CHAPTER 9

GENERAL CONCLUSION

9.1 Conclusion

Acetate buffered QuEChERS (AOAC) was successfully modified and optimized for date fruit matrix extraction and cleanup for pesticide's residue analysis, the factors, and responses for optimization of the extraction and cleanup steps were studied using Minitab 17.0 software and plackett-Burman (P-B) screening design program. The modified method showed high recovery and low matrix effect (< 20) for the three examined pesticides (dimethoate, carbendazim and chlorpyrifos), and it was superior on the original unbuffered method regarding the recoveries of the three examined pesticides.

A simple, low-cost, high-performance liquid chromatography with diode array detector (HPLC-DAD) method was developed and optimized for simultaneous determination of the highest risk pesticides that are frequently reported in date fruits (dimethoate, carbendazim, and chlorpyrifos) using isocratic elution with mobile phase methanol: water (95:5), 5 mM ammonium format. The peaks of dimethoate, carbendazim, and chlorpyrifos appeared at retention times of 12.79, 13.60, and 23.53 min, respectively, and they were identified by comparison with the peaks of the authentic samples. The linearity of the method was confirmed by the correlation coefficients of linear calibration curves which ranged from 0.983 to 0.999. The limit of quantification (LOQ) for each examined pesticide were found equal or lower than of the EU-MRLs which indicates the suitability of the method. The validation principles, including the accuracy (average recoveries), precision (RSDs), linearity and LOQ

values, provided evidence that the method is acceptable for the analysis of objective aimed of this work.

The health hazard index (HHI) was determined using the mean pesticide residue concentration in each induvial date fruit sample. Despite the fact that the majority of the samples surpassed the EU-MRLs of the pesticides tested, there was no health risk associated with any of the pesticides tested since no sample above the tolerable HHI limit (1) for adults or children. However, chronic disorders might be predicted if such contaminated date fruits are consumed on a regular basis.

However, excessive date fruits consumption may result in bioaccumulation, particularly for pesticides present at amounts over the MRL. As a result, the preharvest intervals (PHI), ADI, and MRL values for date pesticides used in date producing countries should be changed, and a constant and thorough monitoring regime should be implemented to restrict these residual levels.

The effect of some washing solutions including detergent, tap water, acetic acid, NaCl, $NaHCO_3$, H_2O_2 and $KMnO_4$ on pesticide residues removal from date fruit was explored and optimized regarding the solution concentration, temperature, and contact time.

The effect of the solutions on % removal of pesticide residues in date fruits was ranged from 13 – 100 % depending on the type of pesticide and the conditions of the experiment represented in the nature and concentration of the washing solution as well as temperature and washing duration. The results showed that the greater role of temperature and washing duration in increasing the capacity of solutions which may be due to the ability of heat to stimulate the dissolution and removal of the waxy layer that contains most of the residues, except $KMnO_4$ and H_2O_2 which showed slight decrease of pesticide % removal with temperature increasing, this may be attributed to the

exothermic nature of oxidant agents' reactions which can be reversed by increasing the reaction temperature.

However, carbendazim showed a relatively low removal percentage with all the tested solutions, which may be attributed to its ability to penetrate into the deep layers of dates and cannot be washed out under the applied experimental conditions. The behavior of carbendazim on the surface of date fruits should be investigated in a future study. If the experiments confirmed the ability of carbendazim to penetrate deeply into the layers of date fruits making the washing process difficult or impossible, it may be necessary to stop its use or reduce the concentration used in order to protect the health of the consumers. Furthermore, pesticide residue levels in fruits should be considered carefully. Certain organophosphorus pesticides containing P = S bonds, such as chlorpyrifos, when they encounter oxidative reagents such as H_2O_2 and $KMnO_4$, it may produce their corresponding oxygen analogues. Such analogues are more potent mammalian acetylcholinesterase inhibitors than the original parent pesticide. In terms of pesticide residue levels in fruits, such chemicals should be avoided unless absolutely necessary.

The effect of washing and optimization conditions including washing solution concentration, washing time, and washing temperature, on the nutrition value of date fruit, specifically total sugars (TS), total phenolic (TP), and antioxidant capacity (DPPH inhibition activity), was investigated. As solution concentration, contact time, and washing temperature increased, all washing solutions had a negative effect on total sugar content. The effect of washing solution on TS for 2 and 5 min seemed to be in descending in order as follows: H_2O > detergent >NaCl > H_2O_2 >$KMnO_4$ >$NaHCO_3$ >acetic acid while at 10 min, concentration of 3% $KMnO_4$ or H_2O_2 caused destructive decreasing in TS contents >33 %.

The effect of wash treatment on total phenolic contents (TP) was studied, all treatments resulted in gradual decreasing in TP contents. Increasing in contact time, temperature or concentration of washing solution led to more decreasing in TP contents. Result revealed significant differences ($p < 0.05$) in phenolic content between samples washed for different time intervals, different temperatures while change the solution concentration showed less effect. The effect of temperature and concentration is greater than the effect of contact time. In general, the washing solutions effect on TP contents was in order as follows: $KMnO_4$ > H_2O_2 > AA > NaCl >$NaHCO_3$> detergent > tap water.

The effect of washing on antioxidant activity (AC) followed a pattern similar to that of TP. This is due to the fact that AC is commonly used to refer to total phenolic contents. The presence of constituents with high free radical scavenging activity, such as phenolics and flavonoids, is well known to be the primary cause of date fruit antioxidant activity. Many researchers have discovered and reported a strong relationship between TP content in fruits and vegetables and their AC content (Allaith, 2008; Biglari et al., 2008). Finally, the observed result indicates that to minimize the effect of washing on date fruits nutrition value, it should be carried out at temperature of 25 °C or lower, the contact time should be maintained between 2-5 min and the solution concentration should be 1- 2 %. This issue should be investigated in further study.

A wet digestion technique for digestion of date fruit matrix was devised and improved in terms of digestion mixture, temperature, and digestion duration to assess the heavy metals level in the analyzed date fruit samples. Using a GFAAS spectrophotometer, the developed technique was used to measure eight heavy metals (Fe, Al, Ni, Pb, As, Cd, Cu, and Sn) in date fruit flesh and adherent dust. The observed

recoveries for all tested heavy metals at low and high concentrations ranged from 80.50 to 114.50 %, with RSDs ranging from 0.42 to 9.55 %, indicating that the established approach is suitable.

The contents of the essential and nonessential heavy metals in the examined date (Table 7.5) samples were found similar with global values. Though Pb and arsenic showed slightly higher concentrations in some samples, the estimated daily intake (EDI) calculated from the means of heavy metal concentrations in date fruit samples showed that all examined heavy metals are lower than the PTDI published by JECFA (2011). The HQ less than one for all evaluated heavy metals suggested that the intake of these dates poses no health hazards to the consumers. However, the HQ for arsenic, lead, and cadmium were 0.7, 0.18, and 0.013, respectively, indicating that exposure to those heavy metals may be a reason for worry for consumers when the consumption of these toxic elements from other foods and beverages is taken into account.

The statistical analysis showed 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, and Al, which are positive and statistically significant ($P < 0.05$). The positive and significant correlation between Cu and Ni may refer to the fact that both elements are included in fertilizers and paper products (Thakur & Parmar, 2013). The positive and significant 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 which may be because all of them are related to air contamination (Soleimani et al., 2018).

The effect of water washing on various heavy metals deposited on date fruits was investigated. Washing for around 90 seconds at room temperature greatly reduced heavy metal levels in all samples, and the results matched those published in the

literature (Aldjain et al., 2011). The washing effect on heavy metal removal rates ranged from 34.78 % to 0.95 %. The study found that washing date fruits in water can remove significant amounts of heavy metals adhered to them, lowering the risk to consumers' health.

9.2 Recommendations

1. To improve and optimize the HPLC- DAD developed method using experimental design software to study the correlation between all possible parameters to the level of pesticide concentration.
2. To improve and optimize using the experimental design software the date fruit digestion method for determination of all essential and non-essential heavy metals in date fruits.
3. The behavior of carbendazim on the date fruits after spraying should be investigated to verify the rate of its penetration into the surface of the date fruits.
4. To optimize the efficiency of washing solutions at lower temperature between 4 -20 °C and shorter duration on order to decrease the washing effect on the nutrition value of date fruits using experimental design software.