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Microbial Contamination of Various Nuts Stored in Commercial Markets in Jeddah

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ABSTRACT. Microbiological and chemical studies have been carried on some widely consumed nuts stored in Jeddah markets. Results indicated that mould count was generally higher than bacterial count specially on shelled nuts. Walnuts showed the highest average of microbial contamination including mould, bacteria, proteolytic and lipolytic microorganisms comparing with other kinds of nuts. While the lowest counts were found to be on pistachio-nuts and cashewnuts even shelled or unshelled. Mould isolates occurring most frequently were *Aspergillus, Penicellium, Rhizopus, Mucor* and *Cladosporium*. Regarding bacterial flora, G + ve sporeforming bacilli, G + ve cocci and G – ve short rods were the most dominant isolates. The microbial contamination which plays an important role in the spoilage of various nuts was generally affected by the composition of nuts specially proteins, lipids and water content.

Introduction

Various nuts are used as a raw material in many industries as well as for a direct consumption. The relatively high cost of animal protein as well, compared with plant protein suggest an increasing market for the latter and various nuts have attracted interest as a potential source of supplementary protein for human food. They contain an important amount of protein and fat and their products have wide acceptance as food throughout the world[1].

Due to the extremely high fat, protein and low water content of various nuts such as hazelnut, almonds, walnuts, pistachio and cashewnut, these products are quite refractory to spoilage by bacteria. Moulds can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation[2,3 and 4].

Therefore, it was found of interest, in this study to investigate the microbial contamination of various nuts stored in commercial markets in Jeddah influencing their nutritional values.

Material and Methods

Sampling

Samples of various nuts (shelled and unshelled) were collected from different stores in Jeddah market in 5 replicates. The nuts include almonds *(Pruns amygdalus)*, cashewnuts *(Anacardium accidentale)*, hazelnuts *(Corylus avellana)*, pistachio-nuts *(Pistacia vera)* and walnut *(Juglans nigra)*. Sampling plan based on a random sample taken throughout the lot, and based also on the hypothesis that the defect is distributed randomly throughout the lot. The samples used incorporate portions taken from various units of the lot. Any sample unit submitted for laboratory testing, contains at least 100 g of product[5]. All the nuts were collected shelled and unshelled except cashewnut which was stored as shelled nut only.

Microbiological Analyses

A twenty-five g analytical unit taken from the 100 g sample unit was used in aerobic bacterial count, mould count, proteolytic and lipolytic microorganisms[5], Plating for total bacterial count was carried on standard nutrient agar and for moulds on Sabourad dextrose agar[6]. Isolated colonies were picked up on slants after incubation period of 3 days at 28°C for bacterial count and 7 days at the same temperature for mould count.

Determination of proteolytic microorganisms was carried on gelatin agar medium plates, which were flooded with solution containing 15 g Hg Cl_2, 20 ml HCl and 100 ml distilled water, after incubation period of 3 days at 28°C[7]. Colonies showing proteolytic activity were distinguished by clear zone around the colonies due to protein decomposition.

The count of lipolytic microorganisms was carried on nutrient agar medium after adding 10% sterilized corn oil. Incubation period was 3 days at 28°C. The plates were flooded with copper sulphate solution 20% to detect lipolytic microorganisms[8]. Colonies showing lipolytic activity were distinguished by blue-green zones around the colonies due to lipids decomposition.

The fungal isolates were identified to the genera according to Thom and Raper[9], Raper and Thom[10], Raper and Fennell[11] and Subramanian[12] while bacterial isolates were grouped according to morphological shape, gram-staining and spore formation[13].

Chemical Analyses

Nut meal was prepared by removing the shells and teguments for each sample, and grinding the seed in coffee grinder. The obtained nut meal was dried at 60°C under

vacuum. The oil was then extracted with petroleum ether (40-60°C) using a soxhlet-type extracter. Fat content was determined as (%) in the nut meal[14].

Crude protein was determined in nut meal using modified semimicrokjeldahl method of Cadahia[15]. The resulting ammonium was estimated using method of Chibnall *et al.*[16] modified by Naguib[17] Nitrogen was converted to crude protein using the factor 5.18 as recommended of this product by FAO[18].

In all cases the data shown represent triplicate analyses using three samples of meal, and are expressed in g of protein 100 g^{-1} of dry nut meal.

Moisture content was determined as a fresh weight basis for each sample from the weight of 50 kernel sample before and after oven drying for 48 hr at 86°C[3].

Results and Discussion

Densities of microorganisms including viable moulds and bacteria were determined for several samples of hazelnuts, almonds, walnut, pistachio and cashewnut either shelled or unshelled.

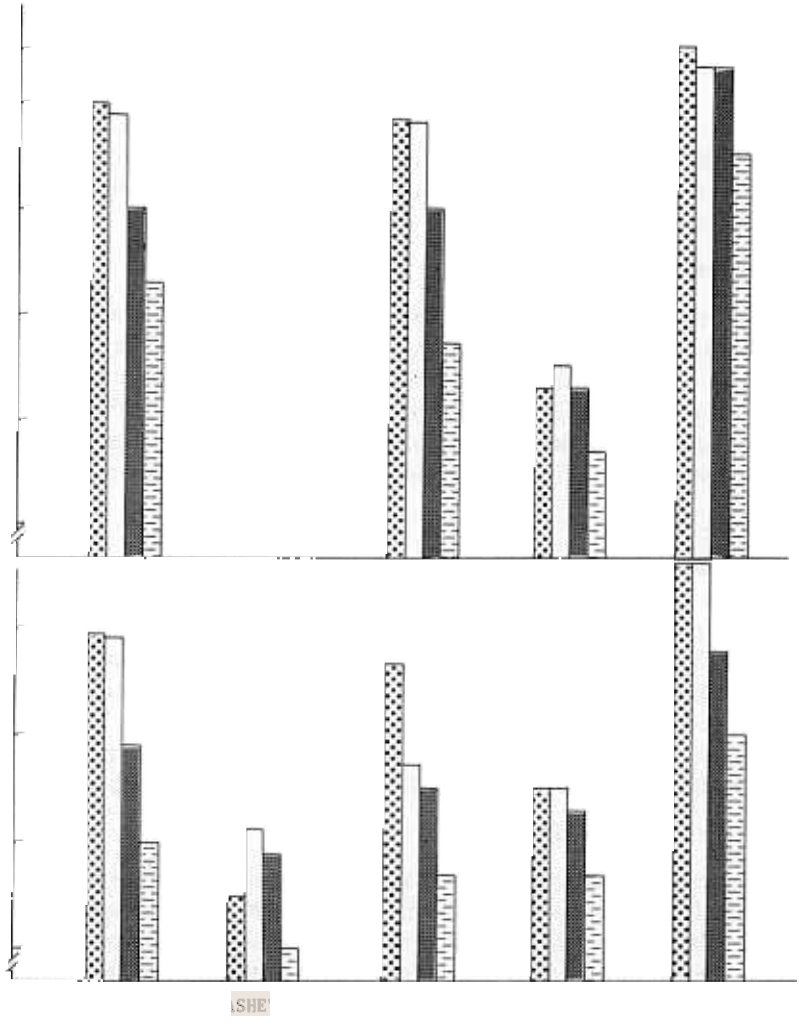
a) Total Microbial Load

Data illustrated by Fig. 1 show the densities of moulds and bacteria in both shelled and unshelled nuts. It is clear from data that the removal of the shell specially walnut resulted in an obvious decrease of microbial count being nearly the tenth for viable moulds or even bacteria. Densities of viable moulds were 300, 105, 95 and 0.2×10^3 organisms/g on the unshelled walnuts, almonds, hazelnut and pistachio respectively, while the corresponding figures for shelled nuts were 30, 9 4.8 and 0.3×10^3 organisms/g.

The bacterial counts showed the same general trend as observed in Fig. 1. Their densities were 200, 95, 80 and 0.3×10^3 organisms/g of unshelled walnut, almonds, hazelnut and pistachio respectively. Removal of the shell decreased the bacterial load reaching 30, 8, 5, 0.3×10^3 organisms/g nut in the respective value. Bacterial densities, generally, showed slightly lower counts than mould densities contaminating the investigated nuts. Walnuts (shelled and unshelled) showed the highest microbial load comparing with the other nuts. On the contrary, the lowest microbial load, including moulds and bacteria, was found in pistachio and cashewnuts.

b) Proteolytic Microorganisms

The densities of proteolytic microorganisms showed the same general trend as the total microbial load (Fungi and bacteria) (Fig. 1). Their densities reached 200, 10, 4.5 and 0.2×10^3 organisms/g unshelled walnuts, almond, hazelnuts and pistachio respectively. On the other hand, the densities of this group of microorganisms were lower in shelled nuts being 6, 0.8, 0.3 and 0.2×10^3 organisms/g in the respective value. It is clear from data that most of the microbial load (Fungi and bacteria) on walnuts could be considered as proteolytical microorganisms. While they reached a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considerable numbers of the total microorganisms contaminating the other tested nuts.

c) Lipolytic Microorganisms

Data illustrated by Fig. 1 showed that densities of Lipolytic microorganisms were generally lower in the tested nuts comparing with proteolytic group. The counts of lipolytic microorganisms were 30, 2, 0.5 and 0.05 $\times 10^3$ organisms/g on unshelled walnuts, almonds, hazelnuts and pistachio, respectively. On the other hand, the densities of this group was generally lower in the shelled nuts being 1, 0.1, 0.05 and 0.05 $\times$ 10^3 organisms/g in the same respect. It is clear from data that densities of lipolytic microorganisms followed the same trend of the total contaminated fungi or bacteria.

It is clear from data that walnuts was highly contaminated with microorganisms than other tested nuts in general. This support the finding of Jay[2] and Mislivec *et al.*[19]. They reported, due to the extremely high fat and low water content of products such as walnuts, this product is quite refractory to spoilage by bacteria and moulds.

The high figure of proteolytic microorganisms may indicate the high activities of this group on the nut which contain high percentage of protein[2,14].

Distribution of Different Genera of Fungi in Various Nuts

Moulds of many genera may be found on examing nut meats which are picked up by the products during collecting, cracking, storing, packaging, etc.[2]. It is clear from data illustrated by Fig. 2 that mould genera, occurring most frequently in shelled and unshelled nuts, were *Aspergillus, Penicillium, Rhizopus, Mucor* and *Cladosporium*. *Aspergillus* and *Penicillium* species predominated in all the tested shelled nuts being (32 and 39%) *Aspergillus* and (25 to 31%) *Penicillium*. While *Aspergillus* was dominant in all the unshelled nuts (41-50%). Other genera were found to be in low percentage *(Rhizopus, Mucor* and *Cladosporium*). These results are in accordance with Smith and Arends[5]and Mislivec *et al.*[19] who stated that *Aspergillus, Penicillium, Rhizopus, Mucor* and *Cladosporium* represented the common genera in nuts.

Distribution of Different Groups of Bacteria in Various Nuts

Data illustrated by Fig. 3 show the qualitative and quantitative distribution of different groups of bacteria present in the shelled and unshelled nuts. It was found that Gram positive spore forming bacilli, Gram positive Micrococci, Gram positive rods and Gram negative short rods were generally contaminating the samples. Their quantitative distribution varied through the tested nuts. The most dominant bacteria appearing in the unshelled nuts was Gram positive spore forming bacilli being 55 and 68% in shelled and unshelled walnuts respectively. While Gram positive Micrococci were 24 and 29% in the respective value. The dominant groups of bacteria presented in shelled nuts showed nearly the same trend as those on unshelled nuts. The distribution of Gram negative short rods were found very low in the nuts (2-10%), comparing with the other groups of bacteria. These results confirm the work of King *et*

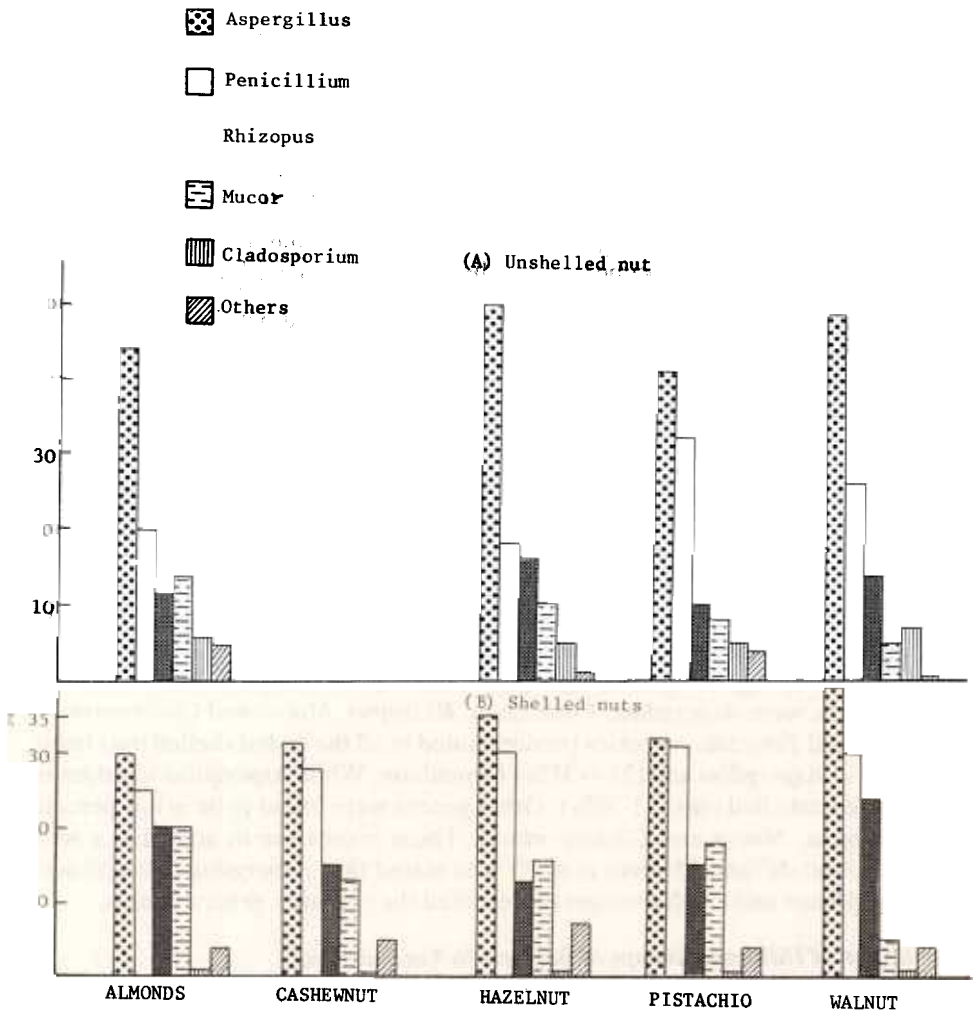
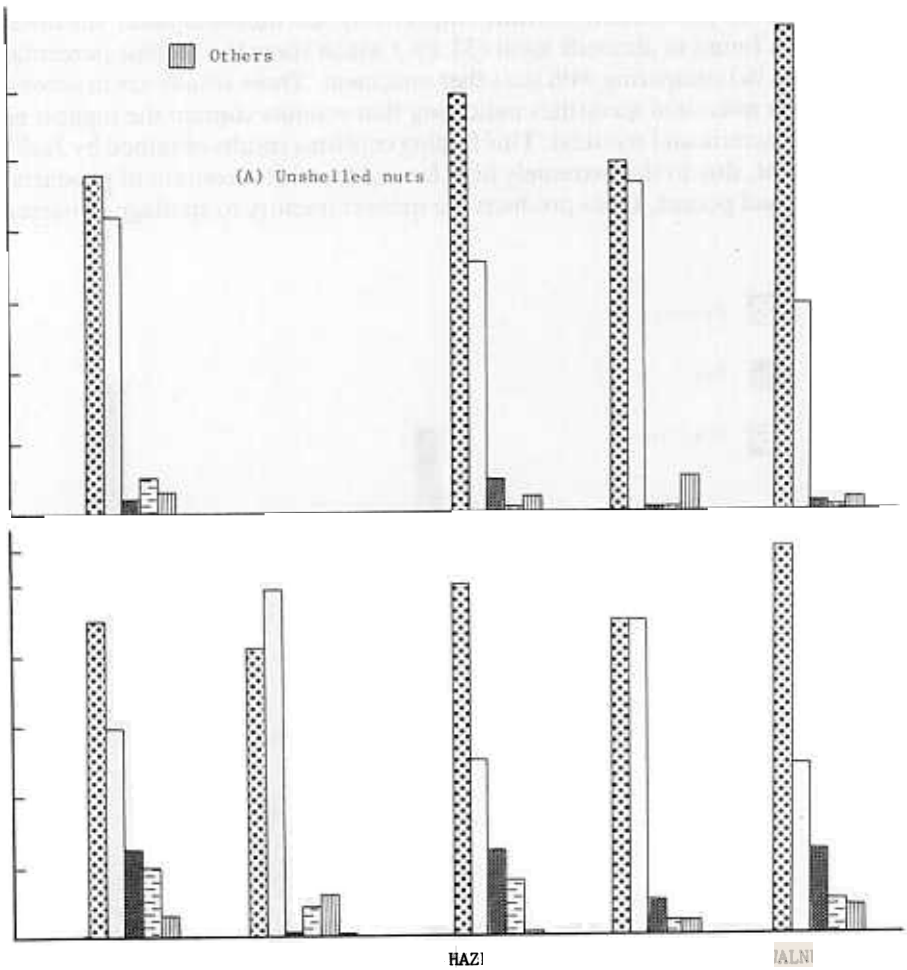


FIG. 2. Distribution of different genera of fungi in various shelled and unshelled nuts.

al.[20] who reported that microorganisms associated with commercially shelled nuts were numerous and varied. They added that the genera of bacteria isolated from almonds include *Bacillus, Micrococcus, Streptococcus, Brevibacterium, Escherichia* and *Aerobacter*. These results are also in harmony with Hall[21] and Chipley and Heaton[22] who found that genera of Gram negative short rods as *Pseudomonas, Escherichia, Leuconostoc, Proteus* and *Aerobacter* were found associated with commercially shelled nuts.



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Chemical Components of Various Nuts

Data illustrated by Fig. 4 show the different composition of various nuts such as hazelnuts, almonds, walnut, pistachio and cashewnut. It is clear from data that nut meat contain high amounts of crude protein and fat and low water content. It is obvious that walnut meat contains extremely high fat and low water content being 71.5% and 2.5% respectively. While its content of protein was low (12.5%) comparing with the other tested nuts. Similar results were found in hazelnut which contain 65.9, 14.4 and 3.4% of fat, protein and moisture respectively. On the other hand, the lowest fat content was found in almonds meat (34.1%) which show the highest percentage of moisture (4.%) comparing with the other nuts meat. These results are in accordance with the nut microbial quantities indicating that walnuts contain the highest microbial load (bacteria and moulds). This finding confirms results obtained by Jay[2] who reported that, due to the extremely high fat and low water content of products such as walnuts and pecans, these products are quite refractory to spoilage of bacteria.

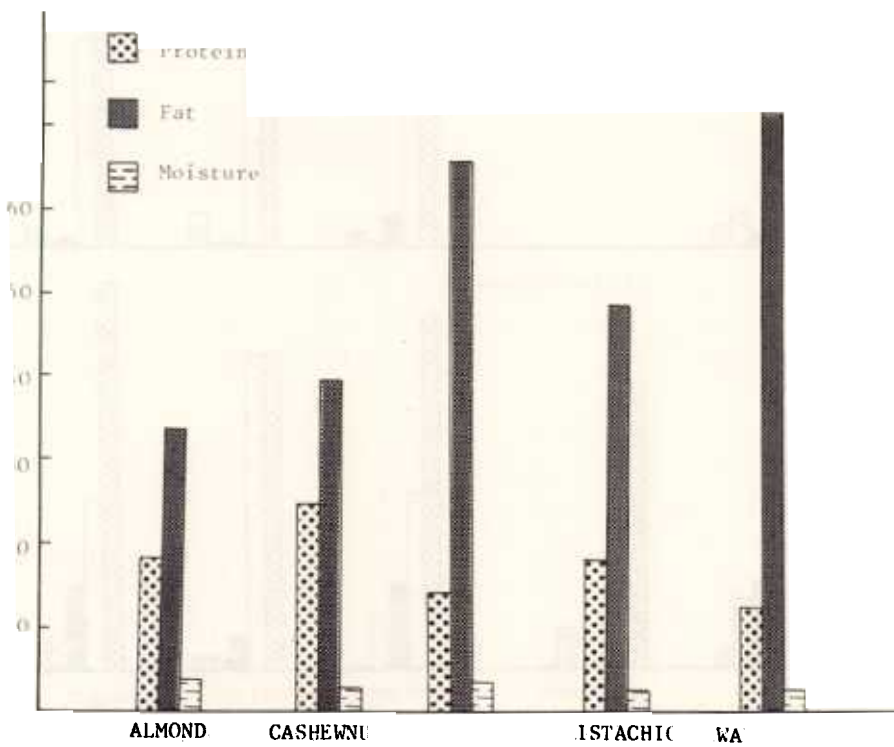

FIG. 4. Percentage composition of various nuts.

As it had been stated before, the spore-forming bacilli and Gram positive micrococci were the dominant isolated bacteria, while *Aspergillus* and *Penicillium* were the dominant isolated fungi from the tested nuts, which have extremely high fat and moderate protein content. These results were in accordance with Lee[24] who reported that proteolytic species are common among the genera *Bacillus, Clostridium, Pseudomonas, Proteus* and *Micrococci.* Frazier and Westhoff[25] and Phillips *et al.*[3] added that *Aspergillus* is the most dominant genera of fungi decomposing protein or even lipids substances. They concluded that *Micrococcus* and *Pseudomonas* are genera contain lipolytic species.

On the other hand, Phillips *et al.*[3] stated that *Aspergillus flavus* and *Aspergillus parasiticus* are widespread on almonds and may produce aflatoxins if environmental conditions are favorable. They added that *A. flavus* has been noted frequently on almond hulls and kernels. Supported by Lillard *et al.*[26] who also found that 85 isolates of *A. flavus* group were shown to produce aflatoxin from pecans. Schade *et al.*[27] detected aflatoxin in almond.

Generally, it could be concluded that microorganisms persisting in various nuts such as almonds, walnuts, hazelnuts ... etc, drive from damaged or cracked nuts, insect infestation, infected or diseased nuts and contamination during processing.

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التلوث الميكروبي لبعض أنواع الجوزيات المعروضة في أسواق جدة

منصور جميل سجيني ، فخري محمد ثابت و محمد كمال الدين الشايب
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أجـريت دراسـات ميكـروبيولوجية وكيميائية على بعض الجوزيات (المكسرات) الشائعة الاستهلاك والمعروضة في أسواق جدة . وقد أظهرت النتائج أن العد الكلي للفطريات كان أعلى من العد الكلي للبكتريا خاصة على الجوزيات المقشورة . كما أظهرت عينات الجوز المختبرة أعلى درجة من التلوث الميكروبي سواء من الفطريات أو البكتريا أو محللات البروتين والدهون ، مقارنة بعينات الجوزيات الأخرى . في حين أظهرت النتائج أن أقل عدد من هذه الميكروبات كان على صنفي الفستق والكاشو . كما أن أهم العزلات الفطرية المنتشرة على الجوزيات تابعة لجنس أسبرجلّس ، بنسليوم ، أما أهم عزلات البكتريا السائدة فقد شملت العصـويات المتجـرثمـة والكـرويات الموجبة لصبغة جرام . كما وجد أن التلوث الميكـروبي عامـة ، الذي يؤدي دوراً هاماً في فساد الجوزيات المختلفة ، يتأثر باختلاف مكونات الجوزيات خاصة البروتين والدهون والمـاء .