

Physicochemical and Microbiological Study of “Raisin”, Local and Imported (Morocco)

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Abstract: This study aimed to evaluate the quality of raisin (local and imported) in Rabat- Casablanca markets and study the *Enterobacteriaceae* strains isolated from samples, physicochemical characterization (moisture content, acidity, pH and electrical conductivity). Microbiological analyses included (Standard plate count, germs pathogens, lactic acid bacteria, yeasts and moulds). Results showed the physicochemical properties of samples were normal with averages 16.3, 0.7, 4.14 and 136.6 for moisture content, acidity, pH and electrical conductivity. Microbiological analyses showed higher counts of microbial flora in all samples tested, with an average 2.8×10^7 , 3×10^3 , 2.3×10^3 , 3×10^3 and 4.6×10^4 CFU/g for standard plate count, total coliforms total, fecal coliforms, yeasts and moulds respectively, while lactic acid bacteria were detected in low count. *Staphylococcus aureus*, *Streptococcus fecal*, *Salmonella*, *Shigella* and *Clostridium* were not detected in all samples. Identification results of *Enterobacteriaceae* isolates showed that the genus *Enterobacter* was the most frequent (56%) with four species: *E. Sakazakii* (68%), *E. Amnigenus* (10%), *E. Agglomerens* (14%) and *E. aerogenes* (8%). Other genus, *Klebsiella* (25%) and *Serratia* (19%) have a lower percentage. Antibiotic susceptibility of *Enterobacteriaceae* isolates showed high level of resistance to Ampicillin, Amoxicillin, Amoxicillin+Ac clavulanic, Cefalotin, co-trimoxazole and Ticarcillin. *Serratia marcescens* recorded high prevalence of resistance to antibiotics.

Key words: Raisin • Quality • Enterobacteriaceae • Susceptibility profile

INTRODUCTION

Raisins are dried grapes, prepared from some varieties of grapes (*Vitis vinifera*). The history of consumption of Raisin is very old, the Bible provides the first written mention of raisin around 1000 B.C. [1].

Raisin is a source of carbohydrates and it contains large amounts of iron, vitamins and minerals [2] Raisins are usually included in breakfast, cereals, dairy and bakery as well as confectionery products and more recently in nutritional bars [3].

Drying is one of the oldest methods of food preservations; the main objective of these operations is reducing the moisture content to level which allows safe storage without spoilage. Drying grape either by open sun drying, solar drying or mechanical dry produces raisin [4].

Contamination of raisin by different microorganisms can occur during harvest of grape, drying pressed, handling, transports and during the exposure of the product in the markets, especially when it is made in open bags [5-7].

Many varieties of bacteria can be transferred from the environment to the dried fruit as raisin, *Enterobacteriaceae* is the most important of these bacteria and thus it's used as indicator of fecal contamination in food also poor hygiene and sanitation treatment [8].

Microbiological studies on raisins are rare. Some of them concern only the part fungal and toxicological [9-13]. The present study investigated the physicochemical and microbiological quality of raisin, local and imported and susceptibility profile of *Enterobacteriaceae* isolated from this product.

MATERIELS AND METHODS

Sampling: Between November 2009 and April 2010, two hundreds and ten samples of raisins were collected from Rabat -Casablanca regions. The varieties of raisins were from Morocco and imports from different countries: Iran, Turkey, China and India. The collection of samples was replicated three times during the period of study.

Methodologies

Quality Hygienic Characterization: Physicochemical characterization including moisture content, acidity, pH and electrical conductivity were evaluated according to AOAC [14]. Microbiological characterization including standard plate count, germs pathogens, lactic acid bacteria, yeasts and molds were determined according to Harrigan and McCance [15].

Isolation and Identification of Enterobacteriaceae Isolates:

Red colonies were transferred from Desoxycholate Lactose agar, reculture on nutrient agar many times until purified, all strains were tested by Gram stain and oxidase activity and isolates identification was performed by determination of the biochemical reaction profile using API 20E kit commercial (Biomérieux, Marcy l'Etoile, France).

Antimicrobial Susceptibility Profile of Isolates:

Antimicrobial sensitivity testing of the isolates to antibiotics was performed by agar well diffusion methods according to NCCLS [16], using following antibiotic disks (Oxoid Ltd., England): AMP: Ampicillin 25g, AMX: Amoxicillin 25µg, CTX: Cefotaxim 30µg, AMC: Amoxicillin+Ac clavulanic 20µg, CF: Cefalotin 30µg, GM: Gentamicin 10µg, CIP: Ciprofloxacin 5µg, STX: cotrimoxazole 10µg, CRO: Ceftriaxon 30µg, TIC: Ticarcillin 75µg, NA: Nalidixic acid 30µg and CN: Cefalexin 30µg.

RESULTS AND DISCUSSION

Physicochemical Characterization: Results of physicochemical analysis of raisin samples from different countries are presented in Table 1. The pH values were ranged between 3.80 to 4.43; similar results were reported by Freeto [17] who mentioned that, the pH range of raisins is 3.5 to 4.0 which indicate the level of acidity in raisins. The results obtained showed the acidity values were in the range of 0.64 - 0.81, these values are considered normal because raisins are rich with organics acids, especially tartaric acid (TA), also raisin acidity correlates much more closely to raisin type than does the titratable acidity of fresh grapes [18]. According to Spiller *et al.* [19] investigation, raisins contain many organics acids, TA is the mostly and to a lesser extent malic acid, it's contains 2 - 3.5g/100g of TA.

Electrical conductivity (EC) values varied from 123.3 to 151.1, with an average 4.15 mS/cm, the highest value was obtained in raisin Moroccan type black, however the lowest values was showed in raisin Indian. EC explains the presence of ions and organic acids in solution [20].

Table 1: Physicochemical characterization of the samples according different countries

Varieties	N.S.	PH	EC (mS m ⁻¹)	AC	M%
Moroccan black	30	4.43	123.30	0.67	16.2
Moroccan yellow	30	4.21	127.50	0.81	17.1
Iranian large	30	4.15	140.20	0.72	16.6
Iranian small	30	4.1	142.03	0.64	15.9
Turkish	30	4.2	130.60	0.66	16.0
Chinese	30	4.1	141.70	0.74	16.8
Indian	30	3.80	151.10	0.66	15.8
Total/ Average	210	4.14	136.60	0.70	16.3

N.S: Number of samples, EC: Electrical Conductivity, AC: Acidity, M: Moisture

Results of moisture content in the samples studied showed a range from 15.8 to 17.1 with an average 16.3%, similar results were found by Karimi *et al.*[21]. Moisture level in raisins is an important indicator of storage ability, the lower their content reflects the long ability of storage [22]. Our results of moisture content were within the standard values (13-18%) of raisins according to national standard [23], Furthermore, in this levels of moisture, the *aw* of raisins typically ranges from 0.50 to 0.65, specialty some varieties of raisins have *aw* up to 0.80, which have high moisture levels [17].

Microbiological Characterization: Results of microbial analysis of the investigated samples are presented in Table 2. The counts of the studied microbial groups were high in the most varieties of raisins comparing with the international microbial limits [24], the highest standard plate count (SPC) value was 8.5×10^7 CFU/g found in Iranian small type and the lowest value 6.1×10^6 CFU/g in Indian type. In quality microbiology, SPC is considered as the first index of quality of food [25].

Results obtained showed the count of total coliforms and fecal coliforms in high level, which might explain the poor condition of raisins, methods of preparation, transports, marketing and the exposed method in the markets [7]. Several reports have confirmed, the relation between the condition of preparation of raisins and microbial quality, as the good practice of raisins preparation, the high quality obtained, also the place of storage of raisin have an important roles on this quality [26, 27]. In addition, various bacteria have ability to growth in the dry environments, as on the surface of a raisin and can survive on raisin and dry surface for months [28-30].

The results showed the presence of yeasts in the samples of raisins tested with high counts, ranged from 6.3×10^5 to 2.44×10^4 with an average 3×10^5 CFU/g. Elhalouat

Table 2: Results of microbial analysis of the investigated raisins samples

Varieties	N.S	Pathogens germs						
		SPC UFC/g	TC UFC/g	FC UFC/g	SA/SF/Sal/ SH/CL	LAB UFC/g	Y UFC/g	M UFC/g
Moroccan black	30	2.6x10 ⁷	1.5x10 ⁴	5x10 ³	abs	8	4.9x10 ⁵	7.5x10 ⁴
Moroccan yellow	30	2.4x10 ⁷	3.7x10 ³	3.5x10 ³	abs	abs	2.4x10 ⁵	1.9x10 ⁵
Iranian large	30	2.6x10 ⁷	3.8x10 ³	2.5x10 ³	abs	10	4.4x10 ⁵	3.6x10 ³
Iranian small	30	8.5x10 ⁷	1.8x10 ³	1.2x10 ³	abs	9	6.3x10 ⁵	10 ³
Turkish	30	1.5x10 ⁷	6.4x10 ³	8.2x10 ²	abs	13	2.3x10 ⁵	5.8x10 ⁴
Chinese	30	1.1x10 ⁷	3.3x10 ³	1.7x10 ³	abs	abs	2.4x10 ⁴	abs
Indian	30	6.1x10 ⁶	1.8x10 ³	1.1x10 ³	abs	abs	3.6x10 ⁴	abs
Total /Average	210	2.8x10 ⁷	3x10 ³	2.3x10 ³	abs	5.7	3x10 ⁵	4.6x10 ⁴

SPC: Standard Plate Count, TC: total coliforms, FC: fecal coliforms, SA: *Staphylococcus aureus*., SF: *Streptococcus fecalis*, Sal: *Salmonella*, SH: *Shigella*, CL: *Clostridium*, LAB: Lactic acid bacteria, Y: Yeasts, M: Molds, abs: Absent

and Debevere [11] showed the presence of yeasts in raisins with low count. Yeasts can be to grow in a wide range of pH values and temperatures; it has also the ability in many species to grow at reduced water activity (*aw*) [31].

Some of samples raisins contained high level of molds, molds and their mycotoxins in raisins has been studied by several investigations in different parts of the worlds, in Morocco by Elhalouat and Deberre [11], in Egypt by Abdel- Satar and Saber [12] and Zohri and Abdel Gawad [10], in Yemen by Saeed *et al.* [32], in Australia by Alisa *et al.* [33], in Spain by Valero *et al.* [34], in Brazil by Beatriz *et al.* [35] and in Argentina by Romero *et al.* [36]. The level of molds in dried fruit depends on many factors; the type of fruit when it support growth and sporulation of molds before or after drying, molds spores that may be present in large number in the dried fruit and if the drying is made by traditional method or on plates not clean, in addition, the contamination by molds can also occur during handling, transports and marketing [7, 27].

Isolation and Identification of Enterobacteriaceae

Isolates: All strains isolated from raisins samples of different countries were Gram negative, short rods and oxidase negative. The identified isolates of *Enterobacteriaceae* are presented in Figure (1).

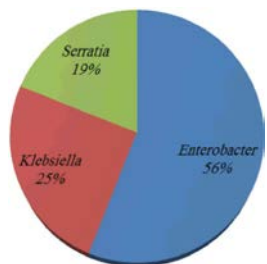


Fig. 1: Identified *Enterobacteriaceae* isolates from the studied raisin samples

The most frequently isolated strains were *Enterobacter* (56%), which contain four species: *E. Sakazakii* (68%), *E. Amnigenus* (10%), *E. Agglomerens* (14%) and *E. aerogenes* (8%). The presence of *Enterobacter* in food is an indicator of fecal contamination and it was recorded by Farmer *et al.* [37]. *Enterobacter* cause many infection in children and adults [38]. *E. Sakazakii* has been isolated from different foods, including powdered infant formula milk [39, 40].

The *Klebsiella* genus was predominantly represented by *K. pneumoniae* (75%), *K. oxytoca* (12.5%) and *K. ornithinolytica* (12.5%). *Klebsiella pneumoniae* is the most important species in *Klebsiella* genus; it causes many problems to human including nosocomial infection, urinary tract infection, diarrhea and other diseases. As other species of *Enterobacteriaceae*, the presence of *K. pneumoniae* in food indicates the pollution of fecal origin [41]. Several works reported the presence of *K. pneumoniae* in a wide range of foods including: orange juice concentrate, home-canned tomatoes, traditional fast food, ready to eat rice sold, tuna sashimi, fruit juices and powdered of children [41, 48].

Serratia was the third genus isolated from raisins samples, this genus was identify by one species *Serratia marcescens* with percentage (19%). Many studies has been reported, *S. marcescens* is one of causes of many infections including urinary tract infection, septicemic meningitis, respiratory tract infection and wound infections [49-51]. Contamination of foods by *S. marcescens* is rare, but it may occur as a cross contamination [52].

The possibility of raisins contamination by *Enterobacteriaceae* occur during drying processing; especially in traditional methods, in addition, the marketing practice contributes to the increase the level of contamination, the most important of this practice is the contact of product by the hands of the vendor and the consumers [26, 53].

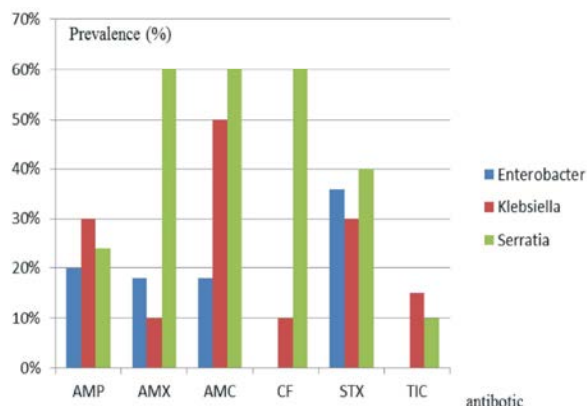


Fig. 2: Antibiotic resistance of *Enterobacteriaceae* isolates

Antimicrobial Susceptibility Profile of Isolates: Results of antibiotic resistance are presented in Figure 2, 100% sensibility to Cefotaxim, Gentamicin, Ciprofloxacin, Ceftriaxon, Nalidixic acid and Cefalexin was recorded on all isolates. *Serratia marcescens* recorded the following resistance rates to the used antibiotics: Ampicillin (24%), Amoxicillin (60%), Amoxicillin+Ac clavulanic (60%), Cefalotin (60%), co-trimoxazole (40%) and Ticarcillin (10%). Ball *et al.* [54] reported resistance of *Serratia marcescens* to ampicillin and the group of cephalosporins, for this, the infection by these bacteria may be difficult to treat. Resistance of *S. marcescens* to antibiotics may occur by two methods; production of chromosomal AmpC cephalosporinases combined and ability to hydrolyse carbapenems by β -lactamases synthesis [55, 56].

Klebsiella isolates showed resistance to Ampicillin (30%), Amoxicillin (10%), Amoxicillin+Ac clavulanic (50%), Cefalotin (10%), co-trimoxazole (30%) and Ticarcillin (15%). Resistance of *Klebsiella* has been reported by Ajayi and Egbebi [44] in Nigeria and Xianteng *et al.* [47] in China. Several researches reported the mechanism of antibiotic resistance in *Klebsiella* isolates as the result of extended spectrum β -lactamases (ESBLs) [57-59].

Results showed the resistance of *Enterobacter* isolates was in low percentage comparing with other genera, they presented resistance to Ampicillin (20%), Amoxicillin (18%), Amoxicillin+Ac clavulanic (18%) and co-trimoxazole (36%). As other *Enterobacteriaceae*, the resistances to antibiotics in *Enterobacter* occur by ESBLs mechanisms [60, 61].

The problem of resistance to antibiotics has increased in recent years worldwide, increasing public health problems, for this, the results of antibiotic resistant bacteria should be taken in strategic plans by the health authorities.

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