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Research Paper / Araştırma Makalesi

Effect of Processing Type and Storage Time on Some Pesticide Residues in Strawberries

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ABSTRACT

In this study, 9 active substances that could be analysed by Gas Chromatography-Mass Spectrometer (GC/MS) were used. Changes in these pesticide residues were determined after strawberries were washed, pasteurized, stored in cold and washed on different days. In addition, strawberry puree was stored at -18±2ºC and pasteurized puree was stored at different temperatures. The highest and lowest processing factors in the pasteurization process were determined in tebufenpyrad (Pf:1.20) and tetraconazole (Pf:0.81), respectively. During cold storage, kresoxim-methyl degradation was found statistically significant ($p<0.05$). At the end of cold storage, penconazole was below the quantification limit while pyrimethanil and tebufenpyrad did not change; however, azoxystrobin, boscalid, tetraconazole and kresoxim-methyl residues decreased by 3.8, 10.9, 25.0 and 36.4%, respectively. Washing process did not reduce tebufenpyrad residues. On the other hand, reduction rates were 3.8, 4.2, 4.5 and 9.1% for azoxystrobin, pyrimethanil, bupirimate and kresoxim-methyl, respectively while they were 10.9% for boscalid and 16.7% for tetraconazole. During long-term storage, the highest and the fastest decrease in pesticide residues was observed at 20±2ºC, and this decrease slowed down with the effect of pasteurization. Pesticide degradation rates at the end of one-year storage period in the samples stored at -18±2 ºC following pasteurization were 20, 23, 26, 27, 37 and 41% for tetraconazole, pyrimethanil, azoxystrobin, kresoxim-methyl, boscalid and bupirimate, respectively. As a result, it was found that pesticide degradation was dependent upon the chemical nature of pesticides, initial concentration, agricultural commodity, processing and storage conditions.

Keywords: Strawberry, Pesticide Residue, Processing, Storage, Washing

Çileklerdeki Bazı Pestisit Kalıntıları Üzerine İşleme Türü ve Depolama Süresinin Etkisi

ÖZ

Bu çalışmada Gaz Kromatografisi-Kütle Spektrometresi (GC/MS) ile analiz edilebilen 9 aktif madde kullanılmıştır. Çileklerin yıkanması, pastörize edilmesi, soğukta muhafaza edilmesi ve farklı günler yapılan yıkama işlemi sonrasında kalıntı değişimleri belirlenmiştir. Ayrıca çilek püresi -18±2ºC'de, pastörize püre ise farklı sıcaklıklarda muhafaza edilmiştir. Pastörizasyon işleminde en yüksek ve en düşük işleme faktörü sırasıyla tebufenpirad (Pf:1.20) ve tetrakonazol (Pf:0.81) için hesaplanmıştır. Soğuk muhafaza esnasında kresoksim-metil degradasyonu istatistiksel olarak önemli bulunmuştur ($p<0.05$). Soğuk muhafaza işlemi sonunda penkonazol hesaplama limitinin altına düşmüştür. Pirimetanil ve tebufenpirad miktarlarında değişme olmamıştır. Azoksistrobin, boskalid, tetrakonazol ve kresoksim-metil kalıntılarının sırasıyla %3.8, 10.9, 25.0 ve 36.4 azaldığı belirlenmiştir. İlaçlı çileklerin yıkanması ile tebufenpirad kalıntılarında azalma olmamıştır ancak azoksistrobin %3.8, pirimetanil %4.2, bupirimat %4.5, kresoksim-metil %9.1, boskalid %10.9 ve tetrakonazol kalıntıları %16.7 oranında azalmıştır. Uzun süreli muhafazada pestisit kalıntılarında en çok ve en hızlı azalmanın 20±2ºC'de olduğu ve pastörizasyon işleminin etkisiyle bu azalmanın

yavaşladığı belirlenmiştir. Pastörizasyon işleminden sonra -18±2ºC'de muhafaza edilen örneklerdeki pestisit degradasyonu bir yılın sonunda tetrakonazol %20, pirimetanil %23, azoksistrobin %26, kresoksim-metil %27, boskalid %37 ve bupirimat %41 olarak belirlenmiştir. Sonuç olarak degradasyonun pestisitin kimyasal özelliklerine, başlangıç konsantrasyonuna, tarımsal ürüne, yapılan işleme ve muhafaza koşullarına bağlı olarak değiştiği belirlenmiştir.

Anahtar Kelimeler: Çilek, Pestisit Kalıntısı, İşleme, Depolama, Yıkama

INTRODUCTION

Fruits are susceptible to insect and disease attacks; therefore, pesticides are widely used. Besides the many benefits of pesticide use, the intensive or unconscious usage during the growing period or after, causes the active substance itself or its breakdown products to remain in the agricultural products [1]. Even when strict maximum residue levels are implemented and pesticides are used properly, pesticide residues are sometimes unavoidable and can be found even in processed agricultural products. For this reason, it is important to carry out residue studies in processed plant products as well as the plants to evaluate pesticide residue behaviour [2-3].

Plants and plant products are subjected to many processes from simple washing to more complex steps in the home and industry as a requirement to obtain a consumable product, prolong shelf life, and increase variety, flavour and nutritional value [4]. Washing or cleaning, peeling, blanching, baking and pasteurization are some of these. Many studies suggest that different techniques and methods reduce pesticide residues. However, several studies have shown that some processes, such as drying and oil production, cause an increase in pesticide residues [5].

The residue level in the products before and after processing is expressed by processing factor. Processing factor (Pf) >1 or <1 indicates increased or reduced residue levels after processing, respectively [6-7]. Processing factor is a conversion factor which indicate the determined residue content was within permitted levels or not. Both in Türkiye and in other countries maximum residue limits in fresh fruits and vegetables were subject to legislations. However in processed fruits and vegetables, such as apple juice or in prunes there is no legislation available. Therefore, there is a necessity for determination of processing factors on the basis of each pesticide and each product.

Strawberry (*Fragaria* sp.) is one of the most widespread fruit species grown in many parts of the world. Because of the health and nutrient value and economical benefit, its production continues to increase [8]. According to FAO, strawberry production increases every year in the World and Türkiye. World strawberry production was 8,893,598 ton and 9,175,384 ton in 2020 and 2021, respectively. Also strawberry production in Türkiye was increased from 546,525 ton to 669,195 ton in same time of period [9]. Strawberry; with its taste, vitamin and mineral substances has become a part of the diet of millions of people around the world. Besides, it has antioxidant substances and ellagic acid that has benefits to health [10-11].

The objectives of this study were to evaluate the effects of washing, pasteurization and cold storage in the reduction of pesticide residues in strawberries and to find out residue degradation in puree storage at different temperatures. In this study tebufenpyrad, etoxazole, kresoxim-methyl, boscalid, pyrimethanil, tetraconazole, bupirimate, azoxystrobin and penconazole were used. The selection of pesticides was based on whether they were registered Türkiye, availability of maximum residue limits in European Union and the usage of them for diseases and pests seen in strawberries.

MATERIALS and METHODS

Materials

Azoxystrobin (99.3%), bupirimate (99.9%), boscalid (99.9), etoxazole (99.4%), penconazole (99.1%), pyrimethanil (99.9%), kresoxim-methyl (96.0%), tebufenpyrad (99.9) and tetraconazole (99.5%) standards were purchased from Sigma Aldrich (St. Louis, USA).

The chemicals used were all analytical grade and the suppliers were as follows: Bondesil-PSA was from Varian (Palo Alto, CA, USA); sodium chloride, anhydrous magnesium sulphate, acetonitrile (LC and GC grade) and acetic acid were purchased from Sigma-Aldrich. High purity nitrogen gas (99.99%) was from Linde (Ankara, Türkiye). Stock standard solutions (1000 mg L^{-1}) of the pesticides were prepared in acetonitrile and stored at -18±2°C. Working standard solutions of different concentrations were prepared by diluting the stock standard solutions to the required concentrations in acetonitrile. All prepared working solutions were kept at -18±2°C until analysis.

Sample Preparation

'Rubygem' variety strawberries were purchased from a vegetable-fruit market in Ankara (Türkiye). Strawberries were analysed before experiments to make sure that they were free from any of the pesticides to be evaluated in this study. Before pesticide spraying, sepals were hand-removed, fruits were washed under running water, and excess water was dried by keeping them on a filter paper.

Strawberry samples were sprayed at a concentration of 30% more than the maximum residue level (MRL) of pesticide active substances. The MRLs were taken from the Pesticides Database of the European Union [12]. To determine the amount of solution to be used in spraying, 1 kg of strawberries was placed in a strainer and water was sprayed on until it dripped well from the underside.

In this way, the approximate amount of water required for 1 kg of strawberries was determined as 100 mL [13]. Strawberry samples were sprayed as two groups with pesticide mixtures:

Group I: Pyrimethanil, tetraconazole, bupirimate, etoxazole, azoxystrobin
Group II: Penconazole, kresoxim-methyl, tebufenpyrad, boscalid

After pesticide treatment, each group was kept overnight at 20ºC to dry. Pesticide-sprayed strawberries were analysed to determine the initial residue amount, and the following applications were made for the remaining part.

Analyses performed within the first 24 hours after spraying were accepted as day 0 analysis. Experiments were set up in three replicates, and a total of 33 kg of strawberries were used, in each pesticide group. Experiments were carried out separately for each active substances group, and 66 kg of strawberries were used in total. Recovery experiments and pesticide analyses were done as five and three replicates, respectively. The experimental design is summarised in Figure 1.

Washing

Strawberries (1 kg) were divided into two equal parts. The first part was washed in a stainless steel basket in an ultrasonic water bath containing 2 L of deionised water at 22±2ºC for 5 minutes and the water on the surface was allowed to dry; then, strawberries were homogenised and analysed for pesticide residues. The second part was homogenised without washing and analysed after homogenisation.

To determine the effect of cold storage and washing during different days on pesticide degradation, some parts of pesticide-treated strawberries were kept at 4±0.5ºC and washed after four days as explained above.

Pasteurization

Pesticide-applied strawberries were homogenized, packed in hot-water-washed 210-mL glass jars with the lids closed and pasteurized at 96ºC for 5 minutes in boiling water. The pasteurization process was repeated for washed strawberries.

Cold Storage

To reveal the effects of cold storage on pesticide degradation, 1000 g of pesticide-sprayed strawberries for each replicate were kept in (4±0.5°C) and analysed on days 0, 1, 3 and 6 without washing.

Puree Preparation and Storage

After the surface of the pesticide-sprayed strawberries dried, they were divided into two parts (Figure 1). Part 1: Strawberries were homogenized and again divided into 2 parts. The first part was immediately frozen in 150 mL polypropylene sample containers and stored at -18±2ºC ($Aa_{(-18)}$ samples). The other part was pasteurized at 96ºC for 5 minutes after being placed in 210 mL glass jars and sealed. Pasteurized samples are divided into 3 parts. The first part is stored in the deep freezer (-18±2ºC) ($Ab_{(-18)}$ samples), the second part is in the refrigerator (4±0.5ºC) ($Ab_{(+4)}$ samples), and the third part is at 20±2ºC ($Ab_{(+20)}$ samples). Pesticide analysis was performed in all samples on different days, and the changes that occurred were determined. Part 2: The sprayed strawberries were washed and homogenized after the water on them dried. The procedures for section 1 were carried out separately for the washed strawberries, and sample codes are given in Figure 1.

Sample Extraction

Pesticide residues on strawberries were extracted according to method of [14-15]. After homogenization of strawberries in a blender, 10 g of the homogenized sample was weighed into a 50 mL polypropylene tube, and 10 mL of acetonitrile was added. The tube was capped and shaken vigorously by hand for 1 min. Then, 4 g anhydrous $MgSO_4$ and 0.5 g NaCl were added. The tube was capped and shaken vigorously by hand again for 1 min, then centrifuged at 6000 rpm for 5 min. 2 mL of acetonitrile extract was transferred to a 15-mL polypropylene tube containing 500 mg anhydrous $MgSO_4$ and 150 mg PSA. The extract was mixed using a vortex mixer for 1 min and centrifuged at 6000 rpm for 5 min. The organic phase was filtered through a polytetrafluoroethylene (PTFE) filter with a 0.45 µm pore size and analysed directly in GC/MS.

Instrumental Analysis

Pesticides were analysed by GC with MS detector. The system (Agilent 6890N Series GC with a 5973 Mass Selective Detector) consists of an HP-5MS column (5% phenyl methyl siloxane, 30 m × 0.25 mm × 0.25 µm film thickness) (Agilent 19091S-433). The carrier gas (helium) flow rate was set at a constant 2.0 mL min^{-1}. A splitless injection of 2 µL was carried out at 250°C. The oven temperature program was 70°C (2 min), 25°C min^{-1} to 150°C (0 min), 3°C min^{-1} to 200°C (0 min) and 8°C min^{-1} to 280°C (10 min). The acquisition mode was scan (50-450 AMU) and SIM, solvent delay time was 8.00 min, Electron Multiplier offset was 400 (1882 EM voltage), transfer line was 280°C, MS quadrupole was 150°C and MS source was 230°C. Chromatograms were evaluated with MSD ChemStation E.02.02.1431 software

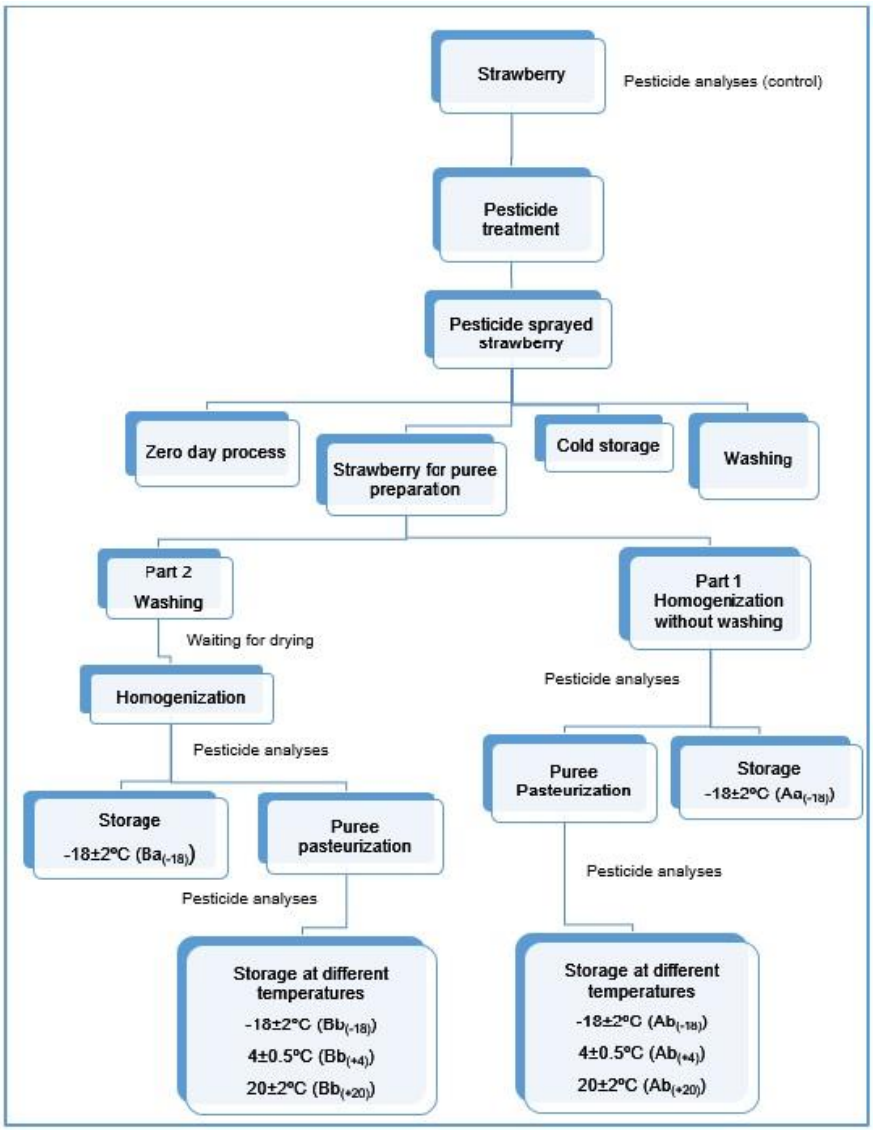

Figure 1. Experimental design

Method Validation

The applied method was tested to assess linearity, mean recovery (as a measure of trueness or bias), precision (as repeatability RSD) and Limit of Quantification (LOQ) according to the guidance document on analytical quality control and validation procedures for pesticide residue analysis in food and feed [16].

Linearity (r^2) was determined with standard pesticide solutions prepared in strawberry matrix. 8-point (0.01, 0.025, 0.050, 0.1, 0.2, 0.5, 0.7, 1.0 mg kg^{-1}) calibration curve was used for pesticides studied by GC/MS. Samples were spiked with appropriate volumes of stock mix solution at different concentrations (0.05 and 0.1 mg kg^{-1}). Five replicates were prepared for each concentration, and they were extracted according to the aforementioned procedure. Acceptable mean recoveries were those within the range 70-120%, with an associated repeatability RSD ≤ 20% for all compounds. The method LOQ was accepted as the lowest spike levels (0.05 mg kg^{-1}). Retention times, maximum residue levels (MRLs) and method validation characteristics of pesticides are summarized in Table 1.

Table 1. Performance characteristics of method

Pesticide	Retention time (min)	Molecular mass (g mol^{-1})	SIM parameters	Fortified level (mg kg^{-1})	Recovery (%)	RSD (%)	r^2	LOQ (mg kg^{-1})	MRL (mg kg^{-1})
Pyrimethanil	14.81	199.25	198, 199, 200, 77	0.05	78.4	8.0	0.9918	0.05	5
				0.1	112.0	1.4			
Tetraconazole	20.51	372.15	336, 338, 171, 337	0.05	100.7	7.6	0.9996	0.05	0.2
				0.1	106.9	8.9			
Bupirimate	25.18	316.42	273, 166, 274, 316	0.05	111	6.9	0.9989	0.05	2
				0.1	95.3	13.8			
Etoxazole	29.50	359.41	141, 204, 300, 187	0.05	78.2	5.9	0.9985	0.05	0.2
				0.1	81.6	12.7			
Azoxystrobin	37.31	403.40	344, 388, 345, 372	0.05	73.7	1.0	0.9937	0.05	10
				0.1	72.6	2.7			
Penconazole	21.74	284.2	248, 159, 161, 250	0.05	71.0	6.1	0.9992	0.05	0.5
				0.1	100.0	3.4			
Kresoxim-methyl	25.27	313.35	116, 131, 206,132	0.05	76.1	5.5	0.9990	0.05	1.5
				0.1	73.3	5.0			
Tebufenpyrad	29.70	336.86	318, 333, 171, 276	0.05	70.2	2.8	0.9967	0.05	1
				0.1	74.0	13.0			
Boscalid	32.88	343.21	140, 342, 344, 112	0.05	94.2	3.0	0.9996	0.05	6
				0.1	99.4	1.5			

Statistical Analysis

SPSS 21.0 package program was used for statistical analysis. Analysis of variance (One-way-ANOVA and general linear model univariate analyses) was performed to determine whether there was a significant difference between the samples. In these analyses, 95% confidence level ($p<0.05$) was taken into account. In addition, the t-test was performed for washings on the 0 and 4 days. Results were expressed as mean±standard errors.

RESULTS and DISCUSSION

Validation

In our study, the lowest correlation coefficient was 0.9918 when pyrimethanil was used. Since the correlation coefficients of all analysed pesticide active substances were greater than 0.99 (Table 1), it was determined that the linearity of the analysis method we used was good. Different methods have been applied for the extraction of pesticide active substances from different matrixes and the applied methods have been validated by the researchers. Researchers have developed and validated methods, for 151 pesticides in strawberries [17], also 16 pesticides in peppers [18] and 51 pesticides in tomatoes [19]. In all three methods, the calibration curves were prepared as matrix-matched to eliminate the matrix effect, and the correlation coefficient of each active substance were 0.99 and above. Thus, good linearity was achieved in all.

Repeatability control (as a measure of trueness or bias), was performed with spiked strawberry samples at two different concentrations (0.05 and 0.1 mg kg^{-1}) with 5 replicates and obtained recoveries (%) and RSD (%, as precision) were shown in Table 1. As seen from this, the recoveries were in the range 70.2–112%, and the RSD percentages varied within 1–13.8%. All of the results were within the acceptable range of the validation assays. In addition, LOQ (0.05 mg kg^{-1}) was accepted as the lowest concentration of spiking level. In a study pesticide-free pepper samples were spiked at two different concentrations (LOQ and 10× LOQ; 10 and 100 µg kg^{-1}) with 7 replicates. They reported that recoveries were in the range 70–110% and the RSD results were below 20% [18]. In another study, conducted on apple samples, recoveries (0.5, 1 and 10 mg kg^{-1}) and RSDs for pyrimethanil, fludioxonil, cyprodinil and kresoxim-methyl were found 97–105% and 2.6-6.2% respectively. The calibration curve for each active substance was prepared in five different concentrations in the matrix. Correlation coefficients were greater than 0.9953 and the lowest spiked level (0.5 mg kg^{-1}) taken as the limit of quantification that meets the method validation criteria [20]. Recovery for azoxystrobin analysis in strawberries was 96–111%, and the RSD was below 11% [21]. The selectivity of the method was evaluated by analysing the unspiked samples. The absence of any signal at the same elution time as the target pesticide suggested that there was no matrix interference.

Residues

Among the active substances studied, etoxazole was not assessed because the amount of etoxazole was below the LOQ on the zeroth day.

Pesticide Residue Changes with Different Treatments

Variations in pesticide residues related to washing, pasteurization and washing+pasteurization are given in Table 2. If initial concentrations were assumed as 100, the amount of decrease in active substances were as follows with washing: tetraconazole %16.7, boscalid %10.9, kresoxim-methyl %9.2, bupirimate %4.5, pyrimethanil %4.2 and azoxystrobin %3.8. Tebufenpyrad amount did not change, and penconazole fell below the LOQ (Table 2).

In our study bupirimate and tetraconazole residues decreased by 4.5% and 16.7%, while in another study bupirimate and tetraconazole residues in strawberries decreased by 56.6% and 84.5%, respectively, by washing [22]. Differences in these studies may be due to the differences in washing efficiency of the water

baths, water amount used in washing and the initial content of residues.

Pasteurization led to different changes in pesticide residue amounts. If initial active substance concentrations were assumed as 100, the amount of decrease in active substances were tetraconazole (16.7), azoxystrobin (5.8) and boscalid (6.5). It is thought that the decrease in pesticide residues with pasteurization may be due to the degradation and hydrolysis caused by the heat effect. Bupirimate, kresoxim-methyl and penconazole amounts remained unchanged while pyrimethanil (4.2) and tebufenpyrad (20) increased (Table 2). While there was no change in some pesticide residues with pasteurization, there was an insignificant ($p>0.05$) increase in some of them. Considering that the system is closed and there is no water loss, it is thought that the increase in the concentrations of some active substances is because the related active substances have been more extracted from the tissue softened by the effect of heat.

Pyrimethanil residue was reduced with washing by 4.2%, and pasteurization did not lead to a significant reduction (Table 2). In one study, azoxystrobin and fenhexamid residues were reduced by washing, while pyrimethanil residue remained unchanged [23]. In both studies, similar results were obtained for pyrimethanil.

Table 2. Changes of pesticide residues with different treatments, cold storage (4±0.5ºC) and washing in different days (mg kg^{-1}) (mean±standard error) (n=3)

Pesticide	Zeroth day different treatments				Cold storage			Washing different days		
	Process	Concentration	PF	P*	day	Concentration	P	Washing day	Concentration	P
Pyrimethanil	Unprocessed	0.47±0.031	-	0.653	0	0.47±0.031	0.433	unwashed	0.47±0.031	0.015
	Pasteurization	0.49±0.026	1.04		1	0.40±0.034		0	0.45±0.005	
	Washing	0.45±0.005	0.96	0.340	3	0.44±0.026		4	0.41±0.009	
	Washing+past.	0.48±0.030	1.02		6	0.47±0.042				
Tetraconazole	Unprocessed	0.12±0.003	-	0.058	0	0.12±0.007	0.001	unwashed	0.12±0.003	0.193
	Pasteurization	0.10±0.007	0.81		1	0.09±0.002		0	0.10±0.003	
	Washing	0.10±0.003	0.81	0.892	3	0.09±0.002		4	0.09±0.001	
	Washing+past.	0.10±0.003	0.80		6	0.09±0.001				
Bupirimate	Unprocessed	0.22±0.002	-	0.660	0	0.22±0.002	0.041	unwashed	0.22±0.002	0.184
	Pasteurization	0.22±0.008	1.00		1	0.17±0.021		0	0.21±0.003	
	Washing	0.21±0.003	0.95	0.286	3	0.20±0.009		4	0.20±0.007	
	Washing+past.	0.21±0.005	0.95		6	0.24±0.014				
Azoxystrobin	Unprocessed	0.52±0.009	-	0.219	0	0.52±0.009	0.035	unwashed	0.52±0.009	0.021
	Pasteurization	0.49±0.018	0.94		1	0.52±0.042		0	0.50±0.012	
	Washing	0.50±0.012	0.98	0.297	3	0.38±0.042		4	0.43±0.016	
	Washing+past.	0.48±0.018	0.92		6	0.50±0.013				

Table 2. . Changes of pesticide residues with different treatments, cold storage (4±0.5ºC) and washing in different days (mg kg^{-1}) (mean±standard error) (n=3) (continued)

Pesticide	Zeroth day different treatments				Cold storage			Washing Different days		
	Process	Concentration	PF	P*	day	Concentration	P	Washing day	Concentration	P
Penconazole	Unprocessed	0.06±0.004		0.757	0	0.06±0.004	0.663	unwashed	0.06±0.004	**
	Pasteurization	0.06±0.000	1.00		1	0.05±0.006		0	0.06±0.004	
	Washing	<LOQ***		**	3	0.05±0.004		4	<LOQ	
	Washing+past.	0.05±0.002	0.83		6	<LOQ				
Kresoxim-methyl	Unprocessed	0.11±0.005	-	0.501	0	0.11±0.005	0.004	unwashed	0.11±0.005	0.004
	Pasteurization	0.11±0.005	1.00		1	0.12±0.010		0	0.10±0.005	
	Washing	0.10±0.005	0.91	0.406	3	0.08 ±0.004		4	0.06±0.003	
	Washing+past.	0.09±0.005	0.82		6	0.07±0.005				
Tebufenpyrad	Unprocessed	0.10±0.003	-	0.405	0	0.10±0.003	0.253	unwashed	0.10±0.003	0.340
	Pasteurization	0.12±0.015	1.20		1	0.10±0.010		0	0.10±0.003	
	Washing	0.10±0.003	1.00	0.507	3	0.09±0.006		4	0.08±0.014	
	Washing+past.	0.10±0.003	1.00		6	0.10±0.001				
Boscalid	Unprocessed	0.46±0.032	-	0.610	0	0.46±0.032	0.387	unwashed	0.46±0.032	0.455
	Pasteurization	0.43±0.034	0.93		1	0.44±0.036		0	0.41±0.033	
	Washing	0.41±0.033	0.89	0.664	3	0.38±0.025		4	0.38±0.021	
	Washing+past.	0.42±0.002	0.91		6	0.41±0.024				

*: P values calculated for 95% confidence level (α=0.05) (P values lower than 0.05 indicates that the considered parameter is significant). **: Since it is a single value, statistical evaluation has not been made. ***: <LOQ: below limit of quantification.

In our study, bupirimate decreased by 4.5% with washing but, it did not change by pasteurization as mentioned before. In a study it was reported that bupirimate residues in apricot decreased 26.7% by pasteurization, and 20.7% by washing. In the same study, bupirimate residues in peach samples were removed by washing and pasteurization by 50.7% and 100%. Same pesticides were reduced in varying amounts in different fruits [24].

Washing with a following pasteurization process also had different effects on pesticide concentration (Table 2). While tebufenpyrad did not change, pyrimethanil increased by 2.1%. In addition, the amounts of other active substances decreased at varying rates. These

decreases were as follows in reduced order: bupirimate 4.5%, azoxystrobin 7.8%, boscalid 8.7%, penconazole 16.7%, tetraconazole 16.7% and kresoxim-methyl 18.2%.

In this study, azoxystrobin residues decreased by 7.8% with the washing+pasteurization treatment and 5.8% with pasteurization treatment (Table 2). In a study, azoxystrobin residue in winter jujube kept in boiling water at 100°C for 15 minutes decreased by 26% [25]. In another study, azoxystrobin residues in rapeseed oil decreased by 47.0% after 20 minutes of cooking at 80-100ºC in a pan [26]. It is thought that the decrease in the amount of azoxystrobin at varying rates is due to the different exposure to heat and heating duration of the samples.

In the cold storage of pesticide-treated strawberries, pyrimethanil and tebufenpyrad amounts did not change. Penconazole residues decreased over time, and fell below the LOQ on day 6th. In addition, while the amount of azoxystrobin, kresoxim-methyl, tetraconazole and boscalid decreased over time, the amount of bupirimate decreased on the days 1st and 3rd but increased on day 6th (Table 2). It is thought that this increase may have occurred because of the water released during respiration and perspiration in the product. Released water dissolves highly volatile bupirimate residue on the surface and carries it to the strawberries in the bottom of the case. Moisture and dry weight losses related to respiration have some effects on pesticide concentration [27].

Penconazole residues fell below the LOQ at the end of the cold storage (day 6). It has been determined that the residues of kresoxim-methyl continue to decrease during cold storage and this decrease was also found to be statistically significant ($p<0.05$). Decreases of 27.3% and 36.4% in kresoxim-methyl residues were determined on days 3 and 6 of the cold storage process, respectively. In addition, significant changes were not detected in tebufenpyrad and boscalid residues during cold storage, and statistical p values were higher than 0.05 (Table 2).

Studies have shown that the main mechanism effective in the reduction of pesticide residues in the storage of fruits and vegetables is evaporation and slow acidic hydrolysis. It has been reported that both mechanisms are significantly controlled by penetration [27].

Active substances were evaluated in terms of washing on days 0. and 4., and there was no decrease in tebufenpyrad residues, while other residues decreased at different levels with the washing performed on day zero (Table 2). The amounts of pesticide residues removed by cold storage and washing on different days increased. Kresoxim-methyl (45.4%) was reduced from washing performed on day 4. in pesticides determined quantitatively.

Processing Factors

The residue level in the products before and after processing is expressed by processing factor, as mentioned before. Processing factors for different processes for all active substances are shown in Table 2. All Pf values were 1 or less than 1, except tebufenpyrad (1.20) for pasteurization. It was thought that the increase in the amount of active substance may be due to the increase in extraction efficiency related to the effect of temperature.

Penconazole residues in the washing process fell below the LOQ, and the processing factor could not be determined. The lowest processing factors were obtained in tetraconazole (0.81) for washing and pasteurization. In one study, it was reported that washing strawberries in an ultrasonic water bath using water and different solutions caused different processing factors. All processing factors varied but were smaller than 1 [28].

In our study, it was determined that boscalid residues were reduced by 10.9% by washing in an ultrasonic water bath, and the Pf for this process was 0.89. In a study, it was reported that the boscalid residue in strawberries decreased by 67.5% with a 5-minute washing process in an ultrasonic water bath, and the processing factor for the applied treatment was 0.32 [22]. In another study on boscalid contents in two tomato varieties, the washing factor for the 'Marissa' variety was 0.39, and for the 'Harzfeuer' variety, it was 0.65 for the boscalid in tomatoes washed for one minute under running tap water [29].

Increased pesticide residues exposed through nutrition most probably cause serious health problems [30]. A Pf value equal to 1 or less than 1 is important in terms of showing that the residue on the product does not tend to increase with the applied process, and is also extremely significant in terms of showing that the residue in the product is not completely degraded and continues to be present in the product. Considering that the pesticides are applied at very low concentrations (30% more than their MRL for each active substance), very low residues may not pose a health risk at the moment, but the recommended doses used in practice are generally much higher than used in this study should not be forgotten.

Effects of Different Storage Temperatures on Pesticide Residues

It is very difficult to preserve fresh strawberries, which have a very short shelf life after harvest. Strawberries spoil quickly due to their soft fruit texture and high water content. The most effective and best method that allows the preservation of the appearance, taste, aroma and nutritional values of fresh fruits without deterioration is the freezing method [31-32].

The effect of the day, as a storage time, was investigated for samples stored at -18±2ºC without pasteurization. The changes were statistically significant

in all of the samples washed or not. The interaction of temperature, day and temperature*day was examined in pasteurized and stored at different temperatures. The P values obtained for all pesticides were generally found statistically significant. When the changes in the pyrimethanil residue was examined, it was observed that the pesticide degradation increased with the longer the storage period. At the end of the one-year, pyrimethanil residues decreased by 31.9% in $Aa_{(-18)}$ and by 22.8% in $Ab_{(-18)}$ samples. The amount of decrease in the sprayed samples kept at the same temperature (-18ºC) was higher in the unpasteurized samples (Table 3-4).

The storage temperature, duration and matrix properties of foods affect the amount of pesticide residues [33]. In particular, the physical and chemical properties of pesticides, such as saturated vapour pressure and solubility, directly affect storage stability. Compounds with low vapour pressure and moderate solubility (up to 40 mg/L) are stable, while compounds with high solubility (up to 700 mg/L) or high vapour pressure are unstable [34-35]. Generally, at low concentrations, the half-life of pesticides becomes more dependent on storage conditions (pH, exposure to light, and temperature) [36].

Table 3. Residue changes of active substances in strawberry puree -18±2ºC (mg kg^{-1}) (mean±standard error, n=3)

Active substance	Sample codes	Duration (days)								
		0	15	30	60	120	180	240	300	360
Pyrimethanil	$Aa_{(-18)}$	0.47±0.031	0.45±0.018	0.39±0.011	0.38±0.013	0.25±0.004	0.25±0.011	0.39±0.010	0.36±0.026	0.32±0.026
	$Ba_{(-18)}$	0.45±0.005	0.49±0.040	0.38±0.038	0.41±0.018	0.32±0.019	0.25±0.023	0.40±0.034	0.38±0.009	0.36±0.009
Tetraconazole	$Aa_{(-18)}$	0.12±0.007	0.10±0.007	0.08 ±0.002	0.08 ±0.005	< LOQ*	<LOQ	0.06±0.002	0.08 ±0.005	0.08 ±0.005
	$Ba_{(-18)}$	0.10±0.004	0.09±0.008	0.08±0.002	0.07±0.003	< LOQ	<LOQ	0.07±0.007	0.07 ±0.004	0.08 ±0.003
Bupirimate	$Aa_{(-18)}$	0.22±0.002	0.20±0.007	0.19±0.002	0.18±0.004	0.11±0.004	0.11±0.007	0.18±0.007	0.18±0.003	0.17±0.008
	$Ba_{(-18)}$	0.21±0.003	0.19±0.004	0.18±0.003	0.18±0.001	0.12±0.000	0.11±0.004	0.17±0.003	0.18±0.019	0.16±0.003
Azoxystrobin	$Aa_{(-18)}$	0.52±0.009	0.50±0.019	0.43±0.027	0.42 ±0.006	0.26 ±0.031	0.21 ±0.006	0.29±0.007	0.30±0.040	0.30±0.017
	$Ba_{(-18)}$	0.50±0.012	0.47±0.022	0.45±0.010	0.43±0.029	0.21 ±0.036	0.20 ±0.001	0.33±0.009	0.30±0.046	0.29±0.007
Tebufenpyrad	$Aa_{(-18)}$	0.10±0.003	0.09±0.004	0.07 ±0.003	<LOQ	0.05±0.002	0.05±0.005	0.10±0.006	0.10±0.012	0.11 ±0.007
	$Ba_{(-18)}$	0.10±0.003	0.08±0.004	0.06 ±0.003	<LOQ	0.05±0.003	0.05±0.011	0.09±0.002	0.09±0.003	0.09 ±0.007
Kresoxim-methyl	$Aa_{(-18)}$	0.11±0.005	0.11±0.005	0.11±0.008	0.05±0.006	0.06±0.003	0.06±0.003	0.10±0.005	0.09±0.006	0.09±0.002
	$Ba_{(-18)}$	0.10±0.005	0.10±0.004	0.09±0.003	<LOQ	0.06±0.007	0.06±0.007	0.08±0.004	0.08±0.004	0.07±0.004
Boscalid	$Aa_{(-18)}$	0.46±0.032	0.40±0.013	0.32±0.034	0.17±0.010	0.14±0.016	0.09±0.005	0.28±0.008	0.26±0.020	0.23±0.014
	$Ba_{(-18)}$	0.41±0.033	0.35±0.015	0.28±0.021	0.16±0.007	0.14±0.008	0.08±0.007	0.25±0.028	0.25±0.019	0.22±0.005

*:<LOQ: below the quantification limit.

Table 4. Residue changes of active substances in strawberry puree at -18±2ºC (mg kg^{-1}) (mean±standard error, n=3)

Active substances	Sample codes	Duration (days)								
		0	15	30	60	120	180	240	300	360
Pyrimethanil	$Ab_{(-18)}$	0.49±0.027	0.51±0.006	0.44±0.028	0.37±0.005	0.31±0.021	0.27±0.012	0.44±0.016	0.35±0.022	0.34±0.010
	$Bb_{(-18)}$	0.48±0.030	0.54±0.034	0.42±0.039	0.42±0.019	0.32±0.022	0.31±0.021	0.43±0.012	0.32±0.037	0.30±0.021
Tetraconazole	$Ab_{(-18)}$	0.10±0.003	0.08±0.008	0.08±0.014	0.07 ±0.002	< LOQ*	0.05 ±0.004	0.06±0.003	0.08 ±0.001	0.08±0.003
	$Bb_{(-18)}$	0.10±0.003	0.10±0.005	0.08±0.002	0.07±0.003	< LOQ	0.05 ±0.002	0.05±0.003	0.07±0.007	0.07 ±0.006
Bupirimate	$Ab_{(-18)}$	0.22±0.008	0.20±0.005	0.19±0.007	0.18±0.005	0.12±0.002	0.12±0.002	0.17±0.002	0.14±0.008	0.13±0.019
	$Bb_{(-18)}$	0.21±0.002	0.21±0.002	0.18±0.007	0.17±0.001	0.12±0.003	0.12±0.003	0.17±0.007	0.16±0.006	0.15±0.006
Azoxystrobin	$Ab_{(-18)}$	0.49±0.018	0.54±0.022	0.51±0.012	0.43±0.015	0.29±0.018	0.24 ±0.012	0.37±0.021	0.34 ±0.050	0.36±0.012
	$Bb_{(-18)}$	0.48±0.018	0.55±0.037	0.46±0.007	0.43±0.018	0.28±0.005	0.20 ±0.002	0.34±0.010	0.32±0.014	0.37±0.004
Tebufenpyrad	$Ab_{(-18)}$	0.12±0.015	0.10±0.006	0.07±0.002	0.05±0.004	0.06±0.002	0.07±0.009	0.12±0.017	0.12±0.006	0.12±0.007
	$Bb_{(-18)}$	0.11±0.017	0.08±0.009	0.07 ±0.004	<LOQ	0.05±0.007	0.06±0.009	0.10±0.002	0.08±0.009	0.11 ±0.002
Kresoxim-methyl	$Ab_{(-18)}$	0.11±0.005	0.12±0.006	0.10±0.007	0.05±0.001	0.07±0.004	0.07±0.002	0.10±0.002	0.09±0.003	0.08±0.002
	$Bb_{(-18)}$	0.09±0.005	0.10±0.009	0.09±0.005	<LOQ**	0.06±0.003	0.05±0.001	0.08±0.006	0.08±0.001	0.08±0.001
Boscalid	$Ab_{(-18)}$	0.43±0.034	0.45±0.022	0.34±0.031	0.20±0.016	0.15±0.008	0.11±0.010	0.32±0.022	0.30±0.031	0.27±0.015
	$Bb_{(-18)}$	0.42±0.002	0.37±0.023	0.29±0.035	0.16±0.012	0.12±0.006	0.11±0.009	0.26±0.030	0.28±0.016	0.25±0.029

*:<LOQ: below the quantification limit.

Although microbiological spoilage can be prevented in the freezing process, a negative effect such as the continuation of enzymatic changes, albeit at a slow rate, is eliminated by boiling vegetables to a large extent, but this possibility is often absent in fruits. Enzymes in fruits and vegetables cannot completely inactivated by freezing. The activities of enzymes are slowed down by frozen storage. Especially peroxidase and lipoxygenase enzymes are enzymes that should be considered in the production of frozen fruits and vegetables. The peroxidase enzyme found in the tissue of fruits and vegetables is the main factor especially in oxidation reactions. Lipoxygenase enzyme also causes the breakdown of fatty acids in frozen products [37]. Therefore, the degradation of pesticides continued during the storage of strawberries at -18±2ºC.

When the effect of process and storage time investigated after one-year period we can say that tetraconazole residues decreased by 33.3% in the $Aa_{(-18)}$ samples. Also for bupirimate we can say residues in the samples kept at -18 ºC decreased by 22.7% in $Aa_{(-18)}$ and 40.9% $Ab_{(-18)}$ respectively.

At the end of a one-year period, azoxystrobin residues in the samples kept at -18ºC decreased by 42.3% and 42%, respectively, in $Aa_{(-18)}$ and $Ba_{(-18)}$ samples, while in $Ab_{(-18)}$ and $Bb_{(-18)}$ samples, it decreased by 26.5% and 22.9%, respectively. Statistical evaluation was not

carried out as the penconazole residues were below the quantification limit as of the 15th day. However, it was determined that penconazole residues did not completely disappear in all samples analysed during one year and continued to be found in samples below the quantification limit. At the end of the storage period, no significant decrease was detected in tebufenpyrad residues in samples kept at -18±2ºC, Table 3-4.

In kresoxim-methyl, degradation increased with longer storage time. At the end of one year, the degradation amount of kresoxim-methyl was found to be 18.2% in the $Aa_{(-18}$ samples, and 27.3% in the $Ab_{(-18)}$ samples. Whether or not the strawberry samples were washed, boscalid degradation was found to be higher in unpasteurized samples than in pasteurized samples. At the end of a one-year period, the boscalid residues in the $Aa_{(-18)}$ and $Ba_{(-18)}$ samples were 50% and 46%, and in the $Ab_{(-18)}$ and $Bb_{(-18)}$ samples, 37% and 40% respectively (Table 3-4).

Samples kept at -18ºC after pasteurization if summarized, penconazole fell below LOQ (0.05 mg kg^{-1}) in the 15th day. Comparisons of other active substance analysis performed on the 240th and 360th days of the $Ab_{(-18)}$ samples are given in Figure 2. It can be seen that the amount of tebufenpyrad did not change in the 240th and 360th day samples while tetraconazole 20%, pyrimethanil 23%, azoxystrobin 26%, kresoxim-methyl 27%, boscalid 37% and bupirimate 41% decreased, respectively, from the lowest to the highest pesticide degradation in the 360th day samples.

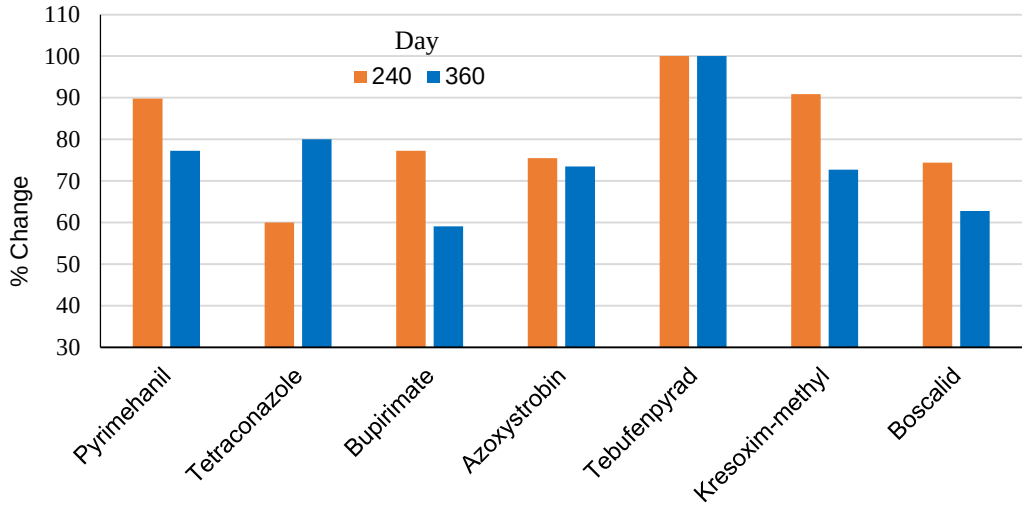

Figure 2. Residue changes of active substances in pasteurized puree without washing $Ab_{(-18)}$ samples (%) (baseline=100)

Effect of pasteurization on pesticide degradation in samples $Ab_{(-18)}$ was examined, the amount of tebufenpyrad did not change, degradation of kresoxim-methyl and bupirimate increased in pasteurized samples, and degradation of pyrimethanil, tetraconazole, azoxystrobin and boscalid was slower in pasteurized samples.

When the degradation at 20±2ºC and 4±0.5ºC investigated, we can say that pesticides degrade the fastest at 20±2ºC. The amount of pyrimethanil degradation in $Ab_{(+4)}$ and $Bb_{(+4)}$ samples were 57.1% and 58.3%, respectively. In addition, degradation was found 57.1% in the samples at 20±2ºC for pyrimethanil (Tables 5 and 6). It is considered that during the keeping of strawberry puree in glass jars and boiling water at 96ºC for 5 minutes, peroxidase and lipoxygenase enzymes are partially inactivated because under normal conditions, faster degradation can occur at 4±0.5ºC to 20±2ºC, but slower degradation occurred. The degradation amount of tetraconazole was 20% in $Ab_{(+4)}$ and 40% in $Bb_{(+4)}$ samples. Also, tetraconazole residues in $Ab_{(+20)}$ samples fell below the LOQ at 180th day. When the bupirimate residues at 20±2 ºC was examined, it was determined that the active substance residues fell below the LOQ in the 2nd month (Tables 5 and 6). In the statistical evaluation, storage time and different temperatures used were found to be effective on pesticide degradation ($p<0.05$). The degradation amount of tebufenpyrad residues was in 25% in $Ab_{(+4)}$ at the 300th day. The degradation amount of kresoxim-methyl in the samples on the 300th day was 45.4% in $Ab_{(+4)}$ and 55.4% in $Bb_{(+4)}$. In addition, the amount of kresoxim-methyl in the washed samples kept at 20±2ºC decreased below the LOQ on the 60th day. The degradation amount of boscalid was found 55.8% in $Ab_{(+4)}$ samples at 300th day.

Comparisons of the 240th day analyses of the samples kept at 4±0.5ºC are given in Figure 3. As the temperature increased, the amount of degradation in pesticides also increased. The amount of degradation in the washed and unwashed samples differed. The amount of decrease determined in $Ab_{(+4)}$ samples is respectively 77% bupirimate, kresoxim-methyl 55%, boscalid 49%, tetraconazole 40%, pyrimethanil 37%, azoxystrobin 37% and tebufenpyrad 17%.

In the samples kept at 20±2ºC after pasteurization, bupirimate decreased below the LOQ on the 60th day, while no active substance peak was found on the 120th day. Penconazole below the LOQ at day 15 but continued to exist in the sample below the LOQ even at day 120. Other active substances (tetraconazole,

azoxystrobin, pyrimethanil, kresoxim-methyl, boscalid and tebufenpyrad) were compared according to the 120th day samples due to their numerical data. Pesticide degradation was determined as kresoxim-methyl, tetraconazole, boscalid, azoxystrobin, pyrimethanil and tebufenpyrad, respectively, from the highest to the least.

Table 5. Residue changes of active substances in strawberry puree at 4±0.5ºC (mg kg^{-1}) (mean±standard error, n=3)

Active substances	Sample codes	Duration (days)								
		0	15	30	60	120	180	240	300	360
Pyrimethanil	$Ab_{(+4)}$	0.49±0.026	0.40±0.006	0.36±0.006	0.38±0.019	0.26±0.006	0.26±0.016	0.31±0.014	0.28±0.013	0.28±0.019
	$Bb_{(+4)}$	0.48±0.030	0.39±0.025	0.40±0.023	0.39±0.034	0.28±0.016	0.27±0.017	0.32±0.011	0.28±0.013	0.29±0.018
Tetraconazole	$Ab_{(+4)}$	0.10±0.003	0.08±0.008	0.08±0.001	0.07±0.002	<LOQ*	0.05 ±0.004	0.06±0.003	0.08 ±0.001	0.08 ±0.003
	$Bb_{(+4)}$	0.10±0.003	0.07±0.002	0.07±0.001	0.07±0.003	<LOQ	<LOQ	<LOQ	0.06 ±0.001	0.06 ±0.002
Bupirimate	$Ab_{(+4)}$	0.22±0.008	0.16±0.010	0.14 ±0.004	0.12±0.012	0.06±0.010	nd**	0.05±0.001	0.05±0.001	nd
	$Bb_{(+4)}$	0.21±0.002	0.17±0.003	0.14±0.003	0.12±0.007	0.06±0.006	nd	0.05±0.001	nd	nd
Azoxystrobin	$Ab_{(+4)}$	0.49±0.018	0.46±0.033	0.46±0.016	0.42±0.006	0.26±0.006	0.25±0.004	0.31±0.012	0.30±0.020	0.24±0.009
	$Bb_{(+4)}$	0.48±0.018	0.43±0.028	0.44±0.017	0.39±0.005	0.24±0.004	0.22±0.006	0.26±0.014	0.27±0.019	0.24±0.006
Tebufenpyrad	$Ab_{(+4)}$	0.12±0.015	0.10±0.006	0.07 ±0.002	<LOQ	0.05±0.02	0.06±0.006	0.10±0.003	0.09±0.003	***
	$Bb_{(+4)}$	0.11±0.017	0.08±0.003	0.06 ±0.001	<LOQ	0.05±0.002	0.05±0.004	0.08±0.007	0.09±0.006	0.09 ±0.004
Kresoxim-methyl	$Ab_{(+4)}$	0.11±0.005	0.12±0.013	0.10±0.001	0.05±0.005	0.05±0.002	<LOQ	0.05±0.004	0.05±0.004	***
	$Bb_{(+4)}$	0.09±0.005	0.09±0.005	0.07±0.004	<LOQ	<LOQ	<LOQ	0.05±0.004	0.05±0.004	0.05±0.004
Boscalid	$Ab_{(+4)}$	0.43±0.034	0.46±0.050	0.32±0.011	0.15±0.021	0.16±0.014	0.12±0.005	0.22±0.008	0.24±0.011	***
	$Bb_{(+4)}$	0.42±0.002	0.35±0.009	0.25±0.025	0.13±0.005	0.14±0.010	0.10±0.008	0.21±0.003	0.20±0.004	0.18±0.009

*: <LOQ: below the quantification limit, **: nd: Not determined, ***: evaluation could not be made (not evaluated due to problem in the sample)

Table 6. Residue change of active substances in strawberry puree at 20±2ºC (mg kg^{-1}) (mean±std error, n=3)

Active substances	Active substances	Duration (days)					
		0	15	30	60	120	180
Pyrimethanil	$Ab_{(+20)}$	0.49±0.026	0.39±0.029	0.33±0.025	0.35±0.028	0.22±0.002	0.22±0.007
	$Bb_{(+20)}$	0.48±0.030	0.39±0.038	0.39±0.024	0.38±0.044	0.30±0.005	***
Tetraconazole	$Ab_{(+20)}$	0.100±0.003	0.06±0.004	0.07±0.001	0.07 ±0.006	<LOQ	<LOQ
	$Bb_{(+20)}$	0.10±0.003	0.06±0.002	0.07±0.003	0.06±0.005	<LOQ	***
Bupirimate	$Ab_{(+20)}$	0.22±0.008	0.13±0.016	0.06±0.002	<LOQ*	nd	nd
	$Bb_{(+20)}$	0.21±0.002	0.12±0.009	0.06±0.001	<LOQ	nd	***
Azoxystrobin	$Ab_{(+20)}$	0.49±0.018	0.38±0.041	0.38±0.002	0.30±0.024	0.19±0.007	0.16 ±0.011
	$Bb_{(+20)}$	0.48±0.018	0.35±0.024	0.33±0.039	0.28±0.006	0.18±0.005	***
Tebufenpyrad	$Ab_{(+20)}$	0.12±0.015	0.08±0.005	0.07±0.001	***	***	***
	$Bb_{(+20)}$	0.11±0.017	0.08±0.004	0.07±0.001	0.05±0.002	0.05 ±0.005	***
Kresoxim-methyl	$Ab_{(+20)}$	0.11±0.005	0.08±0.003	0.07±0.003	***	***	***
	$Bb_{(+20)}$	0.09±0.005	0.08±0.010	0.06±0.001	<LOQ	<LOQ	***
Boscalid	$Ab_{(+20)}$	0.43±0.034	0.34±0.014	0.26±0.011	***	***	***
	$Bb_{(+20)}$	0.42±0.002	0.31±0.025	0.27±0.016	0.12±0.004	0.10±0.004	***

*: <LOQ: below the quantification limit, **: nd: Not determined, ***: evaluation could not be made (not evaluated due to problem in the sample)

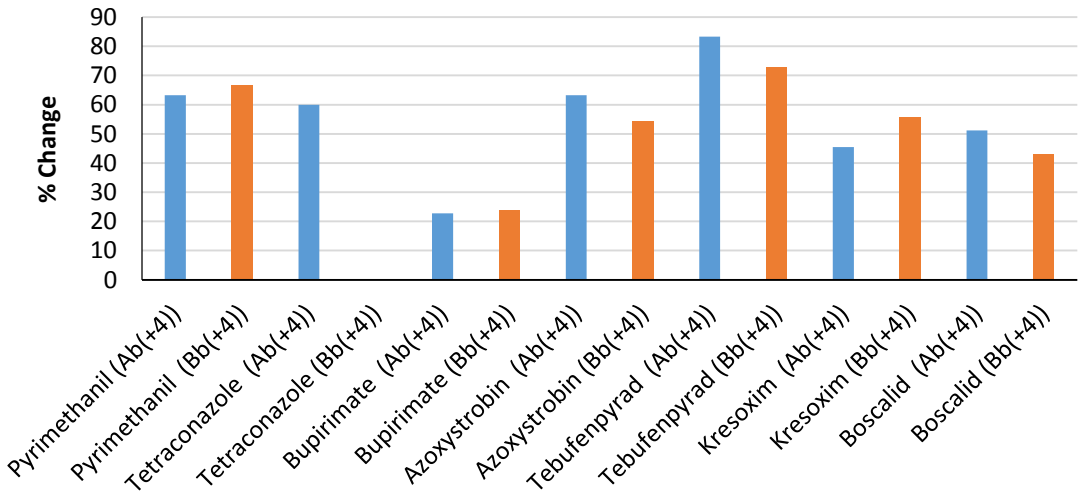

Figure 3. Residue changes of active substances in pasteurization without washing $Ab_{(+4)}$ and with washing $Bb_{(+4)}$ samples at day 240 (baseline=100)

CONCLUSION

The analytical method was validated to determine residues of nine active substances in strawberry. Recovery, r^2, RSD and LOQ were all in the acceptable range of validation assays. Pesticide degradation occurred during washing, pasteurization, cold storage and long term storage processes. Ultrasonic washing decreased the pesticide residues in the range of 3.8–16.7%. Pasteurization removed the pesticide residues too. At the end of the cold storage process, penconazole residues were below the LOQ. The amount of residue removed by washing on different days increased. Processing factors were calculated for the washing, pasteurization and washing+pasteurization processes. The highest and lowest processing factors in the pasteurization process were for tebufenpyrad (Pf:1.20) and tetraconazole (Pf:0.81), respectively. In addition, it was determined that the pesticide residues decreased at varying rates during the storage of pasteurized and unpasteurized strawberry puree at different temperatures.

Considering how low the concentration used in the spraying is (30% of the MRL of each active substance) and the length of the analysis time it has once again been demonstrated that pesticide residues are not completely removed, and that varying amounts of pesticide can be remain in the product after processing. The fact that many factors such as the structure of the agricultural product, the applied process, the temperature of the environment, the acidity level, the chemical structure of the pesticide, and its solubility have effects on the residue variation. Our results also explain why generalization should not be made in this regard and why studies on processing factors should be carried out separately on the basis of product and pesticide.

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REFERENCES

[1] Jeong, L.S., Kwak, B.M., Ahn, J.H., Jeong, S.H. (2012). Determination of pesticide residues in milk using QuEChERS-based method developed by response surface methodology. *Food Chemistry,* 133, 473-481.

[2] Commission, E. (1997). Appendix E. Processing Studies. Directorate General for Agriculture. Retrieved from https://food.ec.europa.eu/system/files/2016-10/pesticides_mrl_guidelines_app-e.pdf

[3] Aguilera, A., Valverde, A., Camacho, F., Boulaid, M., Garcia-Feuntes, L. (2014). Household processing factors of acrinathrin, fipronil, kresoxim-metyl and pyridaben residues in green beans. *Food Control,* 35, 146-152.

[4] Bajwa, U., Sandhu, K.S. (2014). Effect of handling and processing on pesticide residues in food-a review. *Journal of Food Science and Technology,* 51(2), 201-220.

[5] Yigit, N., Velioglu, Y.S. (2020). Effects of processing and storage on pesticide residues in foods. *Critical Reviews in Food Science and Nutrition,* 60(21), 3622-3641.

[6] Zhao, L., Ge, J., Liu, F., Jiang, N. (2020). Effects of storage and processing on residue levels of chlorpyrifos in soybeans. *Food Chemistry,* 150, 182-186.

[7] BfR. (2019). *BfR data collection on processing factors. Updated communication No: 034.* Retrieved March 21, 2021, from https://www.bfr.bund.de/cm/349/bfr-data-collection-on-processing-factors.pdf

[8] Kafkas, E. (2017). Strawberry growing in Türkiye: current status and future prospects. *Acta Horticulture, 1156*, 903-908.

[9] FAOSTAT. (2021). *Production data of Food and Agriculture Organization of the United Nations*. Retrieved 18 March, 2021, from Food and Agriculture Organization of the United Nations: http://www.fao.org/faostat/en/#data/QC

[10] Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Oiles, J.L., Mezzetti, B., Battino, M. (2012). The strawberry: composition, nutritional quality, and impact on human health. *Nutrition,* 28, 9-19.

[11] Giampieri, F., Alvarez-Suarez, J.M., Battino, M. (2014). Strawberry and human health: effects beyond antioxidant activity. *Journal of Agricultural and Food Chemistry,* 62, 3867-3876.

[12] Commission, E. (2017). *EU Pesticides database*. Retrieved January 25, 2022, from European Commission: https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database_en

[13] Kırıs, S., Velioglu, Y.S. (2016). Reduction in pesticide residue levels in olives by ozonated and tap water treatments and their transfer into olive oil. *Food Additives and Contaminants Part A,* 33, 128-136.

[14] Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of Association of Official Analytical Chemists International,* 86(2), 412-431.

[15] Yigit, N., Bayhan-Öktem, A., Yentür, G. (2012). Development of multiple residue analysis method by high pressure liquid chromatography (HPLC) for the analysis of pesticide residues in some fruits and vegetables. *Plant Protection Bulletin,* 52(4), 375-394.

[16] SANTE. (2019). Guidance document on analytical quality control and method validation procedures for pesticide residues analysis in food and feed

SANTE/12682/2019. Retrieved January 26, 2019, from https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf

[17] Bolaños, P.P., Moreno, J.L., Shtereva, D.D., Frenich, G.A., Vidal, J.L. (2007). Development and validation of a multiresidue method for the analysis of 151 pesticide residues in strawberry by gas chromatography coupled to a triple quadrupole mass analyzer. *Rapid Communications in Mass Spectrometry,* 21, 2282-2294.

[18] Morales, A., Ruiz, I., Oliva, J., Barba, A. (2011). Determination of sixteen pesticides in peppers using high-performance liquid chromatography/ mass spectrometry. *Environmental Science and Health, Part B: Pesticides, Food Contaminants and Agricultural Wastes,* 46, 525-529.

[19] Pizzutti, I.R., Dias, J.V., Kok, A., Cardoso, C.D., Vela, G.M. (2016). Pesticide residues method validation by UPLC-MS/MS for accreditation purposes. *Journal of the Brazilian Chemical Society,* 27(7), 1165-1176.

[20] Jiang, W., Chen, X., Liu, F., Pan, C. (2019). Residue distribution, dissipation behavior and removal of four fungicide residues on harvested apple after waxing treatment. *Journal of Agricultural and Food Chemistry,* 67, 2307-2312.

[21] Shokr, A.S., Malhat, F., Saber, E.S., El-Gammal, H.A., Ahmed, M.T. (2019). Dynamic distribution of azoxystrobin residues in strawberry (Fragaria x ananassa Duchesne) using liquid chromatography tandem mass spectrometry: Putative evaluation of dietary intake. *International Journal of Environmental Analytical Chemistry,* 101(15), 2479-2490.

[22] Lozowicka, B., Jankowska, M., Hrynko, I., Kaczynski, P. (2016). Removal of 16 pesticide residues from strawberries by washing with tap and ozone water, ultrasonic cleaning and boiling. *Environmental Monitoring Assessment,* 188(1), 51.

[23] Angioni, A., Schirra, M., Garau, V.L., Melis, M., Tuberoso, C.I., Cabras, P. (2004). Residues of azoxystrobin, fenhexamid and pyrimethanil in strawberry following field treatments and the effect of domestic washing. *Food Additives and Contaminants,* 21(11), 1065-1070.

[24] Cámara, M.A., Cermeño, S., Martínez, G., Oliva, J. (2020). Removal residues of pesticides in apricot, peach and orange processed and dietary exposure assessment. *Food Chemistry, 325*, 126936

[25] Peng, W., Zhao, L., Liu, F., Xue, J., Li, H., Shi, K. (2014). Effect of paste processing on residue levels of imidacloprid, pyraclostrobin, azoxystrobin and fipronil in winter jujube. *Food Additives & Contaminants Part A,* 31(9), 1562-1567.

[26] Jiang, Y., Shibamoto, T., Li, Y., Pan, C. (2013). Effect of household and commercial processing on acetamiprid, azoxystrobin and methidathion residues during crude rapeseed oil production. *Food Additives and Contaminants Part A,* 30(7), 1279-1286.

[27] Amvrazi, E. G. (2011). Fate of pesticide residues on raw agricultural crops after postharvest storage and food processing to edible portions. In *Pesticides-formulations, effects, fate. Edited by M. Stoytcheva.* IntechOpen. Retrieved May 20, 2021, from https://www.intechopen.com/chapters/13027.

[28] Kwak, S.Y., Lee, S.H., Jeong, H.R., Nam, A.J., Sarker, A., Kim, H.Y., Kim, J.E. (2019). Variation of pesticide residues in strawberries by washing and boiling processes. *Korean Journal of Environmental Agriculture,* 38(4), 281-290.

[29] Jankowska, M., Kaczynski, P., Hrynko, I., Lozowicka, B. (2016). Dissipation of six fungicides in greenhouse-grown tomatoes with processing and health risk. *Environmental Science and Pollution Research,* 23, 11885-11900.

[30] Shah, R. (2020). Pesticides and Human Health. In *Emerging Contaminants. Edited by A. Nuro.* IntechOpen. Retrieved March 30, 2022, from https://www.intechopen.com/chapters/73921

[31] Skrede, G. (1996). Fruits. In *Freezing Effects on Food Quality. Edited by E.J. Lester.* Marcel Dekker Inc. New York, 432p.

[32] Ancos, B., Ibañez, E., Reglero, G., Cano, P. (2000). Frozen storage effects on anthocyanins and volatile compounds of raspberry fruit. *Journal of Agricultural Food Chemistry,* 48(3), 873-879.

[33] Afridi, I. A., Parveen, Z., Masud, S.Z. (2001). Stability of organophosphate and pyrethroid pesticides on wheat in storage. *Journal of Stored Products Research,* 37(2), 199-204.

[34] Barceló, D.A. (1996). A review of sample storage and preservation of polar pesticides in water samples. *Chromatographia,* 42, 704-712.

[35] Aboulfadl, K., De Potter, C., Prévost, M., Sauvé, S. (2010). Time-dependent integrity during storage of natural surface water samples for the trace analysis of pharmaceutical products, feminizing hormones and pesticides. *Chemistry Central Journal,* 4, 1-10.

[36] Domingues, V., Cabral, M., Alves, A., Delerue-Matos, C. (2009). Use and reuse of SPE disks for the determination of pyrethroids in water by GC-ECD. *Analytical Letters,* 42(4), 706-726.

[37] Demiray, E., Tülek, Y. (2010). Donmuş muhafaza sırasında meyve ve sebzelerde oluşan kalite değişimleri. *Akademik Gıda,* 8(2), 36-44.