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PHYSICAL AND MICROBIAL DISCRIMINATION BETWEEN FOOD GRADE SODIUM CHLORIDE AND EL-SAYAHAAT SALT.

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ABSTRACT

The problem at hand is dealing with the possibility of discrimination between El-sayahaat salt and other edible food grade sodium chloride sources. Such trend was considered due to the harmful effect of El-sayahaat salt which related to the presence of higher concentration of heavy metals. To achieve such goal, instrumental analysis based on X-ray fluorescence, X-ray diffraction and Infrared technique were considered in order to realize and identify the heavy metals as well as the functional groups of the investigated samples. The obtained data were statistically analyzed according to the principal component methods by which El-sayahaat salt was successfully discriminated in relation to the other NaCl sources. It was also found that cadmium (Cd) was only identified in El-sayahaat salt (2.3ppm) accompanied with high concentration of lead (Pb, 20ppm), Iron (Fe, 13ppm) and mercury (Hg) that reached 1.0 ppm represents ten times that of the non-private sector (NPS). With respect to the IR spectra the most pronounced functional group that could act as a finger print of El-sayahaat salt was identified at wave number 1540.84 cm^{-1} of 32.2% related to the covalent sulfate. On the other hand El-sayahaat salt sample was found to be microbially contaminated; while the other four investigated NaCl salt sources were considered to be safe from any microbial contamination.
Keywords: Sodium chloride -X-ray– Infrared – Heavy metals - Microbial aspects

INTRODUCTION

Table salt (sodium chloride) is considered to be one of the strategic important commodities in the world, whether from the alimentary point of view or the industrial one. It is also one of the serious elements since it is necessary for balancing the salt solution of the body systems to do their biological functions in a best-fit way. On the other hand, common edible salt has an important role as food additive in preparation and processing of food products. Its function includes one or more of the following aspects: flavoring, preservation, formation of a desirable texture by solubilization of food proteins, controlling the rate of fermentation and reduction of water absorption in bread and bakery products (Crocco,1982 and Schmidt, 1988).

Controlling of NaCl levels in human diets is very important since excessive consumption of salt has been related to the disorder of physiological conditions such as high arterial pressure (Herrador *et al*., 1998).

Toxicological studies have also indicated that NaCl may have mutagenic, clostogenic and cytotoxic effects in laboratory animals (Fujie *et al*., 1990).

Zidan *et al*. (1998) reported that food grade salt should be free from contaminants that may be harmful to the consumer health. In particular the following maximum limits recommended by Codex Alimentarius (1991) should not be exceeded in the produced NaCl: As (0.5), Cu (2), Pb (2), Cd (0.5) and Hg (0.1) mg/kg.

The Egyptian standard specifications of the edible sodium chloride, (1996) indicated that the following maximum limits should not be exceeded: Fe (10), Cu (2) and Pb (2) ppm.

El-Sayahaat salt could be defined as a surface salt layer that usually precipitated in deep ground points which covered with saline like solutions. Such source of salt may be produced from a mixture of seawater, lake and under ground water, as well as the drained water of agriculture lands. The sodium chloride that withdraws from the aforementioned sources is not passing through the usual required technological treatment carried out for producing the edible food grade NaCl. Subsequently, the precipitated salt layer of this mixed water may contain several dissolved substances, contaminants, toxic material and heavy metals, which lead to a real harmful effect on consumers health.

The present work focuses on the resolution of a map of heavy metals and trace elements that may be presented in the edible table salt (NaCl) collected from different sources. Such tool beside the microbial load were used to discriminate between the investigated edible food grade NaCl and El-sayahaat salt. Instrumental analysis such as X-ray diffraction, X-ray fluorescence and infrared spectra will be applied to reach such goal.

MATERIALS AND METHODS

Materials

Four commonly used samples of edible food grade NaCl produced by different manufacturers and marketed in Egypt were collected randomly from different places:

- A sample of Non-Private Sector (NPS) that produced table salt for local production (net weight 500g) were collected from Alexandria Governorate.
- A Private Sector sample (PS) of edible food grade salt of local production was obtained from 6-October city, Giza Governorate.
- Imported table salt (samples A and B from Jordan and Saudi Arabia, respectively) which repackaged in Egypt under the Egyptian standard specification No.2732/96 and marketed in Cairo Governorate.
- El-sayahaat salt sample was obtained from a local market of Port Said Governorate.

Analytical methods

1. X-ray diffraction

X-ray diffraction was performed according to the method of Whiston (1987) using X-ray apparatus available at the Central Services Laboratory of the National Research Center, Cairo, Egypt.

X-ray diffractograms were obtained with a computer controlled X-ray diffractometer (formally made by Diano Corporation, USA). The range of "2θ" measuring was from –20 to +150°. The reproducibility "2θ" was ±0.01° and the diameter of the measuring circle is 401mm. The detector was scintillation counter with a dead time of less than 10^{-6} second. Iron filtered cobalt radiation was used and the X-ray tube was energized at 45 KV and 10 m.A. The X-ray diffraction patterns were recorded automatically using XPEX software which indicating in each pattern the "2θ" values of each diffraction line and its corresponding "d-value" with its relative intensity "I/Io".

2. X-Ray fluorescence

X-ray fluorescence of the investigated five samples was performed using the X-ray apparatus Model 84200/10 produced by the Applied Research Laboratory, Switzerland. Such technique was used to determine heavy metals and minerals. Experiments were carried out at the chemical department of the X-ray flourescence; laboratory of the Egyptian Organization for Standardization and Quality Control, Ministry of Industry and Technology (Whiston, 1987).

3. Infrared spectrum

The infrared spectra of the investigated NaCl samples were recorded after being hold on a potassium bromide disk of the Philips Pu 9712 Infrared Spectrophotometer available at the Central Services Laboratory of the National Research Center,Cairo,Egypt .

The functional groups of the investigated samples were identified according to Pomeranz and Meloan (1994).

4. Microbiological analysis

Twenty-five gram samples of each of the tested NaCl salts were suspended under aseptic conditions in 225ml of peptone water (0.1%). A serial decimal dilution was made using the same medium and plated onto growth media in duplicate. Microbial groups (Aerobic mesophilic bacteria, Lactic acid bacteria, Yeasts and Moulds, Salmonella spp., staphylococci, Coliform bacteria, Halophilic bacteria and Spore forming bacteria were determined according to American Public Health Association for food stuff examination (APHA, 1992).

5. Statistical analysis

Statistical analysis of the data in the form of principal component analysis was carried out at Food Science Dept., Faculty of Agric., Ain Shams University, Cairo, Egypt according to Piggott (1986) using the "CSS" program given by Statsoft (1991).

RESULTS

1- X-ray analysis

X-ray analysis of the investigated NaCl salt samples was performed by the more precise two methods. The first one was based on the crystal structure within diffraction pattern of the X-ray from which specific values of "d-spacing", angle of diffraction, I/Io and count per second were compared as illustrated in Figs. (1-3). The second approach of the X-ray analysis was given in terms of X-ray fluorescence from which a major number of 16 elements were clearly identified as given in Tables (1 and 2).

1.a- X-ray diffraction

With respect to X-ray diffraction, the available data given in Figs. (1, 2 and 3) indicated the main following points:

X-ray diffraction of El-sayahaat salt sample was characterized by the presence of 10 "d-spacing" starting from 5.6273 at angle (2θ = 18.31) with a count per second of 73.87 and 0.2 for I/Io. The corresponding value of the "d-spacing" 1.1519 being as follows: 101.99, 72.10 and 0.5 as seen in Fig. (3). The 100 level of I/Io was found to be at angle 37.09 with a count per second of 15389.31 and specific "d-spacing" of 2.81459.

The X-ray diffraction of the non-private salt (NPS) sample was characterized by a "d-spacing" of 2.8209 at angle 2θ = 37.01 with the level of 100% of the I/Io value and 1111 3.56 count per second (Fig.1).

The private salt (PS) sample was the only one that indicated a "d-spacing" of 4.92387 at angle 2θ = 20.95 with a value of 0.3 for I/Io and 60.80-count/ sec. The 100% of I/Io were also detected at the angle 2θ = 37.03 with a value of 2.81876 for "d-spacing" and 18637.95 count per second.

The imported sodium chloride (samples A and B) realized the highest 100% of the I/Io at angles of diffraction of 2θ = 36.99 and 37.03 with a "d-spacing" of 2.82162 and 2.81850, respectively as shown in Fig. (2). There was also a real difference between the values of count per second of the two previous samples being about 19330 for the former sample and only 9681 for the latter one. Once the aforementioned analysis and the data illustrated in Figs. (1,2 and 3); the angle of diffraction which possess the 100 I/Io was found to be of a highest angle of diffraction (2θ = 37.09) for the El-sayahaat salt sample and approximately similar to of all the other tested salt samples. In other words, the crystal structure of the four NaCl salt sources under investigation in addition to that of El-sayahaat was too close to the level that differentiation between the samples was very complicated. Subsequently, X-ray fluorescence was applied to detect the mineral content of these samples.

1.b- X-ray fluorescence

It is well known that some minerals are used in relatively large amounts, up to 100mg/day such as sodium, potassium, and calcium. Others are required in amount of only few milligrams/ days, and are usually termed trace elements such as zinc, copper and chromium. Minerals that are present in the body in relatively large amounts and have a well-defined physiological function include calcium, iron, iodine, potassium, sodium and phosphorus. A second group are those which acting as parts of enzyme system (Ranken, 1984).

Fig1

fig2

fig3,4

With respect to the data given in Table (1) and with the exception of El-sayahaat salt, the following heavy metals: Fe, Cu, Pb, As, Hg and Mn were found to be within the acceptable limits in the other four NaCl salt samples collected from local market (either produced locally or those imported one) according to the Egyptian Standard specification of edible NaCl salt (1996).

triplicate determinations.

It is clearly noticed from Table (1) that El-sayahaat salt sample contained high levels of heavy metals being 20 ppm for lead and 13 ppm for iron such pattern indicates the hazard and risk of using such salt, especially for human consumption or within any food processing purposes. From the available data of the same Table cadmium (Cd) was not detected in local (NPS and PS) as well as in the imported NaCl salt samples; while El-sayahaat salt contained 2.30 ppm of cadmium. On contrary, a different trend was noticed concerning the concentration of Zn which was found in all of the edible salt samples to be of higher concentration but in El-sayahaat sample reached the lowest value being 5.00 ppm. It is of importance to clarify that zinc and cadmium tend to have a consistent relationship to each other and generally have contrasting effects in experimental animals. Schroeder and Buckman (1967) reported that administration of zinc to rats reversed the hypertension induced by elevated cadmium intakes. They suggested that an increased ratio of cadmium to zinc in the renal cortex is associated with the increment of blood pressure in rats and humans. In the present investigation it is worthy to notice the increase of Cd/Zn ratio in El-sayahaat salt sample (2.30: 5.00); could strengthen the presence of hazards and risks of consuming such type of salt.

Table (1): Identified heavy metals* (ppm) in the investigated NaCl samples Based on X-ray fluorescence.

Heavy metals (ppm)	Sources of NaCl samples				El-sayahaat salt
	Local Production		Imported		
	(NPS)	(PS)	A	B	
Fe	7.50	3.50	4.90	1.70	13.00
Cu	1.30	1.10	0.17	1.20	4.00
Pb	0.15	0.45	0.12	0.18	20.00
As	0.55	0.28	0.59	0.43	2.00
Hg	0.14	0.39	0.46	0.26	1.00
Mn	4.90	4.80	18.00	3.90	10.00
Cd	--	--	--	--	2.30
Zn	13.80	16.00	14.00	17.00	5.00

NPS: Non-private sector PS : Private Sector * = Average values of triplicate determinations

Data in Table (2) assured the presence of high values of Na^+ cation and Cl^- anion in the edible NaCl salts collected from local and imported samples i.e. ranged from 41.30 - 44.30% for (Na^+) and from 55.40 - 58.00% for (Cl^-). On contrary, concentration of sodium chloride in El-sayahaat sample was not exceed 94.10%; a result which was less than 98% that reported in the Egyptian Standard No. 2732/1996. This finding was in accordance and coincides with those obtained by El-Ahwal *et al*. (1998).

2.Infrared spectrum

Identification of materials by means of their infrared spectra is so certain and convenient. Its chief advantages lie in the certainly of the conclusion even with slightly impure materials. It is also well known that although the molecular vibration is responsed to the whole molecule, it is sometimes related to one section of the molecule which if occurred in different items, the associated infrared absorption will re-appear at the same frequency with approximately the same intensity. This happens so reliably that the presence or absence of the functional chemical groups could be assured (Pomeranz, and Meloan, 1994).

The functional groups of the investigated samples are given in Table (3) which showed the following trends: The functional groups that appeared only in the non-private NaCl (NPS) were noticed at wave numbers of 111, 121, 152, 169, 345, 1103, 1637 and 3419 cm^{-1}. With the exception of the functional group at 152 cm^{-1}, the other aforementioned groups are not present in El-sayahaat salt as seen in the same Table. The numbers of the functional groups are 16 in the private NaCl sample (PS) against 12 for the imported NaCl sample (A) and 18 for the other imported salt; sample (B).

El-sayahaat salt is characterized by the presence of four functional groups. Two of which possess a weak absorption at wave numbers 308.55 and 3417.24 cm^{-1} and the most probable functional group for the latter wave number is sulfonic acid; $R\text{-}SO_3H$ as indicated by Pomeranz and Meloan (1994). On the other hand, there are other two groups of 8.3 and 8.8 transmittance % at wave numbers 1141.65 and 1635.34 cm^{-1} corresponding to SO_4 (Ionic sulfate) and $R\text{-}SO_3$ (Ionic sulfonate). The most pronounced functional group that could act as a finger print of El-sayahaat salt was identified at wave number 1540.84cm^{-1} of 32.2 transmittance % which related to covalent sulfate ($R\text{-}O\text{-}SO_2O\text{-}R$) as seen in Table (3).

2

3

4. Microbiological analyses

Standard parameters that matched with good quality of sodium chloride showed the sample to be un-infected with halophilic bacteria, low in calcium, magnesium and iron content of good white color and free from foreign matter (Ranken 1984).

The counts of the different microbial groups which expected to contaminate the investigated NaCl samples are shown in Table (4). The local “NPS” and “PS” as well as the imported sodium chloride were free from salmonella, staphylococci, coliform, halophilic, lactic acid bacteria, spore forming bacteria, yeasts, moulds and total viable cells. These results are in agreement with Crocco (1982) who proved that high salt concentrations were lethal to many pathogens such as salmonella, staphylococci. Marshall and Odame-Darkwah (1995) reported that, the growth of microorganisms was poor at a level of 80g NaCl/ L (broth media) and there was no growth at 90-100 g/L. Rozes and Peres (1996) indicated that L. planetarium cells tolerated 4% (W/V) NaCl, but addition of 6% (W/V) caused an increased long phase in cell growth and 8% (W/V) was found to be totally inhibitory to cell growth. In addition, it is well known that sodium chloride was used as antimicrobial as well as a chemical preservative agent.

The only sample, which was found to be microbially contaminated, was El-sayahaat salt. The counts of total viable cells as well as spore forming bacteria are $5x10^2$ CFU/g and $8x10^1$ CFU/g respectively.

Table (4): Average of microbial counts (CFU/g) in the investigated NaCl samples and El-sayahaat salt.

Microbial Groups	Sources Of NaCl Samples				
	Local production		Imported		El-sayahaat
	(NPS)	(PS)	(A)	(B)	
Total aerobic bacteria	--	--	--	--	$5x10^2$
Spore forming bacteria	--	--	--	--	$8x10^1$
Yeasts and Moulds	--	--	--	--	--
Lactic acid bacteria	--	--	--	--	--
Coliform bacteria	--	--	--	--	--
Salmonella spp.	--	--	--	--	--
Staphylococcus spp.	--	--	--	--	--
Halophilic bacteria	--	--	--	--	--

NPS: Non-private sector **PS: Private sector**

This result is going parallel with those mentioned by Zidan *et al*. (1998). Although, the microbiological analysis was only positive for El-sayahaat salt but it is recommended and important to stress upon the application of the applied physical trend beside the microbial analysis to achieve the goal and assure the differentiation between edible NaCl and El-Sayahaat salt.

DISCUSSION

The investigated sodium chloride sources were analyzed for their heavy metal and element constituents within a randomized arrangement according to the experimental design. By comparing the available data of Table (1) it could be stated that El-sayahaat salt exhibit the lowest content of Zn (5 ppm) and the highest level of the other identified heavy metals i.e. Fe (13.0 ppm) and Pb (20.0 ppm) in relation to the other tested samples. Such trend of analyses is surely related to the way by which El-sayahaat salt was produced. In other words, El-sayahaat salt contains contaminants of heavy metals which may lead to the fact that it contains only 94.1% sodium chloride (61.7% Cl^- and 32.4% Na+). The presence of 4.21%Mg and 0.729% S and 0.548 % Ca that reported in Table (2) assured the previous conclusion. Subsequently, on the basis of analyzing Cl^- and Na+ content of the tested salts, it could easily differentiate between El-sayahaat salt (94.1% NaCl) and the other salt sources in which the corresponding concentration exceeds 97% as seen in the same Table.

A successful method was created to distinguish between El-sayahaat salt and the other edible food grade NaCl. The method was based on the performance of data matrix in which the different salt sources was organized randomly to format the chemometric way of discrepancy published by (Piggott, 1986). In such a case, each salt sample is considered to be an assembly of eight descriptors of the identified heavy metals given in Table (1). By using the statistical program 'CSS' given by Statsoft (1991) the data trend was visualized by Principal Component Analysis (PCA) as given in Table (5).

Table (5): Principal Components Analysis (PCA) of the tested heavy metal.

Identified heavy Metals	Percent of variance	Cumulative percentage
Fe	81.7085	81.7085
Cu	14.3393	96.0478
Pb	3.5044	99.5521
As	0.4479	100.0000
Hg	0.0000	100.0000
Mn	0.0000	100.0000
Cd	0.0000	100.0000
Zn	0.0000	100.0000

These two principal components explained up to 99.55 of the data variance and when "PC I" and "PC II" were illustrated in scores plot, it is easily succeeded to differentiate between El-sayahaat salt and other edible salts as seen in Fig (4). Such type of illustration succeeded and enables the possibility of discrimination between El-sayahaat salt and the other NaCl sources. As it can be observed from the data of Table (6) El-sayahaat salt that completely separated to the left corner in Fig. (4) having a value of 0.019241 for the PC I and the PC II reached 0. –99268 as seen in Table (6).

It is of interest to discriminate between the different salt sources on the multiple R^2 based on the principal components; PC I and PC II. All of the tested NaCl samples having a value of R^2 which is greatly higher than El-sayahaat which had only R^2 of 0.5725 inspite of its higher content of heavy metals.

This result indicated that the NaCl sources are characterized by a natural and native matrix of their mineral constituents leading to the presence of a higher correlation co-efficient. Such pattern is out of control in El-sayahaat salt and so, leading to a lower R^2 value.

Table (6): Factorial Analysis Of The Different Salt Sources And Their Heavy Metal Contents.

Salt Sources	Principal Component Based on Salt Sources	
	Factor I	Factor II
IMP_A	0.829865	-0.177765
NPS	0.959947	-0.070249
El-sayahaat	0.019241	-0.992686
IMP_B	0.963629	0.123747
PS	0.987396	0.045557

	Communalities of Principal Components		
	From I Factor	From II Factors	Multiple R-Square
IMP_A	0.688676	0.720277	0.901184
NPS	0.921498	0.926433	0.994059
El-sayahaat	0.000370	0.985795	0.572498
IMP_B	0.928582	0.943895	0.999356
PS	0.974951	0.977026	0.999646

Heavy Metals	Principal Component Based On Heavy Metals	
	Factor I	Factor II
F	0.152309	-0.91158
Cu	-0.472025	0.50565
Pb	-0.652135	-1.81273
As	-0.575234	0.76350
Hg	-0.602336	0.91128
Mn	0.613910	-0.67019
Cd	-0.660396	0.72509
Zn	2.195906	0.48898

IMP A = Imported Salt Sample A. IMP B = Imported Salt Sample B.NPS = Non-private Sector. PS = Private Sector.

CONCLUSION

The obtained results based on instrumental analysis; i.e. X-ray diffraction, X-ray fluorescence and infrared spectra succeeded in discriminating between El-sayahaat salt and the other investigated four NaCl samples. The point that should be concerned is, the place from which El-sayahaat salt is obtained. Such point of view is related to the area of El-sayahaat as well as the other environmental surroundings and conditions that will affect on the pattern (type and concentration) of the tested heavy metals.

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التفرقة الطبيعية والميكروبية بين ملح الطعام المتداول (كلوريد الصوديوم) وملح السياحات

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يهدف البحث إلي إمكانية التفرقة بين ملح الطعام المتداول وملح السياحات وقد أخذ هذا الاتجاه في الاعتبار نظرا للتأثير الضار لملح السياحات٠

ولإنجاز هذا الهدف تم إجراء التحليلات بواسطة الأشعة السينية (X-ray diffraction) ، (X-ray fluorescence) وكذلك الأشعة تحت الحمراء IR وذلك للتعرف علي المعادن الثقيلة والمجموعات الوظيفية المختلفة لعينات ملح الطعام المتداول والمأخوذ من مصادر مختلفة وعينة ملح السياحات٠

وتم تحليل النتائج المتحصل عليها إحصائيا تبعا لطريقة ال Principal Componentحيث أمكن تفرقة ملح السياحات بنجاح عن باقي عينات ملح الطعام الأخري٠

وأوضحت النتائج أن عنصر الكادميوم Cd وجد فقط في ملح السياحات بتركيز ٢,٣ جزء في المليون مصاحبا لتركيزات عالية من : الرصاص Pb (٢٠ جزء في المليون) ، الحديد Fe (١٣ جزء في المليون) والزئبق Hg بتركيز (١ جزء في المليون) يمثل حوالي ١٠ أمثال تركيزه في عينة الملح المنتجة محليا (Non-private sector (NPS ٠

وبالنسبة لقياسات الأشعة تحت الحمراء ال IR فإن المجموعة الوظيفية التي تمثل بصمة مميزة وواضحة لملح السياحات تم التعرف عليها عند طول موجة قيمته ٨٤،١٥٤٠ سم-١ بمعدل انتقال (Transmittance) ٢،٣٢% وهي تتعلق بمجموعة ال Covalent Sulfate ٠

كما وجد أن ملح السياحات ملوث ميكروبيا بينما الأربع عينات ملح الطعام المتداولة الأخرى تعتبر آمنة من أي تلوث ميكروبي .

Table (2): Identified minerals* in the investigated sodium chloride (NaCl) samples and El-sayahaat salt based on X-ray fluorescence.

Identified Minerals	Sources of NaCl Samples												El-sayahaat Salt		
	Local Production						Imported								
	(NPS)			(PS)			(A)			(B)					
	a	b	c	a	b	c	a	b	c	a	b	c	A	b	c
Cl	105.9	117	58.0	110.06	123.0	56.8	110.39	124.0	55.4	104.5	119.0	56.0	101.18	116.0	61.7
Na	171.62	84.9	41.9	183.15	93.2	43.0	194.13	99.2	44.3	176.16	87.8	41.3	121.01	60.6	32.4
Mg	-	-	-	0.718	0.383	0.177	1.223	0.683	0.305	10.655	5.44	2.56	19.47	7.89	4.21
S	-	-	-	-	-	-	-	-	-	0.728	0.263	0.124	3.944	1.37	0.729
Ca	0.386	0.235	0.116	-	-	-	-	-	-	-	-	-	1.671	1.03	0.548
K	-	-	-	-	-	-	-	-	-	-	-	-	0.543	0.640	0.342
I	0.029	0.045	0.022	-	-	-	-	-	-	-	-	-	0.029	0.050	0.027
Si	0.029	0.016	0.008	-	-	-	-	-	-	-	-	-	0.043	0.021	0.011

a = Net intensity as KCPS. **b = Actual concentration (%).** **c = Normalized concentration (%)**
NPS = Non private sector. **PS = private sector.** **- = Not detected.** *** = Average values of triplicate determinations.**