

Extractability of Incurred Residues using QuEChERS



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EURL-SRM

EU Reference Laboratory for Pesticides
requiring **Single Residue Methods**

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3rd Joint EURL-Workshop
Freiburg, 27 - 28 Sep 2011

Use of QuEChERS

EUPT	Share of Labs employing QuEChERS (rough numbers)
EUPT-FV7 (2005)	1 out of 8 Labs
EUPT-FV8 (2006)	1 out of 5 Labs
EUPT-FV9 (2007)	1 out of 4 Labs
EUPT-FV10 (2008)	1 out of 3 Labs
EUPT-FV11 (2009)	1 out of 2 Labs
EUPT-FV12 (2010)	3 out of 5 Labs
EUPT-SRM5 (2010)	Fluazifop: 3 out of 4 labs Fenbutatin Oxide: 4 out of 5 labs Abamectin: 4 out of 5 labs

Private sector: 70-80% of labs employ QuEChERS (Source QS)

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EU Reference Laboratory for Pesticides
requiring **Single Residue Methods**

QuEChERS Validation

>165 000 recovery figures on **SPIKED** pesticides
in ~120 different commodities

(Method Validation Database of EURL-DataPool)

but ...

How well
do we extract
INCURRED RESIDUES?

Incurring Residues

not always easily accessible

Can...

- be enclosed in cells, vacuoles, starch or wax particles
- undergo strong non-covalent interactions with matrix

Various Parameters can influence Extraction-Efficiency:

- ❖ Solvent Type,
- ❖ Extraction Temperature,
- ❖ Extraction Time,
- ❖ Sample Comminution Grade (particle size)



QuEChERS

- MeCN / Water: 1-phase extraction system
- 1 min extraction by shaking

MeCN and MeCN-Water mixtures are quite weak in dissolving lipids and penetrating waxes

Extraction **Time** and **Temperature**

☞ expected to have a strong effect

Question 1:

What are the **minimum agitation times** required to extract incurred residues quantitatively ?



Question 2:

How do longer extraction times impact recoveries of spiked pesticides ?

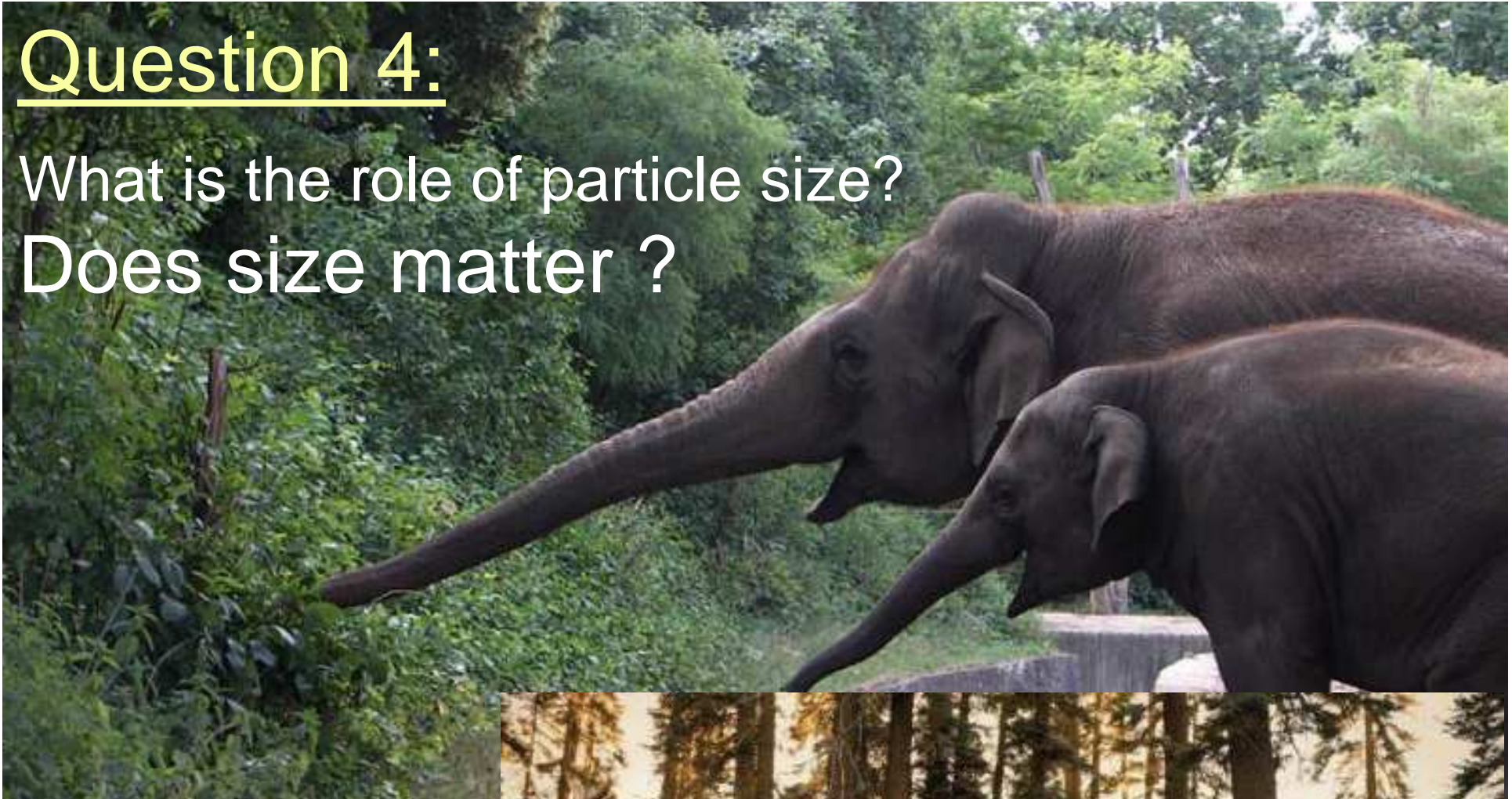
Question 3:

What is the impact of Temperature?



Question 4:

What is the role of particle size?
Does size matter ?



Extraction/ Homogen. equipment employed in this study:



**Geno Grinder
Spex Sample Prep**



Biosan Multi RS-60



Horizontal shaker



Rotation shaker



Heidolph Multi Reax



Agytax®

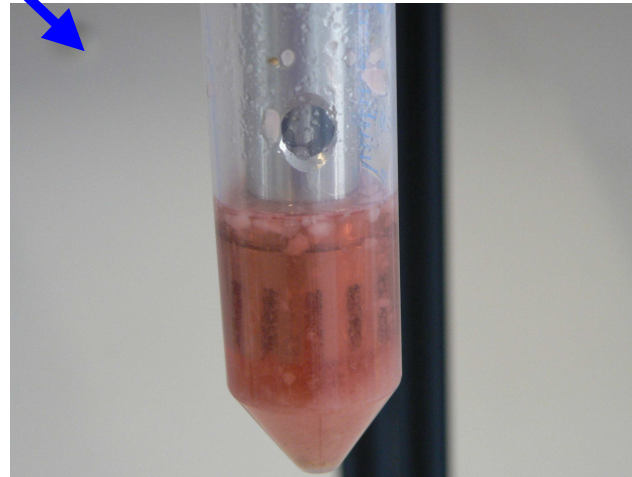
Ultra Turrax



Ultrasonic probe

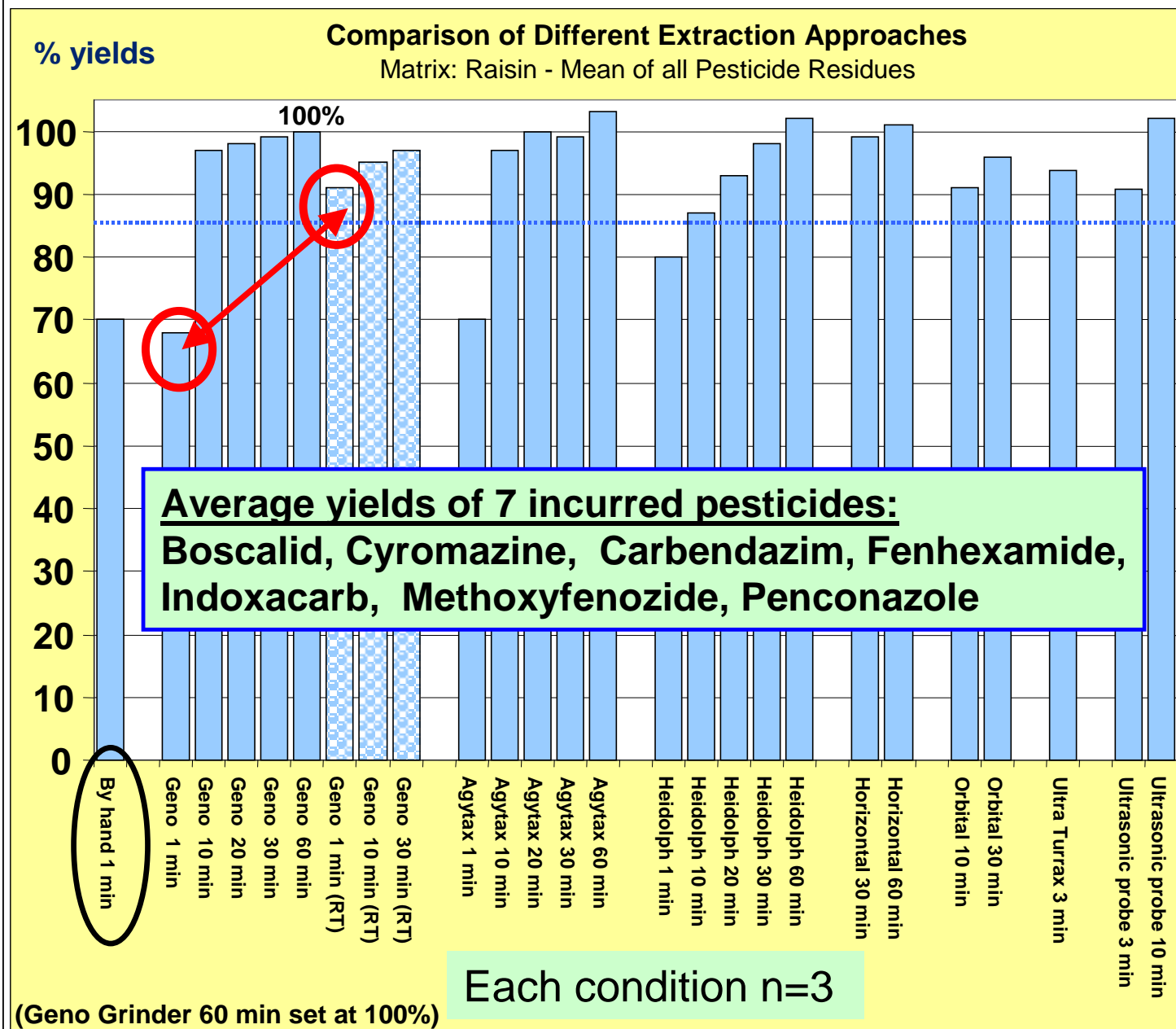


By hand



Comparison of Different Approaches

Sample: RAISINS

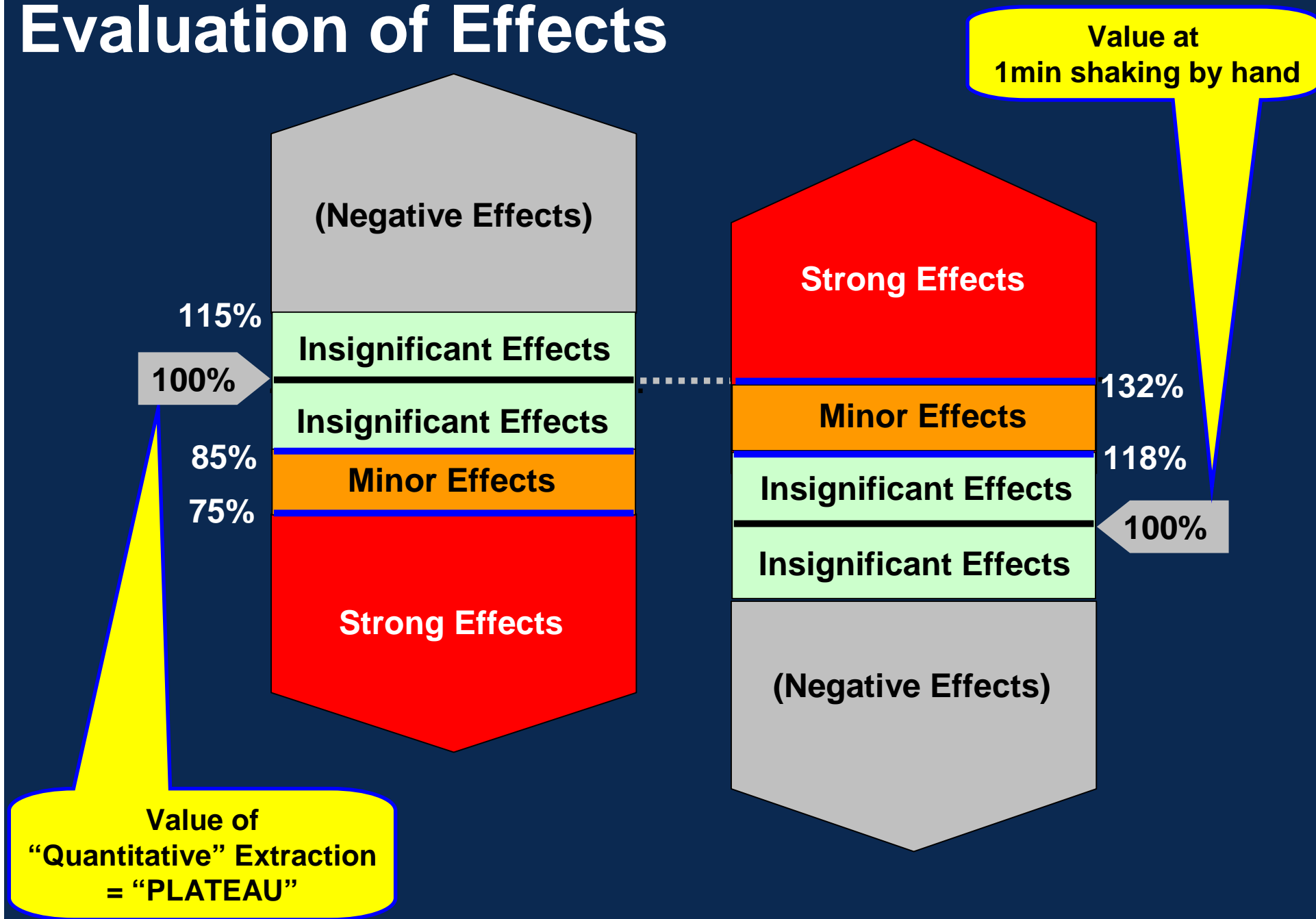


Frozen Material:
10-15 min extraction times seem sufficient for >90% yields

Material at RT:
much shorter times needed

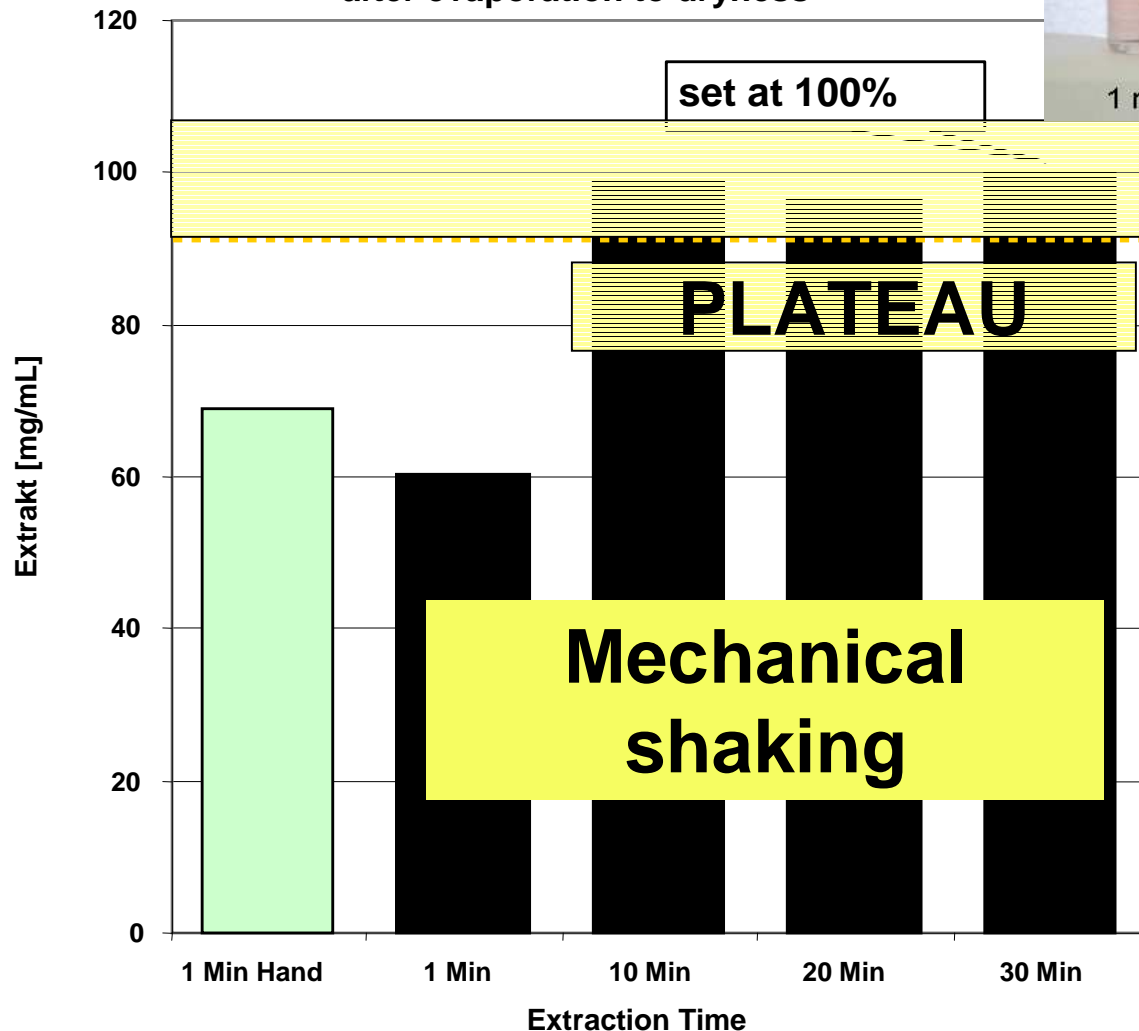
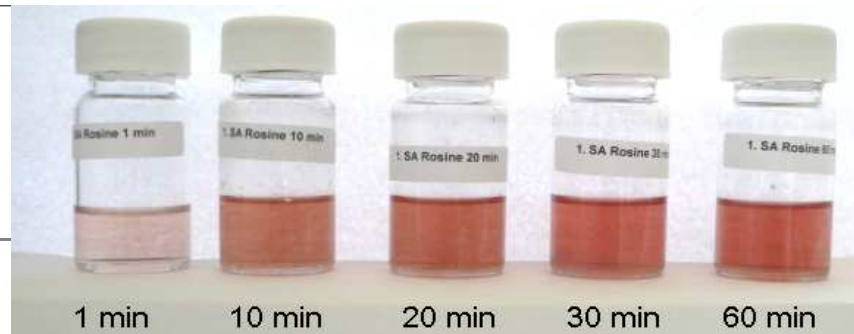
NOTE:
Times refer to 1st extraction step

Evaluation of Effects



Co-extracted Matrix

Residual extract
after evaporation to dryness

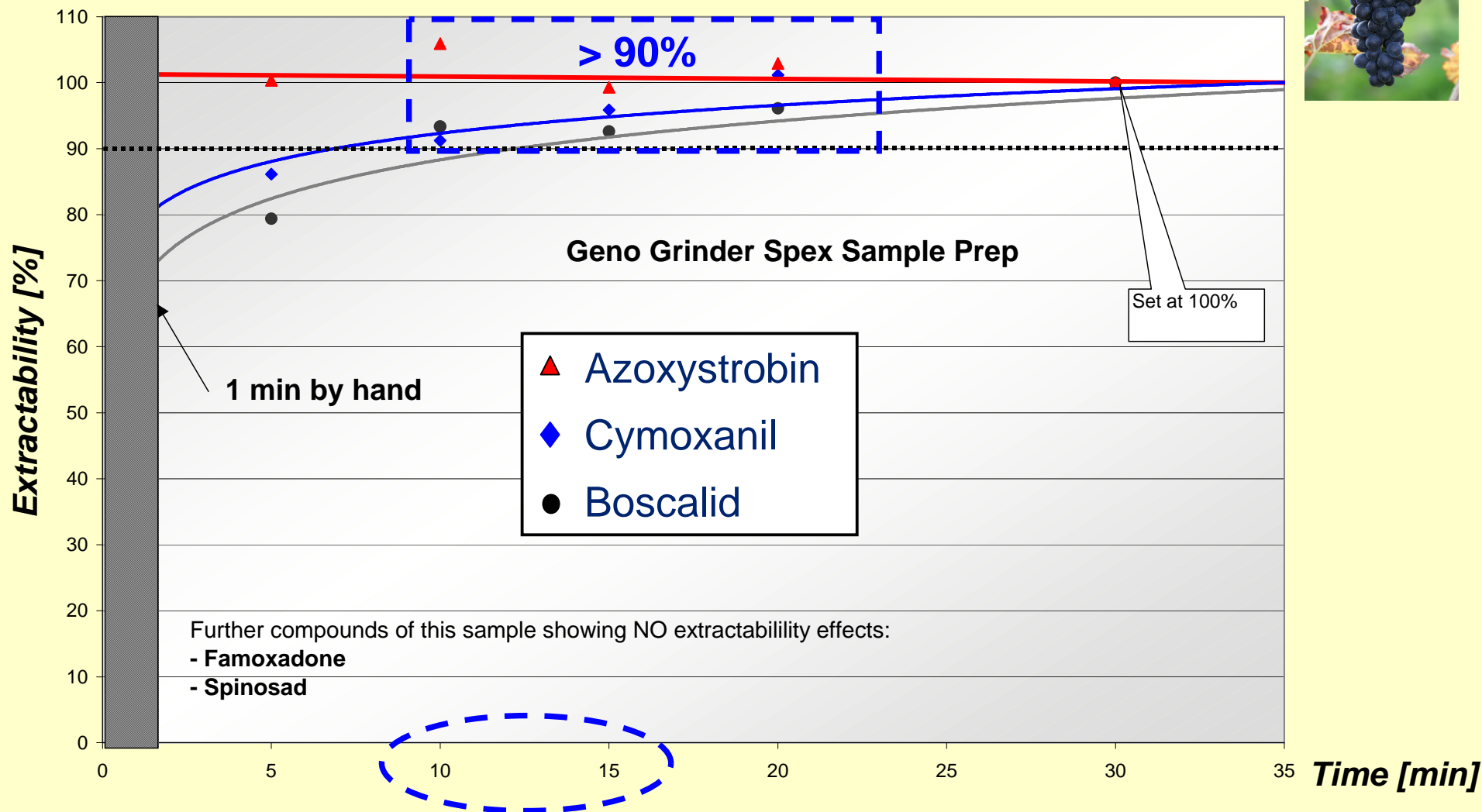


Raisins

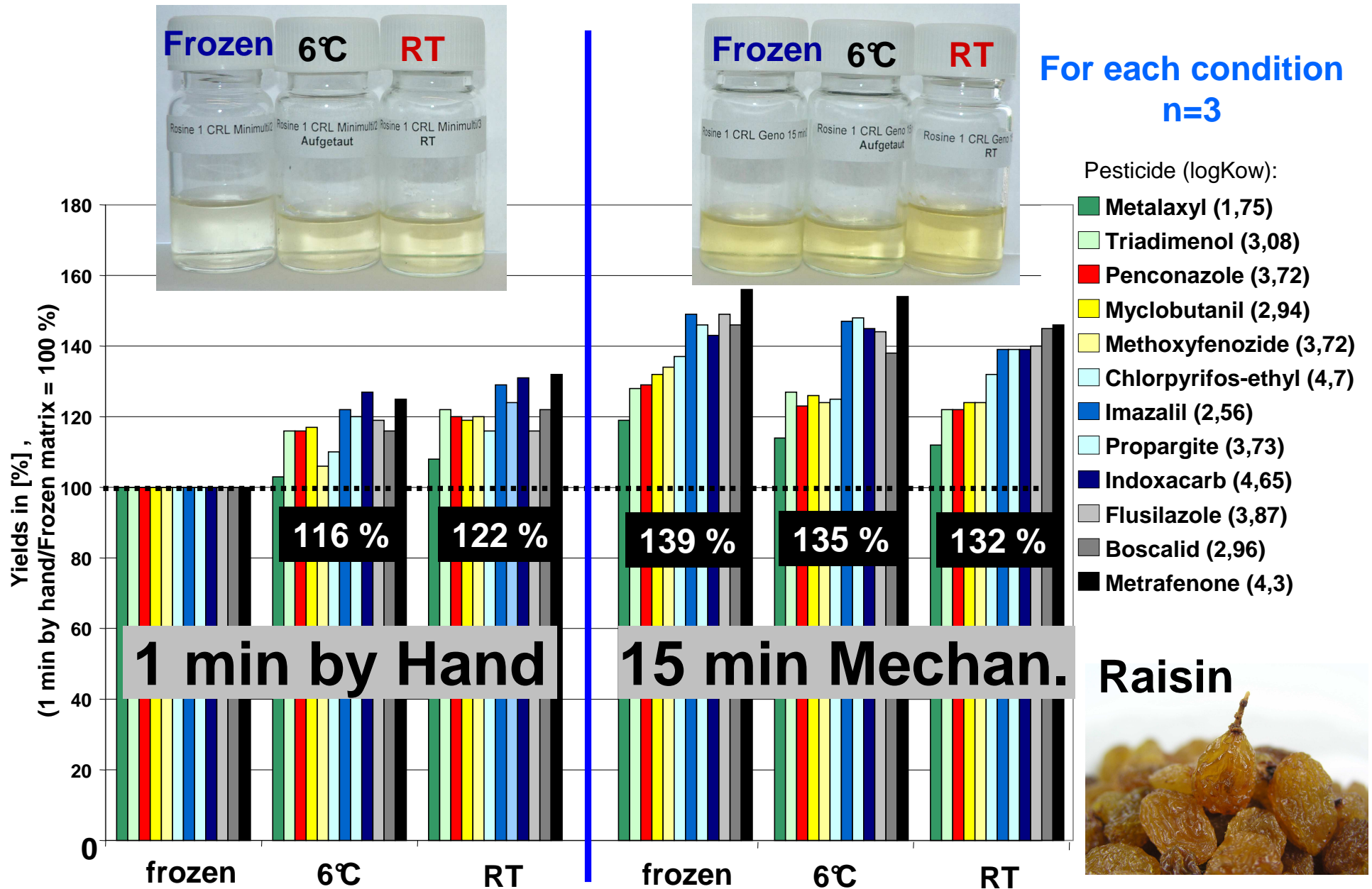


Extracted with Geno Grinder

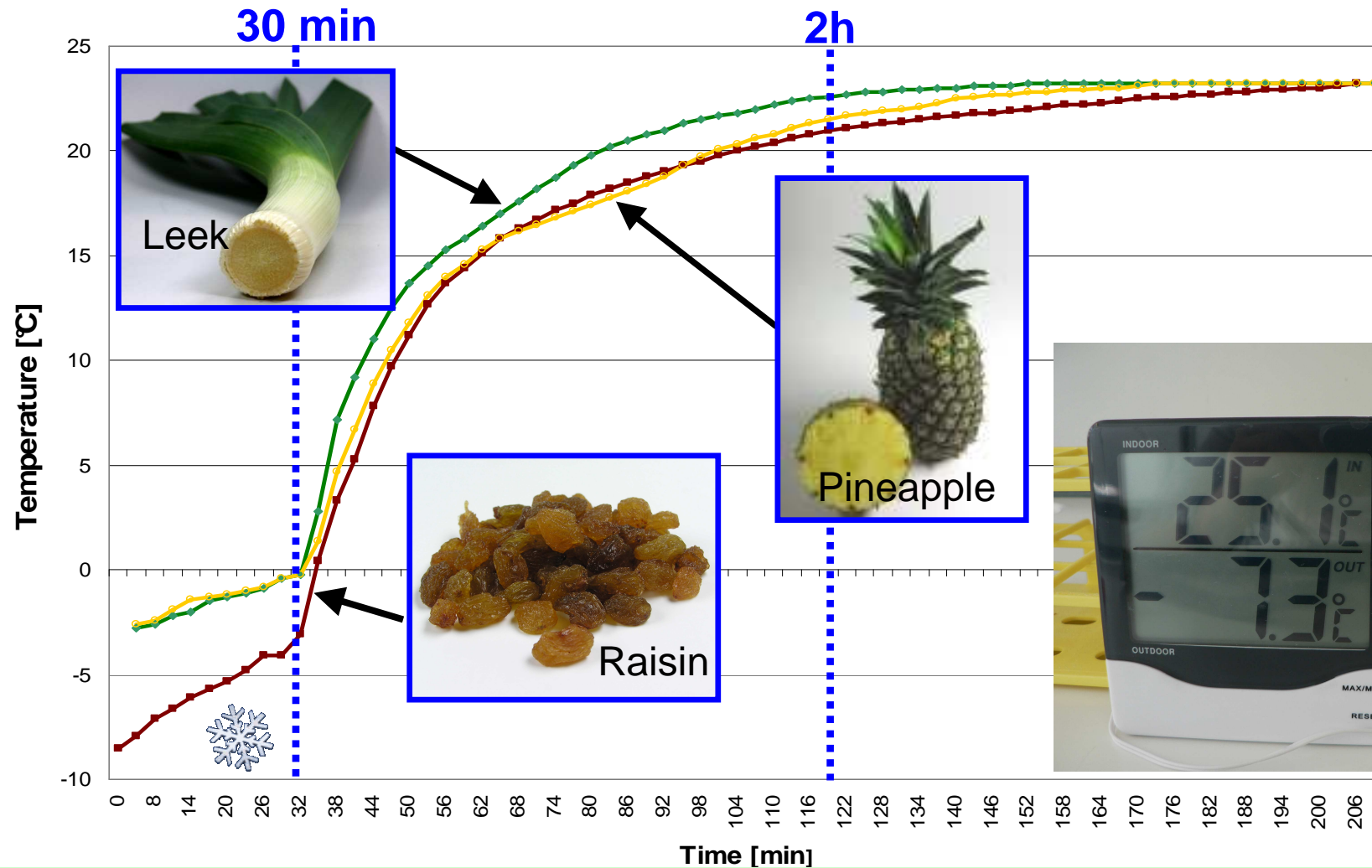
PT-Grape w. incurred residues



QuEChERS-Extractability: Influence of Sample Temperature



Temperature development while thawing

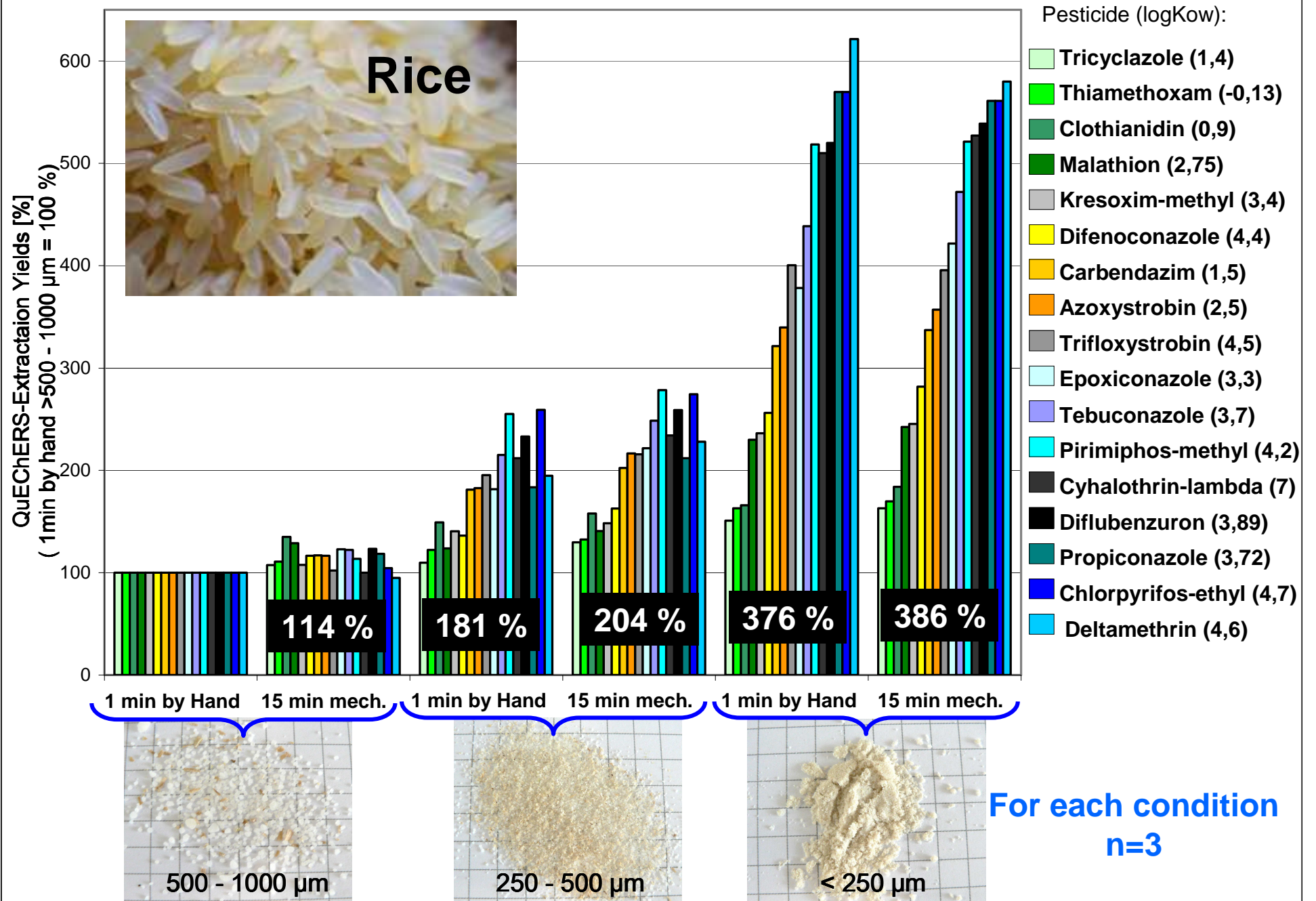


Waiting times:

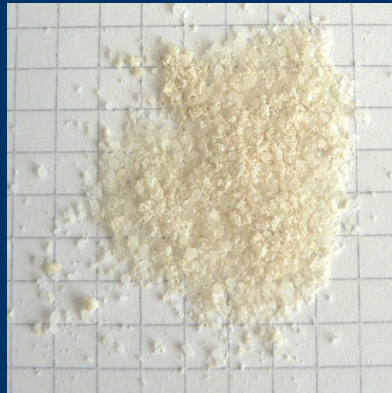
- 30 min for Thawing
- 2h for RT (would be faster in water bath)

→ 15 min extraction of frozen sample overall much faster

QuEChERS-Extractability: Influence of Particle Size



Different fractions – different plant parts?

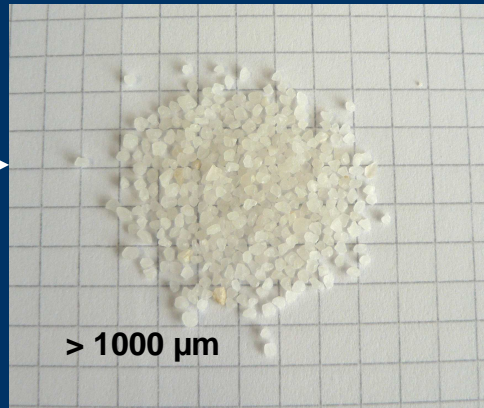


Rice homogenate
(Real sample)



Sieving

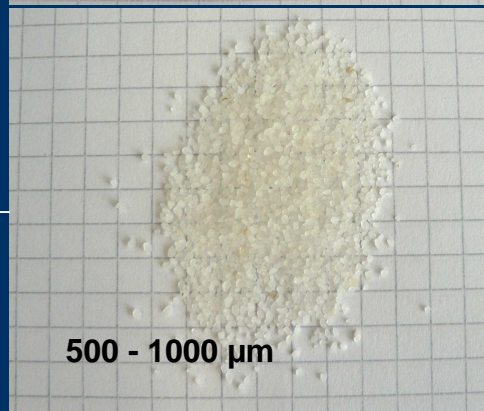
Fractions with different particle sizes



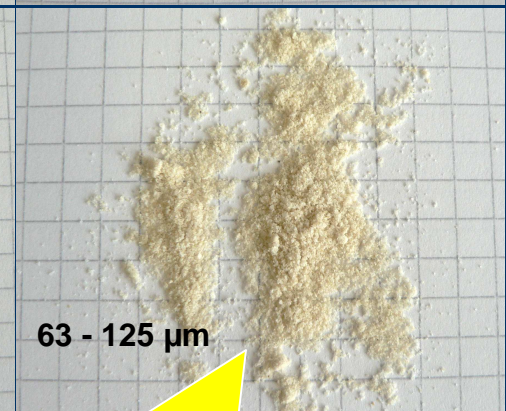
> 1000 µm



125 - 250 µm



500 - 1000 µm

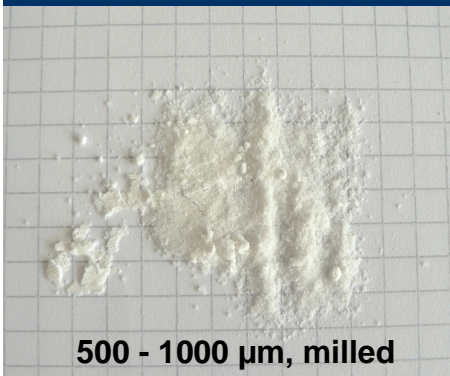


63 - 125 µm

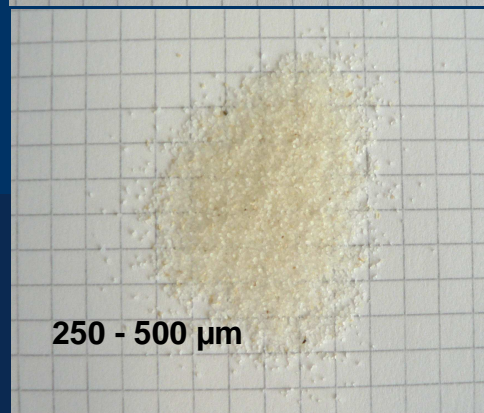
Further Milling



No increased values for incurred Tricyclazole and Tebuconazole



500 - 1000 µm, milled



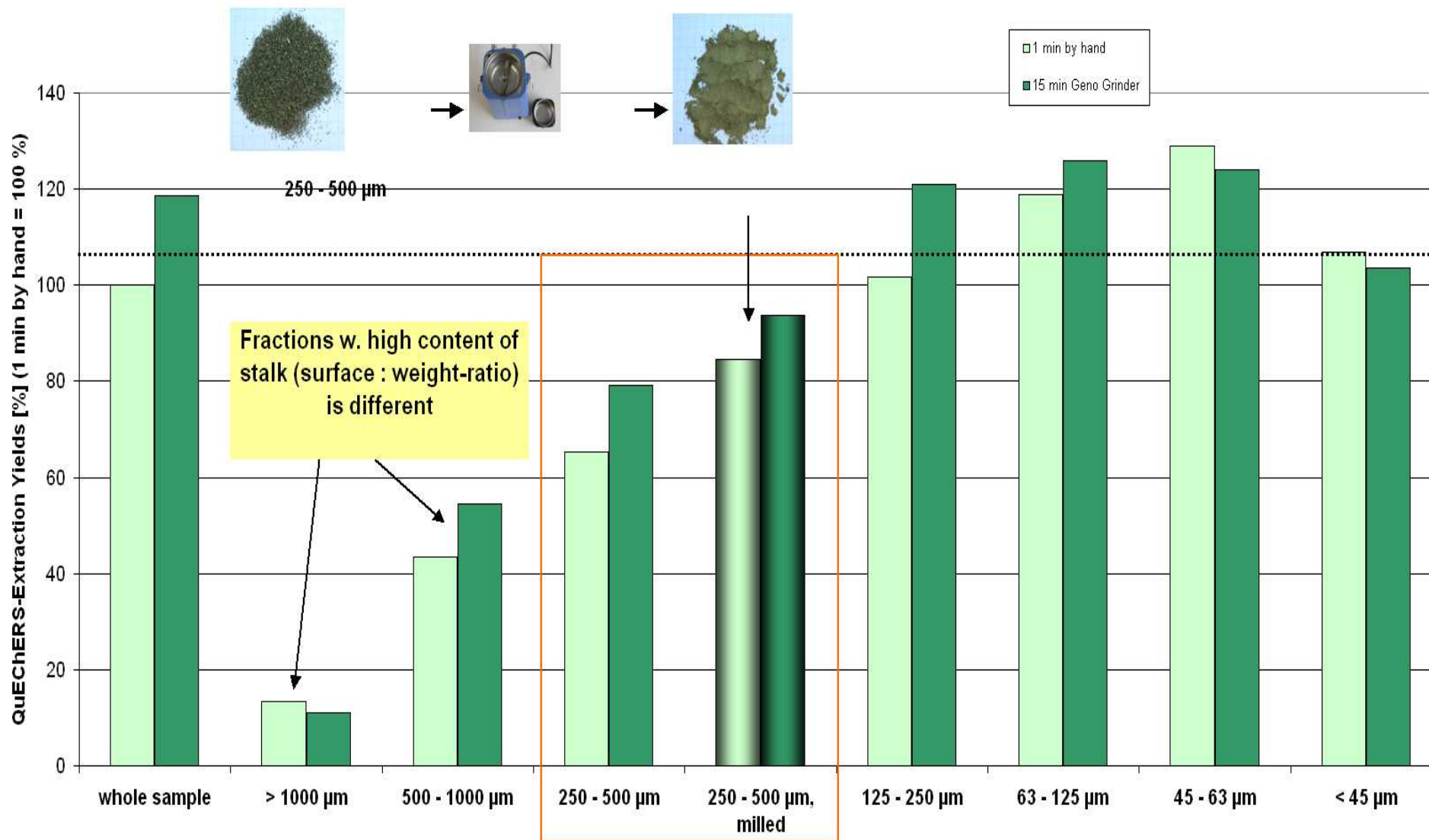
250 - 500 µm

Fractions with small particle size visibly contained more peel particles

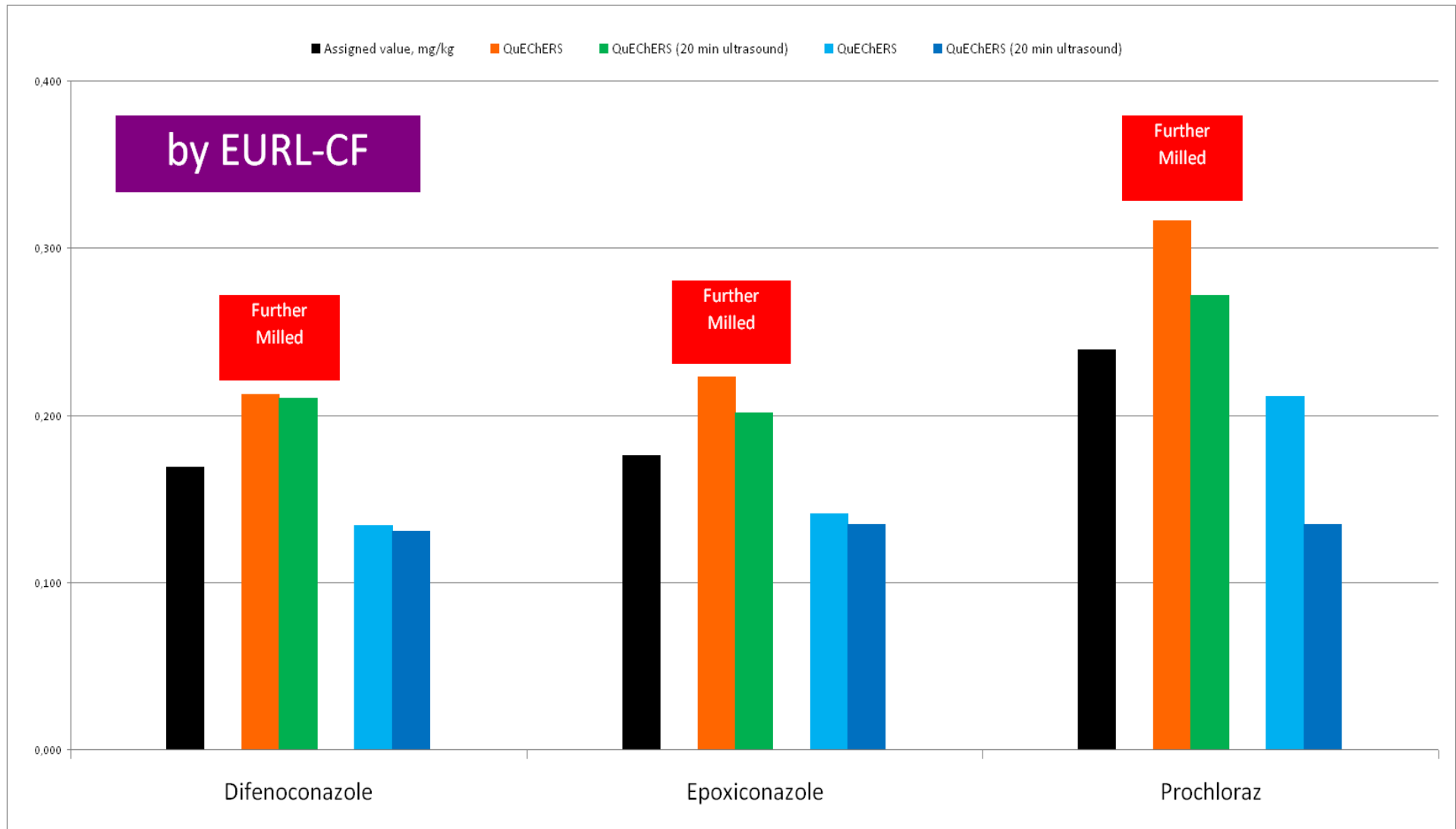


Influence of Particle Size on extraction yields ("QuEChERS")

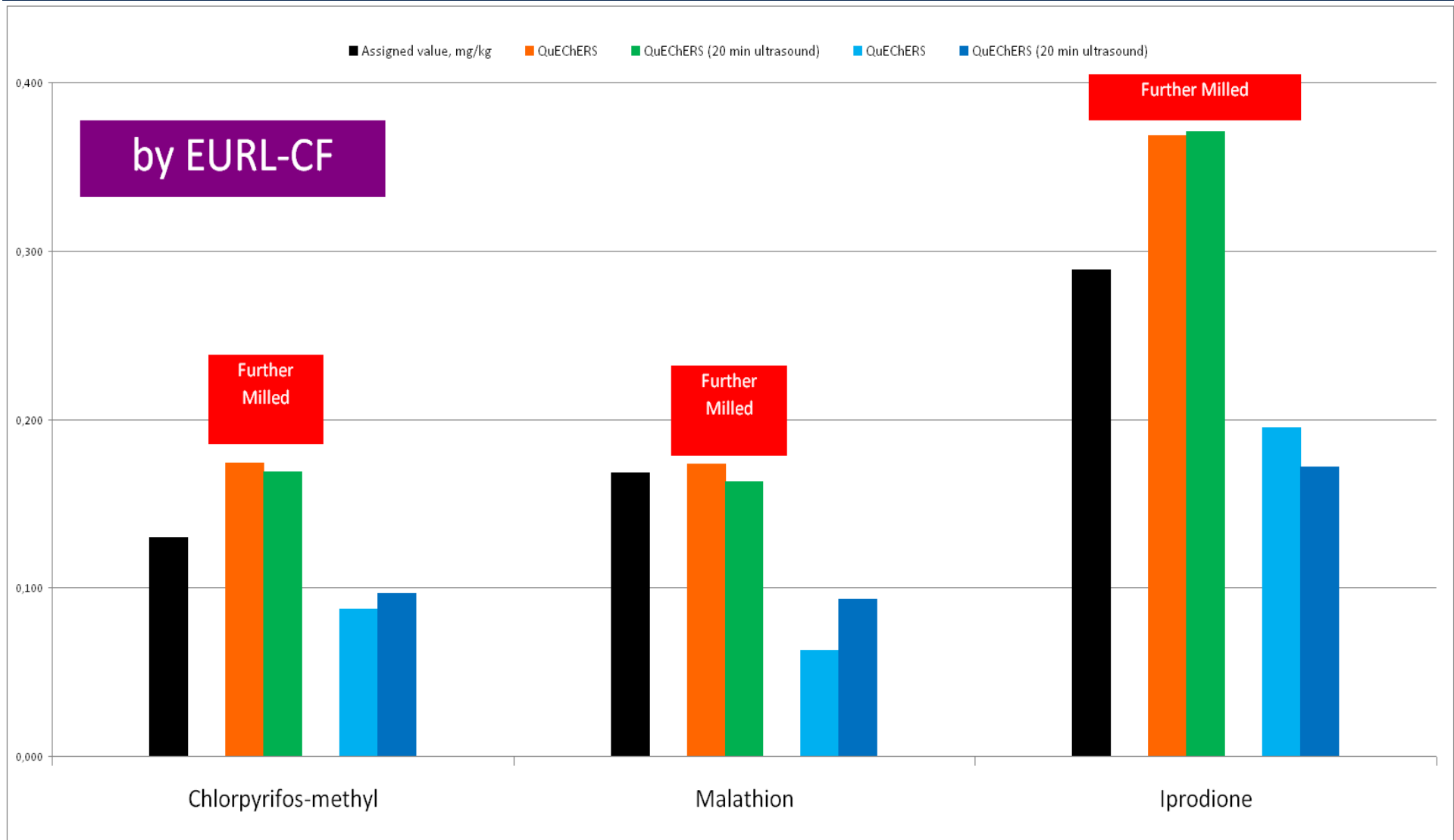
- Fenpropathrin in currant leaves -



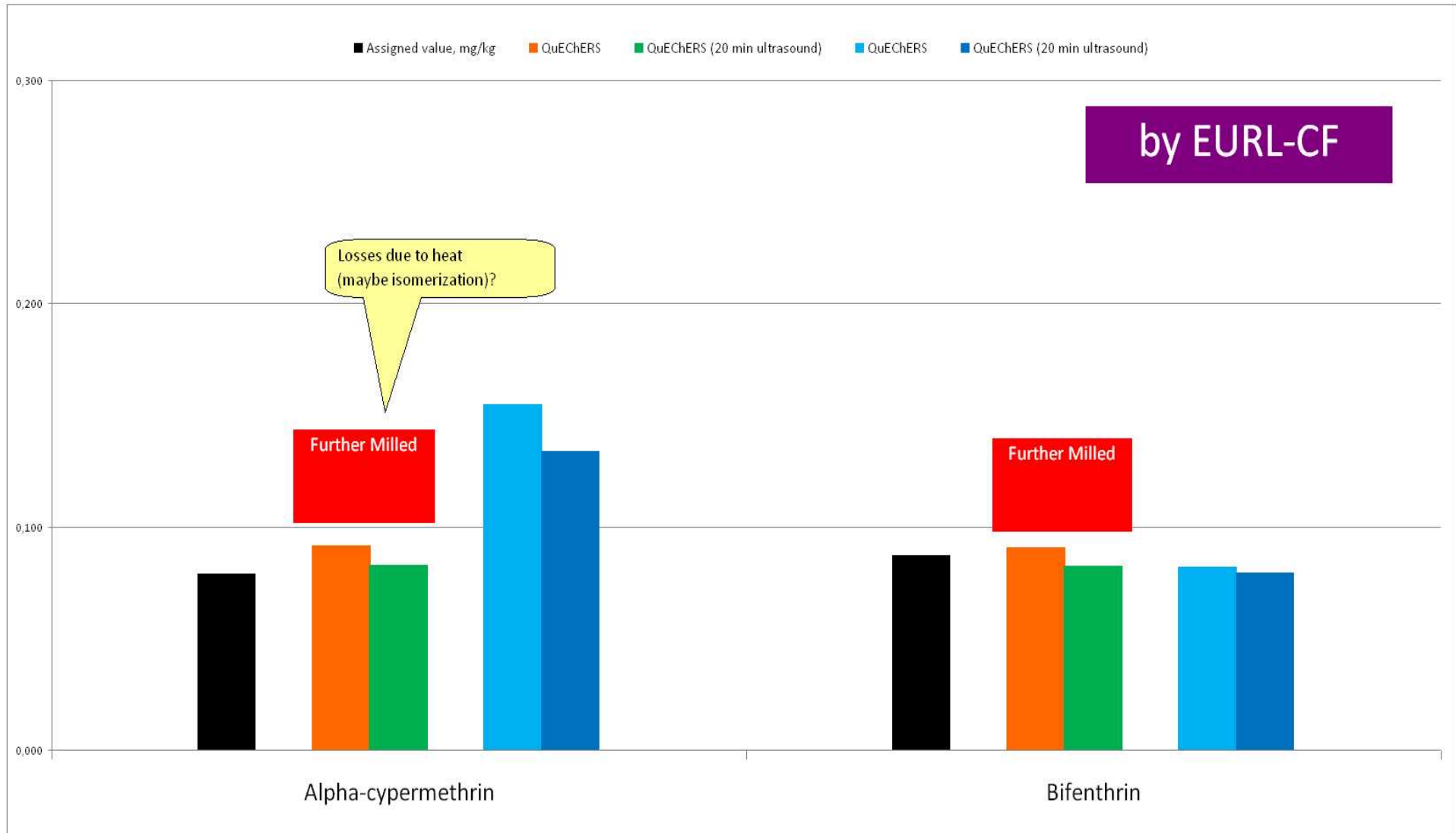
Impact of particle size (EUPT2-Wheat further milled)



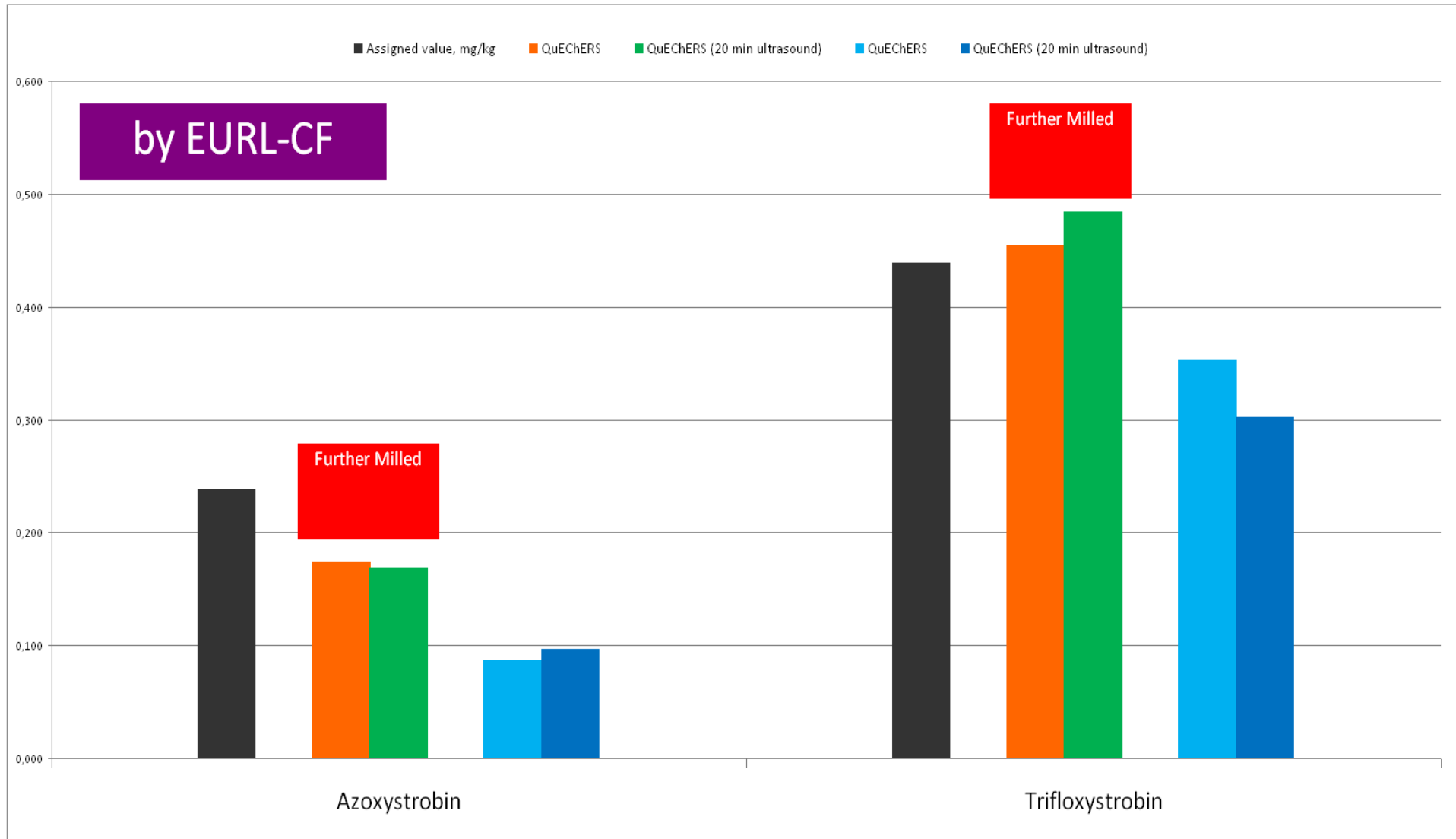
Impact of particle size (EUPT2-Wheat further milled)



Impact of particle size (EUPT2-Wheat further milled)



Impact of further Comminution (PT-sample)



Beeswax with incorporated Pesticides - Preparation

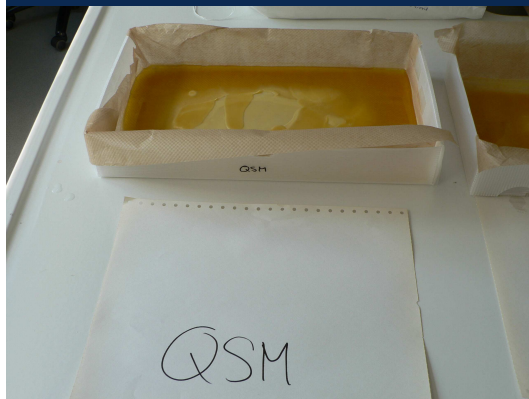
1) Spiking of the beeswax



2) Heating beeswax (+ 80 °C) until melting



3) Cooling down to room temperature



4) Overnight storage at - 80 °C



Beeswax with incorporated Pesticides - Preparation

5) Mincing procedure

4.0 x 1000 rpm

t = 2 min



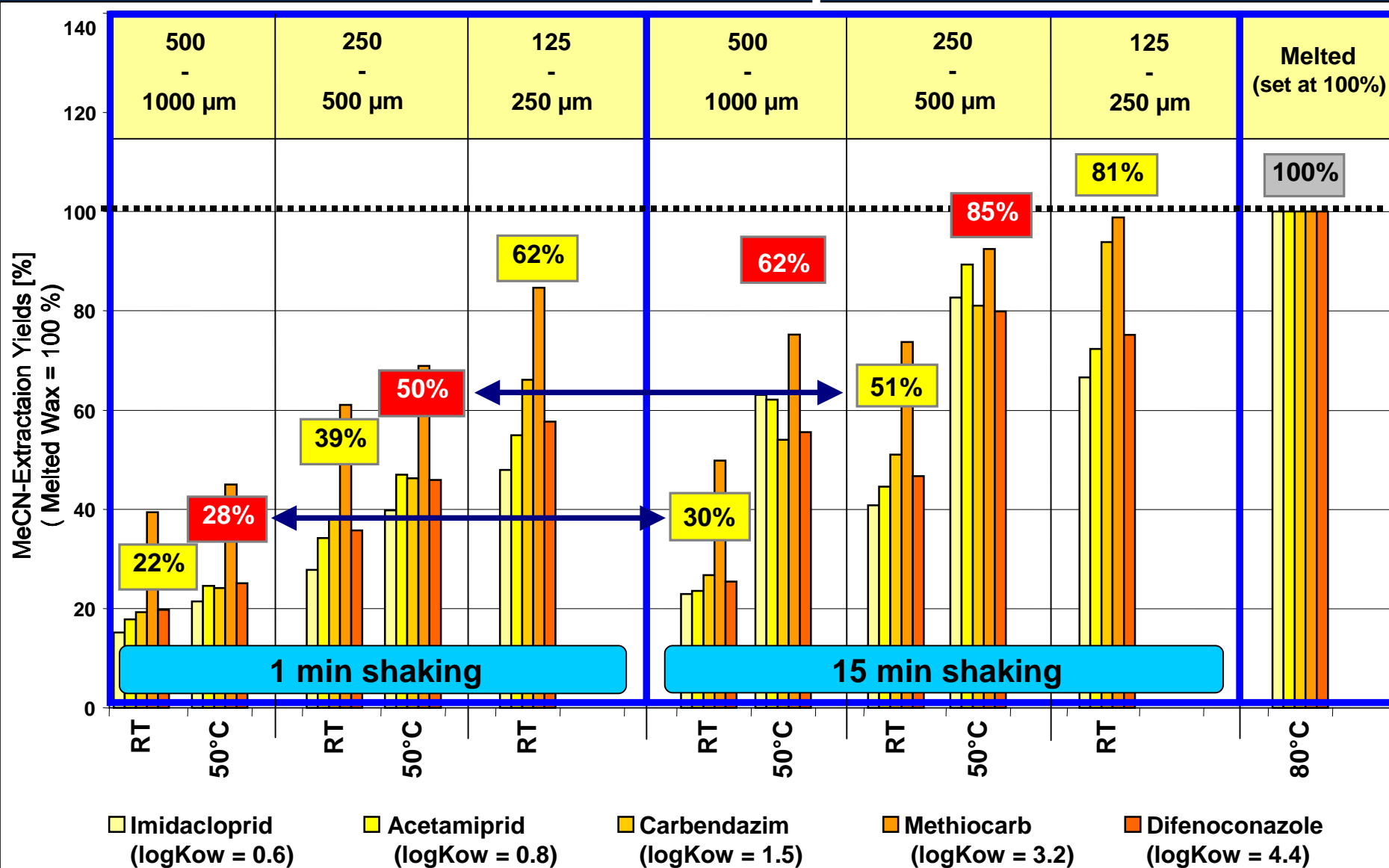
6) Sieving procedure

3 fractions; particle sizes:

- 500 – 1000 μm
- 250 – 500 μm
- 125 – 250 μm



Extraction from Beeswax – Impact of Particle Size



Correlation between Mean Pesticide Recov. and Extract Weight after evaporation (Melted=100%)

RT /1min : 500-1000μm (22% vs. 20%); 250-500μm (39% vs. 36%); 125-250μm (62% vs. 59%)

RT/15min : 500-1000μm (30% vs. 28%); 250-500μm (51% vs. 52%); 125-250μm (81% vs. 80%)

First Conclusions

1) SHAKERS

- Shaking approach (intensity) less important

2) TEMPERATURE AND TIME

- Frozen Samples: ca. 10-15 min shaking time sufficient
- Shaking at RT accelerates extraction (~2 min sufficient)

3) ULTRA-TURRAX AND ULTRASOUND-PROBE

- Accelerate extraction, but...
... impractical for routine work (sequential approach)

4) PARTICLE SIZE

- The smaller the faster the extraction and the higher the yields

STUDY OF REAL SAMPLES (with incurred residues)

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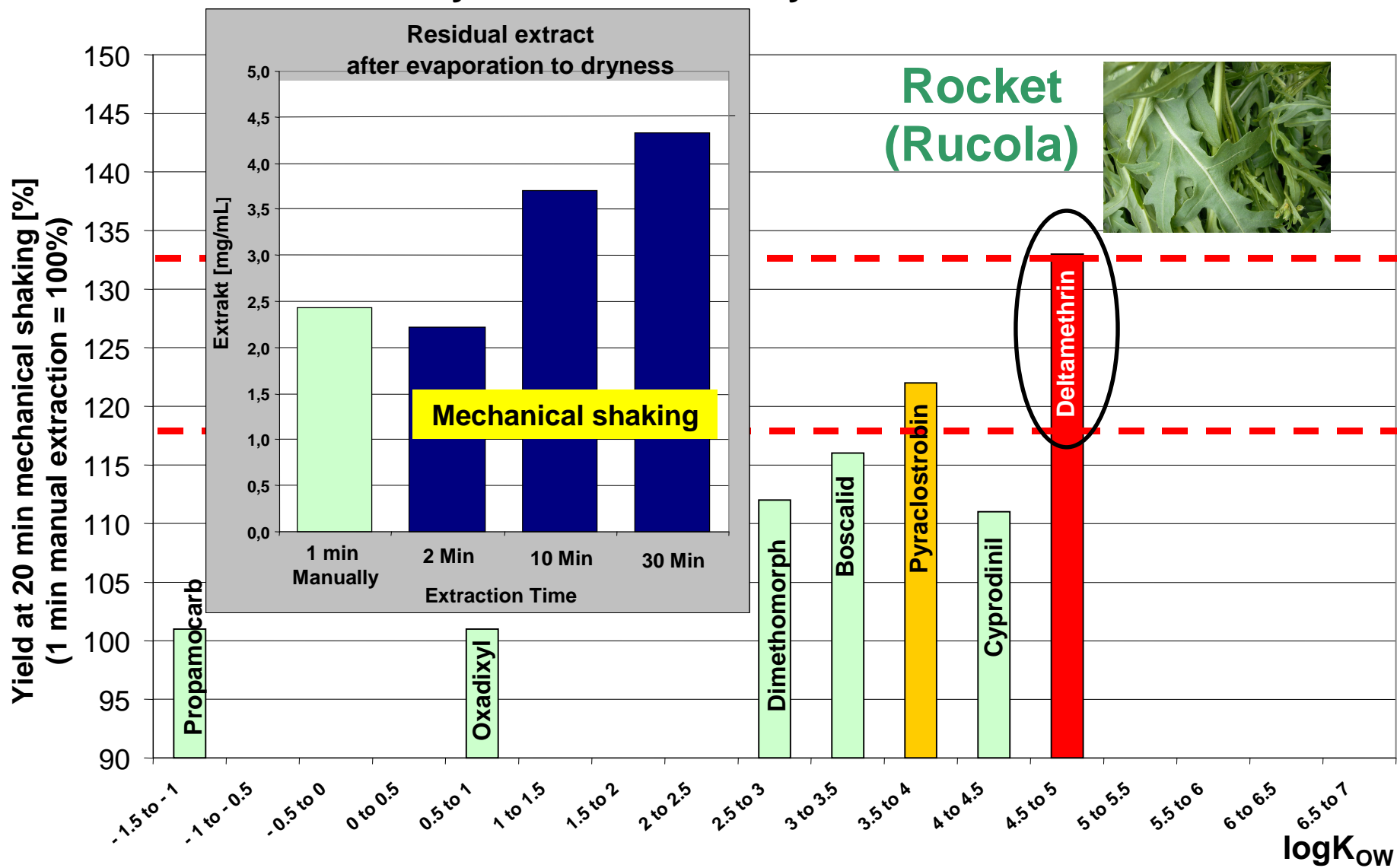
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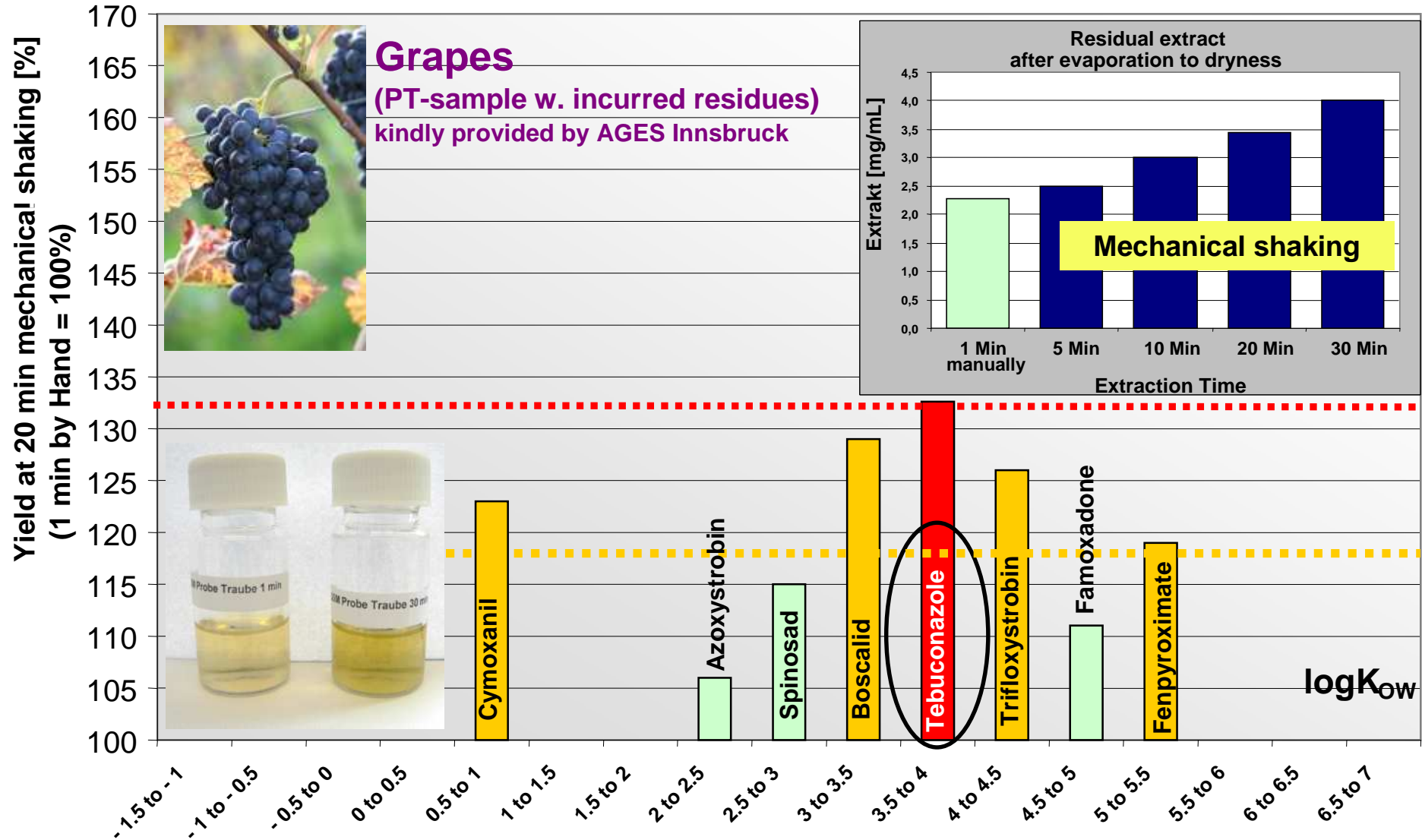
Comparison of extractability using QuEChERS

1 min by hand vs. 20 min by automated shaker



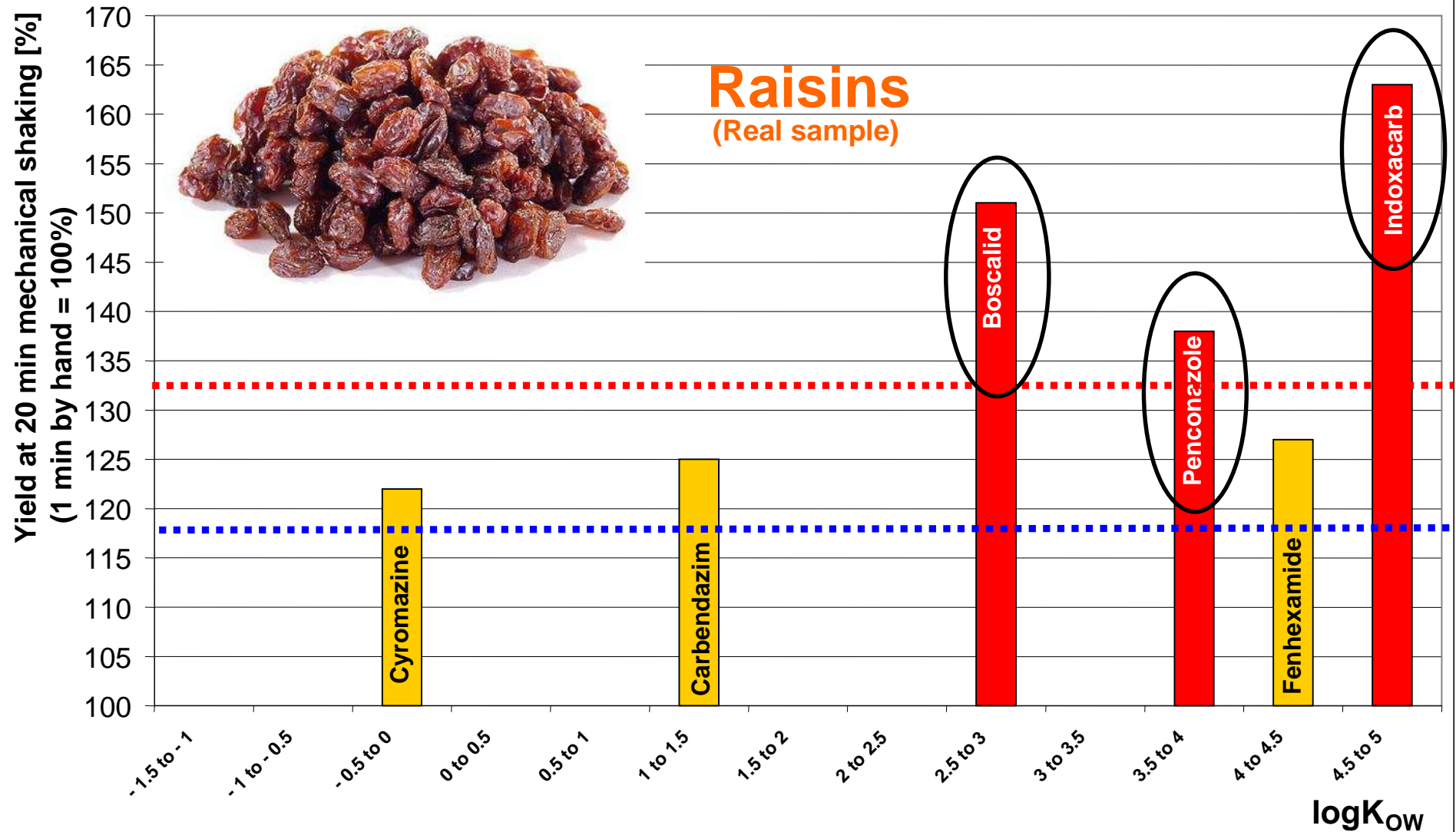
Comparison of extractability using QuEChERS

1 min by hand vs. 20 min by automated shaker (Geno Grinder)



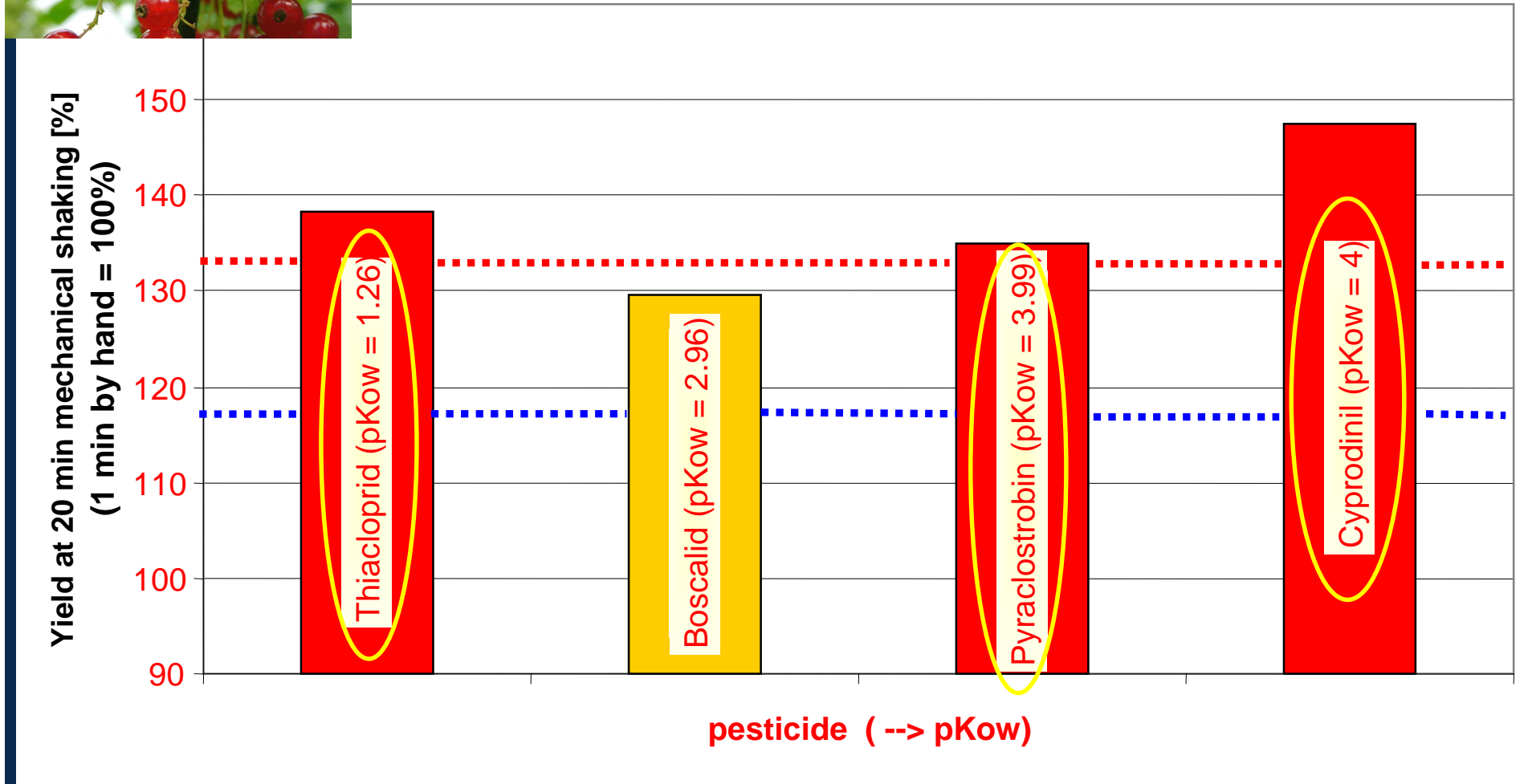
Comparison of extractability using QuEChERS

1 min by hand vs. 20 min by automated shaker

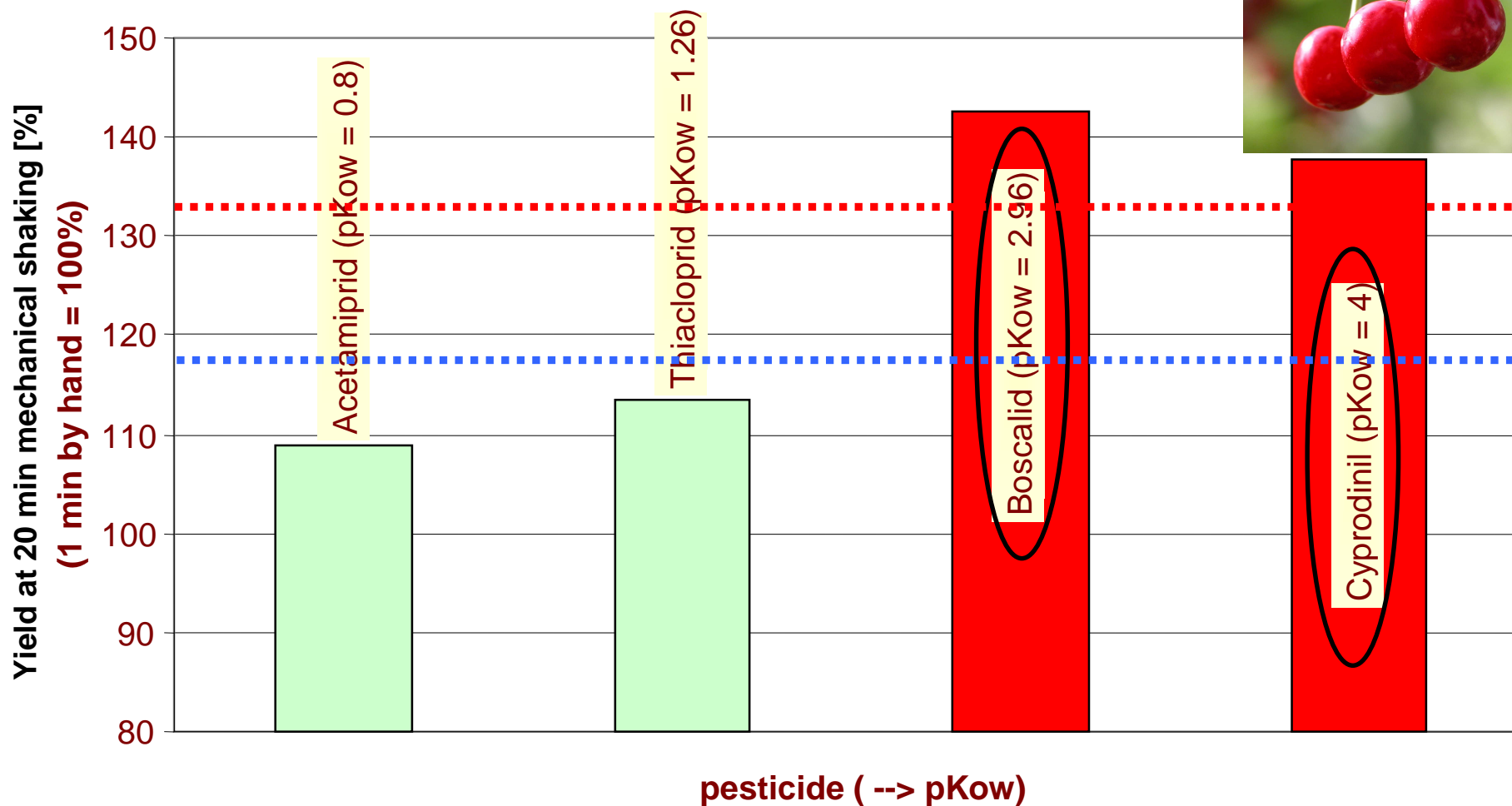




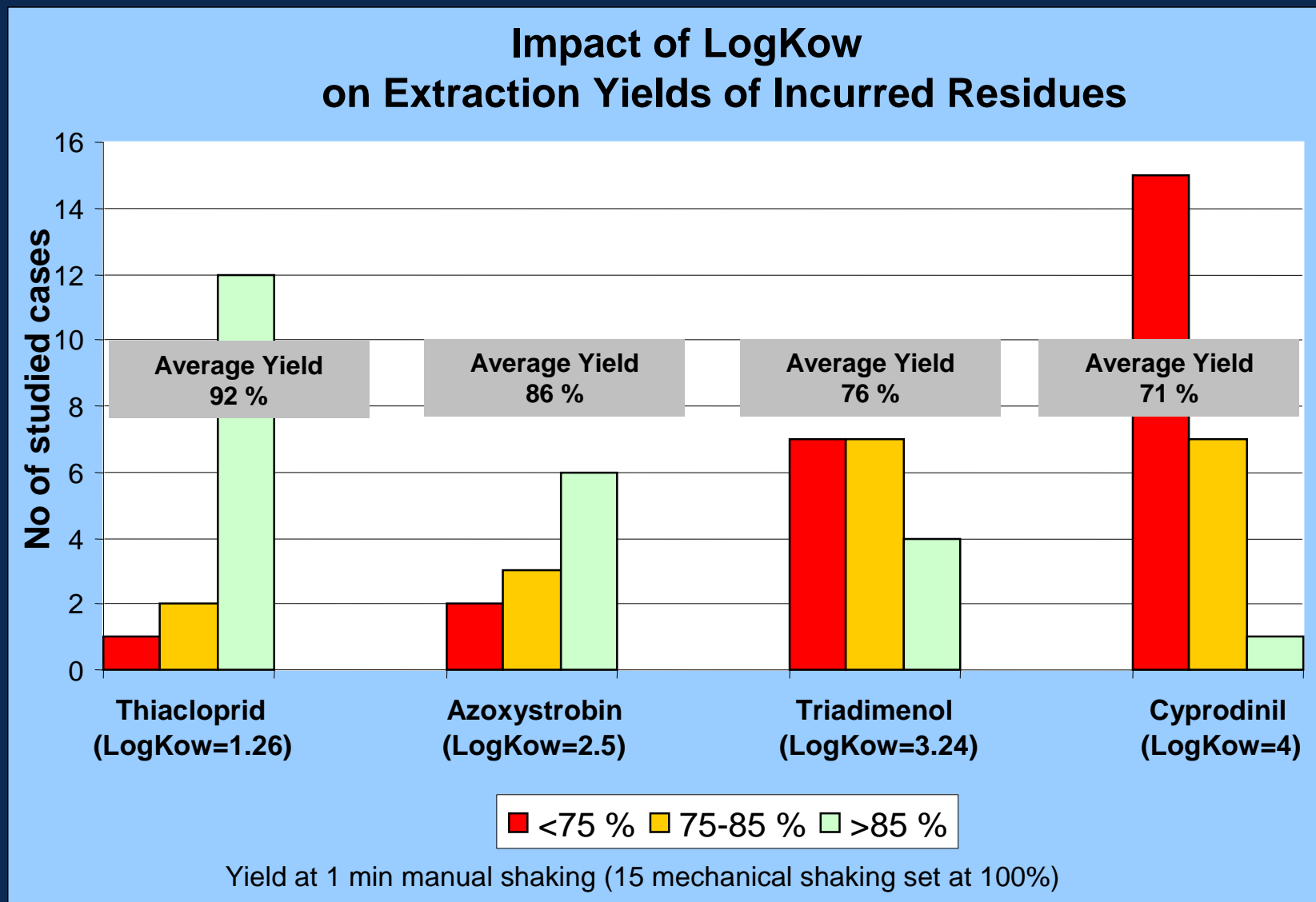
Currant (real sample)
1 min by hand vs. 15 min by automated shaker



Cherry (real sample) 1 min by hand vs. 15 min by automated shaker



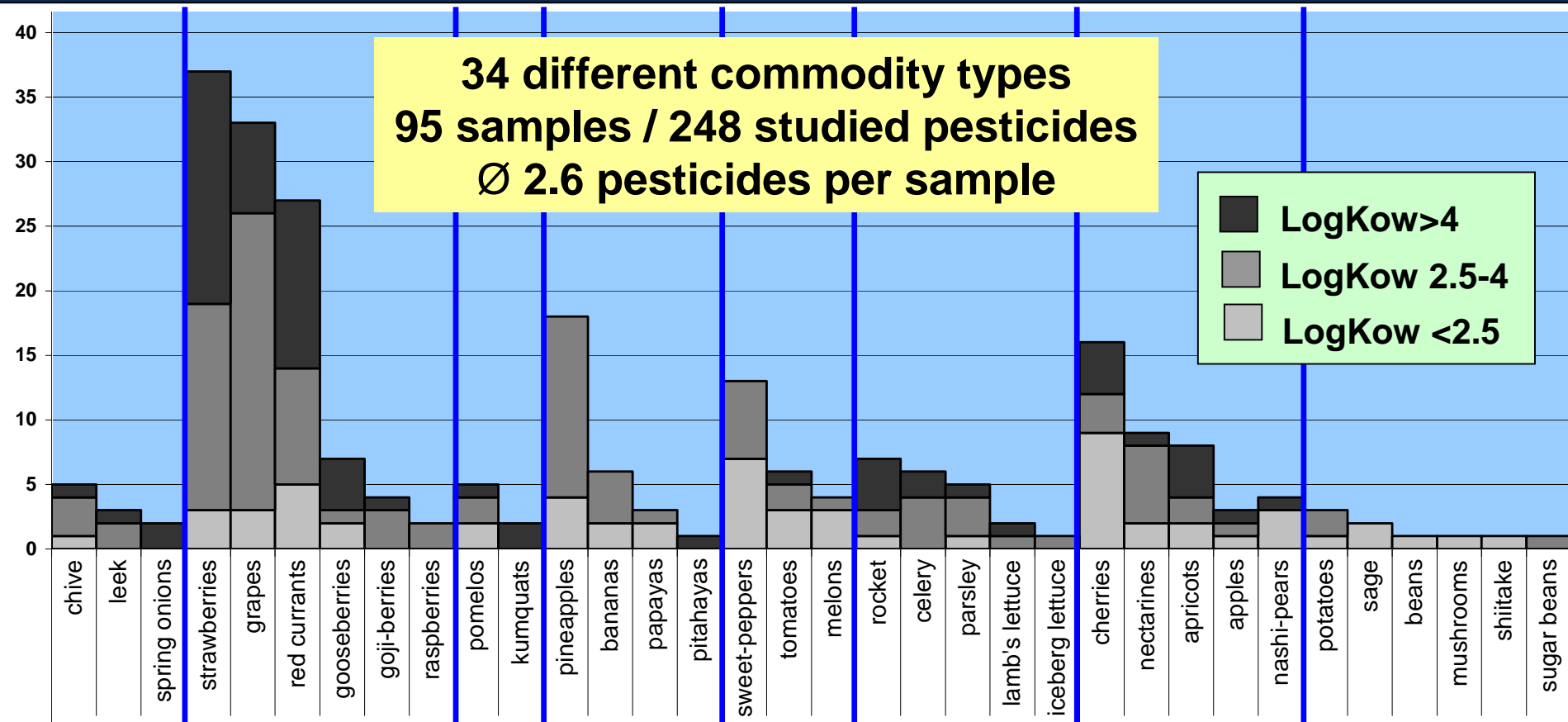
Impact of LogKow on Extraction yields



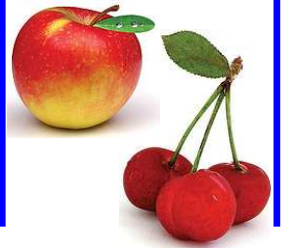
Tested Commodities

34 different commodity types
 95 samples / 248 studied pesticides
 Ø 2.6 pesticides per sample

LogKow >4
 LogKow 2.5-4
 LogKow <2.5

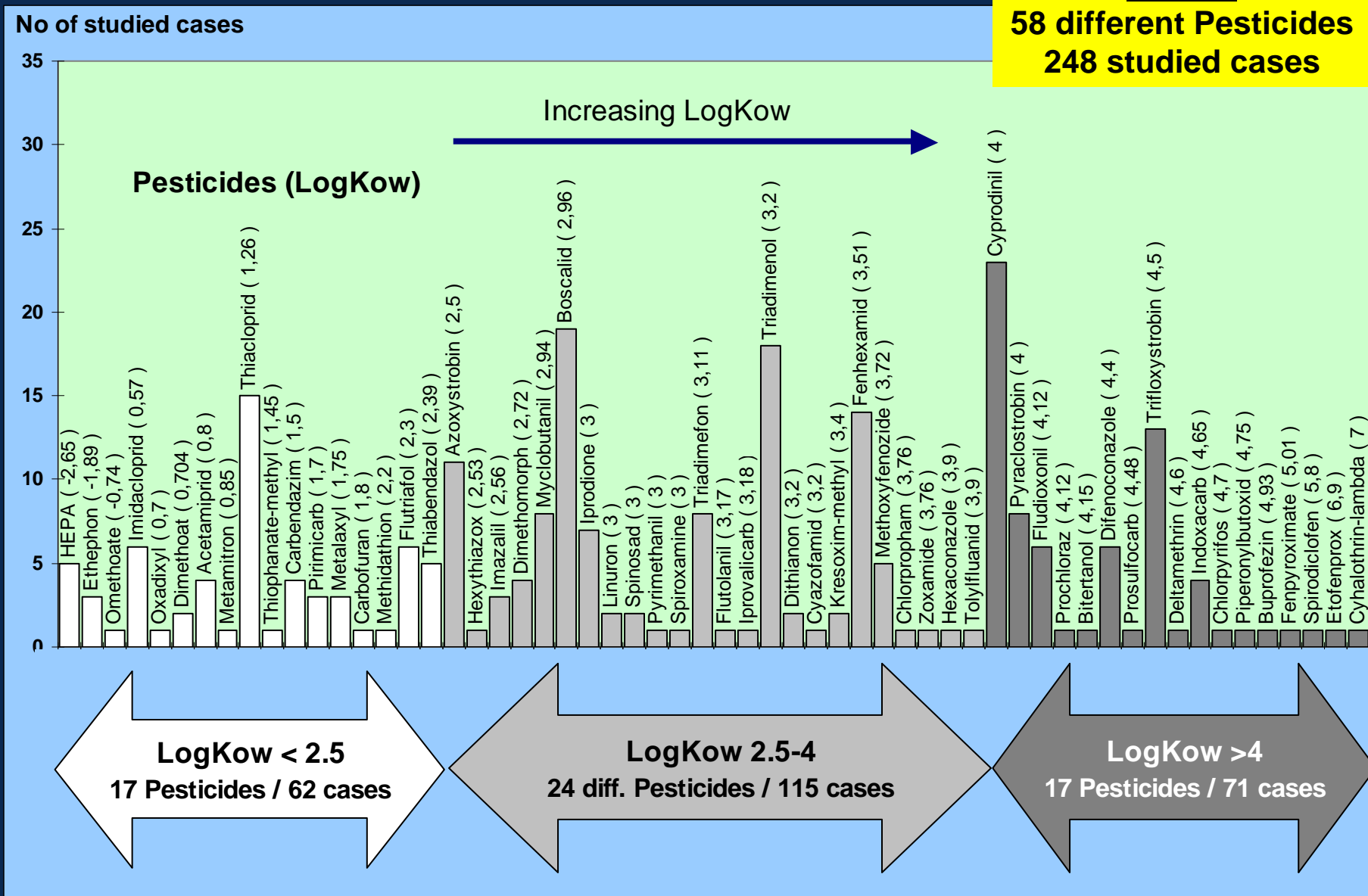


Allium Crops
Berries
Citrus Fruit
Exotic Fruit
Fruiting Veg.
Leafy Vegetables
Pome+Stone Fruit
Other Crops

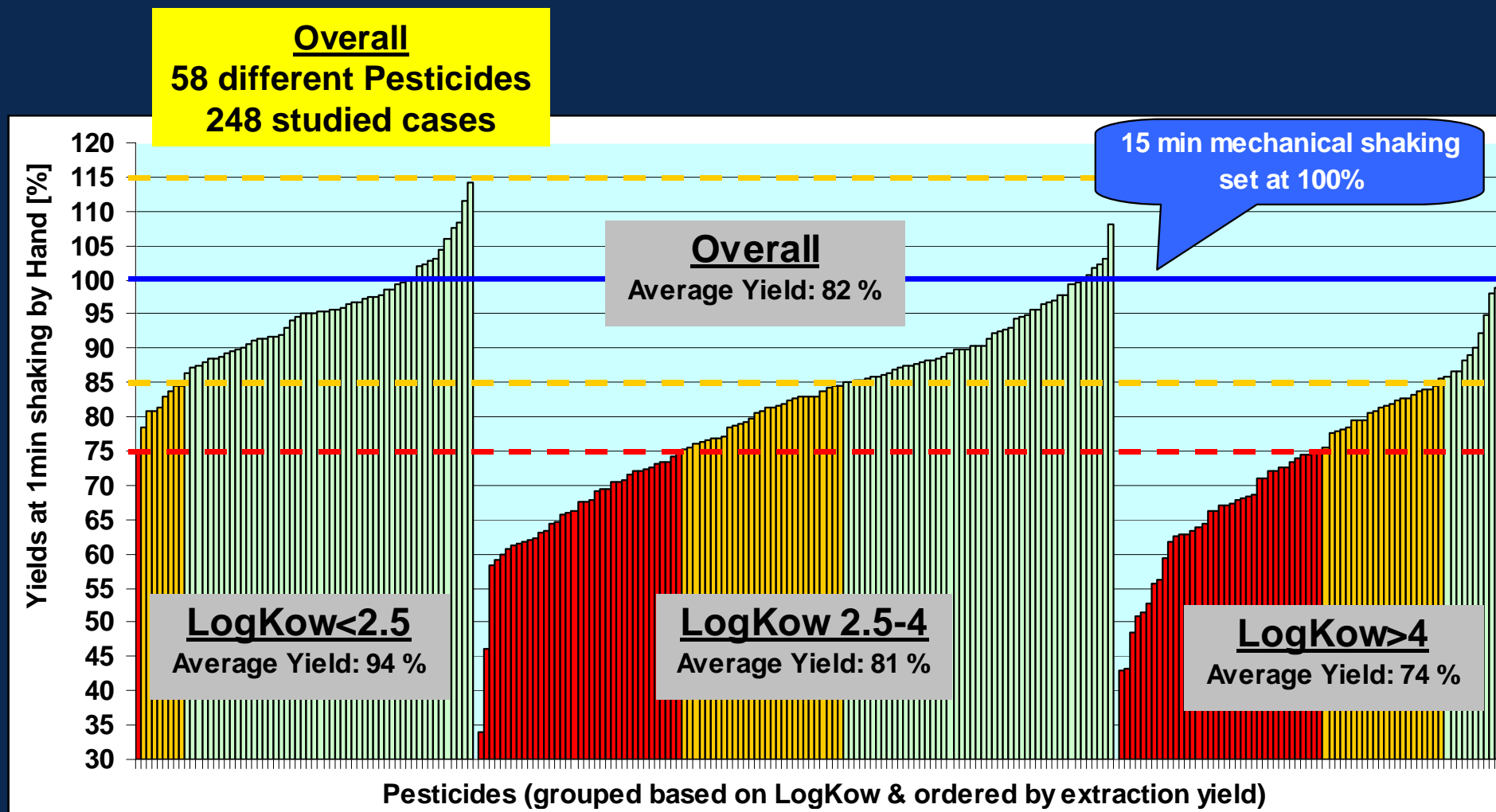


Scope of studied pesticides

Overall
58 different Pesticides
248 studied cases



Extractability of incurred residues – Role of LogKow



Impact of LogKow on Extraction yields

Pesticide	LogKow	No of studied cases	Average Yield at 1 min shaking (15 min set at 100%)
HEPA	-2,65	5	93
Ethephon	-1,89	3	99
Imidacloprid	0,57	6	95
Acetamiprid	0,8	4	97
Thiacloprid	1,26	15	92
Carbendazim	1,5	4	91
Pirimicarb	1,7	3	91
Metalaxyl	1,75	3	103
Flutriafol	2,3	6	91
Thiabendazol	2,39	5	90
Azoxystrobin	2,5	11	86
Imazalil	2,56 3,82 (pH9)	9	88
Dimethomorph	2,72	4	87
Myclobutanil	2,94	8	80
Boscalid	2,96	19	81
Iprodione	3	7	78
Triadimefon	3,11	8	74
Triadimenol	3,2	18	76
Fenhexamid	3,51	14	83
Methoxyfenozide	3,72	5	86
Cyprodinil	4	23	71
Pyraclostrobin	4	8	63
Fludioxonil	4,12	6	86
Difenoconazole	4,4	6	79
Trifloxystrobin	4,5	13	74
Indoxacarb	4,65	4	82

Differences between commodities

Bananas	72
	73
Melons	69
Lemon	95
Lemon	103
Grapefruit	99
clementine	96
clementine	91
clementine	98

cherries	51
grapes	51
lamb's lettuce	72
parsley	81
red currants	43
	63
	64
rocket	80

gooseberries	52,7
	68,7
grapes	56,2
red currants	55,8
	67,2
	72,1
	72,2
strawberries	80,5
	84,1
	86,7
	86,7
	90,1
	92,1

Impact of **Commodity Type** on Extraction yields of incurred residues

- Residue retardation seems more pronounced in certain commodities
- But ...available Data not sufficient to draw safe conclusions

Possible factors influencing extractability:

- Presence of natural surface-wax (slow penetration by extractant)
- Time between pesticide application and residue extraction (**unknown**)
- pH (in case of acids and bases)

In any case:

78% of **SAMPLES** showed yields <85% for at least 1 pesticide

52% of **SAMPLES** showed yields <75% for at least 1 pesticide

Results of EU-PTs samples

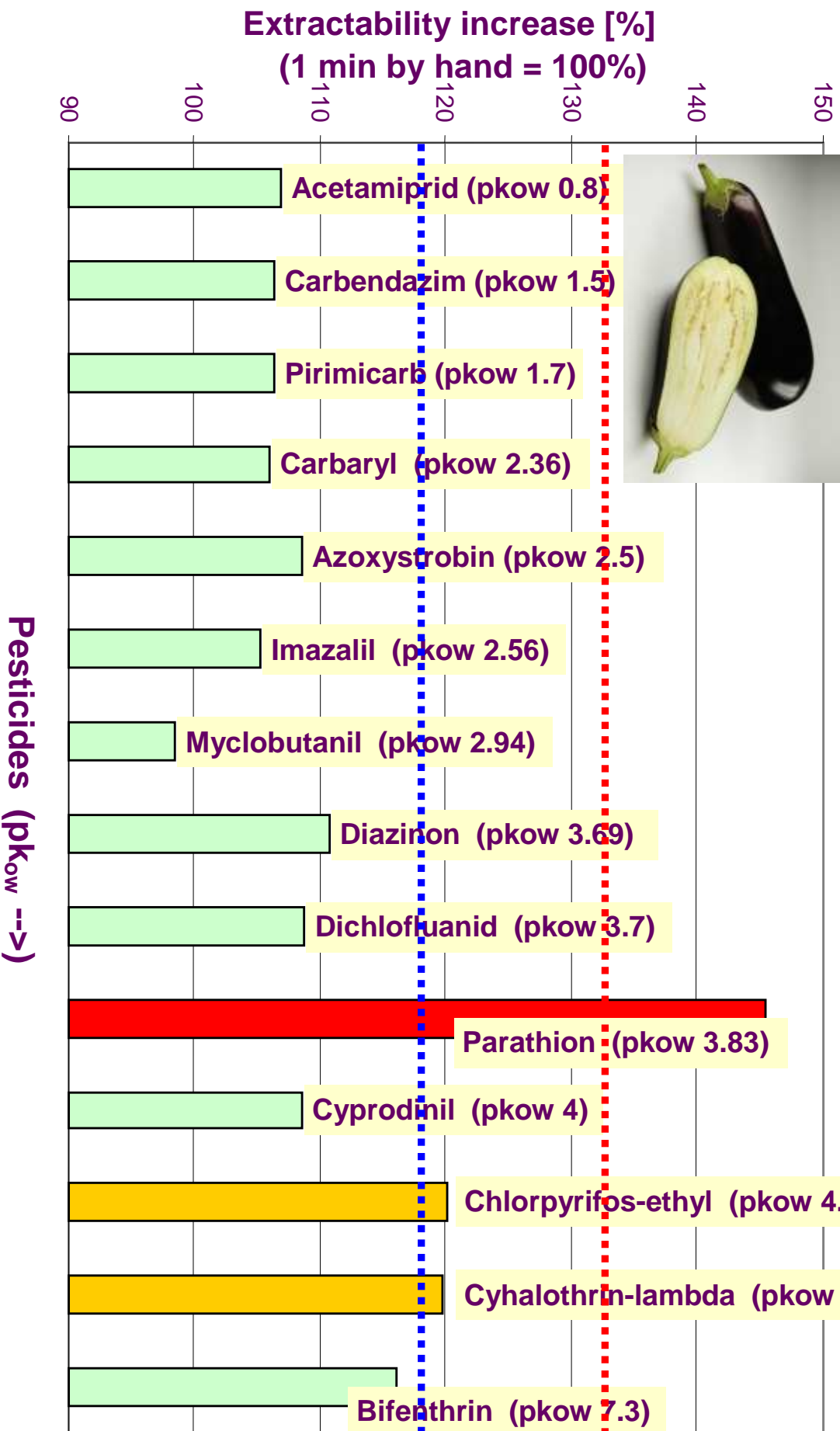
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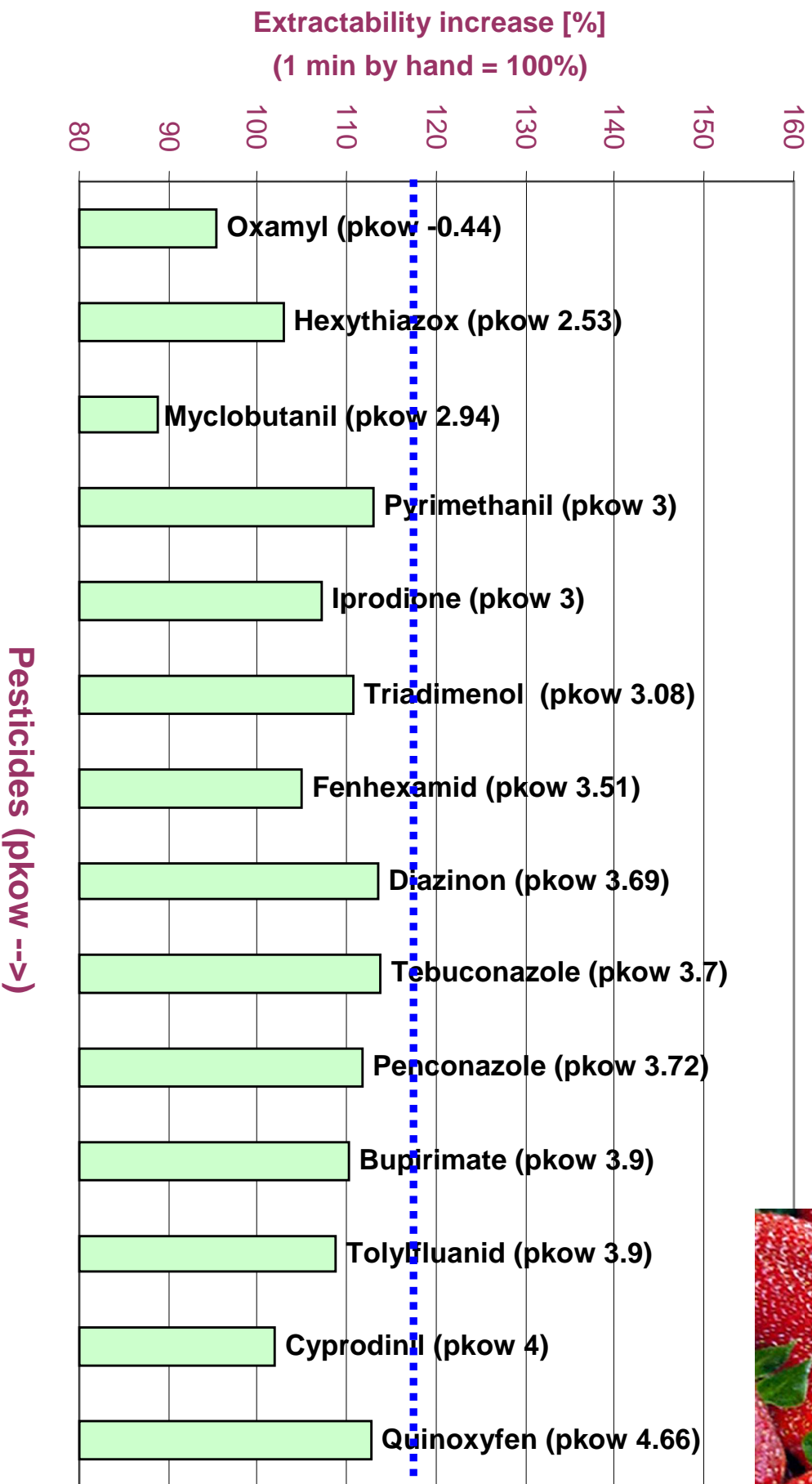
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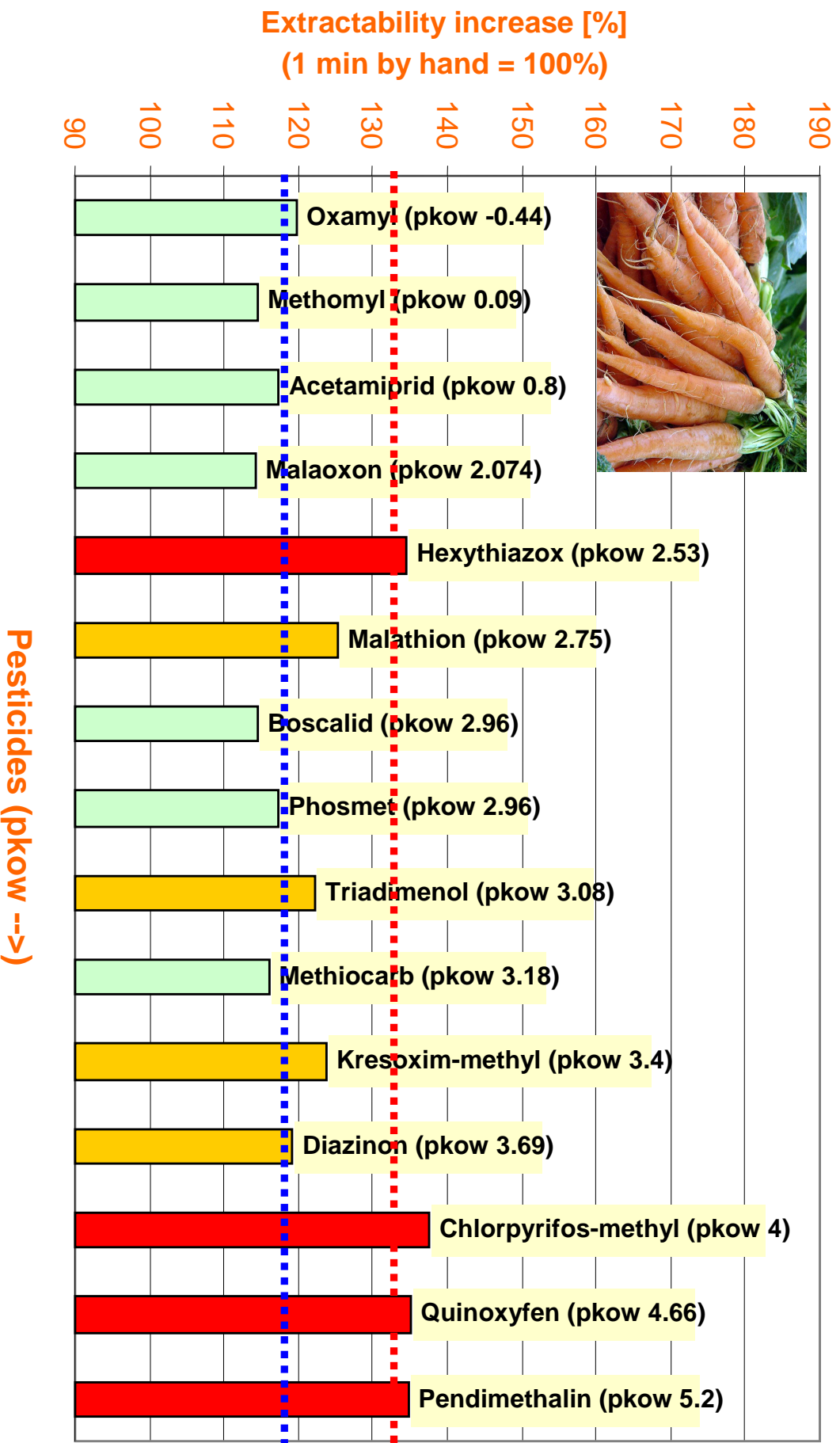
Aubergine (EUPT FV 8)
1 min by hand vs. 15 min by automated shaker



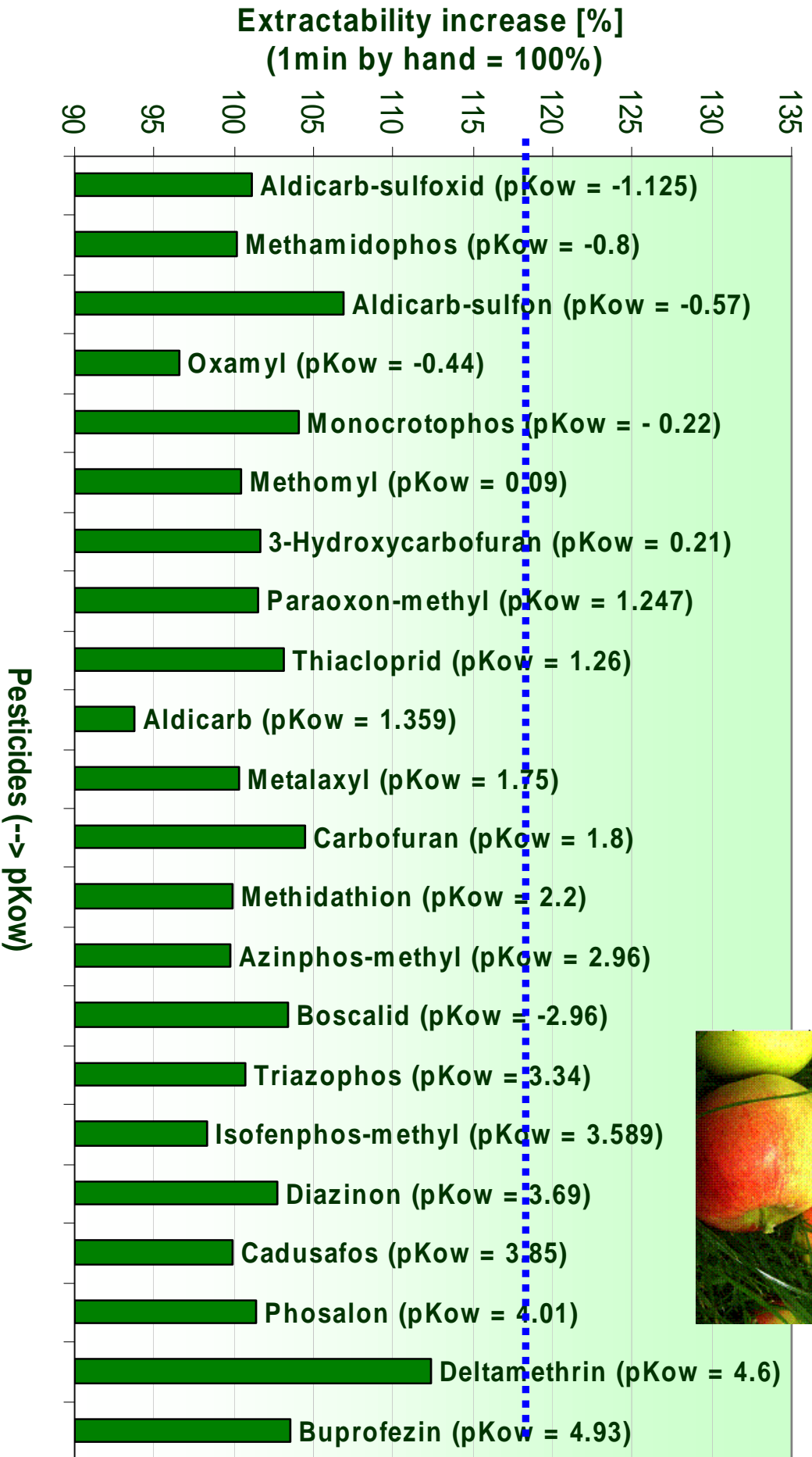
Strawberry (EUPF FV 9) 1 min by hand vs. 15 min by automated shaker



Carrot (EUPT FV 10) 1 min by hand vs. 15 min by automated shaker

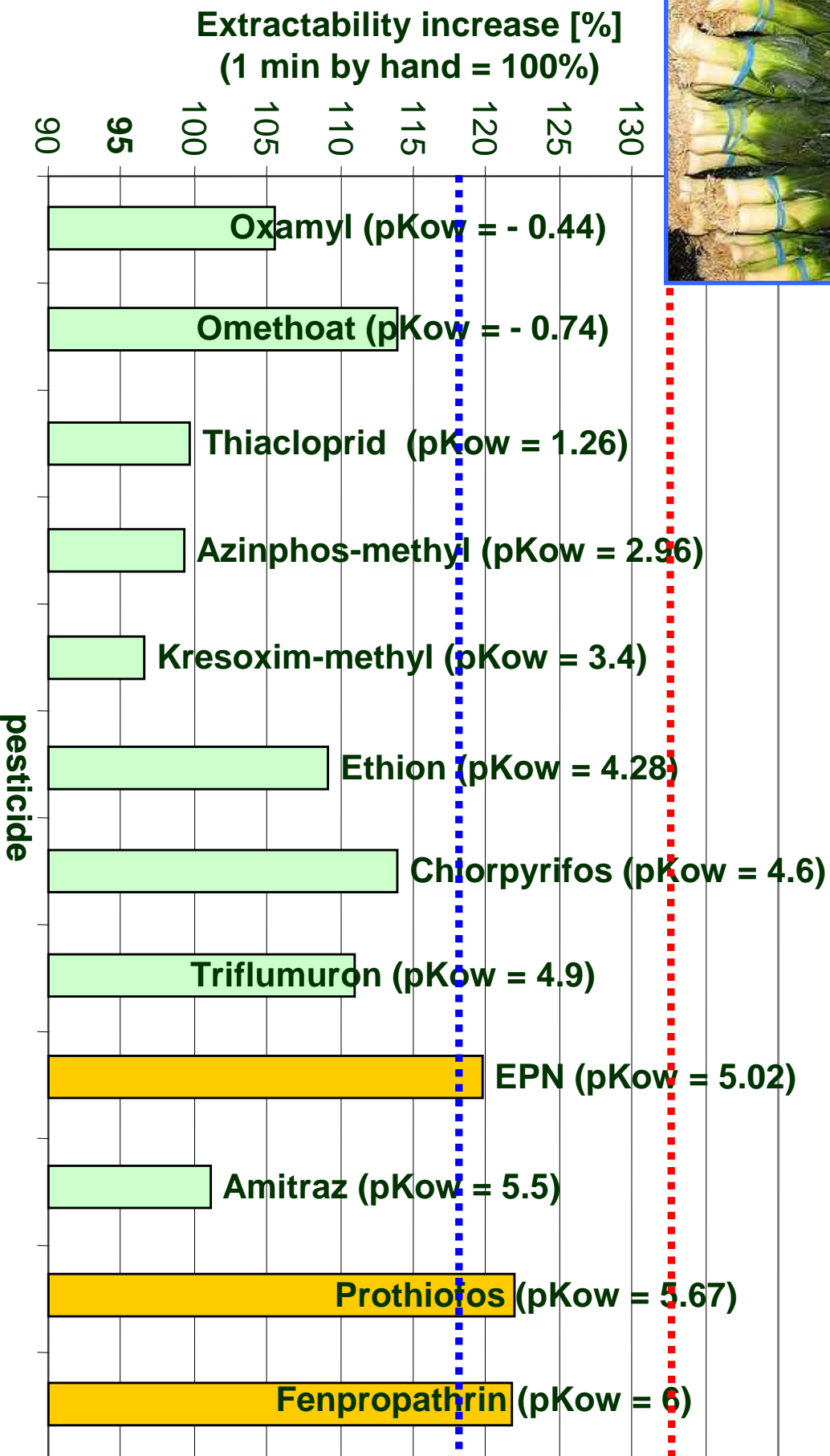


Apple (EUPF-FV 11)

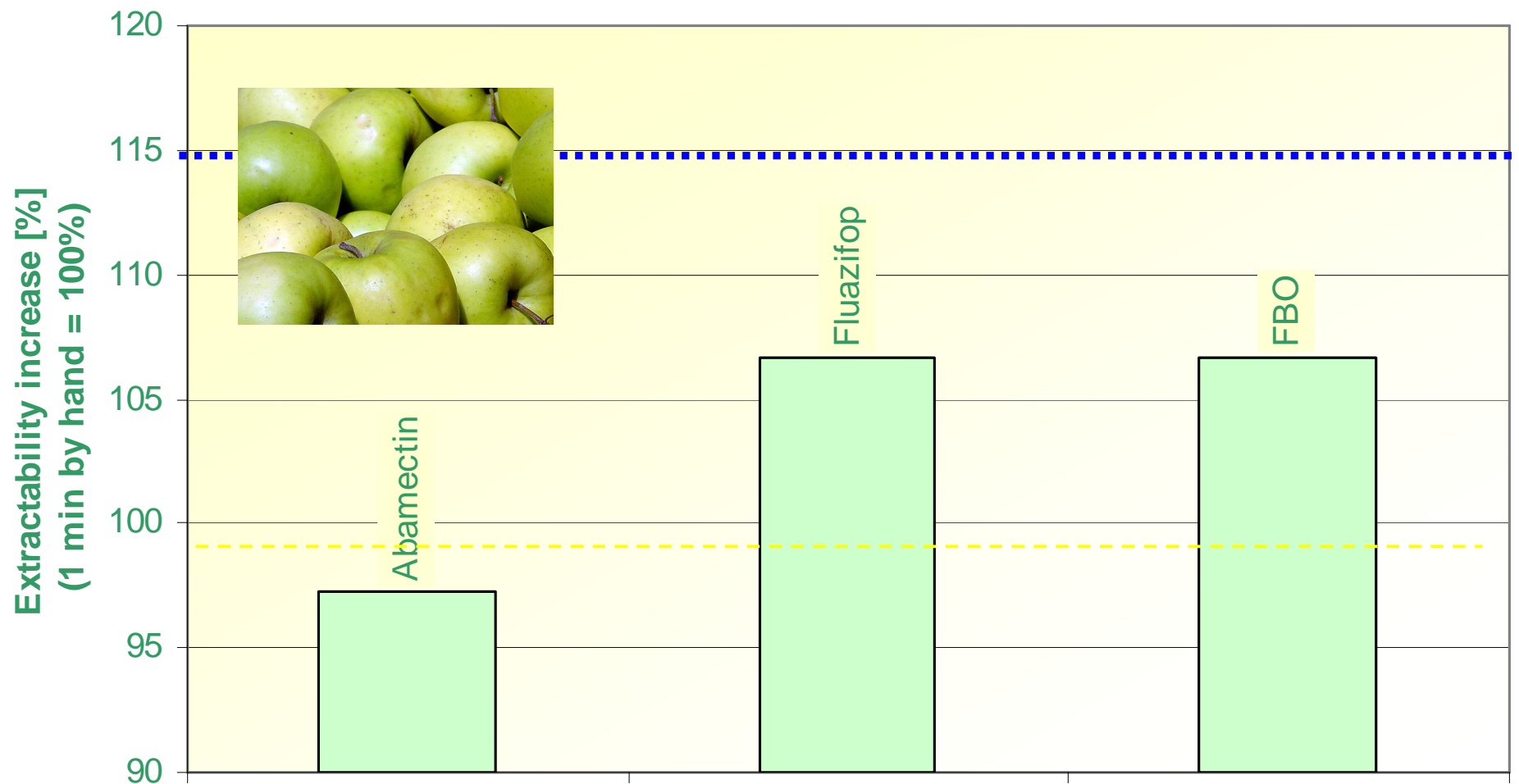




Leek (EUPF FV 12)
1 min by hand vs. 15 min by automated shaker

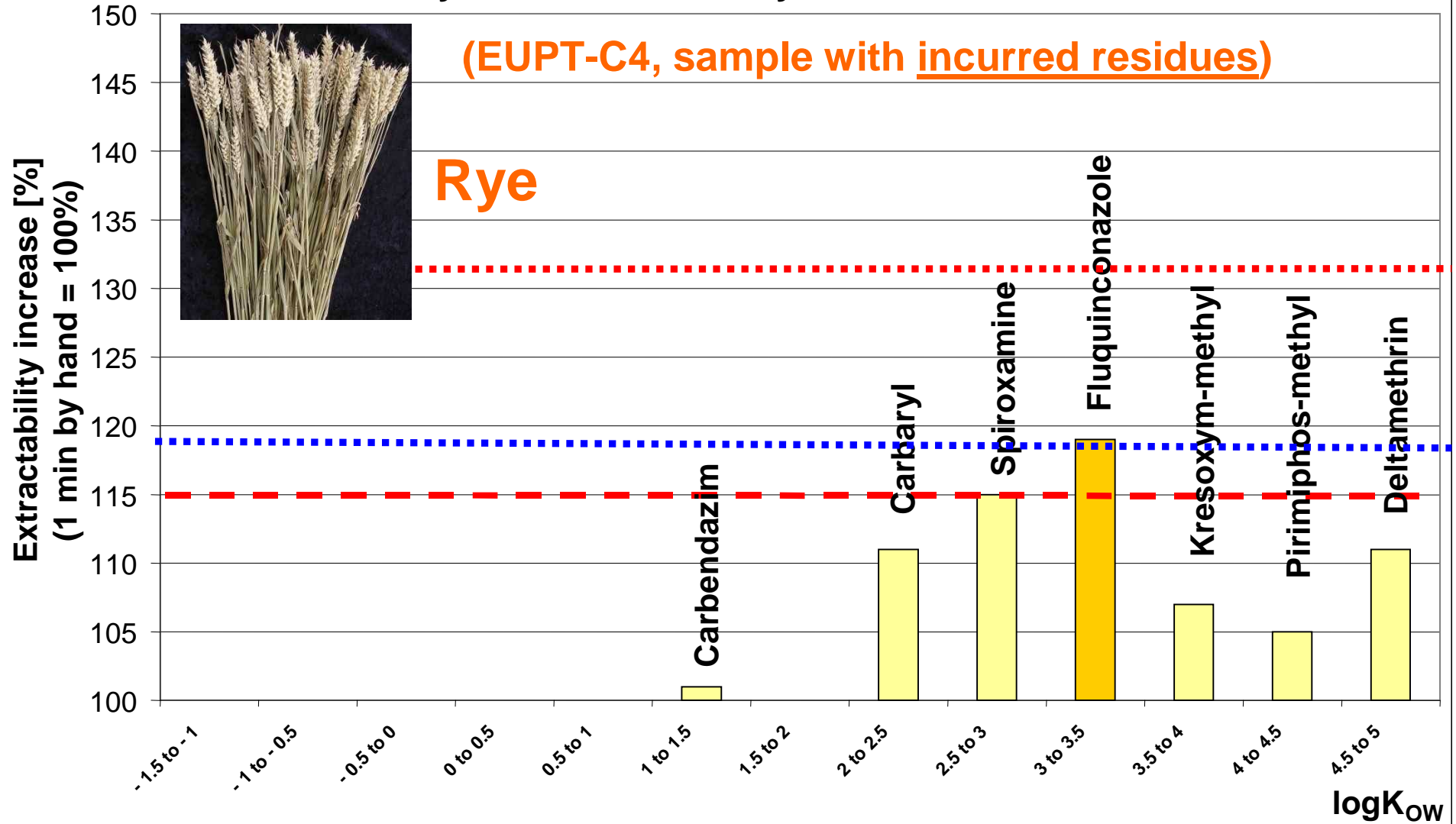


Apple puree (EUPT SRM 5) 1 min by hand vs. 15 min by automated shaker



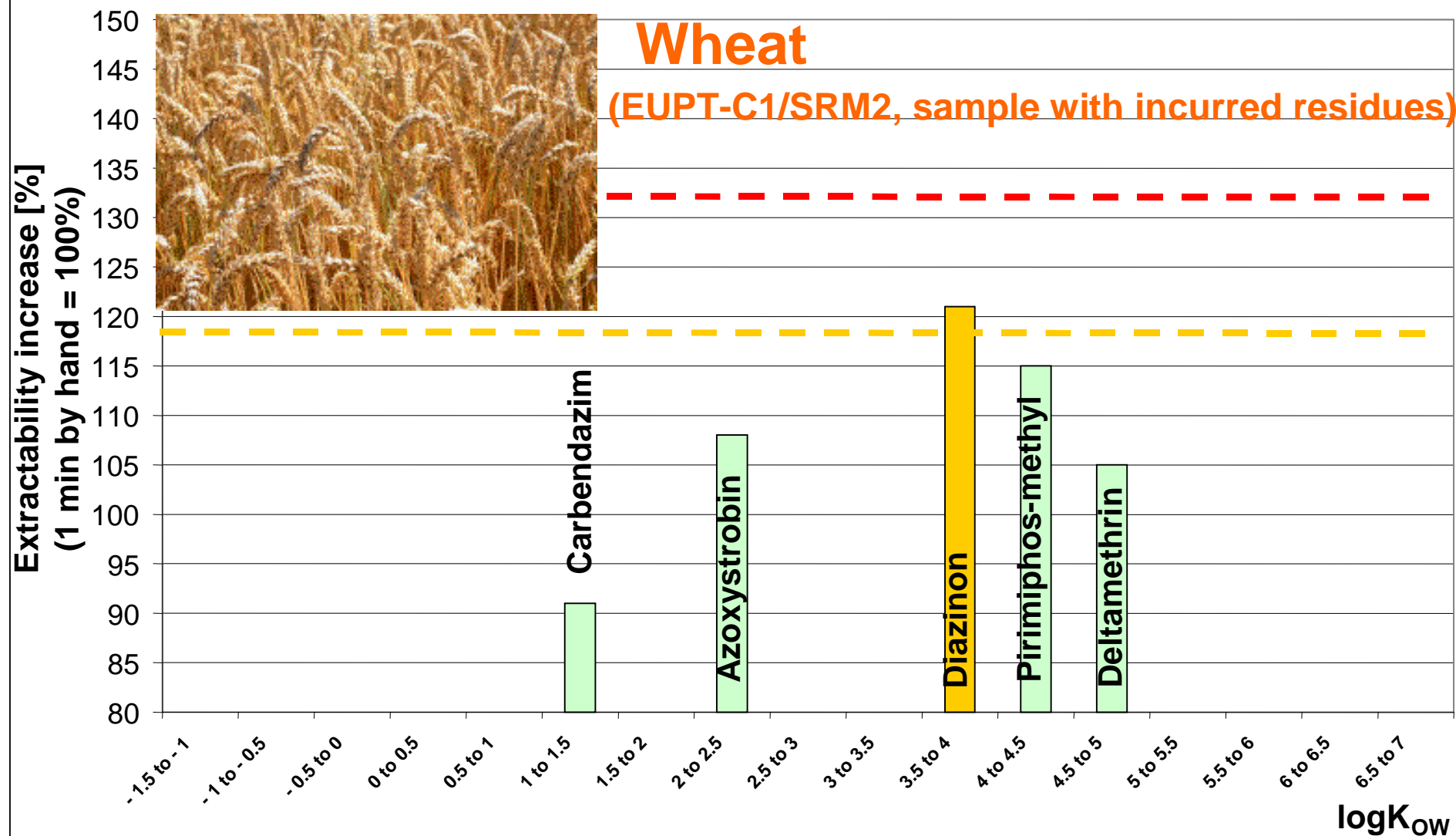
Comparison of extractability using QuEChERS

1 min by hand vs. 20 min by automated shaker



Comparison of extractability using QuEChERS

1 min by hand vs. 20 min by automated shaker



How was Data-Reliability ensured?

We have studied...

IMPACT OF EXTENDED EXTRACTION TIMES ON...

a) RECOVERIES

b) MATRIX EFFECTS

Also:

Only sample-compound combinations with good repeatability included in the study (**RSD <12 % at n=5**)

☛ good homogeneity,

☛ little influence of measurement uncertainty

At least n=3 for each experimental settings

IMPACT OF EXTENDED EXTRACTION TIMES

a) Check for Matrix Effects...

Raisins

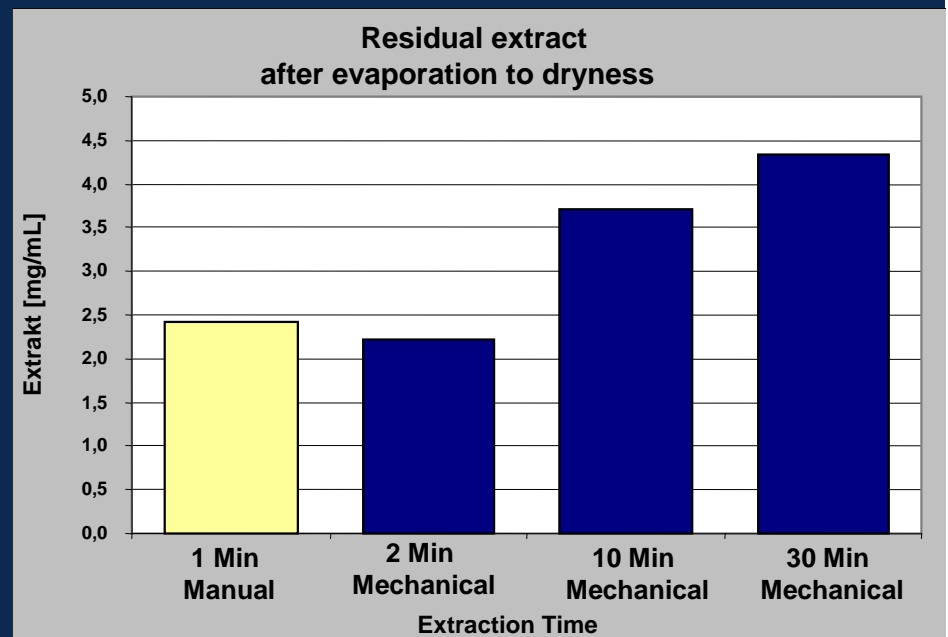
Extended extraction times resulted in :

- a) deeper coloured extracts
- b) higher dry residue

However...

➔ Impact on matrix-effects was insignificant

(compounds responsible for matrix effects are obviously rather quickly extracted)



IMPACT OF EXTENDED EXTRACTION TIMES

b) on Recoveries ...

- 430 pesticides tested whether they degrade during prolonged extraction times (30 min.) (125+ Pesticides tested on 15 min.) ...ongoing...
- Commodities used represent lower and upper end of the physiological pH range
- Only very few pesticides showed a clear degradation effect e.g. captan, folpet, dichlofluanid, chlorothalonil in the higher pH-range.

Cucumber

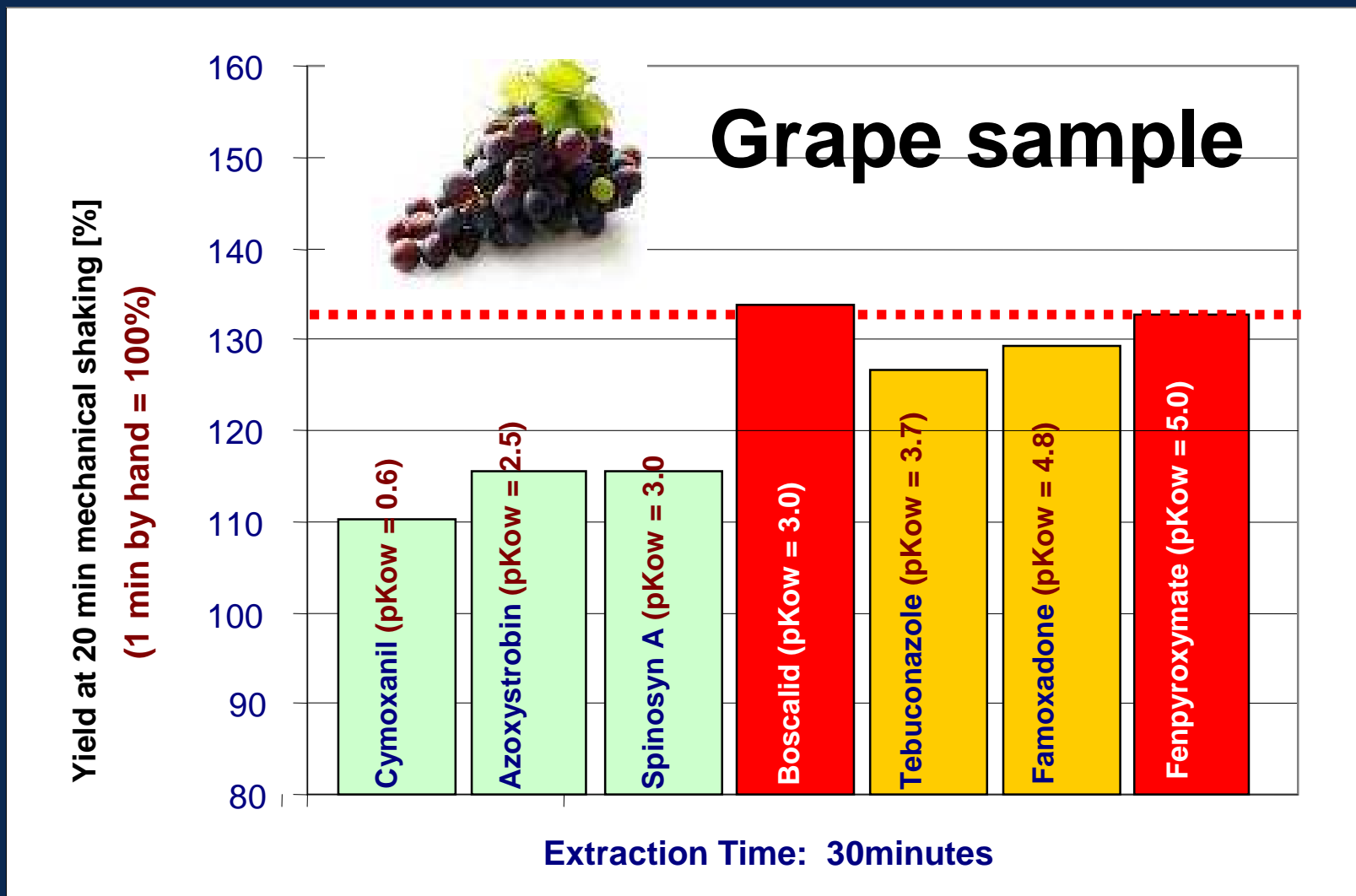


Matrix weight (g): 10
Matrix details: Matrix employed frozen
Preextraction details: no water addition

Pesticide				Recovery (in %)					Instrument Information			
	N =	Mean	RSD	a	b	c	d	e	Chromato- graphy	Interface	Extraction Step 1, time (min)	GC- Injection- Details
Dicloran	3	95,3	3,7	91	99	97			GC-MS/MS		2	
Pyrimethanil	3	102,6	0,6	102	103	102			GC-MS/MS		2	
Pirimicarb	3	96,5	0,6	96	97	97			GC-MS/MS		2	
Vinclozolin	3	107,7	1,7	106	110	107			GC-MS/MS		2	
Chlorpyrifos-methyl	3	100,2	1,0	100	102	99			GC-MS/MS		2	
Metalaxyl	3	102,1	2,7	99	105	102						
Pirimiphos-methyl	3	104,2	2,1	102	107	104						
Malathion	3	101,1	1,1	100	103	100						
Chlorpyrifos	3	102,5	1,7	101	105	102						
Pendimethalin	3	105,0	2,0	102	105	107						
Cyprodinil	3	100,7	0,7	100	100	102			GC-MS/MS		2	
Quinalphos	3	102,2	1,5	103	104	100			GC-MS/MS		2	
Procymidon	3	105,0	0,7	104	105	106			GC-MS/MS		2	
Mepanipyrim	3	99,4	1,0	100	100	98			GC-MS/MS		2	
a-Endosulfan	3	100,8	0,6	101	100	102			GC-MS/MS		2	
Fludioxonil	3	100,1	1,8	100	102	98			GC-MS/MS		2	
Myclobutanil	3	105,6	3,3	102	110	104			GC-MS/MS		2	
Kresoxim-methyl	3	104,4	1,9	102	106	106			GC-MS/MS		2	
Buprofezin	3	101,5	2,0	103	103	99			GC-MS/MS		2	
b-Endosulfan	3	100,9	1,3	99	103	101			GC-MS/MS		2	
Trifloxystrobin	3	97,3	2,7	95	101	96			GC-MS/MS		2	

**More Recovery Data in
EURL-DataPool
Soon!**

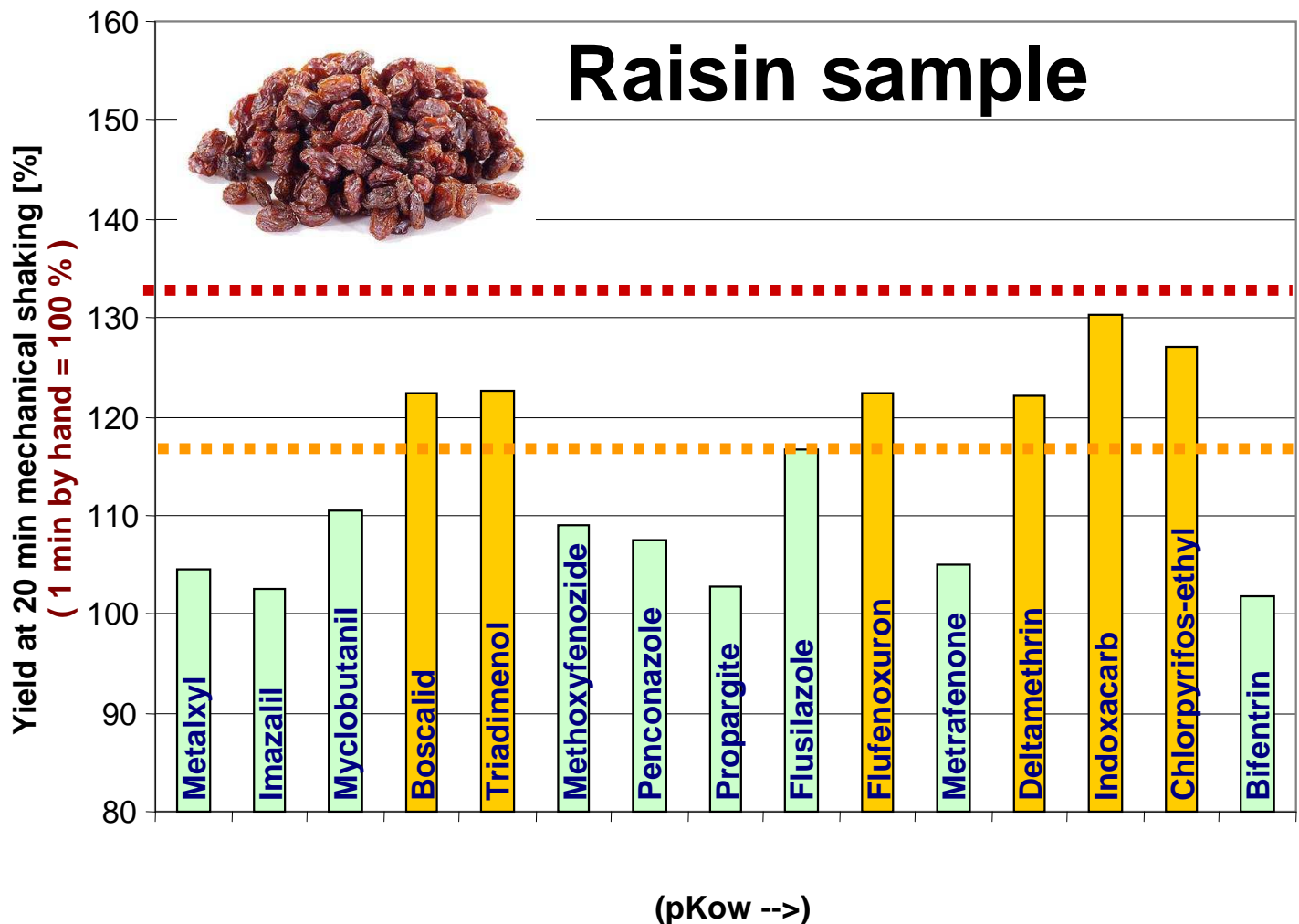
Effects with Ethyl acetate Method



Effects with Acetone Method



Raisin sample



Pesticide	pKow
Metalxyl	1.75
Imazalil	2.56
Myclobutanil	2.94
Boscalid	2.96
Triadimenol	3.20
Methoxyfenozide	3.72
Penconazole	3.72
Propargite	3.73
Flusilazole	3.74
Flufenoxuron	4.00
Metrafenone	4.30
Deltamethrin	4.60
Indoxacarb	4.65
Chlorpyrifos-ethyl	4.70
Bifentrin	6.00

Comparison of Methods



QuEChERS (ACN:Water 1:1)	Triadimefon	Triadimenol
1 min by Hand (Frozen)	80	70
1 min by Hand (RT)	95	110
1 min Ultra Turrax (Frozen)	94	93
5 min Mechanical (Frozen)	95	90
15 min Mechanical (Frozen)	100	100
15 min Ultrasound (Frozen)	100	107
Ethylacetate (EtAc:Water 1:1)	% (15 min = 100%)	
	Triadimefon	Triadimenol
1 min by Hand (Frozen)	86	94
1 min by Hand (RT)	103	124
1 min Ultra Turrax (Frozen)	122	143
5 min Mechanical (Frozen)	96	101
15 min Mechanical (Frozen)	100	100
15 min Ultrasound (Frozen)	91	102
Acetone (Acetone:Water 3:1)	% (15 min = 100%)	
	Triadimefon	Triadimenol
1 min by Hand (Frozen)	90	84
1 min by Hand (RT)	89	87
1 min Ultra Turrax (Frozen)	98	94
5 min Mechanical (Frozen)	94	91
15 min Mechanical (Frozen)	100	100
15 min Ultrasound (Frozen)	93	97

Interlaboratory Comparison

All labs using QuEChERS

1 min by hand vs. 15 min automatic shaking

(1 min by Hand = 100%)



Pineapple with
Incurred residues

	Triadimefon	Triadimenol
Lab 1	134	137
Lab 2	132	125
Lab 3	126	131
Lab 4	138	144
Lab 5 (thawed)	112	102
Lab 6 (thawed)	102	103

OUTLOOK

- Perform additional **recovery experiments** and collect recovery data from other labs (datapool)
- Study **additional commodities** w. incurred residues to elucidate the role of commodity type
- Organize an **inter-laboratory comparison** with real samples
- Study impact of **pH** on extractability of ionizable compounds
- Further study impact of **particle size** (dry commodities)
- Further study effects in the case of **ethylacetate**- and **acetone**-based procedures
- Study extractability of **microencapsulated** pesticides

SUMMARY

Experiments with real samples have shown the need for **PROLONGED EXTRACTION TIMES** (e.g. 10-15 min) to increase the recoveries of incurred residues.

The use of **MECHANICAL SHAKERS** is indicated

GOOD COMMINUTION (SMALL PARTICLE SIZES) is essential to improve accessibility of incurred residues and achieve high extraction yields.



Thank you very much...

EURL-SRM

EU Reference Laboratory for Pesticides
requiring **Single Residue Methods**

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3rd Joint EURL-Workshop
Freiburg, 27 - 28 Sep 2011