

## Effect of Food Processing on Residue of Imidacloprid in Strawberry Fruits

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### ABSTRACT

An experiment was conducted to evaluate the effects of some technological processes on the residual levels of imidacloprid in strawberry fruits and products. According to their half-life ( $t_{1/2}$ ) values, strawberry fruits can be safely harvested for human consumption 7.4 days after spraying. The removal ratio of imidacloprid residue in strawberries was in the range of 9.9–30.55% by washing with tap water. The average amount of imidacloprid residue in strawberry fruits, juice, and syrup under cold and hot break greatly decreased compared with those in unwashed strawberry fruits. Moreover, the residue of pesticide decreased more in strawberries syrup under hot break than cold break. Imidacloprid residue was concentrated into jam and increased to higher levels than strawberry juice and syrup under cold and hot break. A change in physical and chemical properties of strawberry fruits and products was related mainly to the processing operations.

**Keywords:** Chemical properties, Pesticide, Residues, Technological processes.

### INTRODUCTION

Strawberry is widely cultivated for its delicious juicy flesh and its high nutritive value. Strawberry is a popular raw material for the food industry, for example, the fresh fruits can be made into many kinds of fruit preparations as ingredients for yogurts, milk, ice cream or in the candy industry. The juice or concentrates are used in multi-fruit based juices or beverages and for the production of liqueurs and fruit wines (Will and Kruger, 1999).

The risk to human health resulting from the widespread application of pesticides for many decades is well known. In recent years, attention has been focused on food safety. This is especially true for pesticide residues, and degradation rates on strawberries subjected to field treatments

(Wennrich *et al.*, 2001) and post harvest processing (Will and Kruger, 1999). The dissipation of pesticides after their application depends on various factors, including plant species, chemical formulation, application method, climatic conditions, physical environmental phenomena (mainly volatilization), and chemical degradation, in which sunlight plays a prominent role (Elbert *et al.*, 1999; Mouden *et al.*, 2009). Therefore, dissipation studies for a given crop, under particular conditions in each growing area, are needed to assess the suitability of the established preharvest intervals (PHI) which ensure that residues levels are below the maximum residue limit (MRL). Commercial and household processing such as washing, juicing, blanching, and concentrating can reduce residue level in food, and, consequently, reduce impact on human

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health (Yun et al., 2011). The extent to which pesticide residues are removed by commercial processing depends on a variety of factors, such as the chemical properties of the pesticide, the nature of the food commodity, the processing step, and the length of time the compound has been in contact with the food (Farris et al., 1992; Holland et al., 1994 ; Chavarri et al., 2005).

Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine) is a systemic, chloro-neonicotinyl insecticide that blocks nicotinic neuronal pathways that are abundant in insects (Sheets, 2001). It is commonly used to control various agricultural and horticultural insect pests (Elbert et al., 1991) such as sucking insects, soil insects, termites, and some chewing insects. It is applied to seeds, soil, crops, and structures and is used as a topical flea control treatment on domestic pets (Meister, 2000).

The present investigation was undertaken to study the level and persistence of imidacloprid in strawberry following field treatments, washing, processing to juice, syrup under cold and hot break, and jam.

## MATERIALS AND METHODS

### Field Experiments

Strawberry fruits (*Fragaria ananassa*) were cultivated in plots located in Deer El-Mullak, Abou-Hammad province, Sharkia governorate, Egypt. Common agricultural and fertilization practices were used. In the case of field experiments, a random block scheme was employed. Each block contained 10 rows and tests were carried out in triplicates. Tested blocks were partitioned and isolated from one another by leaving five untreated rows as guard rows. The

pesticide was applied once and the control plot was left without application of the pesticide. Strawberry plants were sprayed on 1<sup>st</sup> March, 2010 with imidacloprid 20% Soluble concentration (SC) formulation at the recommended rate of application, i.e., 100 g ai per feddan (1 Feddan= 4,200 m<sup>2</sup>), equal to 0.238 kg ai ha<sup>-1</sup>. The amount of formulated pesticide required for 1 Feddan was diluted in 400 L of water and applied to plants with foliar spray using a hand sprayer. The fruits were harvested at a suitable maturity stage, either for consumption or for processing. The collected strawberry fruit samples were stored in a freezer at -20°C for further analysis.

### Sampling and Food Processing Operations

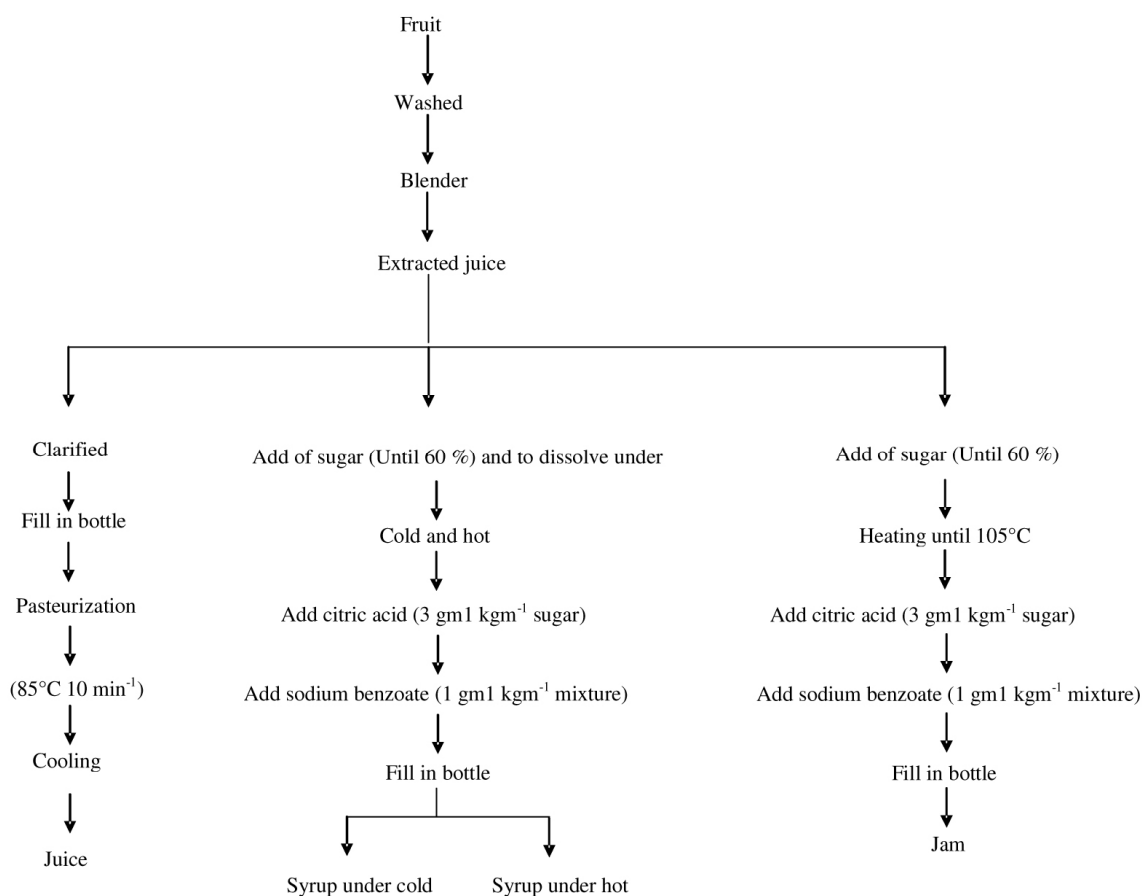
Representative strawberry fruit samples were taken 1 hour after pesticide application to determine the initial deposits of each pesticide. Afterwards, the fruits were collected at intervals of 1, 3, 7, and 14 days after application. Random samples of about 1,500 g were collected from the three plots of treatment. Different samples of strawberries juice, syrup under cold/hot, and jam were prepared as described in Figure 1. Samples of 50 g of each fresh strawberry, washed fruits, jam, and 50 mL of each juice and syrup were taken to determine residues.

### Chemical Analysis

Total soluble solids (TSS), titratable acidity (TA), pH, ascorbic acid (AA), reducing, non-reducing, and total sugars, and total protein were determined according to Association of Official Analytical Chemists, A.O.A.C. (1995).

### Determination of Pesticide Residues

In strawberry fruits and processing products, imidacloprid was extracted and



**Figure 1.** Samples preparation diagrams of strawberries juice and syrup under cold/hot break and jam.

cleaned up according to the method described by Sanyal *et al.*, (2006). Samples were extracted three times with 100 ml of acetonitrile on an electric shaker (1 hour), each time followed by ultrasonic vibration for 5 minutes. After centrifugation at 3,000 rpm for 10 minutes, the extracts were collected and imidacloprid was partitioned into  $\text{CHCl}_3$  (100 ml+50 ml+50 ml). The combined organic extract was put into a 250 ml round bottomed flask and evaporated to dryness with rotary vacuum evaporator with the water bath temperature adjusted to 40°C. The concentrated extract was then subjected to adsorption chromatography over florisil (60-120 mesh) with 10 cm layer of anhydrous sodium sulphate on the top. The column was eluted with 200 ml of acetonitrile: methanol (95:5, v/v). The organic fraction was evaporated to dryness, rinsed with HPLC

grade methanol and filtered (0.2  $\mu\text{m}$ ) for direct HPLC analysis. Samples were determined using HPLC with a UV-detector set at the wavelength of 270 nm. A C18 column was used and the mobile phase was a mixture of methanol/water (60:40, v/v) for imidacloprid. The flow rate was 1.0 mL  $\text{min}^{-1}$ . Under these conditions the retention time of imidacloprid, was 3.36 minutes.

The rate of degradation (K) and half-life ( $t_{1/2}$ ) were obtained from the following equation of Gomaa and Belal (1975):

$$\text{Rate of degradation (K)} = 2.303 \times \text{Slope (1)}$$

$$\text{Half-life (} t_{1/2} \text{)} = 0.693 / \text{K (2)}$$

### Recovery Assays

Samples of untreated strawberries were fortified with appropriate volumes of



standard solutions to reach concentrations of 0.05 and 0.1  $\mu\text{g gm}^{-1}$ . The samples were allowed to settle for 30 minutes before extraction and then processed according to the above procedure. Recovery data were obtained from three replicates for each concentration. Results were corrected according to the percent mean $\pm$ SD recoveries.

### Statistical Analysis

In this study, all statistical analyses were performed with CoStat 6.311CoHort Software). Significant differences between unwashed strawberry fruits and treatment samples were determined by the One Way ANOVA test.

## RESULTS AND DISCUSSION

### Analytical Determination

Calibration curve was obtained by plotting peak areas on 'y' axis against concentrations of the pesticide on 'x' axis within the investigated range (0.01– 0.25  $\text{mg L}^{-1}$ ) of concentrations. Each solution was injected in triplicate. The linearity were good with an excellent coefficient of determination  $r^2=0.999$ . The limit of quantification (LOQ) of imidacloprid in this study was measured to be 0.028  $\mu\text{g gm}^{-1}$ , which was substantially lower than the MRL of imidacloprid in the strawberry (0.5  $\mu\text{g gm}^{-1}$ ). The average percent recoveries obtained were between 84 $\pm$ 3.0 and 93.1 $\pm$ 5.3 (Table 1).

### Effect of Processing

The results of dissipation of imidacloprid in strawberry fruits and processed products are presented in Table 2. The average initial deposits of imidacloprid in the unwashed strawberry fruits 1 hour after application was found to be 3.6 $\pm$ 0.11  $\mu\text{g gm}^{-1}$ . Fourteen days after application, imidacloprid residue was dissipated in the unwashed strawberries to reach 0.11 $\pm$ 0.01  $\mu\text{g gm}^{-1}$ . The total amount dissipated to 96.94% at the end of 2 weeks of application. The dissipation of pesticide residues in/on crops depends on climatic conditions, type of application, plant species, dosage, interval between application, and time of harvest (Khay *et al.*, 2008). It can be seen from the results that the half life value of imidacloprid calculated by first-order reaction, for strawberry fruits was found to be 2.74 days. Half-life ( $t_{1/2}$ ) value of 2.5 days for imidacloprid in tomato fruits was reported by Romeh *et al.* (2009). The  $t_{1/2}$  of imidacloprid was found to be 2.31 and 2.18 days for brinjal, when applied at 42 and 84  $\text{g ai ha}^{-1}$ , respectively (Kousik *et al.*, 2010). Half-life for degradation of imidacloprid in cucumber was observed to be 3.40 and 2.70 days at the single and double dosages, respectively (Hassanzadeh *et al.*, 2012). The MRLs ( $\mu\text{g gm}^{-1}$ ) of imidacloprid was found to be 0.5  $\mu\text{g gm}^{-1}$  for strawberry fruits as adopted by the FAO/WHO Codex Alimentarius Commission (CAC, 2008). Residues of imidacloprid in strawberry fruits were less than the MRL value after 7.6 days of its application at the recommended dosage. Consequently, a waiting period of 7.6 days is suggested for safe consumption of

**Table 1.** The fortified recovery of imidacloprid in strawberry.

Spiking level ( $\mu\text{g gm}^{-1}$ )	Recovery percentages (Mean $\pm$ SD, %)						LOD <sup>a</sup>	LOQ <sup>b</sup>	RSD (%)
	Unprocessed	Juice	Cold syrup	Hot syrup	Jam				
0.1	91.0 $\pm$ 4.2	93.1 $\pm$ 5.3	92.3 $\pm$ 4.0	90.7 $\pm$ 3.0	86.5 $\pm$ 2.87	0.011	0.028	1.6	
0.05	90.1 $\pm$ 4.1	92.7 $\pm$ 5.1	90.3 $\pm$ 4.3	88.0 $\pm$ 3.8	84 $\pm$ 3.0				

<sup>a</sup> Limits of detection; <sup>b</sup> Limit of quantification, MRLs = maximum residue levels, RSD: relative standard deviations

**Table 2.** Residues of imidacloprid in strawberry fruits and processing

Treatments	Days after treatment				
	Initial <sup>a</sup>	1	3	7	14
Unwashed strawberry fruits					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	3.6 ±0.11	2.4±0.22	1.6±0.25a	0.77±0.31a	0.11 ±0.01
% loss	0.0	33.33	55.56	78.61	96.94
Washed strawberry fruits with tap water					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	2.5±0.18	1.8±0.3	1.3±0.4c	0.64±0.41c	0.1±0.02
% loss	30.55	25.0	18.75	16.88	9.09
Strawberry juice					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	-	-	1.2±0.39d	0.52±0.17d	-
% loss	-	-	25.0	32.45	-
Strawberry cold syrup					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	-	-	0.97±0.15e	0.43±0.11e	-
% loss	-	-	39.37	44.16	-
Strawberry hot syrup					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	-	-	0.92±0.26 f	0.38±0.13f	-
% loss	-	-	42.5	50.64	-
Strawberry jam					
Mean±SD ( $\mu\text{g gm}^{-1}$ )	-	-	1.32±0.24b	0.65±0.32b	-
% loss	-	-	17.5	15.58	-
Significantly	-	-	***	***	-
$T_{1/2}$ (Days <sup>-1</sup> )			2.74		
$Kr^b$ (Days <sup>-1</sup> )			0.253		
AUC <sup>c</sup> mg l <sup>-1</sup> (Days <sup>-1</sup> )			13.21		
Maximum residue levels ( $\mu\text{g gm}^{-1}$ )			0.5		

<sup>a</sup> One hour post treatment; <sup>b</sup> The disappearance rate constant, <sup>c</sup> Areas under the curve.

strawberry fruits. Information on residue study of imidacloprid on strawberry fruits in Egypt is not available to our knowledge. However, some information is available on residue of imidacloprid on other vegetables. Imidacloprid residues in tomato from treatment 250 g ai ha<sup>-1</sup> required a preharvest interval (PHI) of 7 days The Egyptian Agricultural Pesticide Committee (2012). From the results obtained in the present study, it is evident that, if imidacloprid is used on strawberry fruits at the dosage recommended for consumption of strawberry fruits (238 g ai ha<sup>-1</sup>), a PHI of 7.6 days should be observed.

The disappearance rate constant (kr) and the Area under the curve (AUC) that represent compound concentration during the period of study were found to be 0.25 days<sup>-1</sup> and 13.21 mg L<sup>-1</sup> (days<sup>-1</sup>), respectively (Table 2).

Washing the pesticide treated strawberry fruits with tap water for three minutes induced considerable relative reductions in pesticide residues within the range of 9.9–30.55% for imidacloprid. The effects of different food processing techniques on pesticide residue levels in fruits washed by a non-toxic solution can decrease the concentration of pesticide residues in the vegetable and fruit samples (Shweta *et al.*, 2010). Washing has been found to reduce pesticides that are loosely attached to the surface of the fruits or vegetables (Abou-Arab and Abou-Donia, 1999). The removal extent depended on the physical and chemical properties of the pesticide, method of application, as well as the nature of the cultivated plant. Residues may get dried on the surface, adsorbed (bound) to waxy material in the outer portion of the fruit or vegetable, or translocated into the inner tissues of the plant with increasing difficulty



of removal (Ripley and Edgington, 1983). The removal of pesticide residues by washing has also been found to depend on the age of the chemical (Guardia-Rubio *et al.*, 2007). Also, depend on imidacloprid-specific reduction factors like in Juraske *et al.*, (2009) who found that 22% imidacloprid was reduced due to washing with tap water. The average removal percentages of imidacloprid residues in strawberry fruits, juice, and syrup under cold and hot break are higher than that of unwashed strawberry fruits. Moreover, strawberries syrup under hot break reduced the amount of the pesticide residues compared with strawberries syrup under cold break (Table 2).

Statistically analyzed data using ANOVA proved that decontamination of imidacloprid residues by various treatments was significant as compared to the control samples (Table 2).

Results revealed that residues of imidacloprid after juicing operation reached the levels of  $1.2 \mu\text{g mL}^{-1}$  on day 3 with dissipation rates of 25%. Only  $0.52 \mu\text{g mL}^{-1}$  of imidacloprid residues were recorded on day 7 with dissipation rates of 32.45%. These results indicated that imidacloprid residue was concentrated in juice; this may be due to physicochemical properties of imidacloprid such as water solubility ( $514 \mu\text{g mL}^{-1}$ ) and Octanol-water coefficient ( $K_{ow}$ ), 2.7 at  $21^\circ\text{C}$  (Tomlin, 2004). The residue levels in juices from strawberry fruits depend on the partitioning properties of the pesticide between the fruit skin/pulp and the juice. The pulp, which often includes the skin, retains a substantial proportion of lipophilic residues. Thus, moderately to highly lipophilic pesticides are poorly transferred into juices and the residues are further reduced by clarification operations such as centrifugation or filtering (Holland *et al.*, 1994).

After 3 and 7 days, the reduction percentage of imidacloprid residues reached 39.37–44.16% in strawberry syrup under cold break and 42.50–50.64% under hot break (Table 2). The rate of dislodging of

residues due to factors like temperature, duration of the process, the amount of water, food additives, and the type of system (open/closed) (Angioni *et al.*, 2004). The elevated values of pesticide reduction at hot break could be due to, firstly, the heat pretreatment of strawberry fruits, secondly, the removal of the fruit rinds in which the pesticide residues were possibly concentrated during straining and, thirdly, the post-heat preservation treatment ( $90^\circ\text{C}$  for 15 minutes) of the pasteurized resulting juice (Ramadan, 1990).

In Table 2, it is evident that, when strawberry fruits were processed into Jam, imidacloprid was concentrated and increased to higher levels than those in the strawberry juice and syrup under cold and hot break. The relatively high residue levels in juicing by-products can undergo further increases upon drying due to the simple loss of moisture (Holland *et al.*, 1994). This indicates that the concentration process had raised the pesticide residues in the final product. This is quite expected because of the concentration step that transforms the juice into a jam. This was evident despite the possible destructive or reductive effect of both heat treatments carried out to convert strawberry juice into strawberry jam and the final treatment of the jam, after being packed in the containers (bottles, processed at  $90^\circ\text{C}$  for 15 minutes) for the final preservation (Ramadan, 1990). Open systems may result in water loss during heating by evaporation, thereby concentrating the pesticide residues if they are not destroyed by heating (Abou-Arab and Abou-Donia, 1999). The processes that normally occur during cooking are volatilization, hydrolysis, and thermal breakdown (Balnova *et al.*, 2006).

Results of chemical analysis of strawberry fruits and strawberry products treated with imidacloprid after 7 days of spray time and in untreated control are shown in Table 3. After 7 days of spray time, the average total soluble solids (TSS) decreased in strawberry juice compared with other samples. Total sugars were increased in hot syrup and jam

**Table 3.** Changes in physical and chemical characteristics of strawberry products treated with imidacloprid 7 days after spray time.

	TSS <sup>a</sup> (BX)	Reducing sugars (g 100 ml <sup>-1</sup> )	Total acidity (citric)	Ascorbic acid (g 100 ml <sup>-1</sup> )	PH value	Crude protein (g 100 ml <sup>-1</sup> )
Juice						
Control	7 ±0.76	4.25 ±0.21	0.72±0.35	50.4±0.35	3.21±0.11	0.25±0.03
Treatment±SD	7.5± 0.66	4.13 ±0.60	0.79±0.48	60.0±0.61	3.21±0.15	0.24±0.02
Cold syrup						
Control±SD	60 ±0.53	15.50±0.38	1.41±0.53	42.7±0.41	3.3±0.10	0.29±0.02
Treatment±SD	60±0.93	11.63±0.23	1.22±0.43	33.65±0.53	3.3±0.32	0.29±0.04
Hot syrup						
Control±SD	60±0.59	45.90±0.28	1.45±0.40	17.20±0.10	3.3±0.15	0.36±0.18
Treatment±SD	60±0.87	46.51 ±0.32	1.3 ±0.25	11.65±0.15	3.34±0.38	0.31±0.43
Jam						
Control±SD	68±0.66	54.30±0.27	1.51±0.86	18.2±0.13	3.35±0.20	0.57 ±0.02
Treatment±SD	68±0.15	57.00±0.29	1.4±0.21	19.23±0.17	3.35±0.10	0.67 ±0.03

TSS<sup>a</sup>= Total soluble solids

than other treatment. This increment may be explained in hot syrup and jam by dissolving sucrose with citric acid and heating. Total sugars decreased in cold syrup under treatment with imidacloprid compared with the control samples. Otherwise, ascorbic acid content in hot syrup and jam were lower in comparison with the other samples. The heating process exposed ascorbic acid to degradation. Also; differences in ascorbic acid content under treatment with imidacloprid in hot and cold syrup were minor when compared with the control (Table 3). Strawberries are a good source of ascorbic acid (vitamin C), which is a very important nutrient, being essential, e.g. for the synthesis of collagen. Ascorbic acid is also a natural antioxidant used in foodstuff formulations in order to prevent browning and discoloring, and to enhance shelf life (Castro *et al.*, 2004). In general, our data showed that the changes in physical and chemical properties of strawberry fruits and products were related mainly to the processing operations (Cordenunsi *et al.*, 2003; 2005; Sandra *et al.*, 2006).

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## اثر فرآوری غذایی روی باقیمانده های حشره کش Imidacloprid در میوه های توت فرنگی

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### چکیده

این پژوهش به منظور ارزیابی اثر چند فرایند فناوری در مقدار باقیمانده حشره کش Imidacloprid در میوه و فرآورده های توت فرنگی انجام شد. بر اساس نیمه عمر ( $t_{1/2}$ ) مربوطه، میوه توت فرنگی برای مصرف خوراکی را می توان ۷/۴ روز بعد از سم پاشی برداشت کرد. سرعت برخه زدودن باقیمانده ماده مزبور در توت فرنگی با استفاده از آب شیر در حدود ۹/۹٪ - ۳۰/۵۵٪ بود. متوسط مقدار باقیمانده این سم در میوه، آبمیوه و شربت توت فرنگی در شرایط سرد و گرم (cold and hot break) به شدت در مقایسه با توت فرنگی شسته نشده کم شد. افزون بر این، باقیمانده سم مزبور در شربت توت فرنگی در شرایط گرم (hot break) کاهش بیشتری از شرایط سرد (cold break) داشت. باقیمانده های سم در مریبا تغلیظ شده و به سطوحی بالاتر از غلظت آن در آب توت فرنگی و شربت آن در شرایط سرد و گرم (cold and hot break) رسید. به طور کلی، تغییرات فیزیکی و شیمیایی در ویژگی های میوه توت فرنگی و فرآورده های آن به طور عمده ناشی از عملیات فرآوری بود.