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Physicochemical and Microbiological Analysis of Alimentary Products

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Abstract: Food products sold in grocery stores and kiosks near the schools have become a source of alimentation for students during breakfasts and breaks. But lately, these students show from time to time alimentary intoxications. To make sure of the quality of these foods and to search the origin of these intoxications. A qualitative study is conducted on the most consumed products. Physicochemical, microbiological and organoleptic analyses are realized. The physicochemical parameters studied are the pH, the acidity and the temperature. For microbiological species searched are: FMAT, total and faecal coliforms, faecal Streptococci, mold and yeast, *Clostridium botulinum*, *Staphylococcus aureus*, *Escherichia coli*, Salmonella and lactic bacteria. While the organoleptic and olfactory-taste criteria focused on the color, taste and flavor of the products. The results obtained showed that on the microbiological side these products are free of the germs of deterioration and germs pathogens while for physicochemical parameters, the food storage temperature exceeds the norm for 80% of the food products tested. For organoleptic criteria, taste modification has been noted in the liquid products.

INTRODUCTION

The diet of school children has changed in recent decades. They are no longer able to take their breakfast with bread, coffee, butter and olive oil in their homes they are starting to replace it with emballed alimentary products such as cookies, wafers, bimos, yogurts dairy products, juices and other food made from sugar, protein and fat sold in grocery stores and kiosks.

These products, placed in areas where the conditions of hygiene and conservation are not good; the students whose are consuming them from time to time have digestive anomalies (vomiting, diarrhea and allergies, appearance of pimples, itching). To find the origin of these alimentary anomalies, we proceeded by a series of microbiological, physicochemical, organoleptic and olfactogustative analyzes.

MATERIALS AND METHODS

In order to ensure the quality of alimentary products that students consume during their breakfasts and during breaks near the public institutions located in the city of

Kenitra. A study was conducted for 3 months (From April to June, 2017) a period marked by an increase in temperature. The samples taken are the most consumed products (Rhaiem *et al*., 2016).

Sampling: Our approach began with the establishment of the list of alimentary products to be analyzed. The latter is based on the selection of products with the highest consumption rate. The information was provided by the results obtained in a survey of students from 3 schools in the city of Kenitra. The alimentary products to be analyzed are purchased from grocery stores and kiosks near public schools. The analyzed products are: fruit juices, soft drinks (apple and lemon) concentrated tomatoes, kosher, canned pickles, canned olives, soy sauces, yoghurts, candies, chocolates, jams, nectars, cheeses, mayonnaise, ketchups, biscuits, chips and chewing gums.

Sample preparation: Once the products are purchased they are taken to the laboratory in sterile plastic bags and put in a cooler. Each food is taken in 5 copies and the analysis is done is three repetitions. The approach taken for the analysis of these samples is based on the preparation of a composite sample for each food product. All samples are checked before opening by an accurate visual check. Any product with a packing defect is discarded. Samples with a correct appearance were cleaned with sterile cotton with alcohol before taking the samples.

Dilution: For the liquid samples, 1 mL of each sample is taken and added to 9 mL of physiological water. A 10^{-1} dilution is prepared, from which the other dilutions (10^{-1} a 10^{-3}) are successively made by placing 1 mL of the solution to be diluted in 9 mL of physiological waters added, the whole is homogenized by vortex for 2 min. The mother solution obtained is recovered in a sterile bottle and left at laboratory temperature for 45 min for virus inversion (ISO 6687: 1983). The mother solution is at 10^{-1} dilution. The other solutions are obtained by introducing 1 mL of the mother solution directly into 9 mL of physiological water until 10^{-3}.

Microbiological analysis of alimentary products: The microbiological analysis is performed in our laboratory based on the microorganism's culture technique in order to cultivate selectively the desired bacterial strain. This culture depends on the temperature and incubation conditions corresponding to each bacterial strain and selective culture medium.

Used media: The culture media used in this analysis are liquid and solid media for which there is, if needed, addition 15% of agar. The used media are grouped in Table 1. Before, they are sterilized by autoclaving at 121°C during 15 min.

Table 1: The culture media for the germs

Microorganisms	Culture media
FMAT (Total Aerobic Mesophilic Flora)	TSA (Crypto Casein Soy Agar)
Lactic bacteria	MRS (Man Regosa Charp Agar)
Staphylococus aureus	Chapman
Streptococci	Litsky
Faecal coliforms	EMB (Eosine Methylene Blue)
Total coliforms	EMB (Eosine Methylene Blue)
Escherichia coli	EMB (Eosine Methylene Blue)
Salmonelles	SSA (Salmonella Shigella Agar)
Mold and yeasts	Sabouroud
Clostridium botulinum	Clostridium agar

Table 2: Culture condition for the desired germs

Microorganisms	Temperature and incubation time (°C/h)
FMAT (Total Aerobic Mesophilic Flora)	30/48
Lactic bacteria	30/24
Staphylococus aureus	37/24
Streptococci sp.	37/24
Faecal coliforms	45/48
Total coliforms	37/24
Escherichia coli	44/24
Salmonelles	37/24
Mold and yeasts	20/72
Clostridium botulinum	37/48

Microorganisms searched and their condition of culture: The main species searched are: FMAT, total and faecal coliforms, faecal Steptococci, fungal flora (mold and yeasts) *Clostridiul botulinum*, *Staphylococcus aureus*, lactic bacteria, Salmonella and *Escherichia coli*. The microorgnisms are cultivated in specific conditions (T°C and incubation time) (Table 2).

Determination of the FMAT: The FMATs represent all microorganisms able to developing at temperatures close to 30°C and under aerobic conditions. They reflect the global microbial load of the product to be analyzed and there informs us about the level of hygiene during the manufacture of the product. The counting of the FMAT is done on TSA medium which allows the appearance of all the germs of the FMAT. The incubating is performed at 30°C for 48 h.

Determination of faecal and total coliforms: They are germs accustomed to the digestive tract of man and animals. They are considered as indicators of hygienic quality of the product to be analyzed. The culture medium used is the EMB (Ethylene Blue Eosin). The enumeration is performed after 48 h of incubation at a temperature of 37°C for total coliforms and 44°C for faecal coliforms.

Determination of *Escherichia coli*: The culture medium used is EMB (Methylene Blue Eosin). The enumeration is performed after the agar plates are incubated at 37°C for 24 h.

Determination of Salmonelles: The culture medium used is SSA (Salmonella Shigella Agar). The enumeration is done at 37°C/24h.

Determination of *Staphylococcus aureus* and faecal Streptococci: The presence of these germs in a food is a witness of its non-sanitary. The selective medium used for Staphylococci is Chapman. *Staphylococcus aureus* gives black colonies with a clear halo. The selective medium for them is the Litsky medium. Incubation of both groups is at 37°C. Bacteria numbers count after incubation (24 h).

Determination of sulphite-reducing anaerobes: The searched species in these microbiological analyzes is *Clostridium botulinum* which is responsible for alimentary botulism and because he is usually localized in canned foods. A selective medium is Clostridium medium. About 1 mL of the 10^{-3} dilution of the mother solution is inoculated into the medium on anaerobic plate. Colonies typical of botulinum are found after on prolonged incubation (4 days) in the high temperature 35°C (Esmail *et al*., 2014).

Determination of yeasts and molds: Yeasts and molds are acidophilic microorganisms mostly mesophilic that develop at a temperature of 25-30°C for 3 days of incubation. They are a common cause of food spoilage, especially, acid foods such as fruit juices and foods with low water activity. They are not involved in food poisoning. Mold can also play a significant role in food spoilage. The evaluation of their abundance in a biotope is done by cultivation on the Sabouroud environment. The lecture is realized after 5 days for incubation at 25°C (Barrios *et al*., 2013).

Determination of lactic acid bacteria: Lactic acid bacteria are microorganisms of biotechnological utility. The most recognized medium for their counting is the MRS. The colonies corresponding to this group can be counted after 24 h of incubation at a temperature of 30°C (Esmail *et al*., 2014).

Enumeration of bacteria: The petri dishes, after their incubation for the appropriate time and temperature are taken to count the colonies appearing on the surface of the specific culture medium of each strain. Only the petri dishes whose colonies having the characteristic appearance of the bacterium in question and whose number of colonies is between 30 and 300 are selected for counting.

Physico-chemical analyzes (pH, temperature and acidity)

Temperature measurement: We measured the food temperature on the spot using a digital thermometer. The products are taken from the points of sale already mentioned and the displayed values are noted on a notebook.

Table 3: Methods of analysis of organoleptic characteristics

Organoleptic characters	Analysis method used
Taste, flavor, smell and aroma	Sensory and taste evaluation
Color	Visual evaluation

PH measurement: For liquid products, 10 mL is taken and poured into a sterile beaker. While for solid products, 10 mL is taken from the mother solution already prepared. The electrode is calibrated at pH 4 and 7.

Acidity determination: The acidity is determined by titrimetry. 10 mL of the solution is titrated with a 0.1 N NaOH solution using a Mohr burette with a tap. The volume of NaOH stopped for the calculation of the acidity is that read on the column of the burette after turning the color indicator (phenophthalein 1%).

Measurement of organoleptic and olfactory taste criteria: The organoleptic and olfactory taste characteristics (flavor, texture, taste and smell) were realized on site. The following table summarizes the parameters studied (Table 3).

RESULTS AND DISCUSSION

Among 10 sales units of these products, we recorded the non respect of packaging of packaged food at 6 traders following the malfunction of their refrigerators against 4 that meet the standards of food preservation.

Microbiological analysis of alimentary products: The 95 food items were purchased from different points of sale near the school. A product is considered for us as a sample. Each sample is composed of 5 units. The result of an analysis corresponds to the average of the 5 analyzes. At the same time, a reading of the information on the labeling has been done. As a result, all the products analyzed contain food additives with the exception of dairy products. The results of the microbiological analyzes are shown in Table 5. The values obtained are compared with the limit values set by the international standard (Ali, 2011).

Reading the results of physicochemical and microbiological analyzes: The results of physicochemical analyzes for pH, temperature and acidity are shown in the following Table 4. The physicochemical analyzes of the samples taken showed that 20% of the samples are kept at a temperature of 10°C, it is perishable foods such as milk and its derivatives while 80% of the other products tested were in an average temperature. of the order of 21°C. For the pH, the lowest values are recorded for soft drinks and the highest values are

recorded for solid products such as biscuits and chocolates. It appears that 90% of the foods sampled have an acidic pH which favors their conservation against the bacteria of deterioration but that does not prevent that the presence of certain micro-organisms like the yeasts and the molds in food acids. For microbiological analysis, the results are summarized in the following Table 5.

The purpose of this study is to demonstrate the presence or absence of germs in the foods analyzed and if they exist, determines their abundance in each food product. For the conformity of the test, three repetitions are made. The results obtained showed that all the samples analyzed are free of pathogenic bacteria (no total and fecal coliforms) such as *Staphlococcus aureus*, Salmonella and *Escherichia coli*. The presence of this type of germ in a foodstuff could only lead to nuisances for the health of the consumer. The absence of Clostridium in our samples parallels the results found by McGlynn who noted that *Clostridium botulinum* germs are lacking in biotopes where the pH of foods is 4.6 or less. Case of analyzed products.

The other microorganisms are absent in most of the samples and when they are present they are less than the values declared by the microbiological standards they are generally $\leq 10^1$ CFU mL^{-1}). Thus, we are limited to presence or absence of the desired germs regardless of their abundance. For strains of biotechnological interest, case of lactic acid bacteria and yeasts they are present in 20% of food products. For the lactic acid strains they are found with the following values: 0.4×10 CFU mL^{-1} in canned gherkins, 0.3×100 CFU mL^{-1} in canned olives 0.6×100 CFU mL^{-1} in yogurt and 10^1 CFU mg^{-1} in the cheeses. While for yeasts their presence is noted in four products, the values found are: $0.2.10^1$ CFU mL^{-1} in concentrated tomatoes, $0.3.10^1$ CFU mL^{-1} in apple juice and $0.2.10^1$ CFU mg^{-1} in fruit juice, cheeses. Dairy products (cheese and yoghurt) and brine products (olives and pickles) are the biotopes most appreciated by these sprouts. The presence of this type of bacteria is accompanied by a drop in pH ($\leq$4.6). The consequence is an inhibition of the proliferation of harmful microorganisms.

In sugary drinks (in the case of juices and nectars) we noted the presence of traces of yeast (apple juice $0.3.10^1$ CFU mL^{-1} and nectar $0.4.10^1$ CFU mL^{-1}). The presence of the latter has no impact on the health of the consumer. This is confirmed by anther result (Baysal *et al*., 2013). The latter mentioned that fruit juices were biotopes favorable to the multiplication of yeasts. For mesophilic flora, analyzes carried out on packaged foods showed the presence of a small number of FMATs (total aerobic

Table 4: Physicochemical analyzes of foods

Food stuffs (N.S)	T°C	pH	Acidite
Orange juice (5)	20	3.8	1.34
Soda (apple) (4)	10	2.9	0.72
Concentrated tomato (5)	21	4.4	0.56
Apple juice (4)	22	4.2	0.74
Casher (5)	23	3.9	1.05
Soda (lemon) (4)	10	2.7	0.70
Pickle (conser) (5)	20	4.6	0.58
Olives (conser) (5)	22	3.9	0.97
Soya sauce (5)	22	4.6	0.95
Yaourt (5)	10	4.6	0.92
Candy (5)	22	4.2	0.54
Jam (4)	21	3.5	1.07
Nectar (5)	23	3.7	1.09
Cheese (5)	10	3.6	1.31
Mayonnaise (5)	22	4.5	0.64
Ketchup (5)	22	4.5	0.56
Biscuit (5)	22	7.0	0.67
Chips (5)	23	4.5	0.50
Chewing-gum (5)	22	4.6	0.43
Chocolate (4)	20	6.6	0.21

NS: Number of Samples

Table 5: Microbiological analyzes of sampled foods

The analyzed products	FMAT	Clos. B	Sta. A	Sal	Col. F	B. Lact	Strept	YM
Orange juice (5)	$0,2.10^1$	-	-	-	-	-	-	-
Soda (Appel) (4)	-	-	-	-	-	-	-	-
Concentrated tomato (5)	$0,7.10^1$	-	-	-	-	-	-	$0,2.10^1$
Apple juice (4)	$0,1.10^1$	-	-	-	-	-	-	3
Casher (5)	-	-	-	-	-	-	-	-
Soda (lemon) (4)	-	-	-	-	-	-	-	-
Pickle (conser) (5)	$0,7.10^1$	-	-	-	-	$0.4.10^1$	-	-
Olives (conser) (5)	$0,5.10^1$	-	-	-	-	$0.3.10^1$	-	2
Soya sauce (5)	-	-	-	-	-	-	-	-
Yaourt (5)	$0,6.10^1$	-	-	-	-	$0.6.10^1$	-	-
Candy(5)	-	-	-	-	-	-	-	-
Jam (4)	-	-	-	-	-	-	-	-
Nectar (5)	1	-	-	-	-	-	-	4
Cheese (5)	$0,1.10^1$	-	-	-	-	10^1	-	-
Mayonnaise (5)	-	-	-	-	-	-	-	-
Ketchup (5)	-	-	-	-	-	-	-	-
Biscuit (5)	-	-	-	-	-	-	-	-
Chips (5)	-	-	-	-	-	-	-	-
Chewing-gum (5)	-	-	-	-	-	-	-	-
Chocolate (4)	-+	-	-	-	-	-	-	-+

NB: +: Presence, -:Aabsence; CF: Fecal Coliforms; Clos. Bo: *Clostridium botulinum*; FMAT: Mesophilic Flora Total Aerobic ; Staphy: *Staphylococcus aureus*; Sal: Salmonella; Col. F: Fecal Coliforms; Strep: *Streptococus* sp.; Bac. Lac: Lactic acid Bacteria; YM: Yeasts and Molds

mesophiles). The abundance obtained is insignificant and does not even reach the threshold set by the microbiological standards for food products. The values found are of the order of $0.2.10^1$ CFU mL^{-1} in orange juice, $0.7.10^1$ CFU mL^{-1} in concentrated tomatoes, $0.1.10^1$ CFU mL^{-1} in apple juice, $0.4.10^1$ CFU mL^{-1} in canned gherkins, $0.5.10^1$ CFU mL^{-1} in canned olives, $0.6.10^1$ CFU mL^{-1} in yogurts and soy sauces, $0.1.10^1$ CFU mL^{-1} in apple nectars and cheeses. All the values found during the analyze are lower than 101 CFU mg^{-1} or CFU mL^{-1} of the analyzed food products. The percentage of food analyzed and noted negative vis-a-vis the microbiological threshold exceeds 95%. This shows that the origin of food poisoning due to the consumption of food products is not microbiological.

In foods containing lactic bacterial flora, we have noted the presence of either lactic acid bacteria alone or accompanied by fungal flora in contrast to the products free of these lactics, we noted the presence of FMAT. This can be explained by the fact that lactic acid strains, secreting metabolites such as bacteriocins or terpene derivatives in the product medium have an inhibitory effect against germs harmful to these foods. On the organoleptic side, we noted the presence of bad taste in 80% of the tested foods. This change in taste is mainly due to the temperature rise of the storage and the deadline of consumption, that has been recorded in 50% of food and which has been behind the change in the nutritional composition of the food product and subsequently changing his taste.

CONCLUSION

Despite the poor storage conditions of food products in the premises dedicated to the sale and exceeding the deadline for the consumption of these products all the food samples analyzed were 100% free of pathogenic germs and alteration. However, the finding has proved the presence of some alteration microorganisms which although, present do not exceed the thresholds declared by the food safety standard. On the organoleptic side, the impact was marked, especially in terms of taste. While for physicochemical parameters, the measured storage temperature was high, it exceeded 20°C and very likely it was behind the change in taste. As a result, the situation seems to be reassuring for the consumer in terms of the microbiological quality of the products they consume. However, it remains for the traders of these foodstuffs to take account of good conservation practice and verification of expiry dates.

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