CHEMICAL ANALYSESOF SOME FOOD SAMPLES AND EFFECT OF THEIRHEAVY METALS ON MICRO-ORGANSIMS

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ABSTRACT

Heavy metal is a group of metals has atomic density greater than 5.0 g/cm^3, usually associated with pollution and toxicological problems .Six food samples were chosen for this study. These samples are two canned, two frozen and two fresh samples. Rutin chemical analysis was carried out including, moisture, ash, lipid, crude protein and total carbohydrates contents.Elementary analysis was also applied including five elements;Fe , Ni, Co, Cd and Pb . Results indicated that frozen shrimp contained the highest amount of moisture, protein and ash. Average values of 21.0, 54.80 and 15.50 % was detected, respectively. While, canned luncheon showed to have the highest content of Fe, Pb and cadmium to be 416.3, 2.33 and 6.78 ppm, respectively.

The effect of the above mentioned elements on yeast growth (*Saccharomyces cerevisae*) and six bacterial strains, two Grampositive *Bacilluscereus&Staphylococcus aureus* and four Gram negativeof pathogenic bacteria,*Shigellaflexineri* , *Enterobactersakazaki, Salmonella* sp. *and Escherichia coli*, and two concentration from every metal was investigated. In the experiments, yeast and six isolates of bacteria has been cultured in petri dishes which supplemented with different concentration of Fe , Ni , Co, Cd and Pb individual and incubated at 30°C and 37°C/48 h for yeast or bacteria respectively. Yeast and bacterial growth was measured using diameter of inhibition zone. Findings obtained from this study indicated that yeast growth reduced at presence of 1.0mM /L concentration of 3150 ppm and 6300 ppm Fe in comparison with control. Growth of all bacterial isolates were inhibited by Fe . Also Cobalt and Cadmium inhibited the growth of some bacteria while no effect on growth was observed with yeast.

Key words:Chemical analysis, Heavy metals, Toxic effects, Pathogenic bacteria, Inhibition growth of microorganisms.

INTRODUCTION

Heavy metals are spread in the environment, as aresult of both natural and anthropogenic activities andhumans are exposed to them through various pathways, especially food chain**(FDA2001).**

Heavy metals are classified in two classes. The first is essential elements which have vital biochemical or enzymatic activities in human body e.g Fe, Mn, Mo, Cr, V and Zn. The second class is the non essential elements that with no biological,

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chemical and physiological importance to man e.g Cd, Pb, As and Hg **(Itodo*et al.*, 2009)**.Heavy metals become dangerous substance because of bioaccumulation system. Heavy metals Pb and Cd contamination in the digestive tract will be absorbed by the body and it spreads throughout the body tissues by blood and the metal will accumulate in body organs, especially kidneys and liver **(Blanco-Pened*et al.*, 2009)**. The amount of heavy metals contaminated goes into the body is just a little, but when it is accumulated, it will cause various health problems in humans **(Raikwar*et al* ., 2008)**. The risk of heavy metal contamination in meat is of great concern for both food safety and human health due to the toxic nature of these metals at relatively minute concentrations **(Santhi*et al.*, 2008)**.Heavy metals have an important role in different biochemical reactions and are poisonous for cells in high concentrations **(Nies, 1999)**.**Kandeler*et al.*(2000)** found that medium have high levels of metal showed lower numbers of microbes than uncontaminated ones. On the other hand,**(Chamnogpol*et al.,* 2002)** mentioned that high concentration of iron is extremely toxic and may implicate to enhance bactericide effects of antimicrobial agent or toxic substances.Another heavy metal, cadmium which widely distributed in the environment. It can induce multiple toxic effects on tissues and also affected immune response to bacterial pathogens**(Simonet*et al.*, 1984)**. **Damien *et al.,* (2000)** stated that cadmium is known to be a non-essential element and can be toxic at very low concentrations and also ubiquitous in sewage sludges, industrial wastes and mining sites .

These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity **(Roane and Pepper, 2000).**Some of the heavy metals are essential and are required by the organisms as micro nutrients (cobalt, chromium, nickel, iron manganese and zinc etc.) and are known as 'trace elements' **(Bruins *et al.*, 2000)**. Whereas some have no biological role and are detrimental to the organisms even at very low concentration (cadmium, copper, lead, etc.).

These heavy metals not only influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity **(Roane and Pepper, 2000).**Heavy metals may decrease metabolic activity and diversity as well as affect the qualitative and quantitative structure of microbial communities **(Giller*et al.*,1998).**Heavy metals such as cadmium and lead are not readily absorbed or captured by microorganisms. Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions **(Bruins *et al.*, 2000).**Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance **(Poole and Gadd, 1989).**Metals can replace essential metals in pigments or enzymes disrupting their function **(Henry, 2000).**

Iron is an essential nutrient for living agents due to its noticeable activity in electron transport reactions in biological systems, but its insolubility and reactivity lead to problems of poor availability and toxicity, respectively **(Andrews, 1998)**

High concentration of iron is extremely toxic and may implicate to enhance bactericide effects of antimicrobial agent or noxious substances **(Gelvan, 1997, Chamnongpol*et al*., 2002).** Due to insolubility of this element at physiological pH all living agents have involved to use iron transport systems and storage proteins. Bacteria elaborate and secrete high-affinity extracellular ferric chelators (siderophores) and many of them have ferrous iron transporter, to soluble iron prior to transport **(Andrews *et al*., 2003, Köster, 2001).**It was demonstrated that extracellular iron is not the only source of available iron and many bacteria deposit intracellular reserves of this nutrient within iron storage proteins. These iron stores can then be used to enhance growth when external iron supplies are restricted **(Andrews, 1998).**

Vido and coworkers study's indicated that cadmium increases oxidative stress of *S. cerevisiae* and strains which are deficient in antioxidant defense enzymes have a high sensitivity to cadmium **(Vido*et al*., 2001).** Metals are directly or indirectly involve in all aspects of growth, metabolism and differentiation of the biota **(Beveridge and Doyle, 1989).**

The aim of the present investigation is to study the chemical analysis of six food samples as well as estimate five heavy metals i.e. Fe, Ni, Co, Cd and Pb. The effect of the above mentioned elements on some microorganisms was also studied.Seven microbial strains were chosen in this study i.eyeast , two Gram positive and four Gram negative bacteria. These elements were applied on yeast and bacterial strains in effective levels may be similar or higher than that found in the examined food samples i.e canned, frozen and fresh under investigation.

MATERIAL AND METHODS

Source of samples

Six food samples were chosen in this study.Two canned samples i.eluncheon and sauce, two frozen i.e shrimps and molokhia and two freshmackerel and okra. These samples were purchased from local market in Mansoura city, DakahliaProvince, Egypt.

Chemical analyses

Moisture content of the tested samples was determined according to the method described in **AOAC (2005)**. A known weight of powdered sample (2.0 g) was dried at 105°C in an electric oven to a constant weight. Percentage of moisture content was calculated.

Ash content was determined according to **AOAC (2005).** Air dried samples powder (2.0 g) were placed in a silica crucible and ignited at 600ºC in a muffle furnace to obtain white ashed sample. The percentage of ash content was calculated

Crude protein content was determined usingKjeldahl method as described in **AOAC (2005)**. The percentage of total nitrogen was estimated and the crude protein content was calculated by using 6.25 as a factor of protein.

Crude lipid of each powdered sample was determined according to **AOAC (2005)**. A known weight of powdered sample (2.0 g) was extracted in Soxhletapparatus using n-hexane as a solvent for 8 hours. Then the solvent was evaporated and the percentage of crude lipid was calculated.

Preparation of samples and elementary analysis:

Six food samples were subjected to elementary analysis. Some elements were detected in these samples in remarkable amounts.Then, microbiological experiments were made on these elements individually on the form of its mineral salts to be sure that their effect come from this metal only and not from the interference of other components.

Samples were dried in an oven at 60-80°C.Two grams of each dried sample were digested in 10 ml of 1:3 mixture of $HClO_4$: HNO_3 using a hotplate, till clear solution was obtained. The solution was filtered and made up to 50ml with distilled water and kept in aclean plastic bottle until use, **(AOAC 2005).**Elements content were estimated in the clear digested samples using atomic absorption spectrometry(Perkin Elmer Analyst 700).

Preparation of elements

Five elements were chosen in this study i.enickel , cobalt , cadmium , lead and ferrous . These elements were in the form of $Ni\ SO_4 . 10\ H_2O$, $CoSO_4$, $Cd\ Cl_2.2^1/_2\ H_2O$,$C_4H_6O_4$. pb.3 H_2O and $FeSO_4 .7H_2O$ salts. Stock solution(100 ml) was made of each element salt to obtain the concentration of 200 ppm and after that diluted to obtain the required concentration . The corresponding salt weights were 0.0934 , 0.053 , 0.0414 , 0.036 and 3.159 g for the above mentioned salts, respectively.

Microorganisms used

Seven microorganisms were used in this study including *Staphylococcus aureus, Bacillus cereus* (Gram positive), *Escherichia coli, shigellaflexineri* , *Enterobactersakazaki* , *Salmonella* sp (Gramnegative) *and Saccharomyces cerevisiae*(yeast).These strains were obtained from Agric. Microbiol. Dept., Fac., of Agric., Mansoura Univ., Egypt . Bacterial strains were grown in **Skerman (1967)**medium . The medium consists of (g/L) : peptone 5.0 ; beef extract 3.0 ; agar 20.0 and pH adjusted 7.0 . While yeast strains was grown in Lowes et.al,(2000) medium. The medium consists of (g/L): malt extract 30.0; agar 20.0 ;pH 5.5 . These media were autoclaved at 121^{o}C/ 30 min .

Stock cultures were maintained at 4^{o} C on slopes of nutrient agar medium . Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrientbroth media for bacteria and malt brothfor yeast and incubated at 37^{o}C for 24 h for bacteria and 30^{o}C for 24 h for yeast **(Duraipandiyan*et. al.*, 2006)**

Determination of antimicrobial activity

Antimicrobial activity of all concentrations was tested by agar well diffusion method according to **Vaghasiya*et al.*,(2009)**. The fresh inoculums were taken andagar media plates were mixed with 1 ml of microbial suspension and allowed to set . Wells were then made using a sterile cork borer (8 mm)and filled with 0.1 ml of each concentration;25 and 50 ppm for Nickel , 35 and70 ppm for Cobalt , 15 and 30 ppm for cadmium , 25 and 50 ppm for Lead and 3150 and 6300 ppm for Iron . Plates were incubated at 37^{o}C for bacteria and 30^{o}C for yeast for 24 h and the antimicrobial activity was determined by measuring the diameter of inhibition (mm)zone .

RESULTS AND DISCUSSION

Chemical analyses

Table 1 showed values of the chemical analyses of six food samples under investigation. Four parameters were determined including moisture, ash, protein, and fat contents . Results showed that frozen shrimps contained the highest value of 21.0% for moisture content followed by canned sauce to be 17.0 % . However the other samples contained moisture content ranged from 3.0 to 8.5 %.

Ash content followed the same trend as moisture .It had the maximum value of 15.5 % for frozen shrimps sample followed by the canned sauce andfrozemolokhia in values of 13.0and 11.5 %, respectively . However, the lowest ash content was found in fresh mackerel sample. Canned luncheon and fresh okra have nearly the same ash content being 10.5 and 10 %, respectively.The ash content in Table 1 for canned sauce (13.0%) was higher than those mentioned by **Itodo and Itodo, (2010)**, who found a value ranged from 2.0 to 4.0 % for the same parameter in canned sauce. The data in Table 1 showed to have less ash content i.e. 2.5% for fresh mackerel, while **Hend, (2010)** gave more ash content reached to 8.76% in dried prepared fresh fillets.

The obtained results (17.0%)are in disagreement with those mentioned by **Itodo and Itodo, (2010)**,who found average values of 78.0 % for moisture content in canned sauce. Table 1 also revealed that protein content of food samples under investigation ranged from 13.5 to 54.8 % in canned luncheon and frozen shrimps, respectively. Fresh mackerel proved to have high protein content(42.0%).The rest samples contained moderate amounts of protein i.e 28.0, 22.1 and 15.2 % for frozen molokhia, fresh okra and canned sauce, respectively.On this respect, the previous authors gave a lower mean value of 7.88 % for protein content, while the present results was 15.2 % for the same component in canned sauce.

The obtained data Table 1 for crud protein content of fresh mackerel (42.0%) were lower than those mentioned by **Hend, (2010),**who found average value of 77.36 % for the same component in prepared fresh fillets. The obtained results in Table 1 showed also that crude lipid content reached the maximum value of 25 % for fresh mackerel followed by 22.5 % for canned luncheon sample. This value was higher than that mentioned by **Hend, (2010),** who gave 13.3% for fat content in prepared fresh fillets. The other food samples contained lower fat content in comparison with fresh mackerel. Average values of 4.5, 1.0, 0.1 and 0.1 % for frozen shrimps, canned sauce, frozen molokhia and fresh okra, respectively.

Five element were determined in food samples under investigation using atomic absorption apparatus (Model N C 9423- 400- 30042)and obtained results are listed in Table 2.

Obtained values of iron were ranged from 65.30 ppm in frozen molokhia sample to 461.30 ppm in canned luncheon . Frozen shrimps and fresh mackerel showed to have higher values of iron reached 412.70 and 400.90 ppm, respectively. Lower values of 87.70 and 145.80 for the same element were detected for fresh okra and canned sauce Table 2in comparison with the other food samples, respectively.

Results in Table 2 also deals with nickel element which gave values ranged from 1.31 in fresh okra to 5.18 ppm in fresh mackerel. Frozen shrimps and molokhia samples had nearly similar amount of this element i.e 1.68 and 1.66 ppm, respectively. Moderate amounts of 3.12 and 2.92 ppm were found for nickel content in canned sauce and canned luncheon, respectively.

Lead content as detected in Table 2showed little amounts. These amounts showed the minimum values of 1.09 , 1.16 and 1.44 ppm in fresh okra , frozen molokhia and canned sauce , respectively. A maximum value of 2.33 ppm was observed for canned luncheon followed by 2.21 and 2.09 for fresh mackerel and frozen shrimps, respectively.

Cadmium was also estimated and data are recorded in Table2. Canned luncheon and frozen shrimps had the highest values of 6.78 and 5.12 for cadmium, respectively. Three food samples i.efrozen molokhia, canned sauce and fresh okra gave nearly the same cadmium content to be 0.93, 0.88 and 0.87,respectively. Moderate cadmium amount of 3.66 ppm was detected for frozen shrimps.

The fifth element was cobalt which estimated in food samples under investigation as can be seen in the same Table. Frozen shrimps had the maximum cobalt value (14.0ppm), however fresh okra and frozen molokhia samples contained the minimum values of 0.45 and 0.56 ppm for the same element. Moderate values of 12.0,10.0 and8.0ppm for cobalt content were found in fresh mackerel , canned sauce and canned luncheon, respectively.

Data in Table 2 showed lower value for iron content (145.8 ppm) in canned sauce than that mentioned by **David *et al* (2008)** for this element in tomato sauce in Italin product, metallic can (209.58 ppm).

The obtained results in Table 2 for lead content of fresh mackerel (2.21ppm) are in a hormony with those mentioned by **Hend,(2010)** for this element (2.10 ppm) in flesh of marine hammour fish.

On the same trend,**Itodo and Itodo, (2010)**reported that the following values of 2.95 , 0.4, 7.0 ppm for Ni, Cd and Co in expired canned tomato, respectively. The present results gave 3.12, 0.88 and 10 ppm for the same elements in local canned sauce, respectively.

Effect of heavy metals on microbial growth :-

The effect of some heavy metals on bacterial growth were evaluated against seven microorganisms. These effects were assessed by the presence or absence of inhibition zones and obtained diameter in which no growth observed were measured after incubation at 37^{o}C/ 24 h , 30^{o}C / 48 h for bacteria and yeast, respectively.

Two Grampositive bacteria namely*Bacillus cereus and Staphylococcus aureus*were exhibited by different heavy metals. Data showed that *B.cereus*, long rod spore forming bacteria was low resistant than *Staph.aureus*, coccoid cells. *B.cereus* recorded inhibition zone (mm)with the three metals, cobalt(70 ppm),cadmium(30 ppm),iron(3150 ppm) and(6300 ppm).*Staph.aureus* recorded inhibition zone (mm) with metal iron (3150,6300 ppm) as illustrated in Table 3.

Gramnegative bacterial strains, results proved that *Escherichia coli* was more resistant than other Gram negative bacteria towards all the tested metals except iron as can be seen in the Table 4. The higher resistance of Gram negative bacteria with the *E. coli* recorded inhibitionzone of 21 mm. No inhibition on the growth of bacteria were observed at the lower cadmium concentration as reported by **Kim,(1985)** . In case of yeast *Saccharomycescerevisiae,* results proved that the superiority of all metals concentration except iron as shown in Table 5. This results are in agreement with those of**Nies,(1999).**

Generally , two strains of bacteria namely *Bacillus cereus and Enterobacter cloacae* were resistance to high concentrations of cadmium and also have great tolerance to copper , lead , zinc and cobalt **Qing *et. al.*,(2007)**.Further , the Gram positive bacteriaare more sensitive to heavy metals than Gram negative bacteria. These results are in accordance with those of **Vijayalakshmi, *at al.*,(2011).**

Table 1. Chemical analyses of food samples under investigation

Examined Samples	Content values (%)				
	Moisture content	Crude protein	Crude lipid	Ash content	Total* carbohydrates
Canned Luncheon	5.0	13.5	22.5	10.5	48.5
Canned Sauce	17.0	15.2	1.0	13.0	53.8
Frozen Shrimps	21.0	54.8	4.5	15.5	4.2
Frozen Molokhia	3.0	28.0	0.1	11.50	57.4
Fresh Mackerel	8.5	42.0	25.0	2.50	22.0
Fresh Okra	4.5	22.1	0.1	10.0	63.3

*** Total carbohydrates were calculated by differences.**

Table 2. Elementary analyses of food samples under investigation

ExaminedSamples	Values in ppm				
	Fe	Ni	Pb	Cd	Co
Canned Luncheon	461.3	2.92	2.33	6.78	8
Canned Sauce	145.8	3.12	1.44	0.88	10
Frozen Shrimps	412.7	1.68	2.09	5.12	14
Frozen Molokhia	65.3	1.66	1.16	0.93	0.56
Fresh Mackerel	400.9	5.18	2.21	3.66	12
Fresh Okra	87.7	1.31	1.09	0.87	0.45

Table 3. Diameter of inhibition zone of Gram-positive bacterial growth as a result of treatment by some heavy metals

Examinedelements	Concentration (ppm)	Inhibition zone (mm)	
		B.cereus	*Staph. aureus*
Nickel	25	-	-
	50	-	-
Cobalt	35	-	-
	70	4	-
Cadmium	15	-	-
	30	4	-
Lead	25	-	-
	50	-	-
Iron	3150	10	15
	6300	12	17

Table 4. Diameter of inhibition zone of Gramnegative bacterial growth as a result of treatment by some heavy metals

Examined elements	Concentration (ppm)	Inhibitionzone (mm)			
		Escherichia coli	***Salmonella* sp.**	***Enterobacter sakazaki***	***Shigellafle xineri***
Nickel	25	-	-	-	-
	50	-	-	-	-
Cobalt	35	-	5	-	-
	70	-	11	6	-
Cadmium	15	-	-	-	-
	30	-	-	-	3
Lead	25	-	-	-	-
	50	-	-	-	-
Iron	3150	17	9	13	7
	6300	21	12	16	15

Table 5.Diameter of inhibition zone of yeast growth as a result of treatment by some heavy metals

Examinedelements	Concentration (ppm)	Inhibition zone (mm)
		Sacchaeomycescervisiae
Nickel	25	-
	50	-
Cobalt	35	-
	70	-
Cadmium	15	-
	30	-
Lead	25	-
	50	-
Iron	3150	5
	6300	4

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الملخصالعربي

التحاليل الكيميائية لبعض العينات الغذائية وتأثير محتواها من المعادن الثقيلة على الكائنات الحية الدقيقة

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قسم الكيمياء كلية الزراعة – جامعة المنصورة.

في هذا البحث تم دراسة المعادن الثقيلة وهى مجموعة من المعادن لديها كثافة ذرية أكبر من $5.0\ g/cm^3$، وعادة ما يرتبط التلوث ومشاكل السمية. تم اختيار ستة العينات الغذائية لهذه الدراسة .هذه العيناتهي اثنين معلبة واثنين مجمدة واثنين طازجة. وأجري التحليل الكيميائي الروتيني وكان يتضمن الرطوبة والرماد والدهون والبروتين الخام والمحتوى الاجمالى للكربوهيدرات . تم تطبيق التحليلات الابتدائية أيضا وكانت تتضمن خمسة عناصر معدنية هي الحديد، النيكل، الكوبالت، الكادميوموالرصاص .وأشارت النتائج إلى أن الجمبري المجمديحتوى على أكبر قدر من الرطوبة والبروتين والرماد .تم الكشف عن متوسط قيم21.0، 54.80و15.50٪ على التوالي .في حين أظهرت ان اللانشون المعلب به أعلى محتوى من الحديد والرصاص والكادميوم(416.3، 6.78 و 2.33جزء في المليون على التوالي).

في هذا البحث أيضا تم دراسة تأثير العناصر المذكورة أعلاه على نمو الخميرةوستة من العزلات البكتيرية المسببة للأمراض (اثنين من العزلات موجبة لجرام وأربعة عزلات سالبة لجرام)كما تم استخدام اثنين من التركيزات كل عنصر .في التجارب، قد تم زراعة الخميرة وستة عزلات من البكتيريا في أطباق بتري مع امدادهابتركيزات مختلفة من الحديد، النيكل، الكوبالت، الكادميوم والرصاص منفردة وحضنت في 30درجة مئوية و 37درجة مئوية لمدة 48 ساعة لكل من الخميرة أو البكتيريا على التوالي . وقد تم قياس نمو الخميرة و البكتيريا باستخدام قطر منطقة التثبيط .وأشارت النتائج التي تم الحصول عليها من هذه الدراسة أن نمو الخميرة قدانخفض في جود الحديد بتركيز 3150جزء في المليون و 6300 جزء في المليون بالمقارنة مع السيطرة .تم تثبيط نمو جميع العزلات البكتيريةبواسطة الحديد .كما ثبط الكوبالت والكادميوم نمو بعض البكتيريا بينما لم يلاحظ أي تأثير على النمو مع الخميرة.