decrease after 72 hr., cabbage initially containing a large inoculum ($10^8$ cfu/g). On the other hand, populations on cabbage stored at 12ºC, significantly increased within 72 hr. On cabbage, the count of *E. coli* O157:H7 ranged from $2.7\times10^3$- $4.0\times10^2$cfu/g and the pH 7.09 - 5.72 at 5ºC, while at 28ºC the count was $2.5\times10^3$- $1.0\times10^2$cfu/g and the pH 7.09 - 4.15. The count of *E. coli* O157:H7 on lettuce at 5ºC was in the range of $3.6\times10^3$- $5.0\times10^2$cfu/g and the pH decreased from 7.11 to 5.66. At 28ºC, the count was $3.9\times10^3$-$1.0\times10^2$cfu/g and the pH decreased from 7.11 to 4.06.

The decreased numbers of *E. coli* O157:H7 in stored vegetable salad may be due to the effect of decreasing pH during storage and competition with other microorganisms (Uzeh and Adepoju, 2013).

*Salmonella* and *Shigella* counts showed a rate of increase by 13.2% after 2 days of refrigerator storage, then showed a rate of decrease by 40.25% by the end of storage period. Staphylococci counts showed a rate of increase by 3.25% after the first day of storage, then significantly decreased afterwards. These results indicate that most microbial groups consistently increased until days 3-5, then slightly decreased until day 7.

**Effect of refrigerator storage on couscous**

The effect of refrigerator storage on microbial load of couscous sample is shown in Table 4. The results showed that total bacterial count increased by 58.06 % after 6 days of refrigerator storage, then decreased afterwards. Enterococci, Staphylococci, and *Salmonella* and *Shigella* counts showed a rate of increase by 84.1, 71.65 and 66.49% respectively, after 6 days of refrigerator storage, then decreased afterwards. The counts of lactic acid bacteria and yeasts significantly increased ($p<0.05$) during the first three days, then stayed with mostly non-significant changes throughout the remaning days. Fungi counts showed a rate of increase by 5.42 log after 8 days of refrigerator storage, then decreased in day 9. Coliform counts showed a rate of increase by 41.35% after 5 days of refrigerator storage, then showed a rate of reduction by 19.12 % afterwards

comparing with the count at the 5$^{th}$ day. These results indicate that the most microbial groups consistently increased until days 6-8, then slightly decreased until day 9.

**Table 4. Changes in couscous microbial popualtion[a] during refrigerator storage for 9 days**

| Microbial count / Storage time (day) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 6.20 g | 4.83 f | 4.03 g | 5.15 g | 5.03 f | 5.73 e | 3.64 f | 0.00 e |
| **1** | 7.11 f | 5.71 e | 4.67 f | 6.06 f | 5.48 e | 6.49 d | 4.59 e | 0.00 e |
| **2** | 8.12 e | 7.12 d | 5.59 e | 6.74 e | 6.30 d | 7.60 c | 5.10 d | 0.00 e |
| **3** | 9.12 cd | 7.81 c | 7.05 b | 7.42 d | 6.99 ab | 8.53 b | 6.01 c | 0.00 e |
| **4** | 9.24 bcd | 8.00 bc | 7.20 ab | 7.64 cd | 7.34 a | 8.72 b | 6.03 c | 2.78 d |
| **5** | 9.29 abc | 8.18 b | 7.23 ab | 7.93 c | 7.11 a | 9.53 a | 6.19 c | 4.03 c |
| **6** | 9.62 a | 8.71 a | 7.42 a | 8.84 a | 6.67 bc | 9.54 a | 6.20 c | 4.28 c |
| **7** | 9.57 ab | 8.74 a | 6.91 bc | 8.77 a | 6.53 cd | 8.67 b | 6.60 b | 4.88 b |
| **8** | 9.29 abc | 8.81 a | 6.62 cd | 8.31 b | 5.81 e | 8.66 b | 6.75 b | 5.42 a |
| **9** | 8.89 d | 8.84 a | 6.43 d | 7.86 c | 5.75 e | 8.65 b | 7.29 a | 4.67 b |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

**Effect of storage at ambient temperature on koshari**

Data in Table 5 show the effect of room temperature storage on microbial load of Koshari samples. Total bacterial count, lactic acid bacteria, enterococci, coliforms, *Salmonella* and *Shigella* and fungi counts showed the rate of increase reaching 39.49, 270.9, 130.2, 145.7, 620 and 43.47%, respectively, after 24 hr. Staphylococci counts showed a rate of increase by 50.06% after 12 hr., of room temperature storage, then decreased after 24 hours. No count of yeasts was detected during the 24 hours of storage at room temperature. The counts of fungi showed the lowest rate of increase by 43.47%, while lactic acid bacteria showed the highest rate of increase by 270.86%. Other than yeasts, *Salmonella* and *Shigella* showed the lowest counts at zero time, while the total count of bacteria showed the highest numbers at zero time as expected. These results indicate that all microbial counts significantly increased ($p < 0.05$) when stored at room temperature for 24 hours.

The numbers of total counts in this study is much higher than those reported in the study of Ali *et al.* (2008) who found that the bacterial load counted in the cooked rice kept at room temperature (30±2ºC) for 24 hours was about $4.2 \times 10^4$ cfu/g. This may be due to the different in preparation conditions of the food since the rice prepared in their study seems to be prepared at home or in the lab, in much better conditions compared to the public restaurants. Also, the zero time in the current study is not the zero time for koshari preparation, but is most likely several hours after preparation. Furthermore, the chemical composition of koshari contains different variety of nutrients including proteins (from lentil and pasta) and more fats and moisture, unlike rice. Such versatile composition of koshari would make it better medium for microbial growth and proliferation.

**Effect of storage at ambient temperature on vegetable salad**

Data in Table 6 show the effect of room temperature storage (22±2ºC) on microbial load of vegetable salad sample. The microbial counts consistently increased and significantly ($p < 0.05$) during the storage of vegetable salad at room temperature for 36 hours. Total bacterial

**Table 5. Changes in koshari microbial popualtion[a] during storage at ambient temperature for 24 hours**

| Microbial count / Storage time (hour) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 6.33 b | 2.30 c | 3.30 c | 3.75 c | 3.15 b | 0.00 c | 0.00 a | 2.30 b |
| **12** | 9.14 a | 8.01 b | 6.74 b | 7.51 a | 7.68 a | 4.82 b | 0.00 a | 3.30 a |
| **24** | 8.83 a | 8.53 a | 7.60 a | 6.64 b | 7.74 a | 6.20 a | 0.00 a | 3.30 a |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

**Table 6. Changes in vegetable salad microbial popualtion[a] during storage at ambient temperature for 36 hours**

| Microbial count / Storage time (hour) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 6.15 c | 5.15 d | 2.00 d | 3.87 c | 4.74 d | 4.65 c | 4.64 d | 0.00 a |
| **12** | 8.34 b | 7.99 c | 5.99 c | 6.48 b | 6.9 c | 6.09 b | 5.14 c | 0.00 a |
| **24** | 8.67 b | 8.96 b | 6.94 b | 6.79 b | 7.47 b | 7.33 a | 6.62 b | 0.00 a |
| **36** | 9.70 a | 9.60 a | 8.18 a | 7.82 a | 7.85 a | 7.68 a | 9.22 a | 0.00 a |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

count, lactic acid bacteria, enterococci, staphylococci, coliforms, *Salmonella* and *Shigella* and yeasts counts showed an increasing percentages of 57.7, 86.4, 309, 102.06, 65.6, 65.2and 98.7%, respectively. Similar results were obtained by Francis and O'Beirne (2001) who found that the populations of viable *E. coli* O157 : H7, significantly increased on lettuce stored at 21ºC. This may be due to the effect of decrease in pH of the vegetables during storage and competition with other microorganisms present, including lactic bacteria. On the other hand, the current results are contradicts with the findings of Faith *et al.* (1997) who found reductions in bacterial number in pepperoni by 2-4 log cfu/g when stored at 21ºC for 14 days.

No counts of fungi could be detected during the 36 hours of room temperature (22±2 ºC) storage. Moulds are less important in minimally processed vegetables due to the intrinsic properties such as a slightly acid to neutral pH favouring bacteria and yeasts which will overgrow moulds (Magnuson *et al.*, 1990; King *et al.*, 1991; Lund, 1992; Moss, 1999; Gimenez *et al.*, 2003; Tournas, 2005). The counts of *Salmonella* and *Shigella* showed the lowest rate of increase by 65.2%, while enterococci showed the highest rate of increase by 309%. Enterococci showed the lowest numbers at zero time, while the total count of bacteria showed the highest numbers at zero time.

**Effect of storage at ambient temperature on couscous**

Data in Table 7 show the effect of room temperature storage on microbial counts of couscous sample. Total bacterial count, lactic acid bacteria, Enterococci, Staphylococci, coliforms,

**Table 7. Changes in couscous microbial popualtion[a] during storage at ambient temperature for 24 hours**

| Microbial count / Storage time (hour) | Aerobic count of bacteria | Lactic acid bacteria | Enterococci | Staphylococci | Coliform | *Salmonella* and *Shigella* | Yeasts | Fungi (fp)[b] |
|---|---|---|---|---|---|---|---|---|
| **Zero** | 4.03 c | 2.15 c | 3.98 b | 4.84 c | 0.00 c | 0.00 c | 0.00 b | 0.00 a |
| **12** | 7.26 b | 5.90 b | 7.03 a | 7.46 b | 6.14 b | 4.70 b | 4.94 a | 0.00 a |
| **24** | 8.12 a | 6.60 a | 7.18 a | 8.48 a | 7.34 a | 6.62 a | 5.20 a | 0.00 a |

a- Numbers (log cfu/g) are mean values of three replicates. b- fp: Fungal propagules.

*Salmonella* and *Shigella* and yeasts counts showed a rate of increase by 101.48, 206.97, 80.4, 75.4, 734, 662and 520 l%, respectively. No counts of fungi were detected after 24 hours of room temperature storage. The counts of enterococci showed the lowest rate of increase by 320%, while coliforms showed the highest rate of increase by 734%. The results indicated that all microbial counts significantly increased ($p < 0.05$) when stored at room temperature for 24 hours.

The consistent microbial increase for all groups under different conditions had led to natural changes in the koshari shown as deterioration in appearance and unpleasant smell.

In view of the obtained results, it could be concluded that it is higher recommended to follow routine inspections and training of vendors to improve the microbiological quality of food products.

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# التوصيف الميكروبيولوجي لبعض الأغذية المصرية التقليدية وتأثرها بالتخزين

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سلامة الأغذية الجاهزة للأكل هو موضوع هام في الحياة اليومية، سوء التعامل مع الأغذية الجاهزة للأكل قد يؤدي إلي تفشي الأمراض المنقولة بالغذاء، في هذه الدراسة تم اختيار الكشري وسلطة الخضروات والكسكسي كهدف للحصر الميكروبيولوجي لدراستها كأغذية جاهزة للأكل، الهدف من الدراسة هو تقييم الجودة الميكروبيولوجية للكشري وسلطة الخضروات والكسكسي في محلات الزقازيق في محافظة الشرقية، تم جمع ٣٩ عينة من محلات الأغذية في فصلي الشتاء والصيف، وتم اختبارها لوجود الكائنات الحية الدقيقة المرضية التى تسبب التسمم الغذائي (السالمونيلا، الشيجلا، الانتيروكوكاي والاستافيلوكوكاي) والكائنات الحية الدقيقة المفسدة للأغذية (الخمائر والفطريات) والعد الكلي للبكتيريا الهوائية وبكتيريا حمض اللاكتيك ومجموعة الكوليفورم، هذه الدراسة هدفها أيضاً رصد تغيرات الأعداد في المجموعات الميكروبية السابقة أثناء تخزين هذه الأغذية على درجة حرارة الثلاجة والغرفة، بمقارنة الثلاثة أغذية المختبرة، وجد عموما أن سلطة الخضروات بها أعلى أعداد للميكروبات يليها الكشري ثم الكسكسى، أعداد السالمونيلا والشيجلا كانت ٦.١٥، ٥.٤٧، و٤.٨٩ لوغاريتم مستعمرة لكل جرام في الصيف لسلطة الخضروات والكشري والكسكسي على التوالي، متوسط أعداد الميكروبات أيضاً في الصيف هو أعلى بوضوح عنه في الشتاء، عموما، أعداد الميكروبات تستمر فى الزيادة لعدة أيام ثم تبدأ في الانخفاض، في معظم المجاميع الميكروبية كانت ذروة أعدادها في أيام ٥، ٣-٥ و٦-٨ للكشري وسلطة الخضروات والكسكسي، على التوالى، بالنظر إلي تغيرات أعداد الميكروبات في السلطة المخزنة في الثلاجة، الزيادة فى الانتيروكوكاي وصلت إلى ٢.٢٦ لوغاريتم مستعمرة لكل جرام بعد ٥ أيام، الزيادة فى الاستافيلوكوكاي وصلت إلى ٠.١٢ لوغاريتم مستعمرة لكل جرام بعد يوم واحد، زيادة الكوليفورم وصلت إلى ٠.٧٧ لوغاريتم مستعمرة لكل جرام وزيادة السالمونيلا والشيجلا وصلت إلي ٠.٤٥ لوغاريتم مستعمرة لكل جرام، كل هذه المجاميع بدأت في الانخفاض بعد ذلك، في ضوء النتائج المتحصل عليها في هذه الدراسة يمكن أن نوصي بشدة بأهمية متابعة عمليات التفتيش الروتينية والتدريب للباعة والمتعاملين مع الغذاء لتحسين مستويات الجودة في الأغذية الشعبية.

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