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PRELIMINARY STUDY ON THE USE OF OZONATION FOR THE DEGRADATION OF DITHIOCARBAMATE RESIDUES IN THE FRUIT DRYING PROCESS: MANCOZEB RESIDUE IN BLACKCURRANT IS THE EXAMPLE USED

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Abstract: In order to reduce the level of dithiocarbamate fungicide mancozeb residues in blackcurrants, two different ozone treatment procedures were evaluated. The first one entailed washing the plant material with an aqueous solution of ozone. This ozone enriched water solution allowed for a 59% reduction of mancozeb residues, compared with the initial concentration. The latter method was based on the utilization of ozone in a gaseous phase combined with a drying process. In that procedure, samples of blackcurrant fruit were exposed to a 19 ppm ozone concentration, and then the blackcurrants were dried. The utilization of ozone in a gaseous phase permitted a 38% reduction of mancozeb residues, in comparison with the initial concentration. As a result of the combination of both processes; ozonation and drying, a 58% reduction of mancozeb residues was achieved.

Key words: degradation of mancozeb residues, food processing, ozone treatment

INTRODUCTION

Poland is one of the greatest producers of soft fruits, such as berries and apples. About 80% of this production is destined for the food processing industry. The 2011, potential soft-fruit production in Poland was estimated to be: cherry-250, strawberry-200, raspberry-70, and blackcurrant-150 Gg (Podymniak 2006).

Current technologies of agricultural production and product storage require utilization of plant protection agents. There has been improved access to agrochemicals which have lowered persistence and lowered application rates of the active ingredients. Still, residues of such active ingredients in food products exist. Pesticide exposure can be minimized by respecting Good Agricultural Practice standards. For fragile consumer groups such as children, residues levels exceeding 0.01 mg/kg are still an issue. In European legislation, Maximum Residue Levels (MRLs) of pesticides in food products were established. These levels ranged from 0.01 to a few mg/kg, depending on the particular product, and active ingredients. According to the Regulations of the Polish Ministry of Health, from 16 September 2010 (Dz. U. z 2010 r. Nr 136, poz. 914) most toxic agent residue levels in products of plant origin should be lower than 0.01 mg/kg for vulnerable consumer groups.

The growing supply of ecological products is only a partial solution, because of the numerous limitations, i.e. risks connected with oversensitivity to various pests and diseases. Therefore, ecological production cannot substitute for modern agricultural production processes. For this reason, it is important to develop efficient methods of food processing which would enable a reduction in the active ingredient residues.

The conventional ozone treatment of such food products as fruits and vegetables is based on storing the food products in an ozone enriched atmosphere. The aim of this procedure is to maintain food quality in storage facilities by inhibiting pathogen activity (Zhang *et al*. 2005; Tzortzakis *et al*. 2008).

The obvious benefit of the ozonation procedures is the decomposition of ozone to pure oxygen, leaving no toxic or taste changing byproducts. Such byproducts may occur in many other food product treatment methods *i.e.* the chlorine and trihalomethanes (THMs) bath. Another benefit is that ozone can be generated on-site, from surrounding air, by means of commercially available installations. This allows for space in processing plants which would otherwise be required for storage of treatment agents, also the cost of agent transportation would be eliminated. Hwang *et al*. (2002) have reported that in

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the case of the ozone bath, a much lower concentration is used, in comparison to solutions of other agents such as calcium chlorine (I) [Ca(OCl)2] or chlorine (IV) oxide (ClO2), that was used for total degradation of mancozeb and ethylenethiourea in fresh apples and their products (Hwang *et al*. 2002).

The treatment of fruits with a gaseous ozone solution for the reduction of pesticide residues can be considered to be a highly innovative approach. A review of the literature has not found any similar methods that have been recommended. The main objective of this work was to evaluate and compare the two methods of ozone treatment (aqueous and gaseous) for the purpose of reducing or completely eliminating mancozeb residues in the selected plant material (blackcurrants) at the food processing stage.

MATERIALS AND METHODS

Mancozeb is an active ingredient of the commercially named fungicide Dithane NeoTec 75 WG. Blackcurrant fruit samples containing residues of mancozeb were obtained from a conventional farm. Water for the fruit bath was saturated with ozone using a Korona 02/10 C.S.I EKOTECH (Piotrków Trybunalski, Poland) ozone generator. For treatment with ozone in the gaseous phase, a TS 30 generator was used (Ozone Solutions Inc., Hull, IA, USA). Ozone concentrations in the gaseous phase were monitored with the UV-106 M Ozone Analyzer. The chemicals used were supplied by local producers. Potassium iodide (KI) and methylene blue both pure p. a. were supplied by Chempur (Piekary Slaskie, Poland) and tin (II) chloride ($SnCl_2$) pure for analysis (p. a.) was supplied by POCH S.A. (Gliwice, Poland).

Sample preparation and storage

Blackcurrant fruit (*Ribes nigrum* L.) samples were stored at –180°C. All samples used in the experiments were taken from the same plant material. Analytical samples of 100 g of blackcurrant were isolated and later analyzed for dithiocarbamate residues (DTCs).

Detection of mancozeb concentration in blackcurrant fruit

In the analytical samples, levels of DTC residues were determined by their decomposition to carbon disulfide (CS_2) (this reaction was carried out in an acidic environment with a presence of $SnCl_2$). After that, CS_2 was transferred to methylene blue. Finally, the samples were analyzed in the aqueous solution on a Unicam Helios spectrometer at 662 nm wave length (Chmiel 1979; Sadło *et al*. 2003). The residues of DTC were expressed in mg of CS_2 per kg of plant material.

Ozonation treatment procedures

Samples of the blackcurrant fruit were treated with two different methods of ozonation. In the first experiment, plant material was subjected to a bath in ozone solution water. The second procedure used ozone in the gaseous phase.

Washing in the ozone solution

In conventional installations for washing treatment, fruits and vegetables are sprayed with an aqueous solution of a particular agent or passed through a solution on an assembly line. Under laboratory conditions, this process was simulated by washing blackcurrant fruit samples in the aqueous ozone solution, at room temperature for 30 min. The ozone bath was prepared by saturating water with an ozone stream for 20 min (gas flow 18 ml/min, concentration of ozone in gaseous phase was 6 ppm). Then, 100 g of blackcurrant fruit samples were washed for 30 min in a vessel containing 2 dm^3 of the aqueous solution. The ozone concentration of the aqueous solution was 2 ppm. During this treatment, ozone was dosed continuously to maintain a constant ozone concentration in the solution. To compare the effect of ozonation, other blackcurrant samples were washed with pure water only.

Treatment with gaseous ozone

In the second approach, 2,000 g of blackcurrant fruit samples were placed in a chamber with a cross-section of 150 mm. There they were exposed to the gaseous ozone for 1 h. The ozone concentration within the chamber was 19 ppm, and the air stream was 22.4 m^3/h. Afterwards, the chamber temperature was increased and the drying procedure was started.

Determination of ozone concentration in water

Ozone concentration in water solution was determined using the iodometric method. For this purpose, 25 cm^3 of ozonated water was added to a flask containing 0.400 g of KI (*Potassium iodide*). After that, 3 cm^3 of 0.1 M hydrochloric acid (HCl) solution and 1 cm of 5% starch solution were added. In this method, ozone is consumed for the oxidation of the iodide ion to the iodine molecule (I_2) as follows:

$$O_3 + 2KI + H_2O \rightarrow O_2 + I_2 + 2KOH$$

A solution of 0.002 M sodium thiosulfate ($Na_2S_2O_3$) was used for titration of released I_2 (with starch solution used as an indicator) according to:

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2NaI$$

According to the reaction, 1 mol of $Na_2S_2O_3$ was equivalent to 0.5 mol of ozone.

The maximal ozone concentration in a gaseous phase that could be obtained with the Korona ozone generator was 6 ppm. In the aqueous solution, the concentration was lower; reaching not more than 4.5 ppm (Fig. 1).

Drying procedure

The drying procedure for blackcurrant fruit consisted of several steps.

1. During the first stage, which could last for about 10 h, the maximal temperature in the drying chamber was approximately 40°C. As a result, the water content in the fruits was reduced to about 50%. The concentration corresponded to the moment when juice stopped leaking from the fruits.

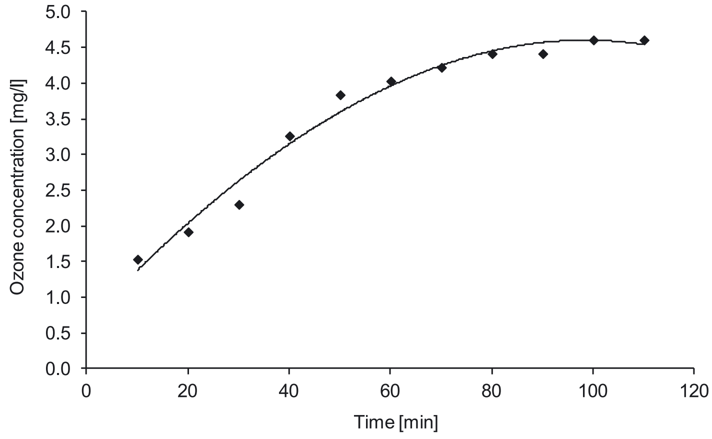

Fig. 1. Course of water saturation with ozone

2. In the second stage, the temperature was increased. After another 12 h of heating at 50°C, the water content was reduced to 30%.
3. In the last stage of the heating, drying was done in temperatures between 70 and 80°C for about 8 h. Next, the drying chamber was cooled until the temperature dropped to 30°C. The sample was tested to make sure no juice leaked from the squashed fruits.

RESULTS AND DISCUSSION

The results of the ozone treatments were successful in terms of pesticide reduction. It is clear that the washing procedures using ozone were more efficient in terms of residue degradation, than washing with water only (Fig. 2).

As a result of the gaseous ozone treatment and the drying process, a reduction of 58% of the active ingredient in blackcurrant fruit could be observed (Fig. 3).

Determined residue levels were summarized in table 1.

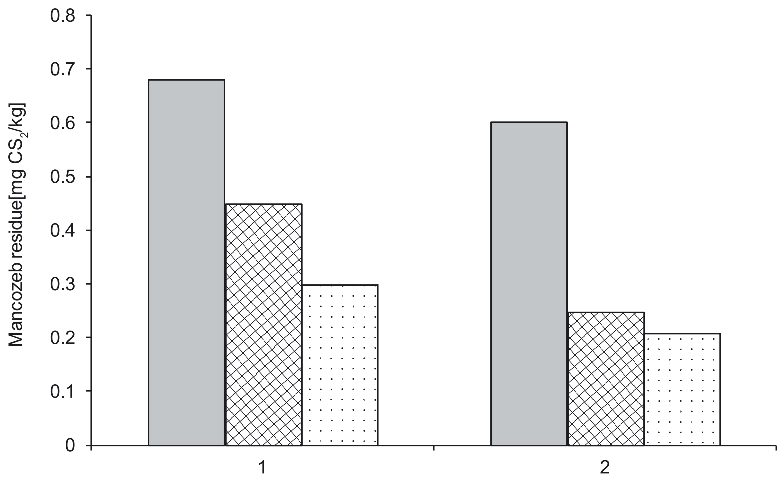

Fig. 2. Mancozeb residue reduction in blackcurrant fruit after washing with water, and aqueous ozone solution: grey – initial residue, grid – residue after water treatment, dots – residue after 0.5 h of treatment using 2 ppm ozone solution in water

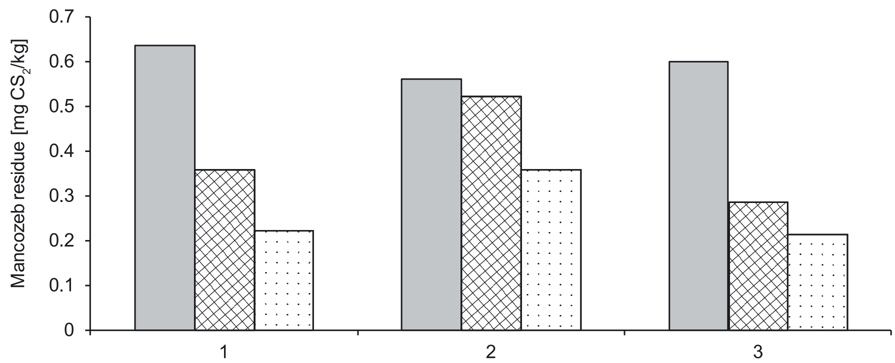

Fig. 3. Mancozeb residue reduction in blackcurrant fruit after the ozonation and drying process: grey – initial residue, grid – residue after 1 h of ozone treatment, dots – residue after the ozone treatment and drying process

Table 1. Mancozeb residue levels before and after the treatment of the tested plant material

Procedure	Initial residue [mg/kg]	Residue after treatment [mg/kg]	Residue reduction [%]
Washing with water	0.615*	0.349	44
Washing with ozone solution		0.254	59
Treatment with gaseous ozone		0.386	38
Treatment with ozone, and drying		0.263**	58

*mean value from all experiments
**values obtained after the calculation of the drying index (DI): DI can be calculated by dividing the initial fruit mass by the fruit mass after water loss. Mancozeb residue levels in fresh fruits were calculated by multiplying the determined residue levels for dried fruit material with DI value

Blackcurrant fruit samples were also subjected to longer ozone treatment (Fig. 4).

In the initial stage, the reduction of the active ingredient can be observed. The reduction of water content during the drying procedure, however, caused an inhibition of the process. In addition, the total mass of the treated plant material decreased due to air stream with ozone exposure. The result was water loss, and the percentage of CS_2 in the total mass of treated fruit material increased. There was no observed effect of the ozone treatment, on the residues in blackcurrant fruit that was dried prior to ozonation. The results of our experiments suggest that water vapor and water within blackcurrant fruit play an important role in this process. There was an indicated involvement of hydroxyl radicals ($^{\bullet}OH$).

The hydroxyl radical is a very strong oxidant (E = 2.8 V) which can be easily generated in the aqueous solution of ozone. Hydroxyl radical reactions with organic compounds are based on hydrogen abstraction, or electrophylic addiction to double bonds (Chiron *et al*. 2000). This radical has low selectivity, which makes it an ideal support for ozone in the oxidation processes of a variety of compounds.

On the other hand, the half-life of ozone in a gaseous solution is higher than in an aqueous solution, which makes its more stable and, therefore, able to be active longer. Furthermore, ozone in air is able to reach targeted compounds much faster due to an enhancement of the diffusion process in the gaseous state in comparison to an aqueous solution (Masten and Davies 1997; Gana *et al*. 2009).

In order to increase the efficiency of the treatment, it seems necessary to maintain the proper level of moisture in fruits during the process. The investigated treatment methods were found to be successful for reducing mancozeb residues in blackcurrant fruit. During the ozonation processes it was possible to eliminate almost 60% of residual content. In conducted experiments, initial levels of mancozeb residue were about 0.6 mg/kg. After both ozonation procedures, fruit quality was verified. Fruit shape, color, and taste were examined, and no changes of those properties were observed.

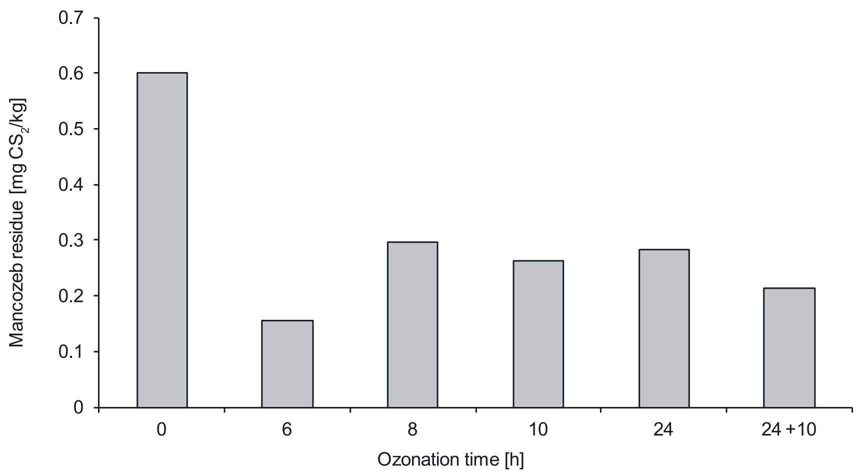

Fig. 4. The course of the ozonation and the drying process

CONCLUSIONS

1. The reduction of pesticide residue levels in fruits with the aid of gaseous ozone can be considered to be a highly innovative approach. The recommended method might be a promising alternative to a washing treatment, especially for fruits designated for drying.
2. Ozonation of fruits can be applied in the food industry for the purpose of producing healthier food for vulnerable groups of consumers, such as infants and young children. Moreover, the process can not only reduce the pesticide residue levels but also improve microbiological purity of the final product.
3. After some minor modifications of the currently used system, the efficiency of the process is expected to improve. This will be the subject of future studies.

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