CHAPTER 7

RESULTS AND DISCUSSION: HEAVY METALS CONTENT IN DATE FRUITS SAMPLES

7.1 Introduction

Eight heavy metals contents including, Al, Ni, As, Cd, Cu, Fe, Pb and, Sn were determined in the examined date fruit samples using wet digestion method described in section 3.8.4. using graphite furnace atomic absorption spectrometer (GFAAS).

7.2 Optimization of Digestion Method

The digestion method was optimized regarding to digestion mixture, time and temperature.

7.2.1 Optimization of HNO_3/H_2O_2 Ratio and Digestion Time

The optimization of the digestion mixture of (HNO_3/H_2O_2) ratio and digestion time was carried out as described in section 3.8.5 depending on the physical appearance of the date matrix. The clear colorless solution considered a complete matrix digestion.

From the results in Table 7.1, the clear colorless solution which indicates the digestion completion was achieved when the mixture ration of HNO_3/H_2O_2 was 15:20 (v/v). The digestion reagents used are chosen based on the sample matrix and the metals being analyzed (Mohammed et al., 2017). The most commonly used acid for oxidation of organic matrices is nitric acid. When combined with other oxidizing agents, such as hydrogen peroxide, its oxidizing strength can be increased. As a result, different ratios of HNO_3 and H_2O_2 were optimized. HNO_3: H_2O_2 (15:20) mixture ratio was found

enough for complete date matrix mineralization. Changing of mixture ratio to 20:20 and 25:20 did not influence the digestion efficiency of date fruits sample regarding the obtaining a colorless solution.

Table 7.1: Data of Optimization of HNO_3/H_2O_2 Ratioand Digestion Time regarding Solution Color at 110°C

30 mL HNO_3/ H_2O_2 ratio (v/v), 110°C	**Digestion time (min)**			
	30	**45**	**60**	**90**
15:10	Yellow	Pale yellow	Pale yellow	Pale yellow
15:15	Yellow	Pale yellow	Pale yellow	Pale yellow
15:20	Yellow	Pale yellow	Clear colorless	Clear colorless
15:25	Yellow	Pale yellow	Clear colorless	Clear colorless

7.2.2 Optimization of Digestion Temperature

Digestion temperature was optimized as described in section 3.8.5 depending on recovery % of four elements namely, Fe (M.P 1538°C), As (Sub.P 615 °C), and Pb (M.P 327.46 °C) and Sn (M.P 231.93 °C). According to results tabulated in Table 7.2, the highest recovery for As and Pb was found at 110°C so, 110°C was chosen as digestion temperature as they are the most sensitive metals for temperature. Digestion temperature is essential factor which can influence digestion efficiency. The boiling points of the acids and the volatility of the analyte determine the digestion temperatures of open acid digestion systems (Mohammed et al., 2004). The average kinetic energy of an acid increases with temperature, resulting in more collisions between the acid and the biological sample, enhancing metal dissolution (Berghof products and instruments 2016). The boiling point of the acid or acids used also limits the digestion process. The most volatile acid boil away first in multi-acid digestion, followed by the next most volatile (Kingston & Jassie,1986). High digestion temperatures lead to the loss of volatilized elements (Mohammed et al., 2014). However, the digestion time, which

governs how long the matrix is exposed to the oxidizing acid, is another important factor. Exothermic processes can be accelerated by increasing exposure time, which increases the degree of solubilization of the target metals from the matrix as well as the loss of these metals through volatilization (Gavriloaiei 2008). The usual time for a wet digestion by open vessel digestion is ranging from one to two hours depending on the experimental conditions (Kingston & Jassie 1986). As a result, selecting the most efficient digestion temperature and time to achieve the best element recovery is critical.

Table 7.2: Data of Optimization Digestion Temperature for Fe, Sn, As and Pb

Time min °C	Recovery %			
	Fe	**Sn**	**As**	**Pb**
70	79.87±1.36	84.31± 5.66	85.72±1.59	68.52 ± 2.83
100	84.39±1.11	79.56± 1.64	93.82±1.65	78.09 ± 2.46
110	97.40±1.45	100.88 ± 4.06	98.02±0.84	89.67± 3.40
120	102±2.72	97.35 ± 1.26	95.44±1.122	89.34 ± 1.99
130	104.98 ± 3.42	95.87± 1.56	94.79±1.10	86.60 ± 1.59

7.2.3 Determination of Heavy Metals' Detection Limit and Quantification Limit

Based on the standard deviation of the response and the slope of calibration curve, limit of detection (LOD) was expressed as: 3x standard deviation of low concentration / slope of the calibration curve. Limit of quantification (LOQ) was expressed as: 10 x standard deviation of low concentration/ slope of the calibration. Result is shown in Table.7.3. Standard calibration curves of the examined elements are attached in Appendix 3 Figure C1 and Figure C2.

Table 7.3: Regression Equations and LOD & LOQ of the Examined Heavy Metals

Element	Regression equation	R^2	SD	(LOD) ppb	(LOQ) ppb
Al	Abs = 0.00057 x C + 0.09177	0.9987	0.0078	41.00	136.80
Ni	Abs = 0.00196 x C + 0.01824	0.9999	0.0003	0.46	1.50
As	Abs = 0.00564 x C + 0.46046	0.9993	0.0011	2.11	7.50
Cd	Abs = 0.05386 x C + 0.18682	0.9682	0.0230	1.28	4.27
Cu	Abs = 0.00470 x C + 0.05747	0.9986	0.0003	0.19	0.64
Fe	Abs = 0.05369 x C + 0.01672	0.9960	0.0002	0.01	0.019
Pb	Abs = 0.00251 x C + 0.03175	0.9969	0.0072	8.60	28.70
Sn	Abs = 0.00226 x C + 0.00539	0.9992	0.0006	0.796	2.65

The calibration curve equations, LOD, LOQ and SD values indicated the high sensitivity and suitability of the method for determination of the examined heavy metals in date fruit matrix especially for Ni, Cu, Fe, Sn, Cd, As, and Pb.

7.3 Recovery Study

The recovery study on heavy metals was done as described in Section 3.4.6. Results in Table 7.4 values measured at two concentrations for each element. All analyzed heavy metals at low and high concentration were recovered in acceptable range from 80.5 -114.5 % with RSDs ranging from 0.42 -9.55 %.

Table 7.4: Data of Heavy Metal Recoveries at Two Different Concentrations (Mean ± SD; n = 3)

Element	Concentration 1 (ppb)		Concentration 2 (ppb)		Recovery %		Relative STD	
	Added	Found	Added	Found	Recovery (1)	Recovery (2)	RSD% (1)	RSD% (2)
Ni	20	19.50	100	110.07	97.5 ± 4.43	110.07 ± 1.70	4.55	1.51
Cd	2	2.29	10	11.45	114.56 ± 5.5	114.53 ± 3.78	4.85	3.30
Cu	20	16.97	100	106.89	84.86 ± 5.17	106.88 ± 4.24	6.09	3.97
Sn	20	19.80	100	105.33	98.99 ± 8.71	105.33 ± 4.41	8.80	4.19
Al	100	101.34	400	410.54	101.34 ± 0.54	102.63 ± 1.42	0.54	1.39
Pb	20	17.90	100	109.79	89.49 ± 8.55	109.79 ± 2.46	9.55	2.24
Fe	1	0.95	5	5.14	94.8 ± 0.40	102.76 ± 1.95	0.42	1.90
As	5	4.02	100	91.11	80.47 ± 5.30	91.11 ± 2.60	6.58	2.85

Table 7.5: Heavy Metals Contents in Date Flesh (µg/g) Fresh Weight

No.	Sample variety	Ni	Cd	As	Cu	Sn	Al	Pb	Fe
1	Ajwa	0.932±0.293	0.024±0.012	0.820±0.089	8.618±1.630	0.130±0.095	7.510±1.125	2.256±.428	11.670± 2.890
2	Deglet Nour	1.111±0.393	ND	0.921±0.034	9.767±2.355	0.083±0.006	9.935±1.130	1.226±0.771	12.630± 3.323
3	Frad	0.971±0.241	ND	1.110 ±0.173	1.817±0.212	0.210±0.035	6.05± 0.653	0.270±0.18	22.95±1.039
4	Khalas-Ihsa	1.102±0.134	ND	1.181±0.172	6.324±0.769	0.203±0.025	14.201±2.16	0.230±0.017	40.300± 3.294
5	Khasouei	0.963±0.344	ND	1.184±0.096	8.782±0.478	0.220±0.036	5.893±1.319	0.224±0.087	30.000 ±1.826
6	Khudri	1.600±0.814	0.033±0.010	1.046±0.066	5.281±2.610	0.180± 0.020	8.622±1.380	0.395±0.107	38.733±2.264
7	Lamry	0.563±0.044	ND	1.126±0.113	8.624±0.498	0.213± 0.040	5.723±2.877	0.205±0.035	29.417±7.786
8	Lulu	1.389±0.281	0.011±0.003	0.979±0.005	9.969±2.162	0.120±0.0300	4.969±1.158	1.350±0.073	29.500± 2.955
9	Mabroom	1.013±0.381	ND	1.001±0.082	6.939±1.706	0.187±0.006	6.650± 1.470	0.290±0.25	27.533±1.623
10	Mariami	1.366±0.383	ND	0.905±0.068	8.076±2.808	0.153±0.076	8.980±5.663	0.156±0.051	8.960±0.289
11	Mashrook	0.746±0.121	0.047±0.006	0.984±0.360	4.700±0.366	0.213±0.012	6.713±3.110	0.335±0.063	49.90± 2.955
12	Medjool	1.533±0.587	ND	0.969±0.177	8.693±1.225	0.168±0.010	8.084±2.210	0.123±0.021	19.617±1.703
13	Nomades (Promel)	2.575±0.780	ND	1.091±0.020	7.490±0.940	0.230±0.017	9.308±2.818	0.242±0.017	28.217±1.559
14	Palam dates	1.044±0.506	ND	1.127±0.072	5.488± 2.767	0.220±0.010	5.821±2.632	0.269±0.030	32.333±5.080
15	Rabi	0.721±0.148	0.006±0.0004	1.181±0.087	9.588±1.360	0.233±0.012	5.526±3.073	0.256±0.087	21.57± 9.545
16	Roshdi	1.028±0.127	0.046±0.017	1.124±0.068	5.598±1.367	0.217±0.060	7.097±2.106	0.451±0.049	26.566 ±3.290
17	Safawi-1	1.402±1.179	ND	1.084±0.157	8.427±0.734	0.220±0.026	5.727±0.534	0.192±0.066	29.400±5.109
18	Safawi-2	1.049±0.953	0.024±0.007	1.161±0.090	5.4810.581	0.207±0.015	6.263±3.167	0.321±.002	45.083±6.141
19	Zahdi -1	2.145±0.668	ND	0.974±0.089	7.75±2.440	0.160±0.010	4.779±0.243	0.181±0.038	16.366±8.740
20	Zahdi. -2	1.573±0.958	ND	1.007±0.043	10.473±0.255	0.200 ±0.046	6.919± 2.92	0.496±0.009	22.450±1.611
Average		1.258±0.443	0.009 ± 0.003	1.048±0.103	7.394±1.253	0.188±0.027	7.238±2.121	0.439±0.116	27.160±3.740
Permitted Level (PL) (FAO/WHO, 2004) (µg/ g)			0.05	0.1-5				1.5	
Malaysian PL (µg/ g) Mohammed et al., (2017)			1.0	1.0				2.0	

The statical analysis of the examined metals in flesh samples Table 7.6 shows that all the correlation coefficients of the elements are negative and statistically insignificant, except for the Cu with Ni, Sn with As, and Fe with As, Sn, Al, which are positive and statistically significant ($p \leq 0.05$). The heavy metals with positive significant correlation are expected to be related to the same source of contamination such as soil and water contamination (Zinkutė, et al., 2011).

Table 7.6: Correlation Analysis of Element among the Flesh of Date Fruit

	Ni	**Cd**	**As**	**Cu**	**Sn**	**Al**	**Pb**	**Fe**
Ni	1							
Cd	-0.158	1						
As	-0.234	-0.035	1					
Cu	**0.340***	-0.438	-0.338	1				
Sn	-0.152	0.078	**0.787***	-0.410	1			
Al	0.062	-0.112	-0.380	0.015	-0.062	1		
Pb	0.020	-0.656	0.207	-0.612	0.142	-0.151	1	
Fe	-0.385	0.060	**0.614***	-0.503	**0.587***	**0.390***	-0.125	1

Table 7.7: Data of Heavy Metals Contents in Date Dust µg/g fresh weight

Sample variety	Ni	Cd	As	Cu	Sn	Al	Pb	Fe
Ajwa	0.134±0.015	ND	0.057±0.011	0.036±0.005	0.011±0.002	3.711±0.908	0.007±0.001	4.709±1.149
Deglet Nour	0.136±.0320	ND	0.071±0.009	0.042±0.008	0.011±0.002	0.736±0.260	0.013±0.004	2.428±0.968
Frad	0.173±0.021	0.016±0.004	0.088±0.010	0.056±0.006	0.021±0.004	3.285±0.651	0.042±0.024	2.341±1.353
Khasouei	0.059±0.011	0.006±0.003	0.066±0.009	0.097±0.032	0.023±0.002	3.033±1.060	0.053±0.017	4.654±0.433
Khudri	0.144±0.012	ND	0.051±0.004	0.039±0.006	0.009±0.0003	2.225±0.423	0.006±0.002	1.902±0.465
Lamry	0.119±0.019	ND	0.076±0.022	0.036±0.008	0.014±0.004	3.027±0.717	0.005± 0.001	1.718±0.543
Lulu	0.166±0.012	0.0035±0.0001	0.065±0.017	0.072±0.022	0.014±0.0003	2.072±0.501	0.027±0.010	4.868±1.289
Mabroom	0.084±0.022	0.0021±0.001	0.054±0.011	0.036±0.002	0.008±0.002	2.142±1.306	0.013±0.007	2.492±0.442
Mariami	0.133±0.028	0.0032±0.001	0.0645±0.013	0.046±0.006	0.010±0.001	2.721±1.534	0.015±0.002	3.107±1.015
Mashrook	0.207±0.021	0.023±0.01	0.140±0.017	0.157±0.032	0.052±0.008	8.232±3.977	0.353±0.351	11.148±7.562
Medjool	0.135±0.035	ND	0.073±0.006	0.059±0.016	0.023±0.003	3.012±0.576	0.008±0.001	3.02±0.161
Nomades (Promel)	0.099±0.032	0.0025±0.0009	0.071±0.007	0.076±0.039	0.016±0.002	2.002±0.475	0.025±0.011	2.309±0.539
Palm dates	0.166±0.036	0.006±0.002	0.091±0.014	0.143±0.110	0.016±0.002	3.112±2.189	0.036±0.014	2.075±0.381
Rabi	ND	0.005±0.001	0.058±0.005	0.070±0.010	0.024±0.001	3.284±0.582	0.053±0.032	2.813±1.157
Roshdi	0.100±0.029	0.0026±0.0019	0.056±0.01	0.102±0.035	0.047±0.011	5.956±2.250	0.045±0.011	3.633±3.328
Safawi-1	0.039±0.008	0.0017±0.0008	0.067±0.017	0.036±0.014	0.013±0.001	1.719±1.036	0.011±0.002	0.941±0.146
Safawi-2	ND	0.013±0.003	0.083±0.007	0.111±0.023	0.028±0.004	5.550±0.692	0.108±0.003	7.966±2.132
Zahdi -1	0.128±0.025	0.0014±0.0005	0.068±0.006	0.07±0.006	0.014±0.004	0.182±0.002	0.012±.002	3.367±2.394
Zahdi- 2	0.149±0.033	0.004±0.0011	0.082±0.007	0.064±0.013	0.014±0.002	3.873±1.110	0.022±0.007	3.520±1.131
Average	0.116±0.021	0.0048±0.002	0.073±0.011	0.071±0.021	0.019±0.002	3.150±1.068	0.045±0.034	3.632±1.399

Table 7.8 shows that all correlation coefficients of the elements in the date fruits dust are positive and statistically significant ($p > 0.05$) except for Cd with Ni, Cu with As, Sn with Cu, Fe with Al, and Pb with Fe which are negative values and not statistically significant ($p > 0.05$). This may be because all heavy metals in dust are mostly related to air contamination (Zinkutė et al., 2011).

Table 7.8: Correlation Analysis of Element among the Dust of Dates

	Ni	Cd	As	Cu	Sn	Al	Fe	Pb
Ni	1							
Cd	0.125	1						
As	0.654*	0.549*	1					
Cu	0.675*	0.685*	0.166	1				
Sn	0.727*	0.558*	0.608*	0.153	1			
Al	0.857*	0.673*	0.611*	0.717*	0.260	1		
Fe	0.766*	0.675*	0.658*	0.651*	0.826*	0.350	1	
Pb	0.863*	0.767*	0.761*	0.740*	0.794*	0.743*	0.320	1

7.4 Risk Assessment of Heavy Metals in Date Fruit Samples

Table 7.9 showed the estimated dietary intake and hazard quotient (HQ) for the investigated metals namely, Cd, Pb, Ni, Al, As, Fe, Cu and Sn in all the 20 samples built on the mean concentration. The results showed the range of EDI (µg/kg bw day) and hazard quotients (HQ) of the tested heavy elements were as the following : Cd 0.0128 µg/kg bw/day (HQ = 0.0128), Pb 0.63 µg/kg bw/day, (HQ = 0.18), Ni 1.497 µg/kg bw/day and Al 8.95 µg/kg bw/day (HQ = 0.0623), As 1.5 µg/kg bw/ day (HQ = 0.699) Fe 64.4 µg/kg bw /day (HQ = 0.081), Cu 7.83 µg/kg bw/ day (HQ = 0.016) and Sn 0.0296 µg/kg bw/ day (HQ = 1.5^{*E-5}). However, the calculated health hazard quotients (HQs) for all elements are less than one (<1) and EDIs of the investigated heavy metals was not exceeded the JECFA values shown in Table 7.8 which indicated that no health risk associated with consumption of the examined date fruits. Taking into account the quantities to heavy metals from other daily food and drinks, health risk should be

expected especially for children. Infants and children are particularly susceptible to the health hazards (acute, subacute, and chronic) of chemicals. Children are more vulnerable to chemical hazards in food than adults because they consume more food per kilogram of body weight. Infants consume twice as much food as adults per unit of body weight. Furthermore, organs and tissues that are in the developing stage are more susceptible to the poisonousness of chemicals. Lead and mercury cause thoughtful harm to the developing brains of fetuses and early childhood, leading to a loss of knowledgeable potential, while mature exposure has no substantial effects on their intelligence (Vračko et al., 2007).

Table 7.9: Risk Assessment of Heavy Metals Calculated from the Mean Contents of Every Element

Element	ED.ml (µg /100 mg/day)	Estimated daily intake EDI (µg/kg/ bw day) for (70kg bw)	(JECFA) STD - PTDI µg/kg.bw/day	HQ (EDI/PTDI)
Cd	0.90	0.013	1.0	0.0128
Pb	43.90	0.63	3.5	0.180
Ni	104.90	1.49	12.0	0.124
Al	626.30	8.95	143	0.0623
As	104.80	1.50	**2.14**	**0.699**
Fe	4508.30	64.40	800	0.081
Cu	548.10	7.83	500	0.016
Sn	2.070	0.03	2000	$1.5*^{E-5}$

Note: ED.ml (µg /100 mg/day) is estimated daily intake per (100g) meal, EDI (µg/kg/ bw day) is Estimated daily intake for (70kg bw), (PTDI) Provisional Tolerable Daily Intake (µg/kg.bw/day).

7.5 Discussion

Although harmful and toxicity of heavy metals on plants are well- known, very little is known about heavy metals, on/in date fruits although dates are highly disposed to heavy metal contamination. Thus, to the best of our knowledge this is one of the few studies in Malaysia investigating more deeply the presence of several heavy metals

on/in date palm fruits and the ability of washing on removing the deposited heavy metals on date fruits.

The mean concentration of heavy metals in date flesh is shown in Table 7.5. The recorded values are similar with global values and appeared to be descending ordered as Fe> Al> Cu> As>Ni> Pb> Sn> Cd.

The average concentration of Fe was (27.16 µg /g). The highest Fe concentration was recorded in Mushroom sample from KSA (49.90 µg/g) and the lowest was found in Mariami sample from UAE (8.96 µg/g). In general, total Fe concentration in all samples were greater in flesh than dust as Fe is essential metal. Iron has several roles in plants, often related to Fe^{2+}, Fe^{3+} ions, such as electron transport, protein structure, enzymes involved in nitrogen and sulphur metabolism (Morrissey, & Guerinot., 2009). Total Fe concentrations in flesh were significantly difference between samples.

Aluminum (Al) is known to cause brain damage, bone disease and anemia (Williams & Pillay., 2005). The highest concentration of Al was found in Khalas-Ihsa sample from KSA (14.20 µg /g), and the lowest concentration was observed in Zahid (4.779 µg /g) from UAE with average of (7.238 µg /g). Al has been studied in the date palm previously. In Egyptian dates, Al was investigated by Awadallah et al., (1999). It was found that (Al) levels were normal and varied from 33.32 - 43.92 ppm, depending on the sample and chemical analysis method. In the study carried out on Omani date palms, the authors considered the leaflets and surrounding soil, as well as the date fruits (Williams et al., 2005). It was found that concentration levels of all examined metals in the date palm flesh were generally, safe and within literature guidelines. An exception was Al in dates in the Kimri stage. The upper limit of 166 µg /g was higher than the literature value of 140 µg/g). Salama et al., (2019) reported Al mean concentration of

5.58 mg/kg in study carried out on seven varieties of date fruits collected from different sites of KSA by applying ICP-OES analysis technique.

Nickel (Ni) is toxic and carcinogenic (Pulford and Flowers, 2006). The average of Ni concentration was 1.258 µg /g, the highest Ni concentration recorded (2.575 µg /g) was in Nomades Promel sample from Tunisia and the lowest concentration was in Rabi sample from Iran (0.721 µg /g). Ni concentration in all samples were in acceptable range and lower than the concentration reported in previous studies. Several studies have been conducted on Ni in the date palm. In 1999, Ni was found in Egyptian dates at a concentration of 0.4 ppm (Awadallah et al.,1999) and fruits of Saudi date palms 0.28-3.89 µg /g (Al-Shayeb, 1995). More recently, Ni in washed dates fruit samples from the type Segae from KSA were found ranging from 8 - 11.75 µg /g by Taha, & Al Ghtani. (2015). Lead (Pb) is a non-essential heavy metal and undergoes many harmful health effects such as neurotoxicity and nephrotoxicity (Kumar et al., 2020). In this study, the average concentration Pb in flesh was 0.439 µg/g. The highest concentration was detected in Ajwa sample from KSA (2.256 µg/g), Lulu (1.35 µg/g) and Deglet Nour (1.22 µg/g) while the lowest concentration found in Medjool sample from UAE (0.123 µg/g). Lead contents in the literature have been reported in the range of 0.84-2.26 mg/kg dry weight in date flesh from KSA (Aldjain et al., 2011; Kamal et al., 2015)

Arsenic (As) is a toxic and carcinogenic for humans (Pulford and Flowers., 2006). The average concentration of arsenic in the examined dates samples was (1.048 µg/g), the highest concentration was found in Khasouei sample from UAE (1.184 µg/g), while Ajwa sample from KSA exhibited the lowest concentration of (0.820 µg /g). Waheed et al., (2004) reported arsenic concentration of 1.6 µg/g in date fruit samples using neutron activation analysis and was considered safe. Salama et al., (2019) reported mean concentration of arsenic of 0.171 µg/g.

Regarding environmental impact, cadmium (Cd) is ranked as one of the most hazardous substances. The average concentration of Cd was 0.009 µg/g, the highest Cd concentrations was found in Mashrook from KSA (0.047 µg/g) while it in not detected in 13 samples namely, Deglet Nour, Frad, Khalas-Ihsa, Khasouei, Lamry, Mabroom, Mariami, Medjool, Nomades (Promel), Palam dates, Safawi-1, Zahid-1and Zahdi-2. The source of Cd in plants is usually phosphate fertilizers which contain ionic Cd as a contaminant, and sewage sludge which is used for soil enhancement (Baird.,1999; Ward., 2000). There have been several studies reported Cd in the date palm. A Turkish group investigated the date palm as a bio monitor of Cd (Aksoy and Ozturk.,1996). They found a good correlation between the concentration of Cd in the surface soil and in washed leaflets. A year later, the content of Cd in dates was investigated by Marzec and Bulinski., (1997). It was concluded that the Cd concentration was within the limits specified by relevant legal standards. Another study carried out in 1999, on Egyptian dates, Cd was found in concentrations of 15.5 - 24.75 ppb, according to the sample and analysis method (Awadallah et al., 1999). In the study carried out by (Mofida, 2002), Cd was reported at an average concentration of 5.65 µg/kg fresh weight in Saudi dates. In the same year, the study curried out by Pillay, et al., (2002), reported that fresh dates (Basir and Rutab) are safe for consumption as Cd levels were below 50 ng/g, but that levels of this heavy metal were a cause for concern earlier in the fruit growing season at the Kimri stage when concentrations of 225 ng/g were reported. In a study carried out by Waheed et al., (2004) in Pakistan using atomic absorption spectroscopy, Cd average level in mature dates was 27 ± 2.8 ng/g. Recently, a Saudi study concluded that Cd levels in dates from around Riyadh were within recommended safe limits (Aldjain et al., 2011). Salama et al., (2019) reported similar mean cadmium concentration of 0.09 mg/kg in dates from KSA which ten times higher than our

findings. The concentrations of Cd in all examined samples in current study are considering in acceptable range.

The average Cu concentration in washed samples was 7.394 µg/g. While highest concentration was found in Zahid sample from UAE (10.473 µg/g), the lowest concentration was found in Fard sample from UAE (1.817 µg/g). our findings agree with the previous studies on date fruits from middle east. The presence of Cu in date fruits was reported in many studies; Yousif et al., (1982) in Oman and the study carried out by Hassan et al., (2017). However, high intake levels of Cu can cause a neurotoxicity known as Wilson's disease (Banum, 1982). Mining, fiber processing, pipe corrosion, printed circuits, and metallurgical processes all contribute to copper pollution in the environment (Thakur & Parmar, 2013). In addition to paper industries, petroleum refining, wood preservation and fertilizers. Furthermore, copper contamination of water and agricultural products is often caused by fungicidal sprays and animal waste (Thakur & Parmar, 2013).

The average concentration of Tin (Sn) in examined samples was 0.188 µg/g, the highest concentration (0.233 µg/g) was found in Rabi sample from Iran while Deglet-Nour showed the lowest concentration (0.083 µg/g). Low levels of inorganic Tin (Sn) can cause fatigue, depression, cardiac problem, low adrenals, breath shortness, asthma, headaches, and insomnia, whereas high levels can cause skin rashes, stomach problems, vomiting, nausea, diarrhea, abdominal pain, and headaches. The toxic effects of organotin such as triphenyl tin (TPT) tributyltin (TBT). tributyltin oxide (TBTO), triphenyl tin acetate (TPTA), trimethyl tin (TMT), triethyl tin (TET) are skin and eye irritation, Respiratory damage, lesion, gastrointestinal irritation, and Neurological effects (Cima, 2011).

Table.7.6 shows that all the correlation coefficients of the elements in date flesh are negative and statistically insignificant, except for the Cu with Ni, Sn with As, and Fe with As, Sn, Al, which are positive and statistically significant $P \leq 0.05$. The correlation between Cu and Ni may refer to the fact that both elements are included in fertilizers and paper products (Thakur & Parmar, 2013). The correlation of Fe, As, Sn and Al may attribute by the soil contents and water contamination by heavy metals.

The mean concentrations of the selected metals in date dust, are given in Table 7.7. In date dust, the Fe total concentration was highest in Mashrook (7.966 mg/kg) and lowest in Safawi (0.941 µg/g). Total Fe concentrations in dust and flesh were significantly different between samples.

The highest concentration of Al in dust was found in Mashrook sample (8.232 µg/g) and the lowest concentration was found in Zahid (0.182 µg/g). The average concentration in flesh (1.258 µg/g). The highest concentration for Ni was found in Mashrook sample (0.207µg/g) and the lowest concentration was in Safawi sample from different sources (0.039 µg/g) while it was not detected in two samples (Rabi and Safawi). The average concentration Pb in dust was (0.045 µg/g).). The highest concentration was detected in Safawi-g (0.108 µg/g) and the lowest concentration found in Lamry sample (0.005 µg/g).

The average concentration of arsenic (As) was (0.073 µg/g), the highest concentration was found in Mashrook sample (0.140 µg/g) while Khudri and Mabroom samples exhibited the lowest concentrations of (0.051 and 0.054 µg/g) respectively. The average concentration of cadmium (Cd) was (0.0048 µg/g), the highest Cd concentrations was found in Safawi (0.013 µg/g) while it was not detected in six samples namely, Ajwa, Deglet Nour, Medjool, Khudri and Lamry.

The average concentration of cupper (Cu) was (0.071 µg/g). The highest concentration was found in Mashrook sample (0.157 µg/g), while the lowest concentration was found in both Lamry and Mabroom samples (0.036 µg/g).

The average concentration of tin (Sn) was (0.0194 µg/g), The highest concentration (0.052 µg/g) was found in Mashrook sample, and the lowest concentration (0.008 µg/g) was found in Mabroom sample.

There was strong correlation (Table7.8) between almost all heavy metals in dust samples. The possible explanation for the observation is that all of them are related to exposed to air contamination that caused by vehicles, nickel-cadmium batteries, sludge, commercial fertilizers and packing materials (Zinkutė, et al., 2011, Yadav et al., 2021). Besides presence of heavy metals such as Pb an Cd in washing residues and flesh of date fruits was reported previously in many studies (Aldjain et al., 2011; Mohebi, 2011, Marzouka & Kassemb, 2011 and Sueleman., 2014). As well as that, the release of heavy metals from oil industries, mining and vehicles may lead to the deposition of these metals on the surface of fruits and vegetables.

The results of risk assessment (Table 7.9) which was calculated from means of metal concentrations in date fruit samples showed that all metals concentrations are lower than provisional tolerable daily intake (PTDI) published by Joint FAO/WHO Expert Committee on Food Additives JECFA (2011). The HQ less than 1 for all the examined heavy metals indicated that the consumption of these dates does not cause health risks for the consumers. However, it is important to note that this study only analyzed one kind of foods and additional studies are needed to verify if the HQ for each of the heavy metals will remain below 1, when the concentration of those metals in other foodstuffs and drinks are involved in the risk calculations. The HQ for arsenic, lead and cadmium were 0.699, 0.18 and 0.0128 respectively, which indicates the

possibility that exposure to those heavy metals could be a cause for concern for the consumers when the intake of this toxic element from other foodstuffs and drinks are considered. In fact, results are similar with that reported by Salama et al., (2019) on heavy metal contents and health risk assessment in seven varieties date of palm fruits collected from different sites of KSA using ICP-OES. The calculated hazard risk index expected that both As and Pb can pose potential health risk to consumers. However, the study showed that Pb contributed greater health risks to consumption compared to arsenic (Salama et al., 2019).

7.6 Conclusion

A method for digestion and determination of eight heavy metals in date fruits flesh and the adhered dust (Fe, Al, Ni, Pb, As, Cd, Cu and Sn) were developed and optimized using GFAAS spectrophotometer. The recovery study was caried out at two different concentrations for each element. All analyzed heavy metals at low and high concentration were recovered in acceptable range from 80.5 to 114.5 % with RSDs ranging from 0.42 to 9.55 % which indicate the suitability of the developed method.

The mean concentration of heavy metals in date flesh are similar with global values and appeared to be descending in order: Fe> Al> Cu>As>Ni> Pb> Sn> Cd. The average concentration of Fe was (27.16 ± 3.74 µg/g) in flesh and 3.632 ± 1.399 µg/g in dust. The average concentration of Al in flesh was 7.238 ± 2.121 µg/g and 3.150 ± 1.068 µg/g. The average concentration of Ni in flesh was (1.258 ± 0.443 µg/g) while in dust, it was found (0.611± 0.021 µg/g). The average concentration Pb in flesh was 0.439 ± 0.116 µg/g while in dust, it was (0.045 ± 0.034 µg/g). The average concentration of As in the examined date flesh samples was (1.048 ± 0.103 µg/g) while in dust was (0.073 ± 0.011 µg/g). The average concentration of Cd in date flesh was (0.009 ± 0.003µg/g)

while in dust it was (0.0048 ± 0.002 µg/g). The average (Cu) concentration in washed samples was (7.394 ± 1.1253 µg/g) while in dust it was (0.071± 0.021 µg/g). The average concentration of Sn in flesh was (0.188 ± 0.027 µg/g). while at dust, it was 0.0194 ± 0.002 µg/g.

The statistical analysis showed that all correlation coefficients of the elements in date flesh are negative and statistically insignificant, except for the Cu with Ni, Sn with As, and Fe with As, Sn, Al, which are positive and statistically significant $P \leq 0.05$. The correlation between Cu and Ni may refer to the fact that both elements are included in fertilizers and paper products (Mukesh and Lokendra Thakur., 2013). The correlation of Fe, As, Sn and Al may attribute to soil contents and water contamination by heavy metals. There was strong correlation between almost all heavy metals in dust samples that may be because all of them are related to air contamination by vehicles that used gasoline fuel, nickel-cadmium batteries, sludge, commercial fertilizers and packing materials (Ishchenko & Vasylkivskyi., 2020)

The risk assessment calculated from the means of heavy metals concentrations in date fruit samples showed that all HQs are lower than PTD1 published by (JECFA) (2011). However, the HQ less than 1 for all the examined heavy metals indicated that the consumption of these dates does not cause health risks for the consumers. The HQ for arsenic, lead and cadmium were 0.699, 0.18 and 0.0128 respectively, indicates the possibility that exposure to those heavy metals could be a cause for concern for the consumers when the intake of this toxic elements from other foodstuffs and drinks are considered.

CHAPTER 8

RESULTS AND DISCUSSION: EFFECT OF WATER WASHING ON HEAVY METAL CONTENTS ON DATE FRUITS

8.1 Determination of Water Washing Effect on Heavy Metals Content

After determination of heavy metals content in all date samples using GFAAS, the water washing effect (reduction % of heavy metals content) was calculated as described in section 3.8.8. using equation 3.9. Result is shown in Table 8.1

Table 8.1: Effect of Washing on Total Heavy Metal Contents in Date Fruits (n = 3)

Element	Average flesh heavy metal contents (µg/g) fruit fresh weight	Average dust heavy metal contents (µg/g) fruit dust	Total contents (µg/g)	Washing effect (%)
Cd	0.009 ± 0.0.003	0.0048 ± 0.002	0.0138 ± 0.005	34.78
Ni	1.258 ± 0.443	0.116 ± 0.021	1.374 ± 0.464	31.02
Al	7.238 ± 2.121	3.150 ± 1.068	10.388 ± 3.980	30.32
Fe	27.160 ± 3.74	3.632 ± 1.399	30.792 ± 5.084	11.79
Sn	0.188 ± 0.027	0.0194 ± 0.002	0.207 ± 0.234	9.35
Pb	0.439 ± 0.116	0.045 ± 0.034	0.484 ± 0.150	9.30
As	1.048 ± 0.103	0.073 ± 0.011	1.121 ± 0.114	6.50
Cu	7.394 ± 1.253	0.071 ± 0.021	7.465 ± 1.274	0.95

8.2 Discussion

The effect of washing on some heavy metals deposited on date fruits was studied using wet digestion technique and GFAAS for analysis. Heavy metal contents in all samples were significantly reduced by washing for about 90 seconds at room temperature, and the results agree with those reported in the literature (Aldjain et al., 2011). The washing effect on dust removing was ranged from 34.78 % to 0.95 % and seemed to be in descending order as follows: Cd> Ni> Al>Fe>Sn>Pb>As>Cu. The highest washing effect was found for Cd (34.78 %) and then Ni (31.02%), Al (30.32 %), Sn (9.35 %), Pb (9.30 %), As (6.5 %) and Cu (0.95 %). Accordingly, studies reported the phenomena of accumulation of dust on date palm trees leaves and the effect of winds, location, and other factors on the quantities of accumulated dust (Qassim et al.,1986, Ibrahim and Nayef., (2001); Al-Anee et al., 2010). Also, in a study carried out by Qassim et al., (1986) on adult palm trees in KSA, the amount of adhered dust on the leaves was varied according to the distance of these trees from unpaved streets and agricultural roads. The mean dust deposit (mg/cm^2) according to the distance between (in meters) the roads through the year 1984 and 1985 was found to be, 0.67, 0.26 , 0.21, and 0.13 mg/cm^2 for distances of 10, 40, 80 and 120 meters, respectively from the roads. The study revealed that, the amount of adhered dust on the leaves was decreased as the trees are located further away from agricultural roads and unpaved streets. The study also revealed that, the accumulation of dust on date palm leaves has negative effect on the quality and quantity of date palm products because of the stress of dust pollution that affects the vital activities and the efficiency of leaves in offering the fruits with the carbohydrates and thus the productivity of fruits. In the study conducted by Ibrahim and Nayef (2001), a relationship was found between the amount of dust deposited on date palm trees cultivated of Hallway variety spread over three regions in the region of Basra

and wind speed and temperature of palm planting area. The amount of dust dropping on the leaves were calculated according to the fruit growth months the study indicated that the production of trees in the study sites decreased, reaching 36 kg in Abu al-Khaseeb area, followed by Al-Hartha region where the average production of palm trees was 32 kg. The variability in date palm yields in the three studied regions may be attributed to variability of the deposited dust on fruit surfaces, as well as deposited dust on the leaves that reduces the leaves' efficiency in providing the fruits with the carbohydrates needed for their growth and development.

Similar results were found in the study of sampled palm leaves from growing trees collected different environments (Al-Anee et al., 2010) including industrial environment, marine environment, agricultural environment, desert environment, and urban environment. The study found three important results: first, that palm trees play a significant role in the absorption and sedimentation of large amounts of heavy metals, and the highest sedimentation of heavy metals was found on leaves taken from the industrial environment and the lowest concentration was found in the agricultural environment and then in the desert. Second, the percentage of heavy elements that are absorbed and deposited by palm leaves (cobalt, chromium, nickel, and lead) was ranged from 22 % to 91 %. Third, palm trees account for 40-80 per cent of the amount of dust suspended in the air and the total amount absorbed by the leaf's ranges from 22 to 91 %. However, the presence of heavy metals in the air is mostly caused by air polluted by vehicles, nickel-cadmium batteries, oil industry, and mining (Chabukdhara et al., 2016). Pesticides and packaging materials may also contribute to the deposition of metals on the surface of fruit and vegetables. Moreover, the presence of heavy metals such as Pb and Cd in wash residues and date fruit was previously reported in several studies, i.e. (Aldjain et al., 2011; Hamid, 2011; Sueleman, 2014).

8.3 Conclusion

The effect of washing on some heavy metals deposited on date fruits was studied using wet digestion technique and GFAAS for analysis. Heavy metal contents in all samples were significantly reduced by washing for about 90 seconds at room temperature, and the results agree with those reported in the literature (Aldjain et al., 2011). The washing impact on dust removal varied from 34.78 to 0.95 % and seemed to be declining in the following order: Cd> Ni> Al>Fe>Sn>Pb>As>Cu. The findings demonstrate that washing can remove a significant proportion of the overall content of heavy metals attached to date fruits, lowering the danger to consumers' health.