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Improvement of Ethoxyquin Yields and Recoveries from Pears through the Addition of Ascorbic Acid

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Abstract

Ethoxyquin (EQ) is a quinoline-based antioxidant widely applied to inhibit superficial scald (formation of brown spots) in pears and apples. EQ typically shows low recoveries when extracted from commodities with low antioxidative potential such cereals, most vegetables, and some fruits (including pears). The impact of adding ascorbic acid (AA) at different stages of the procedure to protect EQ was studied, using pears with incurred EQ residues as well as pears that were superficially spiked in the laboratory. It was shown that extensive degradation occurs during milling, irrespective if conducted cryogenically or at ambient temperature. By adding AA to the sample prior to milling (1 g AA per 100 g sample) EQ losses declined dramatically. In the case of cryogenic milling a single addition of AA prior to milling provided sufficient protection throughout the procedure. During ambient milling, however, AA consumption was much higher requiring supplementary AA addition prior to QuEChERS extraction for sufficient protection. The average EQ recoveries (spiked to pear homogenates at 0.05 mg kg^{-1} and 0.2 mg kg^{-1}) reached 98 % if AA had already been added to the blank pears during milling and 86 % if AA was added directly after EQ spiking. RSDs ranged between 1 % and 8 %. From 2014 onwards 82 pear samples from the local market were analyzed for EQ residues with the analysis involving addition of AA prior to milling. Three pear samples analyzed in 2014 (all from Italy) were found to contain ethoxyquin residues at levels between 0.25 and 0.58 mg kg^{-1}. There have been no further findings of EQ in pear samples since 2015.

Introduction

Ethoxyquin (EQ) is a quinoline-based antioxidant widely used to inhibit superficial scald (formation of brown spots) in pears and apples. Codex Alimentarius classifies EQ as a scald control agent. As scald is often accompanied by fungus infections, EQ is also often listed as a fungicide. For treatment, the fruits are typically dipped into a solution containing EQ, but EQ-impregnated fruit wraps are also often employed. Pre-harvest applications have been reported for apples.

Being an antioxidant, EQ is prone to oxidative losses. Applying the QuEChERS method, notoriously low EQ recoveries are observed in commodities with poor antioxidative potential such as pears, apples, cucumbers and cereals. In contrast, recovery rates from commodities exhibiting strong antioxidative potential such as citrus fruit and berries are typically nearly quantitative. Decomposition may occur both in crops and in the laboratory during most stages of analytical procedures, such as sample homogenization and extraction. Although not part of the current residue definition, EQ transformation products, such as the EQ-dimer, can be used as indicators for the presence of EQ, especially in those cases where routine multi-residue procedures lead to a complete or almost complete decomposition of the parent compound.

In 2011 the EU Commission decided not to include EQ in Annex I of Directive 91/414/EEC, setting 3 Sept. 2012 as the deadline for its use [1], [2]. EU-MRLs are set at the agreed LOQ for all commodities; for instance, the MRL for pears is set at 0.05 mg kg^{-1} (=Codex MRL) [3].

An additional food-related use of EQ concerns spices such as chili, paprika and curcuma powder. Here EQ is applied as an additive to prevent oxidative discoloration of carotenoid pigments during storage (US tolerance 100 mg kg^{-1}). The EU-regulation on additives does, however, not foresee this application.

There are also numerous feed related applications with indirect food relevance. The use of EQ as an animal feed additive often leads to residues in food of animal origin such as poultry, meat, and eggs [6], as well as in farmed fish [7] and shrimp (See also E-Journal: Analysis of Ethoxyquin and its Metabolites in Fish, Using the QuEChERS Method).

Prior to this study it was already observed that EQ degrades at various stages of the analytical procedure for example following spiking to homogenates at room temperature during extraction as well as during the storage of extracts especially when temperatures were high and when dealing with commodities with poor antioxidative potential. Degradation was even noted during the storage of frozen sample homogenates.

In this study the protection of EQ by adding the antioxidant ascorbic acid (AA) at various stages of the analytical procedure was to be tested.

Experimental

Chemicals and Standards

Methanol of gradient grade was purchased from Merck KGaA (Darmstadt, Germany). Water for liquid chromatography was deionized in the laboratory using a Direct-Q 3 UV Ultrapure Water Purification System (Billerica, MA, USA). Ammonium formate (as eluent additive), L-ascorbic acid and sodium ascorbate were supplied by Sigma-Aldrich (Germany). Ethoxyquin (purity > 98.5 %) and the internal standard chlorpyrifos D10 (purity > 98 %) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). All standards were stored at -18 °C. Stock solutions of 1 mg mL^{-1}, considering standard purity, were prepared in acetonitrile. For the internal standard chlorpyrifos D10 a working solution of 20 µg mL^{-1} was prepared in acetonitrile. All solutions were stored in the dark at 4 °C.

Samples and Commodities

The method development focused on pears of different origins. The samples were collected by sampling officers and analyzed by the pesticide residue laboratory of the Chemisches und Veterinäruntersuchungsamt (CVUA) Stuttgart. After arriving at the laboratory the samples were coarsely pre-chopped, put in the freezer overnight and subsequently milled cryogenically using dry ice. The homogenates were stored at -18 °C until extraction. Dried fruits (e.g. raisins) were mixed with cold water at a ratio of 1:1.7, homogenized with the addition of dry ice and the homogenates were deep-frozen at -18 °C until use. Dry samples (e.g. wheat) were ground thoroughly without adding water and were stored at room temperature until extraction.

Apparatus

Frozen samples were comminuted with dry ice using a Prime Cut UM5 universal machine from Stephan Machinery GmbH (Hameln, Germany). The automatic shaking machine Geno Grinder 2010 (SPEX Sample Prep, Metuchen, USA) was used for QuEChERS extraction. The centrifuge Rotanta 460 by Hettich (Tuttlingen, Germany), appropriate to the centrifuge tubes employed in the procedure and capable of achieving 4000×g, was used. Electronic pipettes applicable for volumes of 10–100 µL and 100-1000 µL, respectively, and manual pipettes applicable for volumes of 1–10 mL were from Eppendorf (Hamburg, Germany). Analytical balances capable of weighing to 0.1 mg or to 0.01 g were from Mettler-Toledo (Greifensee, Switzerland). The Fortuna Optifix 10 mL solvent dispenser was used to add solvent to the samples. The automatic dispenser system Opus (20–50 mL; Hirschmann Laborgeräte, Eberstadt, Germany) was used for diluting the working solutions.

50 mL PP (114×28 mm) single-use tubes with screw caps for the sample extraction were from Sarstedt (Nümbrecht, Germany). 1.5 mL LC autosampler vials and 20 mL screw-cap vials

were from Ziemer GmbH (Mannheim, Germany). Salt mixtures for phase separation were purchased from UCT (Bristol, USA).

A Waters Acquity UPLC system (Waters GmbH, Eschborn, Germany) combined with the mass spectrometer Sciex 4000QTrap (Sciex, Framingham, Massachusetts, USA) run in ESI positive mode was used for the analysis of the extracts.

For LC-MS/MS analysis the samples were injected onto a Acquity BEH C18, 2.1x5 mm, 1.7 µm pre-column connected with a BEH C18, 2.1x100 mm, 1.7 µm column (both from Waters GmbH, Eschborn, Germany).

Sample Extraction

For the QuEChERS method 10 ± 0.1 g of the prepared frozen samples were weighed into 50 mL PP tubes. 10 mL of acetonitrile followed by 100 µL of the internal standard working solution of chlorpyrifos D10 were added to the samples. The tube was closed and shaken by a mechanical shaker or by hand for one minute as a first extraction step. The second extraction step entailed the addition of a pre-weighed partitioning salt mixture (4 g magnesium sulfate anhydrous grit, 1 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate) and shaking for 15 min by mechanical shaker. The tube was centrifuged for 5 min at 4000xg and the upper acetonitrile phase was transferred to vials and directly applied to LC-MS/MS measurement without any further cleanup.

In some cases the raw QuEChERS extracts were cleaned up via dispersive SPE by pouring 6 mL of the extract in a vial containing 900 mg $MgSO_4$ and 150 mg PSA sorbent. After shaking for 30 s the vial was centrifuged and the cleaned extract was transferred into vials for analysis.

LC-MS/MS Measurement Conditions

Mobile phase A consisted of 5 mM ammonium formate in purified water and 5 % methanol; mobile phase B contained 5 mM ammonium formate in methanol.

TABLE 1. Gradient program for LC-measurement

Time (min)	**Mobile Phase A** (%)	**Mobile Phase B** (%)
0	95	5
0.5	60	40
2	10	90
5	10	90
5.1	95	5
10	95	5

A gradient program (see Table 1) was used with a flow rate of 0.35 mL min^{-1}. The injection volume was 2 µL. The column temperature was set to 40 °C. The ESI interface was operated in the positive mode was applied. The MS/MS detection was performed in the multiple reaction monitoring (MRM) mode. The ion spray voltage was 4500 V; the nebulizer gas (60 psi) and the turbo gas (50 psi) were synthetic air and the curtain gas was nitrogen at 30 psi; the source temperature was 440 °C.

Three precursor-product ions were monitored for each compound in the multiple reaction monitoring mode (MRM). The transition with the highest intensity was used for quantification, while the second and third most intensive transitions served for identification. Table 2 shows the MRM transitions used for EQ and the internal standard chlorpyrifos D10.

TABLE 2. Mass Transitions of Compounds

Compound	Intensity ranking	Q 1	Q 3	DP	CE	CXP
Ethoxyquin	1	**218**	**148**	86	31	8
	2	**218**	**160**	86	47	8
	2	**218**	**174**	86	43	10
Chlorpyrifos D10	-	**360**	**199**	66	23	12

Results and Discussion

Initial Observations

Initial recovery experiments using QuEChERS for EQ spiked at 0.1 mg kg^{-1} on pear homogenates resulted in very low and strongly variable recovery rates. Recovery experiments on apples (results not shown here) showed a similar trend. No EQ was recoverable (0 %) from cucumbers and wheat flour. In contrast, very good average recoveries were obtained from red currants and rehydrated raisins (103 % and 96 %, respectively). We attribute this to the stronger antioxidative potential of these two commodities. Red currants contain ca. 40 mg ascorbic acid (AA) per 100 g. The AA levels in grapes are comparable to apples and pears and rather low (4-10 mg per 100 g), but grapes contain higher levels of catechins and polyphenols, which also exhibit antioxidative activity.

EQ Protection during Extraction

In preliminary experiments AA was added to the homogenates in a solid form after spiking with EQ. The results (not shown here) were quite variable and very much depending on the initial temperature of the homogenate as well as on the time elapsed between the addition of EQ and the addition of AA.

It was thus decided to conduct several experiments where solid AA is added to the semi-thawed homogenates prior to spiking with EQ. This practice may not reflect the situation when dealing with samples containing incurred residues (where EQ is present prior to AA) but it was considered that it would still give valuable information about the protective capability of AA. For cucumbers, pears, raisins, and red currents 0.5 g of solid AA was added to the analytical portions, followed by vortexing for one minute (to evenly distribute the AA) and by spiking with EQ (onto the semi-thawed material). In the case of raisins 13.5 g of the rehydrated homogenate was used (containing 5 g raisins). In the case of wheat flour 0.5 g of solid AA was added to the dry material followed by spiking with EQ and addition of cold water. The spiked sample was left for 10 min to soak before the extraction was started. In all cases the first QuEChERS extraction step was conducted by mechanically shaking the solution for 15 min. No dSPE cleanup with PSA-sorbent was conducted. Suitable matrix-matched calibration solutions were used.

The addition of 0.5 g solid AA per analytical portion of cucumber, pear, raisin, red currant and wheat before spiking with EQ resulted in satisfactory recoveries (between 95 % and 105 %) and RSDs (< 5 %) in all cases. Recoveries with and without the addition of AA prior to extraction are shown in Figure 1.

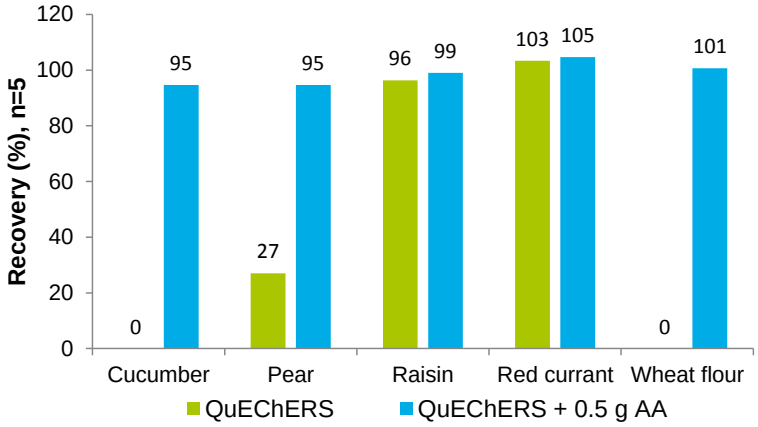

FIGURE 1. Impact of AA-addition on the recoveries of EQ from different commodities

Additional experiments focused on pear and involved a variation of the amount of solid AA added to the pear matrix before spiking with EQ (0.1, 0.25, 0.5 and 1 g). As shown in Figure 2 EQ was sufficiently protected in all cases, with recoveries ranging between 87 % and 97 %. The experiments also involved measurements following dSPE cleanup with PSA as sorbent; the recovery rates were good in all experiments (results not shown). Appropriate matrix-matched calibration solutions were used in all cases.

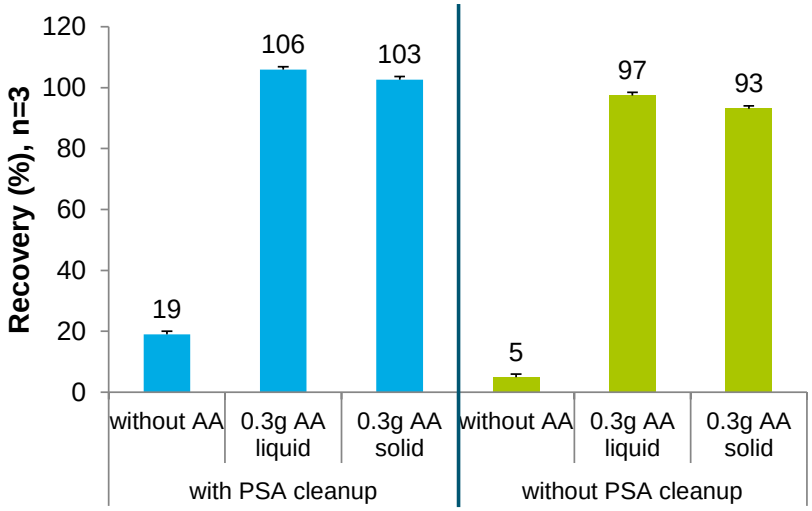

FIGURE 2. EQ recoveries in pears depending on different amounts of AA added to the matrix prior to spiking with EQ

With practicability in mind it was further tested whether AA can be more conveniently added as an aqueous solution. For this experiment a nearly saturated aqueous solution of AA was prepared (containing approx. 0.3 g AA per mL). 1 mL thereof (this corresponds to 300 mg AA) was added to 10 g analytical portions of **pear** before spiking with EQ. As shown in Figure 3, the protection of EQ following the addition of "liquid AA" was also sufficient.

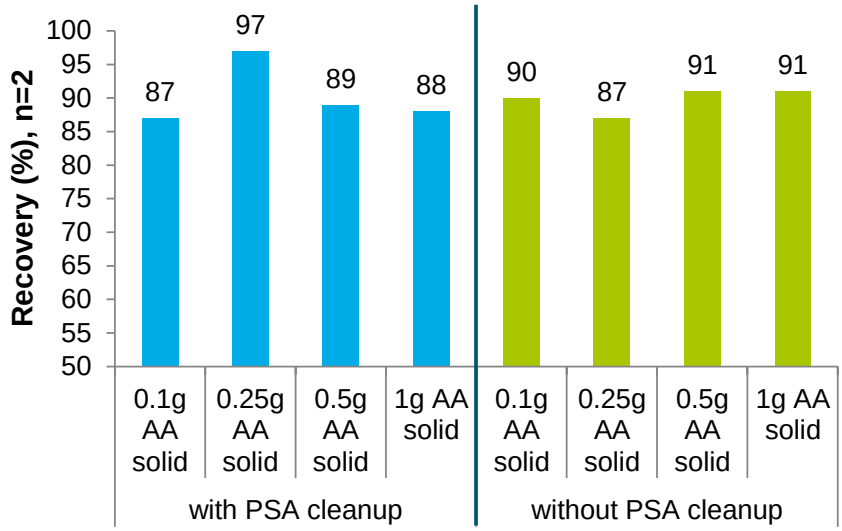

FIGURE 3. EQ recoveries from pear homogenate to which AA was previously added in solid and liquid form, as well as recoveries without AA-addition

EQ Protection during Milling and Extraction

In another experiment the degradation of EQ during milling was studied and checked whether it could be reduced by adding AA prior to milling. For this a large number of whole fresh pears with a solution containing 0.5 mg EQ per mL were superficially spiked and left standing in the dark for 5 days. The spiked fruits were divided into two groups. The first one was intended for cryogenic milling (CRYO-group) and the second one for milling in its fresh condition (RT-group). Before milling, the fruits of both groups were cut into 8 segments each and the segments were randomly divided into two subgroups. The material of the CRYO-group was first put in the freezer overnight and then milled in its frozen condition with the assistance of dry ice. The material of the RT-group was milled directly. One of the two sub-groups of the CRYO- and the RT-Group was milled following the addition of 1 g AA (solid) per 100 g pears and the other one without. From each of the homogenates of the four sub-groups six analytical portions were weighed and put in the freezer until extraction. In each case 3 of the portions were extracted normally using QuEChERS and the other 3 following the addition of 1 mL AA-solution (containing 0.3 g AA per mL). A total of 24 samples were extracted. Measurements were made both directly from the raw extracts as well as from the extracts following d-SPE cleanup with PSA. This resulted in a total of 48 determinations. An appropriate matrix-matched calibration was prepared in each case. The following table gives an overview of the experimental setup.

TABLE 3. Overview of the experimental design for analysis of EQ in laboratory-spiked intact pears, with and without addition of AA

Code	Milling conditions	AA-Addition prior to milling	AA-Addition prior to extraction	dSPE with PSA
CRYO	Frozen samples were milled, dry ice was added and milling was continued (Cryo-milled)	YES	YES	YES
				NO
			NO	YES
				NO
		NO	YES	YES
				NO
			NO	YES
				NO
RT	Room Temperature (RT-milled)	YES	YES	YES
				NO
			NO	YES
				NO
		NO	YES	YES
				NO
			NO	YES
				NO

A summary of the results of this experiment, focusing on the measurements without d-SPE cleanup, is shown in Figure 4. The addition of AA during milling gave the highest degree of protection to EQ. Where milling was done without AA-addition, EQ losses were massive

during both RT- and CRYO-milling. The addition of supplementary AA to the analytical portions just prior to extraction increased the determined EQ levels in these samples. Milling in the absence of AA contributed to roughly 60 % of the overall EQ losses irrespective if the milling was done in cryogenic form or not.

When AA was added during milling, losses of EQ were reduced also during the subsequent extraction step. This protective effect was much stronger in the case of cryogenic milling whereas in RT-milling, considerable losses occurred during the subsequent extraction step. These losses could be minimized by the addition of supplementary AA to the analytical portion prior to extraction. In fact RT-milled samples showed a clearly darker color compared to the CRYO-milled ones indicating that AA had been already consumed to the most part and considerable enzymatic (polyphenol oxidase-catalyzed) browning was underway. Overall, the results showed that the supplementary addition of AA to the test portions was beneficial but could be possibly omitted when comminution was done cryogenically with the addition of AA.

The conduction of d-SPE cleanup with PSA had, in most cases, no clear effect, but it had a clearly positive impact on EQ yields in those cases where no AA was added during extraction (results not shown).

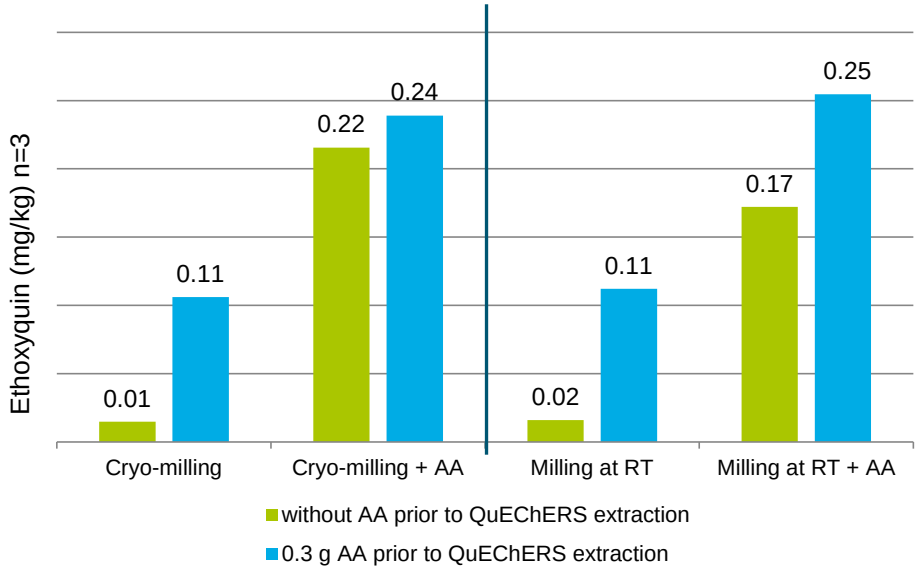

FIGURE 4. EQ levels in pears spiked in the laboratory with EQ prior to comminution with and without AA-addition prior to milling and with and without AA-addition prior to extraction (n = 3 each, measurements directly from the raw extract)

The experiments were repeated using pear samples obtained from the US market with incurred EQ residues but focusing only on cryogenic milling. As expected, the addition of AA prior to milling resulted in a dramatic increase in the determined EQ levels (see Figure 5). When no AA was used during milling the EQ losses ranged between ~ 15 % and ~ 80 %, depending on the sample.

The addition of supplementary AA during extraction proved superfluous in the case of pear 2, of limited value in the case of pear 3 (< 15 % losses when no supplementary AA was added) but of high value in the case of pear 1 (> 25 % losses when no supplementary AA was added). Increasing the amount of AA added prior to comminution is expected to make the supplementary AA addition superfluous in all cases but this option was not tested In general the supplementary AA-addition during extraction is recommended when adding 1 g AA per 100 g sample during comminution..

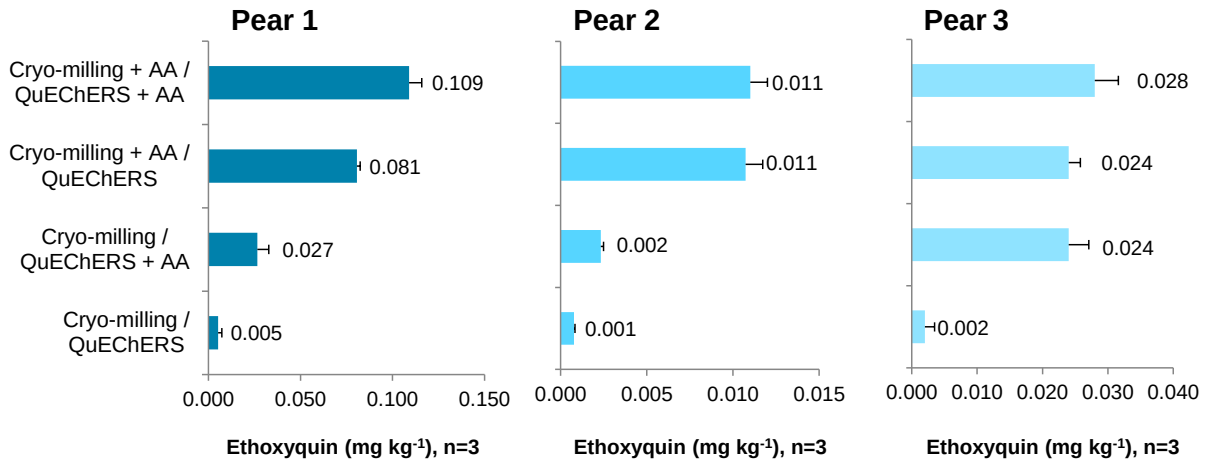

FIGURE 5. Impact of AA on EQ levels determined in pears from the US market with incurred EQ residues

Validation Experiments on Pears

For validation blank pear matrix was cryogenically milled in two ways:

a) following addition of 1 g AA per 100 g sample

b) directly (without AA-addition)

The homogenates were kept frozen until the extraction experiment.

The homogenates containing AA were spiked with EQ and extracted normally (a).

The analytical portions not containing AA were either spiked with EQ and extracted normally (b2), or spiked with EQ followed by the addition of AA (1 mL AA-solution containing 0.15 g AA a per mL water) just prior to extraction (b1). To protect the final extracts of (b2) as well as the matrix-matched calibration solution from further oxidation, 50 µL of AA-mix (containing 0.075 g AA and 0.075 g sodium-ascorbate per mL water) per mL extract was added.

10 g of frozen homogenates were weighed into 50 mL extraction tubes. Spiking with EQ was done at 0.05 mg kg^{-1} (corresponding to the MRL) and at 0.2 mg kg^{-1} by adding 50 µL and 200 µL of a standard solution of c = 10 µg EQ/mL acetonitrile respectively. Extraction was conducted following the addition of 10 mL acetonitrile by mechanically shaking the tubes for

15 min. Phase separation was induced by adding the QuEChERS salts mixture (as described in EN-15662) followed by 1 min shaking and centrifugation.

The results of this recovery study are summarized in Table 4 and Figure 6.

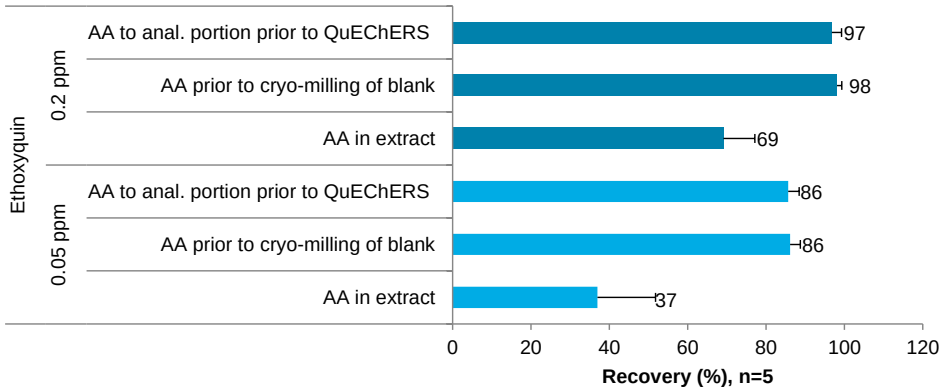

FIGURE 6. Recoveries of EQ in pears (0.05 mg kg^{-1} and 0.2 mg kg^{-1}, n = 5)

The addition of AA prior to cryogenic milling improved the EQ recoveries dramatically, from 37 % to 86 % at 0.05 mg kg^{-1} and from 69 % to 98 % at 0.2 mg kg^{-1}. When AA was added to the samples the variability of the results was general very low (RSD 3 % or lower). When no AA was added RSDs were higher (15 % at the 0.05 mg kg^{-1} level and 8 % at the 0.2 mg kg^{-1} level).

In case of AA being present in the analytical portions used for spiking due to its use during milling of the blank material, the supplementary addition of AA prior to extraction did not further improve the EQ recovery rates.

TABLE 4. Validation Data of EQ from Pears Using Chlorpyrifos-D10 as Internal Standard

EQ Spiking Level [mg kg^{-1}]	Extraction	A	B	C	D	E	Mean	RSD
0.05	**AA in extract**	28	38	39	37	43	**37**	**15**
	AA prior to cryo-milling of blank	83	85	90	86	86	**86**	**3**
	AA-Mix to anal. portion prior to QuEChERS	82	86	88	84	88	**86**	**3**
0.2	**AA in extract**	73	72	71	60	69	**69**	**8**
	AA prior to cryo-milling of blank	99	96	99	98	98	**98**	**1**
	AA-Mix to anal. portion prior to QuEChERS	96	99	98	93	98	**97**	**3**

Analysis of Pear Samples from the German Market

From July 2014 to the end of 2016 a total of 104 pear samples from the local market were analyzed for EQ residues. In all cases AA (1 g AA per 100 g pear) were added to the samples during the comminution step (cryo-milling).

An overview of the results from 2014 concerning 26 samples is shown in Figure 7. Three of the 26 samples (all from Italy) were found to contain EQ residues at levels between 0.25 and 0.58 mg kg^{-1}. In all three cases the EQ levels exceeded the MRL of 0.05 mg kg^{-1} for pears. These findings also indicate a misuse of EQ; the Commission's decision 2011/143/EU required that EQ be withdrawn from all EU member states by September 2012.

Since the end of 2014 there have been no further findings of EQ residues in pear samples from the market.

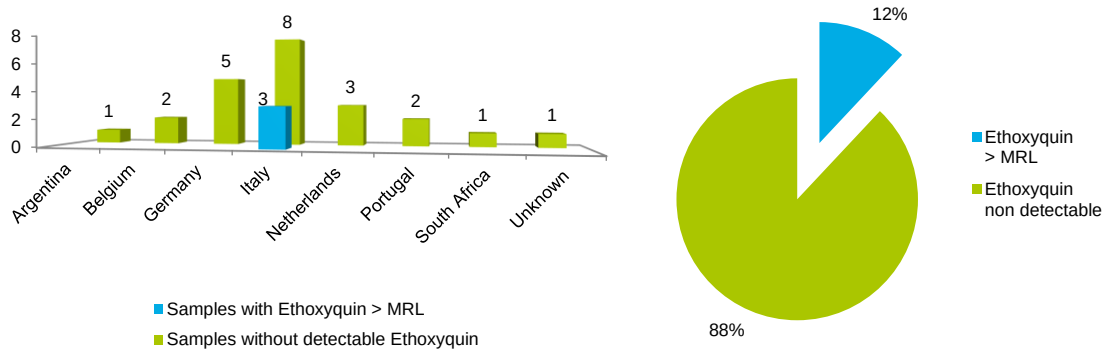

FIGURE 7. Number of samples with and without EQ findings in pears and country of origin of the analyzed pears

All 104 above mentioned pear samples analyzed from 2014 to 2016 were milled in two ways i.e. adding AA and not adding AA prior to milling. Both homogenates were analyzed in parallel for all pesticides within the scope of the CVUA Stuttgart. There have been no signs so far, that other pesticide residues are negatively influenced by the addition of AA.

Conclusions and Outlook

EQ recoveries from pears and apples (and several other crops) using the QuEChERS method are low, due to oxidative losses. The addition of ascorbic acid (AA) to analytical portions prior to extraction proved very helpful in the prevention of oxidative losses and the increase of EQ recoveries. If the commodity to be analyzed does not exhibit antioxidative protection, the addition of AA should occur as early in the procedure as possible (optimally already prior to the homogenization step) to minimize EQ degradation. Using pear samples with incurred EQ residues, as well as pears that were superficially spiked in the laboratory, it could be shown that degradation occurs both during cryogenic and ambient temperature

milling. When conducting cryogenic milling the addition of AA prior to homogenization was in most cases enough to sustain EQ throughout the procedure. When milling pears at ambient temperature, however, a supplementary addition of AA was necessary prior to QuEChERS extraction to minimize degradation. When dealing with pears containing incurred residues, it is recommended adding AA to coarsely cut pieces prior to freezing and cryogenic milling.

References

[1] European Commission, „2011/143/EU Commission Decision of 3 March 2011 concerning the non-inclusion of ethoxyquin in Annex I to Council Directive 91/414/EEC“.

[2] European Commission, „Regulation (EC) No. 1107/2009 of the European Parliament and of the Council“, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC, of 21 October 2009.

[3] European Food Safety Authority, „Reasoned opinion on the review of existing maximum residue levels (MRLs) for ethoxyquin according to Article 12 of Regulation (EC) No. 396/2005. EFSA Journal 2013; 11(5):3231, 25pp.,“ 2013.

[4] A. Hobson-Frohock, „Residues of Ethoxyquin in Poultry Tissues and Eggs“, *Journal of the Science of Food and Agriculture,* Vol. 33, No. 12, pp. 1269–1274, 1982.

[5] P. H. a. R. Ackman, „Residues of Ethoxyquin and Ethoxyquin Dimer in Ocean-Farmed Salmonids Determined by High-Pressure Liquid Chromatography“, *JFS: Food Chemistry and Toxicology,* Vol. 65, No. 8, pp. 1312–1314, 2000.

[6] J. Desmarchelier und Y. Ren, „Analysis of fumigant residues-a critical review,“ *JOURNAL-AOAC INTERNATIONAL,* pp. 1261–1280, 1999.

[7] J. Daft, „Preparation and use of mixed fumigant standards for multiresidue level determination by gas chromatography,“ *Journal of Agricultural and Food Chemistry,* pp. 563–566, 1985.

[8] EURL-SRM, „Datapool of the EU Reference Laboratories for Residues of Pesticides, www.eurl-pesticides-datapool.eu“, 1 Sep 2014. [Online].

[9] D. Heikes, „Purge and trap method for determination of ethylene dibromide in whole grains, milled grain products, intermediate grain-based foods, and animal feeds,“ *J Assoc Off Anal Chem,* Vol. 68, No. 6, pp. 1108–11, Nov–Dec 1985.

[10] EURL-SRM, *Analysis of Dithiocarbamate Residues in Foods of Plant Origin involving Cleavage into Carbon Disulfide, Partitioning into Isooctane and Determinative Analysis by GC-ECD,* Vol. 2, 2008.

[11] Y. Iwata, M. Düsch und F. Gunther, „Use of a sulfuric acid cleanup step in the determination of 1,2-dibromoethane residues in lemons, oranges, and grapefruits,“ *J Agric Food Chem,* Vol. 31, No. 4, pp. 171–4, Jan–Feb 1983.

[12] „http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.380“.

[13] European Commission, „European Union Register of Feed Additives, pursuant to Regulation (EC) No. 1831/2003, Annex I“.

[14] European Commission, *Commission Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive Parliament and of the Council,* Editor 2005, 2005.

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