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Chemical and Organoleptic Investigations of Hazelnut Irradiated with Multiple Disinfestation Doses

Istraživanje kemijskih i senzorskih svojstava lješnjaka ozračenog višestrukim dezinfestacijskim dozama

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Summary

Hazelnut suffers considerable loss during storage due to storage pests. In considering disinfestation of hazelnut by irradiation, a relatively high disinfestation dose requirement for ***Lepidoptera****, as compared to* ***Coleoptera****, as well as a possible requirement for repeated irradiation, must be taken into account. Chemical and organoleptic properties of irradiated hazelnut kernels were followed as a function of irradiation dose, storage temperature and time after irradiation. Low irradiation doses, up to 5 kGy, did not produce any significant increase of the free fatty acid level, or any decrease of vitamin C. Chemical changes were produced in lipophilic components, where an increase with dose of lipid hydroperoxides (up to 240 ppm) and a decrease of α-tocopherol (up to 170 ppm) with increasing irradiation dose were measured. These changes do not result in any detrimental organoleptic properties, as no effects on odor and taste could be detected up to 3 kGy. This means that irradiation of hazelnut with multiple disinfestation doses, up to at least 3 kGy is possible. The fate of radiation-induced free radicals is discussed in terms of the spatial and temporal distribution of radiation chemical events in the lipophilic regions of a hazelnut kernel.*

Sažetak

Skladišni štetnici uzrokuju značajne gubitke uskladištenih lješnjaka. Pri razmatranju mogućnosti dezinfestacije lješnjaka ozračivanjem, treba uzeti u obzir zahtjev za relativno visoku dozu dezinfestacije ***Lepidoptera****, u usporedbi s* ***Coleoptera****, kao i mogući zahtjev za ponovljenim ozračivanjem. Kemijska i osjetilna svojstva ozračenog lješnjaka praćena su kao funkcija doze ozračivanja, temperature skladištenja i vremena nakon ozračivanja. Male doze zračenja, do 5 kGy, nisu uzrokovale nikakav značajan porast slobodnih masnih kiselina ili smanjenje vitamina C. Kemijske su promjene nastale u lipofilnim sastojcima, gdje su izmjereni: porast lipidnih hidroperoksida (do 240 ppm) i smanjenje α-tokoferola (do 170 ppm) s povećanjem doze zračenja. Te promjene nisu uzrokovale nikakva štetna senzorska svojstva jer nisu otkriveni nikakvi učinci na aromu i okus do doze od 3 kGy. To znači da se lješnjak može ozračivati višestrukim dezinfestacijskim dozama, barem do 3 kGy. Sudbina zračenjem induciranih slobodnih radikala razmatra se s gledišta prostorne i vremenske raspodjele radijacijsko-kemijskih događaja u lipofilnim regijama lješnjakove jezgre.*

Introduction

Of the nut crops, hazelnut *(Corylus avellana)* is second in importance only to almond *(1)*. It contains a significant amount of lipids, proteins and vitamins. Hazelnut kernel is a popular snack and a high quality food. It can be used in various forms, as whole, crushed, peeled, paste etc. Besides in households, hazelnut is mostly used in confectionery, and also in pharmaceutical and cosmetics industries.

The industrial uses of hazelnut set industrial requirements on conditions for the appropriate handling and storage of large quantities of nuts *(2)*. Similarly to other nuts, the hull provides a good protection to the kernel, so that properly dried hazelnut can be stored for an extended period of time.

However, hazelnut suffers considerable loss during storage due to storage pests. *Tribolium castaneum* (Herbst) has been identified as the most important storage pest of hazelnut in Croatia *(3)*. It is one of the most frequent pests in stored grains, legumes, nuts and oil seeds *(4)*. It is a cosmopolitan pest, and frequently occurs in imported soybean meal *(5)*. Other insects infesting hazelnut are *Trogoderma granarium* (Everts), which is only a quarantine pest in Croatia, and *Tribolium destructor* (Uyttenboorgaart) which has not been found in Croatia *(3)*. A hazelnut pest recently gaining in importance is *Corcyra cephalonica* (Sta.) *(6)*.

Besides a direct economic loss due to the mechanical damage of nuts, the action of insects is also responsible for the accelerated chemical deterioration *(4)*, as well as for a potential contamination with bacteria and parasites *(3,7)*, all of which eventually compromises the wholesomeness of the stored product. The current methods for insect disinfestation used by the dry fruit and nut industry primarily involve fumigation with methyl bromide *(8)*. In comparison with fumigation, insect disinfestation by irradiation has many advantages, especially regarding the mode of action, versatility, efficacy, absence of residues, inability of insect to develop resistance, safety and time consumption *(9)*. On the other hand, the justification of the irradiation treatment must be established by a careful consideration of the radiation chemistry criteria for the wholesomeness of irradiated food, as required by the Joint Expert Committee on Food Irradiation *(10)*.

The data on the sensory, as well as radiation chemistry aspects of radiation disinfestation of nuts are, in general, rather scarce *(11)*; the data pertaining to hazelnut in particular are even more meager. While adequate entomological data exist on the radiation sensitivity of the major insect pest, *Tribolium castaneum* (Herbst) *(12)*, radiation chemistry aspects of hazelnut irradiation should not be neglected, especially with respect to its high content of unsaturated fatty acids.

Repeated exposure to gas is a common practice in fumigation *(13)*. Codex General Standard for Irradiated Foods also allows re-irradiation of »foods with low moisture content... irradiated for the purpose of controlling insect reinfestation« *(14)*. Should this practice be necessary with hazelnut, the total dose in the product could exceed a single treatment dose by several times. An adequate disinfestation procedure in case of *Corcyra cephalonica* infestation should take into account a significantly (an order of magnitude) higher dose requirement (1 kGy) for the sterilization of this pest which belongs to *Lepidoptera*, as compared to *Coleoptera* (100 Gy), (of which *Tribolium* and *Trogoderma* are members) *(12)*. Based on these considerations, doses of several kGy may be expected.

In view of the increasing economic importance of hazelnut crop in Croatia *(15)*, and in an effort to find a suitable substitute for methyl bromide fumigation, this paper deals with the radiation induced changes as chemical indicators for the evaluation of wholesomeness of irradiated hazelnut.

Materials and Methods

Material

A sample of hazelnut kernels, harvested in Orahovica, Eastern Slavonia, was acquired 1.5 months after harvesting which took place in late August. The kernels were of uniform size and healthy looking with characteristically brown peel. Only kernels with undamaged peel were selected for experiments. The contents of moisture and lipids were determined in 2-g samples withdrawn from the larger quantity of hazelnut kernels (10 g) ground in a household mill for nuts.

Irradiation and Storage

For analyses to follow immediately after the irradiation, 10-g samples in polyethylene pouches were irradiated with gamma rays in the ^{60}Co irradiation facility of the Ruđer Bošković Institute. The dose rate of about 3 Gy/s was determined by the ethanol-chlorobenzene dosimetry *(16)*. After irradiation the entire sample was ground in a household mill for nuts, and 2-g samples were taken for chemical analyses.

For analyses to follow after an extended storage period at a specified temperature, 150-g samples were irradiated and stored in dark. Portions were withdrawn thereof in specified intervals and ground immediately before the analysis.

The storage of samples simulated cool storage at 8 °C (in the refrigerator). The samples for sensory evaluation were stored also at 18 °C to accelerate and make more distinct the eventually occuring degradative processes. The samples were stored for up to 22 weeks.

Chemical Analyses

Chemical analyses of lipophilic components, free fatty acids, lipid hydroperoxides (LOOH) and vitamin E, were performed in the oil obtained by the extraction. Vitamin C was determined in the defatted hazelnut kernels after the extraction.

Moisture content. Determination of moisture was performed by heating 2 g of ground hazelnut kernels at 104 °C for 2 h and an additional period of 40 min *(17)*.

Determination of lipids. The amount of lipids in samples was determined by shaking 2 g of ground hazelnut kernels with a cold deaerated chloroform-methanol mixture of volume ratio 2:1 *(18)*.

Determination of free fatty acids. The hazelnut oil obtained by extraction was suspended in an ethanol-ether mixture of volume ratio 1:1 and homogenized in an ultrasound bath. A few drops of 1 % ethanolic phenolphthalein were added, and the mixture titrated with a 0.01 M standardized aqueous solution of KOH to a faint pink color *(19)*. Since oleic acid is a predominant fatty acid, accounting for about 75 % of hazelnut oil mass fraction *(20)*, free fatty acids were expressed as percent of free oleic acid mass fraction in the oil.

Determination of lipid hydroperoxides. Lipid hydroperoxides formed by irradiation were determined by spectrophotometry using ferric thiocyanate complex. Fe(II) ion was first oxidized by LOOH, and the formed Fe(III) ion complexed with thiocyanate, yielding an intense red color *(21)*. Our modification of this method consisted mainly in the development of the ferric thiocyanate complex and its measurement directly in the extraction medium, a deaerated chloroform-methanol mixture of volume ratio 2:1. In this solvent the complex was characterized by $\lambda_{\max} = 500$ nm and $\varepsilon_{500\ \mathrm{nm}} = 14300 \pm 680\ \mathrm{dm^3 mol^{-1} cm^{-1}}$ *(22)*. Some more commonly used methods for the determination of LOOH, such as the thiobarbituric acid (TBA) test or the measurement of conjugated dienes, were not used because the major unsaturated fatty acid in hazelnut, oleic acid *(20)*, yields no response to these methods, and the results would not be representative.

Determination of α-tocopherol. The hazelnut oil obtained by extraction was hydrolyzed by KOH, diluted with water, and the aqueous layer extracted with diethylether. After evaporating ether, a deaerated methanolic solution of hydrolyzate was made, containing also 7-dehydrocholesterol as an internal standard. The sample was analyzed on a reverse phase column of μ-Bondapak C_{18} and eluted isocratically with 3.5 % (by volume) of methanol in water

Table 1. Free fatty acids (FFA) in irradiated and unirradiated samples of hazelnut stored at 8 °C
Tablica 1. Slobodne masne kiseline (FFA) u ozračenim i neozračenim uzorcima lješnjaka uskladištenim na 8 °C

Storage time/ week Vrijeme skladištenja/ tjedan	FFA[a] / % expressed as free oleic acid in oil izražene kao slobodna oleinska kiselina u ulju				
	0 kGy	1 kGy	3 kGy	5 kGy	10 kGy
0	0.74±0.03	0.71±0.01	0.72±0.02	0.73±0.01	0.81±0.03
20	0.74±0.04	0.76±0.03	0.77±0.02	0.73±0.03	0.78±0.04

[a] Average of 3 replicates ± std. err.
Prosječna vrijednost triju ispitivanja ± standardna pogreška

at 1 mL/min. The spectrophotometric detection was at 280 nm *(23).*

Determination of vitamin C. Defatted hazelnut (residue after extraction of oil) was extracted by metaphosphoric acid and subjected to oxidation with bromine. The osazone resulting after the addition of 2,4-dinitrophenylhydrazine was measured by spectrophotometry at 520 nm using the molar absorbance $\varepsilon_{520\,\mathrm{nm}} = 3940 \pm 150\ \mathrm{dm^3\,mol^{-1}\,cm^{-1}}$ *(24).*

Sensory Evaluation

A panel of six experienced judges was judging odor and taste of unirradiated, as well as of irradiated hazelnut kernels on a hedonic scale between 1 and 5 (1 for the worst, 5 for the best). The nuts were assessed immediately upon acquisition, as well as after 22 weeks of storage. The average score and the pertaining standard error were calculated for each set of variables (dose, temperature, storage time).

Results

The average mass fraction of moisture (three determinations) was 4.74±0.14 %. Hazelnut kernels in the original state contained 54.2±0.3 % lipids (or 56.9±0.3 % expressed per dry matter).

The content of free fatty acids in irradiated hazelnut as a function of irradiation dose and 20-week storage at 8 °C is shown in Table 1. The level of free fatty acids in unirradiated hazelnut was found stable over that period. The irradiation with a low dose (1 kGy) induced a small loss of free fatty acids below the level occurring in unirradiated hazelnut. With increasing the irradiation dose the level of free fatty acids was reestablished to the value found in unirradiated samples, while at very high irradiation dose (10 kGy), higher content of free fatty acids was found. The overall picture was essentially unchanged 20 weeks after irradiation and storage at 8 °C.

The amount of lipid hydroperoxides as a function of dose and storage time is shown in Table 2. Lipid hydroperoxides increased with the irradiation dose in a fashion characteristic for a saturation process. Hydroperoxides formed by irradiation decreased during storage, the decrease being somewhat faster immediately after the irradiation than after an extended period of storage.

The content of α-tocopherol sharply decreased with increasing dose at low irradiation dose (1 kGy), reaching a constant value at higher doses (Table 3). This behavior is a mirror image of the behavior of lipid hydroperoxides. Immediately upon irradiation about 100 mg of α-tocopherol per 1 kg of lipid was destroyed by a 1 kGy dose. Knowing the amount of the chemical change and the absorbed dose, the radiation chemical yield for the destruction of α-tocopherol, G(–α-toc), can be calculated. Radiation chemical yield is the amount of chemical change (mol) brought about by the absorption of the unit of irradiation energy per unit mass, (J/kg):

$$G(-\alpha\text{-toc}) = \frac{\dfrac{0{,}100\ \mathrm{g}}{1\ \mathrm{kg\ L}} \cdot \dfrac{1\ \mathrm{mol}}{416{,}7\ \mathrm{g}} \cdot 0{,}54\ \dfrac{\mathrm{kg\ L}}{\mathrm{kg}}}{1 \cdot 10^{3}\ \dfrac{\mathrm{J}}{\mathrm{kg}}} =$$

$$= 1{,}3 \cdot 10^{-7}\ \frac{\mathrm{mol}}{\mathrm{J}}$$

where L stands for »lipid«, and other symbols have the usual meaning.

The radiation chemical yield for the formation of LOOH in the same dose range (0-1 kGy) is $1.5 \cdot 10^{-7}\ \mathrm{mol\ J^{-1}}$. Relatively high numerical values of the radiation chemical

Table 2. Lipid hydroperoxides (LOOH) in irradiated and unirradiated samples of hazelnut stored at 8 °C
Tablica 2. Lipidni hidroperoksidi (LOOH) u ozračenim i neozračenim uzorcima lješnjaka uskladištenim na 8 °C

Storage time/ week Vrijeme skladištenja/ tjedan	LOOH[a] / mmol/kg L				
	0 kGy	1 kGy	3 kGy	5 kGy	10 kGy
0	0.067±0.005	0.324±0.011	0.580±0.035	0.698±0.140	0.799±0.029
1	0.052±0.004	0.303±0.018	0.547±0.011	0.622±0.043	0.722±0.030
3	0.034±0.002	0.294±0.018	0.513±0.014	0.601±0.017	0.698±0.048
8	0.065±0.004	0.284±0.004	0.505±0.012	0.606±0.031	0.690±0.011
20	0.088±0.007	0.274±0.025	0.408±0.005	0.453± 0.009	0.467±0.015

[a] Average of 3 replicates ± std. err.
Prosječna vrijednost triju ispitivanja ± standardna pogreška

Table 3. α-Tocopherol in irradiated and unirradiated samples of hazelnut stored at 8 °C
Tablica 3. α-tocopherol u ozračenim i neozračenim uzorcima lješnjaka uskladištenim na 8 °C

Storage time/ week Vrijeme skladištenja/ tjedan	α-tocopherol^a / mg/kg L α-tokoferol			
	0 kGy	1 kGy	5 kGy	10 kGy
0	367.6±12.3	271.8±9.9	178.0±11.5	195.2±3.0
4	374.1±4.4	234.5±1.9	171.5±5.2	188.8±8.2
8	382.1±11.6	237.8±7.8	176.2±7.2	177.5±4.0

^{a}Average of 3 replicates ± std. err.
Prosječna vrijednost triju ispitivanja ± standardna pogreška

yields for the formation of LOOH, as well as for the destruction of α-tocopherol, uncharacteristic of the radiation chemical reactions in the solid state, indicate that both reactions might proceed by the chain mechanism. Permanent generation of radicals by irradiation seems necessary, however, for sustaining the decay of α-tocopherol because practically no post-irradiation effect on α-tocopherol was observed up to 8 weeks of storage.

Contrary to α-tocopherol, vitamin C was not destroyed by low irradiation doses immediately after irradiation. A significant decrease of vitamin C occurred only above 5 kGy (Table 4). At this dose α-tocopherol was already reduced to about half of its original level and lipid hydroperoxides were approaching saturation. Therefore vitamin C was not considered to be a particularly informative indicator of radiolytic degradation of irradiated hazelnut, and it was not followed as a function of storage time.

The results of sensory evaluation are shown in Table 5. Due to the relatively large errors of the mean values of attributed marks, rigorous statistical interpretation would not be very informative and might lead to the conclusion that no effects of irradiation and storage could be detected whatsoever. Nevertheless, a cautious attempt to interpret these results relying on minute differences between the individual mean values was made. Taking this approach, it could be speculated that irradiation with a low dose (1 kGy) slightly »improved« odor and taste, while slightly deteriorated odor and taste were found immediately after irradiation with a higher dose (3 kGy). A slight preference for low-dose irradiated hazelnut (1 kGy) was best maintained, and moreover, somewhat enchanced after 22 weeks of storage at 8 °C. Storage at 18 °C almost completely erased the differences between the irradiated and unirradiated samples. In case of higher dose irradiation (3 kGy), not even the storage at 8 °C could stop some minor post-irradiation deterioration of odor and taste.

Table 4. Vitamin C in irradiated and unirradiated samples of hazelnut immediately after irradiation

Tablica 4. Vitamin C u ozračenim i neozračenim uzorcima lješnjaka neposredno nakon ozračivanja

Dose/kGy Doza/kGy	Vitamin C^a / mg/kg
0	65.5±6.6
5	64.0±10.0
10	53.2±6.5
15	50.3±1.4

^{a}Average of 3 replicates ± std. err.
Prosječna vrijednost triju ispitivanja ± standardna pogreška

Discussion

A slight preference expressed by the panel for odor and taste, as found in samples irradiated with 1 kGy and stored at 8 °C, was also reported for dry almond, ground nut, pine nut and walnut *(25)*, irradiated with low-dose (0.5 kGy), as compared to both unirradiated nuts and nuts irradiated with higher doses (0.75 and 1 kGy).

A strong correlation between the sensory ratings of irradiated dry almond, peanut, pine nut and walnut was also reported with peroxide accumulation *(26)*. Although the dose applied in that study did not exceed 1 kGy, and the level of peroxidation was low immediately after irradiation, peroxide content increased during an extended (200 days) storage.

Peroxidation is certainly the most important cause of quality deterioration of lipid-containing foods *(27)*, and deserves special attention. In considering the results of hydroperoxidation measurements, however, it must be borne in mind that different dose-response curves can be obtained by taking different indicators as measures of peroxidation *(28)*, as well as by taking measurements at different times after irradiation *(29)*, which may distort the results.

This explains the variations in the course of accumulation of lipid hydroperoxides with increasing the irradiation dose *(30)*, and post-irradiation changes *(31)* in irradiated peanut kernels, as measured by the oxidation of ferrous to ferric iron, TBA method and conjugated dienes test. The measure of lipid hydroperoxides in the present work is the ability to oxidize ferrous iron. In this way, total lipid hydroperoxides can be accounted for, which otherwise would not be the case with TBA or conjugated dienes tests, which are insensitive to oleic acid hydroperoxides.

Although hazelnut kernels contain indigeneous antioxidants, α-tocopherol and vitamin C, the accumulation of lipid hydroperoxides with the increasing dose in irradiated hazelnut does not exhibit a lag dose. The molar ratio of lipid (expressed as triolein) to α-tocopherol is about 1300. For comparison, α-tocopherol conferred antioxidative protection to linoleic acid micelles irradiated up to 0.2 kGy under a less favorable concentration conditions, at the linoleic acid: α-tocopherol concentration ratio of 4000 *(32)*.

The fractions of the irradiation dose used in this work, however, are too large to allow for the resolution of a lag

Table 5. Results of sensory test of irradiated and unirradiated hazelnut[a]
Tablica 5. Rezultati ocjenjivanja osjetilnih svojstava ozračenih i neozračenih lješnjaka[a]

Storage time/ week Vrijeme skladištenja/ tjedan	Sensoric property Osjetilna svojstva	0 kGy		1 kGy		3 kGy	
		8 °C	18 °C	8 °C	18 °C	8 °C	18 °C
0	Odor	3.4±0.7		3.5±0.7		3.3±0.7	
22	Aroma	3.6±1.0	3.3±1.0	3.0±0.6	3.3±1.0	3.0±1.4	3.3±1.3
0	Taste	3.3±0.6		3.4±0.7		3.2±0.8	
22	Okus	3.7±1.0	3.3±0.9	4.0±0.8	3.1±1.0	2.8±1.2	3.2±1.1

[a]Average scores (scale 1-5) of 6 panel members ± std. err.
Prosječne ocjene (na ljestvici od 1 do 5) 6 ocjenjivača ± standardna pogreška

dose of that magnitude, if there is any at all. Namely, no lag dose was observed in almond, ground nut, walnut and pine nut, all rich in tocopherols, irradiated up to 1 kGy by 0.25 kGy dose fractions *(33)*.

While α-tocopherol was efficiently degraded by low irradiation doses, the degradation of vitamin C was low at low irradiation doses, and became apparent only as the content of α-tocopherol decreased to a steady level at about half of its initial concentration, at about 5 kGy.

This is a completely reverse situation to the temporal sequence of disappearance of vitamin E and vitamin C in the autoxidation of methyl linoleate in tert-butyl alcohol/methanol mixture *(34)*, whereby vitamin C regenerated vitamin E. The synergistic interaction of the two vitamins was first observed in a homogeneous solution, where a rapid repair of vitamin E radical and the simultaneous appearance of vitamin C radical were observed by pulse radiolysis *(35)*. Vitamin C alone was found a poor antioxidant when free radicals were generated initially within the lipid membrane but, nevertheless, acted as a synergist and protected vitamin E when both were present in the membrane *(34)*.

Contrary to homogeneous solutions and liposomal membrane systems which are characterized by a fair mobility of reactive species, in a rigid hazelnut kernel there is no evidence of vitamin C protecting vitamin E. On the basis of the present results we can only hypothesize on the possible mechanism of radiation induced damage in irradiated hazelnut.

Molecules of lipidic substances give on irradiation free radicals which promptly react with a limited amount of oxygen available inside the kernel. This results in the formation of lipid hydroperoxides until available oxygen is consumed, above 10 kGy. Another factor contributing to the saturation of the yield of LOOH is the spatial distribution of free radicals in a rigid matrix: after a certain dose, any newly formed free radical will be formed in the vicinity of some previously formed free radical, and will have an opportunity to recombine with it before reacting with oxygen, which is becoming more sparse.

Lipophilic α-tocopherol is in intimate contact with the formed lipid free radicals and can undergo radical transfer reaction from neighboring radicals. However, α-tocopherol in a rigid matrix can react only with radicals within its interaction radius. Nevertheless, radical transfer reactions are very efficient, as illustrated by the ratio of the respective G-values; $G(-\alpha\text{-toc})/G(\text{LOOH}) \approx 0.9$ means that about 1/2 of formed lipid free radicals is successful in destroying a molecule of α-tocopherol, whereas another half gives LOOH. The reaction is presumably fast. Therefore, no slow post-irradiation reactions are important, which is consistent with the finding that the level of α-tocopherol found 8 weeks after irradiation was practically the same as that immediately after irradiation.

The distribution of the lipophobic vitamin C, on the other hand, is different from that of vitamin E. It is thereby protected from oxidative damage which takes place predominantly in the lipidic regions. It is also prevented from diffusion by a high viscosity of the medium, and thereby prevented from its synergistic interaction with vitamin E. The situation to some extent resembles the one in lipid membranes when radicals are generated within the membranes, and are initially inaccessible to vitamin C *(34)*. The inaccessibility of free radicals in lipidic phase to vitamin C in hydrophilic phase, both embedded in a rigid matrix, however, remains permanent.

Conclusion

Irradiation of hazelnut kernels did not produce any significant increase of free fatty acids, or any decrease of vitamin C up to 5 kGy. Some changes were produced in lipophilic components, where an increase with dose of lipid hydroperoxides and a concomitant decrease of α-tocopherol were measured. These changes did not result in any detrimental organoleptic properties, as practically no effects on odor and taste could be detected up to 3 kGy after an extended storage. Consequently, irradiation disinfestation of hazelnut kernel is possible up to at least 3 kGy, which means that the nuts can be subjected to multiple treatments, and that not only *Coleoptera*, but also the more radiation-resistant *Lepidoptera* storage pests can be eliminated.

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