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Behaviour of some organophosphorus pesticide residues in peppermint tea during the infusion process

Ayse Ozbey, Umran Uygun *

Department of Food Engineering, Hacettepe University, 06800 Beytepe, Ankara, Turkey

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Abstract

In order to investigate dissipation behaviour of malathion, fenitrothion, dimethoate, chlorpyrifos and pirimiphos-ethyl during the infusion process, pesticide-free dried peppermint leaves were spiked with the pesticides. Infusions were prepared according to the usual process of infusion preparation. The effect of the infusion process on the transfer of the pesticides from the spiked peppermint leaves into brew was examined at intervals of 5, 10, 15 and 20 min. Residues were determined using a gas chromatograph equipped with a flame ionisation detector (FID). The decrease in pesticide levels during infusion was found to be statistically significant ($p < 0.05$). Transfer of residues decreased significantly with infusion time. The carryover of the residues of dimethoate, which has the highest water solubility, into the infusion was the highest. Satisfactory relationships were found between water-solubility (Ws), partition coefficient (K_{ow}) and Henry's law constant (H) of the pesticides with the transfer of pesticides to brewed tea. It was observed that not only water solubility or K_{ow} but also H controls the dissipation of the pesticides from water or their air–water partitioning.

Keywords: Malathion; Fenitrothion; Dimethoate; Chlorpyrifos; Pirimiphos-ethyl; Peppermint tea

1. Introduction

Peppermint is among the economically important individual herbs in Turkey. The plant is cultivated for culinary, cosmetic, and medicinal purposes. This herb is widely used as a supplement for dietetic products and especially for "self-medication" in the general population. In recent years, the demand for medicinal herbs is significantly increasing. The World Health Organization has estimated that 80% of the global population relies mainly on traditional medicine for health care (Zuin & Vilegas, 2000).

Both fresh and dried herbs are taken in many different forms such as tea, juices and decoction. The peppermint infusion is commonly consumed by people of different ages and social classes as a traditional medicine. Therefore, it is important to know the risk that their consumption has on health.

In agricultural practice, the large-scale cultivation of herbs is not possible without pesticides. The presence of high levels of residues of such chemicals can have detrimental effects on public health especially when the plants are freshly consumed. In recent years, there has been increasing concern about pesticide residues in green and black tea and hence the safety of tea containing pesticide residues (Jaggi, Sood, Kumar, Ravindranath, & Shanker, 2001; Nagayama, 1996; Tewary, Kumar, Ravindranath, & Shanker, 2005; Wan, Xia, & Chen, 1991). Although there are some studies on pesticide residues in medicinal plants (Abou-Arab & Abou-Donia, 2001; Zuin & Vilegas, 2000), the information on the effect of infusion processing on pesticide residues in herbal teas is limited in the literature. As dried herbs are subjected to infusion prior to consumption, residues of pesticides on the herbs and their transfer in brewing must be investigated.

* Corresponding author. Tel.: +90 312 2977 117; fax: +90 312 2992 123.
E-mail address: umran@hacettepe.edu.tr (U. Uygun).

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Water is a principal solvent and a reactive agent of chemical degradation. Therefore, it is responsible for considerable breakdown of pesticides in infusions (Ramesh & Balasubramanin, 1999). The carryover of pesticides from plant into the infusion depends to a very high degree on, among other factors, the water-solubility (Ws), and the octanol–water (K_{ow}) partition coefficient and the character of the herbs, e.g. their oil content (Nagayama, 1996; Petersen & Jensen, 1986).

The purpose of this study was to investigate the dissipation behaviour of pesticide residues at different time intervals during infusion processing of peppermint tea and to examine the relationship between physical–chemical properties of pesticides and the residue levels in the tea. Pesticide-free peppermint was fortified with malathion, fenitrothion, chlorpyrifos, dimethoate and pirimiphos-ethyl, which are organophosphorus insecticides. They are commonly used and monitored pesticides except pirimiphos-ethyl, which is a highly hazardous compound. Residues were determined using a gas chromatograph equipped with an FID.

2. Materials and methods

2.1. Materials

Pesticide standards, malathion, fenitrothion, dimethoate, chlorpyrifos and pirimiphos-ethyl were purchased from Promochem Ltd. (Germany). The pesticide-free dried peppermint was supplied from the local market.

2.2. Fortification of peppermint with pesticides

The dried herb samples were analytically confirmed for residue of pesticides. Then the pesticide-free samples (2 g) were spiked using the pesticide standard solutions (400 μg ml^{-1}) (solution I: dimethoate (D), pirimiphos-ethyl (P), malathion (M) and solution II: fenitrothion (F) and chlorpyrifos (C)) prepared in ethyl acetate (1 ml). The samples were sprayed by the solutions separately at the concentrations of 40 mg kg^{-1} for each pesticide and the fortified samples were subjected to the infusion process after evaporating the excess of solvent at room temperature in a fume cupboard for 15 min according to Lino and Silveira (1997).

2.3. Infusion process

Infusions were prepared with tap water and using a stainless steel kettle according to a manner similar to the usual process of infusion preparation. Two grams of spiked samples were immersed in 100 ml of boiling water and allowed to stand at boiling temperature for 5, 10, 15 and 20 min, respectively. After brewing, the infusions were filtered through an ordinary stainless steel tea strainer and cooled. Both the infusions and the spent leaves after brewing were examined for the residues separately. The procedure was carried out in duplicate.

2.4. Extraction

All of the spent leaves after brewing were homogenized with ethyl acetate (30 ml) and anhydrous sodium sulphate (10 g) in a high-speed blender for 2 min. The homogenate was filtered and the residues were extracted twice with ethyl acetate (2 × 50 ml). The combined extracts were concentrated to ca. 20 ml in vacuo at 40 °C using a rotary evaporator. Clean-up by gel permeation chromatography was performed as previously described (Uygun, 1997).

The infusion (100 ml) was placed into a 500 ml separating funnel containing 50 ml of dichloromethane. Then, the separating funnel was shaken for 2 min and the dichloromethane phase was collected and dehydrated with anhydrous sodium sulphate (15 g). The aqueous phase was reextracted with 2 × 50 ml of dichloromethane. The dichloromethane phases were concentrated in a vacuum evaporator (40 °C) to 10 ml and then the remaining solvent was vaporized to dryness by purging with nitrogen gas. The residue was dissolved in 10 ml of ethyl acetate and 1 ml of this sample was applied onto the gel permeation chromatography column.

2.5. Recovery study

To evaluate the recovery of pesticide residues in the infusion, 1 ml of a pesticide standard solution (100 mg kg^{-1}) was added to 100 ml of pesticide-free peppermint infusion for two replications. These samples were subjected to the whole procedure (extraction procedure and GPC clean-up).

2.6. Gas chromatography

Gas chromatography was performed using a HP5890 gas chromatograph equipped with a flame ionisation detector and capillary column (Alltech AT-1, 30 m × 0.32 mm ID, 0.25 μm film thickness) using nitrogen carrier gas at a flow rate of 2 ml min^{-1}. The oven temperature program was: initial temperature isothermal at 150 °C for 5 min, then from 150 °C to 250 °C at 10 °C min^{-1}, then hold 15 min at 250 °C. Injector and detector temperature was 250 °C. Quantification of the pesticides was performed by comparing the peak areas to that of a calibration curve of standards. Correlation coefficients were found to be higher than 0.98 in all cases, indicating a good linearity.

2.7. Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA) procedure. When significant differences were found, the least significant difference (LSD) test was used to determine the differences among means.

3. Results and discussion

3.1. General

Analytically confirmed pesticide-free peppermints were spiked with the pesticides for determining the residues in infusions. According to Wan et al. (1991) the extraction rates of pesticides from tea infusion prepared the field samples and the fortified samples were found to be the same.

A multi-residue pesticide analysis was used for determination of the pesticides in the infusions and the spent leaves. The compounds were identified from their chromatogram and confirmed by comparison with authentic standards. In organophosphorus pesticide analysis, dichloromethane is most popular solvent for partitioning against the aqueous acetone extract. In this study using dichloromethane as an extracting solvent gave good results, which as observed from the recovery studies (Table 1). Similarly Tewary et al. (2005) also used dichloromethane as an extracting solvent for pesticides from infusions. The reproducibility of the recovery results, as indicated by the standard deviations, suggested that extraction and cleanup procedure could be considered reliable enough for the routine analysis of the pesticide residues in tea.

Table 1
Recovery mean of the pesticides from peppermint infusions

Pesticides	% Recovery ± sd
Fenitrothion	85.4 ± 4.75
Malathion	92.8 ± 3.19
Pirimiphos-ethyl	95.1 ± 4.43
Dimethoate	97.6 ± 4.29
Chlorpyrifos	109.5 ± 4.22

3.2. Transfer of the pesticides into brew

The effect of infusion processing on the transfer of the pesticides from the spiked peppermint leaves into brew was examined at intervals of 5, 10, 15 and 20 min of infusion. The results of the transfer of the pesticides from leaves to brew are shown in Fig. 1. The residual level was different with each pesticide. The decrease in pesticide levels was found to be statistically significant for malathion, pirimiphos-ethyl, fenitrothion and chlorpyrifos during 10,

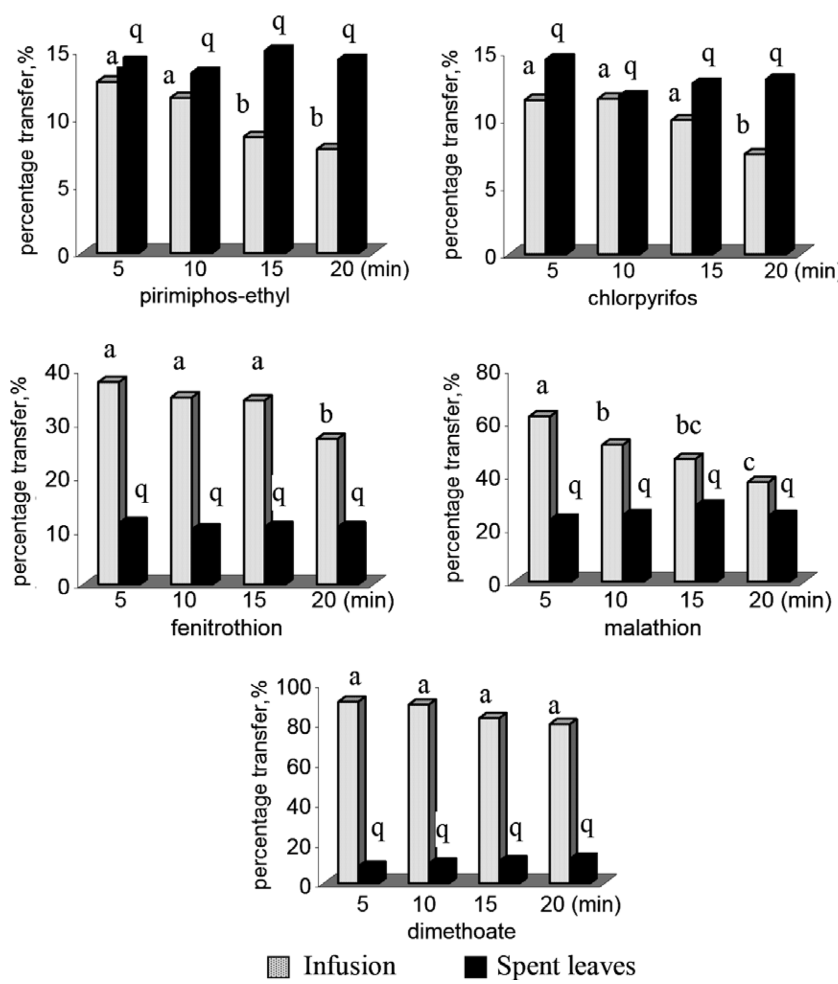

Fig. 1. Effect of time on pesticide residues in peppermint infusion. Data are the mean of four replicates. Values followed by the same letter are not significantly different ($p < 0.05$).

Table 2
Residue levels in peppermint infusion (mg kg^{-1})

Time (min)	Fenitrothion		Chlorpyrifos		Dimethoate		Malathion		Pirimiphos-ethyl	
	Infusion	Spent leaves	Infusion	Spent leaves	Infusion	Spent leaves	Infusion	Spent leaves	Infusion	Spent leaves
5	15.1^{a}	4.57^{a}	4.58^{a}	5.63^{a}	36.5^{a}	3.75^{a}	24.9^{a}	9.33^{a}	5.07^{a}	5.68^{a}
10	13.9^{a}	4.07^{a}	4.59^{a}	4.83^{a}	35.8^{a}	3.80^{a}	20.6^{b}	10.0^{a}	4.60^{a}	5.35^{a}
15	13.8^{a}	4.27^{a}	3.96^{a}	5.01^{a}	33.2^{a}	4.12^{a}	18.5^{bc}	11.5^{a}	3.45^{b}	6.00^{a}
20	10.8^{b}	4.24^{a}	2.96^{b}	5.39^{a}	31.9^{a}	4.74^{a}	15.0^{c}	9.88^{a}	3.09^{b}	5.73^{a}

Data are the mean of four replicates. Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Table 3
Physical–chemical properties of the pesticides under study

Pesticides	Solubility (mg/l)[a] (at 20 °C)	Octanol–water partition coefficient[a] (log K_{ow})	Henry's law constant (Pa m^3/mol)[b] (at 20 °C)	Vapour pressure (Pa)[b] (at 20 °C)
Chlorpyrifos	1.4	4.7	1.75	0.0025
Pirimiphos-ethyl	2.3	5	–	0.00068[a]
Fenitrothion	21	3.43	0.0036	0.00040
Malathion	145	2.75	0.0023	0.0010
Dimethoate	23300	0.7	0.00011	0.021

[a] Tomlin (1994).
[b] Suntio et al. (1988).

15 and 20 min of infusion, respectively (Table 2). It was observed that the carryover of the residues of dimethoate into the infusion was highest (91%), followed by malathion (62%) and fenitrothion (38%), whereas the transfer rates of pirimiphos-ethyl (13%), and chlorpyrifos (11%) were comparatively less during 5 min infusion (Fig. 1). Transfer rates of the pesticides followed the same trend at the rest of the infusion periods. The residues on spent leaves remained generally unchanged.

The high transfer of dimethoate into the infusion mostly depends on the water solubility (Table 3). Pirimiphos-ethyl and chlorpyrifos residues on spent leaves were found to be higher than that of infusion depending upon their low solubility. Although water solubility plays an important role, the results obtained were not necessarily dependent on this factor alone. In this study, satisfactory relationships were found between water solubility (Fig. 2), partition coefficient (Fig. 3) and Henry's law constant (Fig. 4) of the pesticides with the transfer of pesticides to brewed tea. Correlation coefficients were found to be higher than 0.98 in all cases, indicating a good linearity. The pesticides with comparatively larger K_{ow} values showed minimum transfer

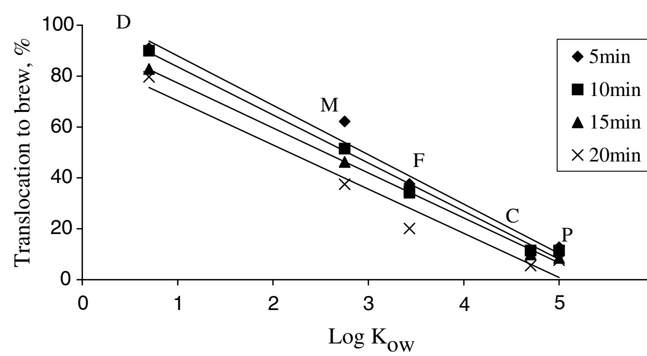

Fig. 3. The relationship between octanol–water partition coefficient and pesticide translocation.

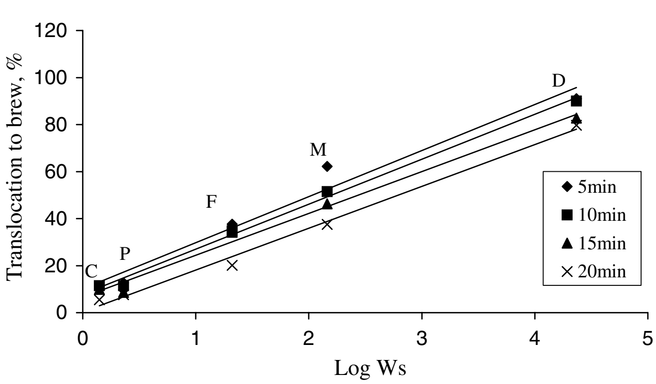

Fig. 2. The relationship between water solubility and pesticide translocation.

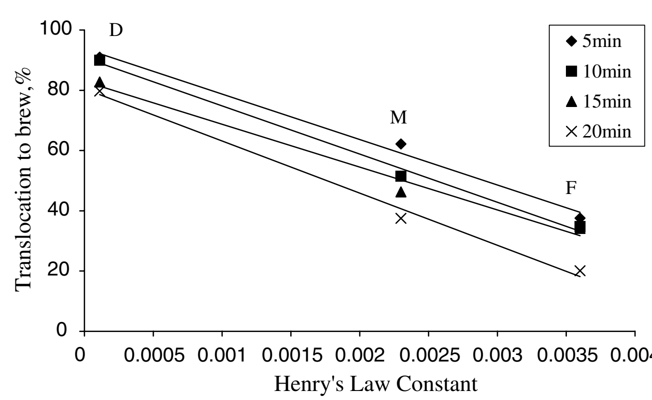

Fig. 4. The relationship between Henry's law constant and pesticide translocation.

to the infusion. Similar relationships were observed with the increased infusion time.

Nagayama (1996) and Jaggi et al. (2001) have explained similar relationships between transfer rate and water solubility and partition coefficient in tea brews. Jaggi et al. (2001) reported that with increased brewing time, concentration of a pesticide in water could increase or decrease depending upon its fugacity and volatility proportions. Fugacity is a measure of the tendency of a component to escape or expand. Many pesticides are known to move, as vapour, between the atmosphere and soil, plant, and water surfaces. The direction of this transfer is dictated by the fugacity of each component of the system, which in turn is controlled by the Henry's law constants (H). Suntio, Shiu, Mackay, and Seiber (1988) have expressed the H value of pesticides, which are calculated from fugacity. They also reported that the pesticide with H value below 1 Pa m^3 mol^{-1} is only slightly volatile and the rate of evaporation falls as H decreases. In the range of 1–25 Pa m^3 mol^{-1}, volatilisation is still slower and may or may not be significant depending on the competitive processes. In the present study, both chlorpyrifos and pirimiphos-ethyl were not given in Fig. 4. Since there is no H value reported in the literature for pirimiphos-ethyl and H value of chlorpyrifos was much higher (several orders of magnitude) as compared the other pesticides under this study. Therefore, it was not possible to include chlorpyrifos in Fig. 4. Chlorpyrifos showed the lowest transfer to infusion as expected from its high H value (>1 Pa m^3 mol^{-1}). On the other side, the pesticide, dimethoate, with the lowest H value showed the maximum transfer to infusion. It is noteworthy that significant correlations were found between the Henry's law constants of the pesticides under study and transfer of residue percentage for each infusion period ($R^2_5 = 0.990$, $R^2_{10} = 0.994$, $R^2_{15} = 0.982$, $R^2_{20} = 0.992$).

4. Conclusion

The present study revealed that during the infusion process, a significant percentage of the residues, particularly pesticides with high water solubility, were transferred to the infusions. The higher dissipation of the pesticide residues occurred in the first 5 min of the infusion process. The increase in infusion time resulted in a lower level of pesticide residue in the brew. Although the transfer of pesticides under study primarily appeared to be dependent on their water solubility, the decrease of residues at longer duration of brewing could be due to the volatilisation of the pesticides.

On the basis of the above findings it can be concluded that the persistence of pesticides in infusions are strongly predictable from the physical–chemical properties of pesticides such as water solubility, octanol–water partition coefficient and Henry's law constant. It should be emphasized that not only water solubility or K_{ow} but also H controls the dissipation of the pesticides from water or their air–water partitioning. The results observed in the present study indicated that the dissipation rate of pesticides residues during infusion was satisfactory, except dimethoate because of its high water solubility. However, to prevent any health problem to consumers, the establishment of maximum residue limits for pesticide residues in herbal teas should be considered.

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