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Hygiene quality of traditional and industrial table olives from markets in Rabat-Salé and Temara cities in Morocco

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Abstract:

Background: Table olives are one of the most important vegetable canning products in Morocco, which is considered one of the world's largest producing countries. Currently, many outlets prepare table olives by different methods that do not comply with standard hygiene practices. Hence, this research was conducted to assess the quality standard of these olives by evaluating their physico-chemical and microbiological properties.
Methodology: A total of 108 samples of table olives (pitted green olives and blacks) obtained from Rabat-Salé and Rabat-Temara markets in Morocco were evaluated. Physico-chemical properties of the olives including pH, oxido-reduction potential (ORP) and titrable acidity were determined using the analytical methods of the Association of Official Analytical Chemists (AOAC). Microbiological analyses including standard plate count (SPC) for total aerobic mesophilic flora (TAMB), total coliforms (TC), faecal coliforms (FC), yeasts, clostridia, *Staphylococcus aureus*, faecal streptococci and salmonella counts, were performed using standard microbiological methods. The identification of yeast isolates was carried out with the commercial API 20C biochemical identification kit.
Results: The average microbial loads for traditional olive samples were $3.2x10^6$ CFU/ml for SPC, $1.7x10^4$ CFU/ml for TC, $8.7x10^3$ CFU/ml for FC, and $2.5x10^6$ CFU/ml for yeast, which were higher compared to the average microbial loads of industrial olives with values of $5.9x10^5$ CFU/ml, $5x10^1$ CFU/ml, 0 CFU/ml and 0 CFU/ml respectively. One hundred percent (56 of 56) of the traditional olives (pitted green and black) from Temara-Rabat markets were contaminated with coliforms while 50% of green and 65% of black olives in Salé-Rabat were contaminated with coliforms. Five percent (5%) each of the traditional green and black olives in Salé-Rabat markets were contaminated with clostridia (spore forming bacteria). No FC or other bacteria and yeasts were present in the industrial olives, and none of the olives was contaminated with *S. aureus*, faecal streptococci and salmonella. Of the total of 8 yeast strains isolated from the traditional olives, 4 (50%) were *Candida guilliermondii*, 2 (25%) *Candida lusitaniae* and 2 (25%) *Candida famata*.
Conclusion: The contamination of olive oil products may be due to different sources such as water, processing materials, storage condition, cleaning, labour and others. There is need for increase awareness and control of these at the points of sale of these traditional olives.

Keywords: hygiene; physico-chemical properties; microbiology; traditional olives; quality

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Qualité Hygiène des olives de table traditionnelles et industrielles des marchés des villes de Rabat-Salé et Témara au Maroc

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Resume:

Contexte: Les olives de table sont l'un des produits de mise en conserve de légumes les plus importants au Maroc, qui est considéré comme l'un des plus grands pays producteurs du monde. Actuellement, de nombreux

points de vente préparent les olives de table par différentes méthodes non conformes aux pratiques d'hygiène standard. Ainsi, cette recherche a été menée pour évaluer le standard de qualité de ces olives en évaluant leurs propriétés physico-chimiques et microbiologiques.
Méthodologie: Un total de 108 échantillons d'olives de table (olives vertes dénoyautées et noires) obtenus sur les marchés de Rabat-Salé et Rabat-Témara au Maroc ont été évalués. Les propriétés physico-chimiques des olives, y compris le pH, le potentiel d'oxydoréduction (ORP) et l'acidité titrable ont été déterminées en utilisant les méthodes analytiques de l'Association of Official Analytical Chemists (AOAC). Les analyses microbiologiques, y compris la numération sur plaque standard (SPC) pour la flore mésophile aérobie totale (FMAT), les coliformes totaux (CT), les coliformes fécaux (CF), les levures, les clostridies, *Staphylococcus aureus*, les streptocoques fécaux et les numérations de salmonelles, ont été effectuées à l'aide de méthodes microbiologiques standard. L'identification des isolats de levure a été réalisée avec le kit d'identification biochimique API 20E du commerce.
Résultats: Les charges microbiennes moyennes pour les échantillons d'olives traditionnelles étaient de $3,2x10^6$ UFC/ml pour le SPC, $1,7x10^4$ UFC/ml pour le TC, $8,7x10^3$ UFC/ml pour le FC et $2,5x10^6$ UFC/ml pour la levure, qui étaient plus élevées par rapport aux charges microbiennes moyennes des olives industrielles avec des valeurs respectives de $5,9x10^5$ UFC/ml, $5x10^1$ UFC/ml, 0 UFC/ml et 0 UFC/ml. Cent pour cent (56 sur 56) des olives traditionnelles (dénoyautées vertes et noires) des marchés de Témara-Rabat étaient contaminées par des coliformes tandis que 50% des olives vertes et 65% des olives noires de Salé-Rabat étaient contaminées par des coliformes. Cinq pour cent (5%) de chacune des olives vertes et noires traditionnelles des marchés de Salé-Rabat étaient contaminées par des clostridia (bactéries sporulantes). Aucune FC ni aucune autre bactérie et levure n'étaient présentes dans les olives industrielles, et aucune des olives n'était contaminée par *S. aureus*, des streptocoques fécaux et des salmonelles. Sur un total de 8 souches de levure isolées des olives traditionnelles, 4 (50%) étaient *Candida guilliermondii*, 2 (25%) *Candida lusitaniae* et 2 (25%) *Candida famata*.
Conclusion: La contamination des produits à base d'huile d'olive peut être due à différentes sources telles que l'eau, les matériaux de traitement, les conditions de stockage, le nettoyage, la main-d'œuvre et autres. Il est nécessaire d'accroître la sensibilisation et le contrôle de ceux-ci dans les points de vente de ces olives traditionnelles.

Mots-clés: hygiène; propriétés physico-chimiques; microbiologie; olives traditionnelles; qualité

Introduction:

Olive tree is a specific tree of the Mediterranean basin whose likely origin is Egypt, India, Syria or Ethiopia. Its cultivation in North Africa existed before the arrival of the Romans and the production of its oil was recognized about 7,000 years ago (1). In Morocco, the national production of table olives is about 2 million tons in year (2) for an area of 957,000 hectare, and the most widely available variety is the Moroccan picholine (Zitoune beldi). The regions in Morocco with highest production of olives are Marrakech, Safi, Beni Mellal, Khénifra, Tangier-Tétouan and Fés-Méknés. The three methods of olive preparations mostly practiced in Morocco are; the Spanish method for green olives, the Californian method for oxidized black olives and the Greek method for black olives, but the method most often used by industrialists is the Spanish preparation method which is an alkaline desamerization treatment of olives.

The presence of staphylococci, faecal streptococci, total coliforms, yeasts and molds contaminating table olives have been reported by many researchers in Morocco, especially in the regions of Rabat and Marrakech (3,4). In addition, the presence of penicillium spores in olive samples (especially black olives) was reported by Maouni et al., (5) and Lamrani et al., (6) in the region of Fez Marrakech. Outside of Morocco, Caggia et al., (7) has isolated and identified *Listeria monocytogenes* in olive samples of traders in Italy, and fecal coliforms, streptococci and reductive sulfite clostridia were isolated from samples of commercial olives in Portugal (8). The objective of this study is to assess hygiene quality and identify the most predominant yeast species in traditional and industrial tables olives sold in the markets of two cities in Morocco.

Materials and method:

Sampling:

A total of 108 pitted black and green olive samples (96 traditional and 12 industrial) from markets in Temara-Rabat and Salé-Rabat in Morocco were collected for evaluation of the hygienic quality of these products for direct consumption. For each of the traditional black and green olive brands, 4 samples were collected from 7 points of sales from markets in both Temara-Rabat and Salé-Rabat. For the industrial olives, 6 samples were collected for each black and green olives (3 per brand).

Samples were delivered to the laboratory directly in a cooler. The maximum time between sampling and sample analysis was one hour. All samples were analysed by and results obtained compared with national and international standards.

Physico-chemical analysis of olive samples

The pH and oxido-reduction potential (ORP) of samples were measured from a 20% dry matter solution using a multi-parameter measurement pH after the device has been calibrated using AOAC method 981.12 (9). The liquid solution of the product was prepared and analyzed by titrimetry at pH 8.1 with 0.1N sodium hydroxide solution (NaOH) using AOAC methods 920.149 (c), 942.15A and 942.15B (9). Total acidity of olives was expressed by convention in grams of citric acid.

Standard plate count (SPC) for total aerobic mesophilic flora

The standard plate count (SPC) for total aerobic mesophilic flora (TAMF) was done after appropriate sample dilutions in peptone water buffered broth and subsequent seeding on the plate count agar (PCA) growth medium and incubation at 30°C for 72 hours (10).

Total and fecal coliform counts

Total coliform (TC) and faecal coliform (FC) counts were done by culturing appropriate sample dilution of olives on MacConkey agar plate and incubating at 30°C for TC and 44°C for FC. After 24 hours of incubation, red colonies were counted (11).

***Staphylococcus aureus* (SA) count:**

Staphylococcus aureus count was performed by inoculating Baird Parker culture medium with appropriate olive sample dilution and incubating aerobically at 37°C for 24 hours (12).

Faecal streptococci (FS) count

Faecal streptococci count was done on Rothe broth and after incubation at 37°C for 24 hours, positive tubes were seeded on Litsky broth and incubated at 37°C for 24 hours (13).

Salmonella count

Pre-enrichment was done by adding 25ml of olive sample to 225ml of sterile peptone water dabbed in a 250ml Erlenmeyer flask, which was incubated at 37°C for 12 hrs. Enrichment was done using two broths; Muller Kaufman and tetrathionate (MKTn) broth (Merck, Germany). MKTn tubes showing positive result were sub-cultured onto XLDA agar for *Salmonella*, where positive colonies appeared green (14). Identification was done by the procedure described by Poelma (15).

Reducing sulfito count for anaerobic spore forming bacteria (SFB)

The count for anaerobic spore forming bacteria (clostridia) was performed on Sodium Sulphite - Polymyxin - Cysteine Sulphite (SPS) medium. The sample solution was first heat-treated at 80°C for 10 minutes, after which SPS medium was seeded and incubated at 30°C for 24-48 hours. Only black colonies were counted (16).

Lactic acid bacteria count

Lactic acid bacteria count was carried out using Man Rogosa and Sharpe (MRS) medium. Incubation was done at 30°C for mesophilic species and 45°C for thermophilic species for 48 hours. Round shape or lenticular colonies were counted (17).

Yeast enumeration and identification

The method used consists of seeding Potato Dextrose Agar (PDA) that has been highly acidified (pH 3-3.5) by lactic acid. The count was carried out after 3 days of incubation at 37°C for yeasts and after 4 days of incubation at 30°C for moulds (18). The identification of yeast isolates was carried out using the commercial biochemical API 20E kit (19).

Results:

The physico-chemical analysis of the traditional green and black olive samples from the different outlets showed average pH, acidity and oxido-reduction potential (OPR) values for green olives of 4.4; 11.8 and 135.5 respectively, while for the black olives, the respective values were 6.3, 8.1 and 8.0. For the industrial olives, the values of pH, acidity and OPR of the black olives are respectively 4.5, 5.5 and 129.5 while the respective values for green olives are 5.9, 8.5 and 92.5 (Table 1).

Microbiological analyses of the black olive samples showed the average microbial loads for traditional olive samples as; $3.2x10^6$ for SPC, $1.3x10^4$ for TC, $8.7x10^3$ for FC, and $2.5\ x10^6$ for yeast, which were higher compared to the average microbial loads of industrial olives with values of $5.9x10^5$, $5x10^1$, 0 CFU/ml and 0 CFU/ml respectively. One hundred percent (56 of 56) of the traditional olives (pitted green and black) from Temara-Rabat markets were contaminated with coliforms (TC and FC) while 50% of green and 65% of black olives in Salé-Rabat markets were contaminated with coliforms.

Five percent (5%) each of the traditional green and black olives in Salé-Rabat were contaminated with clostridia (spore forming bacteria). No FC or other bacteria and yeasts were present in the industrial olives, and none of the olives was contaminated with *S. aureus*, faecal streptococci and salmonella. Of the total of 8 yeast strains isolated from the traditional olives, 4 (50%) were *Candida guilliermondii*, 2 (25%) were *Candida lusitaniae* and 2 (25%) were *Candida famata* (Fig 1).

Table 1: Physico-chemical composition of traditional and industrial pitted olives in Morocco

Type of olives	Point of sale (PS)	Number of sample	pH			Acidity °D			Oxido-reduction potential ORP (mV)		
			Min	Max	Average	Min	Max	Average	Min	Max	Average
Traditional pitted green	PS1 to PS7 (Rabat-Temara)	28	3.34	4.62	4.17	7.7	19.7	11.8	111.6	200.8	144.6
	PS8 to PS12 (Rabat-Salé)	20	-	-	4.60	-	-	-	-	-	123.5
	Total	48			4.35			11.8			135.5
Traditional pitted black	PS1 to PS7 (Rabat-Temara)	28	6.08	7.69	6.90	7.50	9.00	8.07	-35.10	27.60	-4.11
	PS8 to PS12 (Rabat-Salé)	20	-	-	5.54	-	-	-	-	-	24.8
	Total	48			6.3			8.07			8
Industrial pitted green	Brand 1 and 2	6	5.43	6.39	5.91	3.00	14	8.5	17.70	74.83	92.53
Industrial pitted black	Brand 1 and 2	6	4.51	4.57	4.54	4.66	6.33	5.49	127.26	131.86	129.56

Min: Minimum; Max: Maximum

Table 2: Microbial contamination of traditional and industrial olives in Morocco

Olive type	Point of sale (PS)	Number of sample	SPC (10^5 CFU/ml)			TC (10^2 CFU/ml)			FC (10^2 CFU/ml)			StaA 10^3CFU/ml	FStr 10^3CFU/ml	Salm CFU/ml	SFB 10^3 CFU/ml	Lactic acid bacteria 10^5CFU/ml	Yeast 10^6 CFU/ml
			Min	Max	Av	Min	Max	Av	Min	Max	Av						
Traditional green	PS1 to PS7 (Temara-Rabat)	28	8.7	51	32	5.5	327	130	5.2	228	87.5	0	0	0	0	2.53	2.5
							28 (100%) contaminated										
	PS8 to PS12 (Salé-Rabat)	20					10 (50%) contaminated					0	0	0	1 (5%) contaminated		>10
Traditional black	PS1 to PS7 (Temara -Rabat)	28	12	45	30	50	450	171	14	227	82	0	0	0	0	15.2	98
							28 (100%) contaminated										
	PS8 to PS12 (Salé-Rabat-)	20					13 (65%) contaminated					0	0	0	1 (5%) contaminated		>10
Industrial green	Brand 1 and 2	6	2.4	9.4	5.9	0	1	0.5	0	0	00	0	0	0	0	0	0
Industrial Black	Brand 1 and 2	6	1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0

TC: coliforms; FC: fecal coliforms; SPC: Standard Plate Count; FStr: fecal streptococci; SFB: spore forming bacteria; StaA: *Staphylococcus aureus*; Salm: salmonella; Min: minimum; Max: maximum; Av: average; CFU: colony forming unit

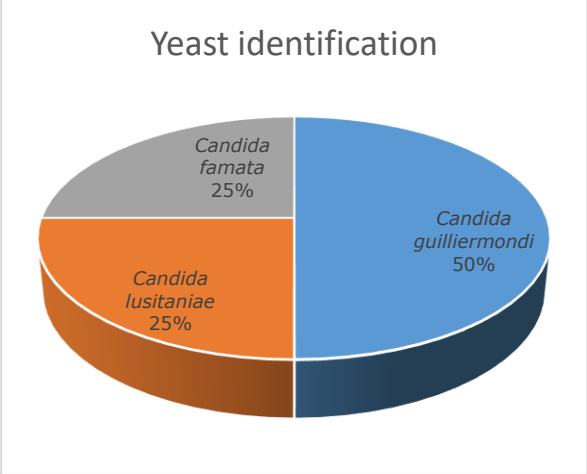

Fig 1: *Candida* species isolated from table olives in Morocco

Discussion:

The oxido-reduction potential (ORP) of traditional green olives in this study was very high compared to that of the black olives of the same type, but almost similar to that of industrial green olives, therefore the pH of green olive is acidic compared to black olive which is close to neutrality. The pH is correlated with the amount of free fatty and organic fatty acids produced by microorganisms (20), and the recommended maximum pH value should be less than 4.3 (21). However, the values obtained in our study are higher than those reported previously (22). The acidity values reported by the IOC (pH 0.3 - 0.5) and those by Kailis and Harris (23), and Ünal and Cevdet (24) were lower than our values, which may relate to the treatment conditions and lactic acid activity of the olives.

Traditional green and black olive samples from Témara-Rabat markets were more contaminated with coliforms (100%) when compared to those from Salé-Rabat markets with 50% and 65% contamination for green and black olives respectively, and 5% of both olive types were contaminated by clostridia. Coliforms and clostridia contaminations have been reported in traditional olives in Portugal (8) and in Marrakech region of Morocco (3). In another study, Maouni et al., (5) reported that household-prepared table olives had more microbial loads than the commercial olives. The high aerobic flora load in the traditional

green olive samples can be explained by the low salt content (less than 6%) which increase acidity, and by prolonged storage at the orchard level, which increase exposure to microbial contaminants. Also, there may be proliferation of microorganisms at the traditional olive preparation and extended open-air storage at room temperature. However, there was no such contamination with the industrial olives, probably the result of heat treatment and good hygiene during preparation, which therefore make them safe for consumption.

For the yeast contents, the values reported in this study exceed the recommended standards, which can lead to the deterioration of the olives with release of CO_2, resulting in bad odors (25). *Candida guilliermondii* was the most commonly identified yeast specie in the contaminated olive samples. This species has been reported as essential for fermentation of traditional olives in Italy (26) and Morocco (27,28). *Candida famata* and *C. lusitaniae* were the other species identified in our study that have been reported as normal flora during fermentation process of olives in Turkey (29).

Conclusion:

Microbiological analyses of the traditional olive samples show the presence of faecal flora especially clostridia in the samples, which is an indicator of poor hygienic conditions in the preparation of these olives. Our findings should inform preparers of the risks associated with poor hygiene in preparation of the olives, and encourage measures such as pasteurization, environmental and instrument cleanliness, availability of water sanitation and hygiene (WASH) facilities, proper packaging of finished products, and cooling, that can help reduce microbial contaminations during preparation.

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