

# Securing safe foods. What is safe – and what is not, looking at analytical results

Árpád Ambrus, Judit Sali, Andrea Zentai  
Hungarian Food Safety Office

Hungarian Food Safety Office  
[www.hfso.hu](http://www.hfso.hu)



## What are ‘safe food’ & food safety?

Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use.

The **food safety** is a specific component of **food quality** which includes:

- the healthy food that is free from any substances or contamination adversely affecting human health,
- the whole food production and distribution chain,
- the (government) institutions controlling the whole process and certify compliance of the product with safety standards.

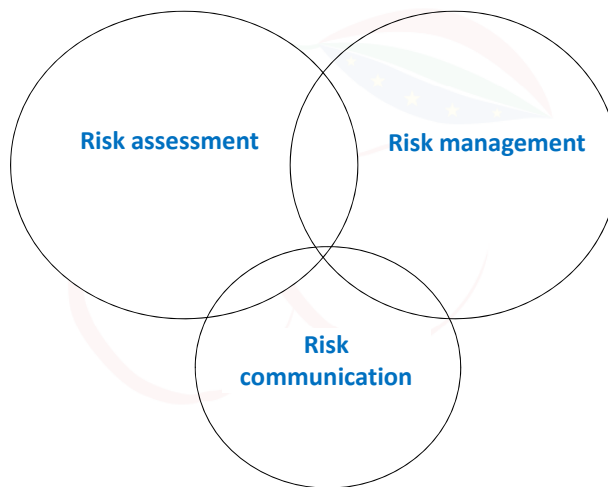


## Basic terms

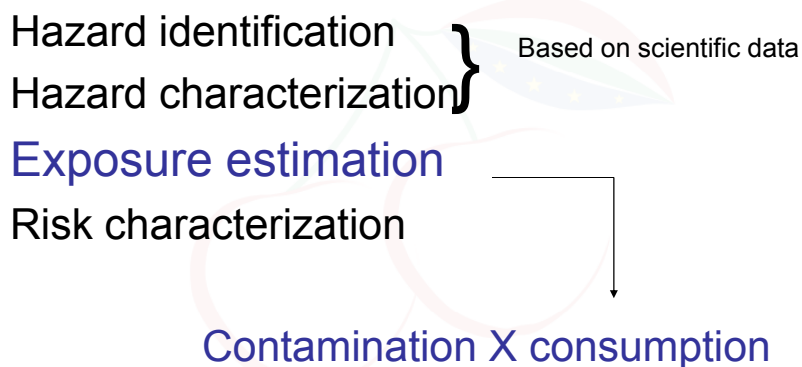
- **Hazard:** potentially harmful substance present in food (e.g. chemical & microbiological contaminants, etc).
- **Risk:** the probability and seriousness of sickness resulted from a hazardous substance.
- **Risk assessment:** scientific method for quantifying the risk.



## Risk analysis



## Risk assessment



## Hazard identification

- **Biological:** bacteria, parasites, viruses, ...  
Inappropriate handling: cross contamination
- **Chemical:** dioxins, mycotoxins, PCB-s, PAH-s, pesticides, antibiotics, heavy metals, impurities, by-products, ....
- **Physical:** glass splinters, metal scraps ....



## Characterisation of chemical hazard

Reference points based on various toxicological tests, and established by international organizations or national authorities: (JECFA, JMPR, EFSA):

- LD<sub>50</sub>, LOAEL, BMDL
- NOEL
- ADI, TDI, TWI, PTWI
- ARfD

**Safe food criteria:**

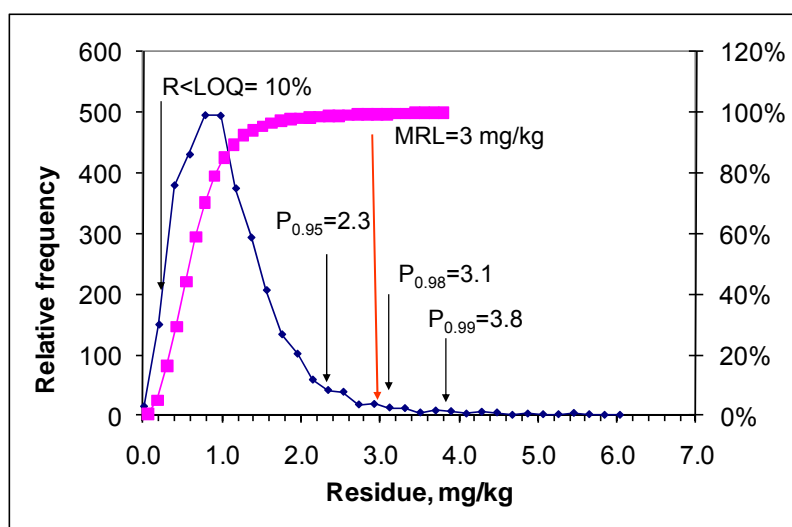
Estimated daily/weekly intake EDI (EWI) ≤ ADI/TDI/TWI

Estimated short term intake ESTI ≤ ARfD

Legal criteria:  $R_{\max} \leq \text{MRL (ML)}$



## Typical distribution of residues



## RISK CHARACTERIZATION

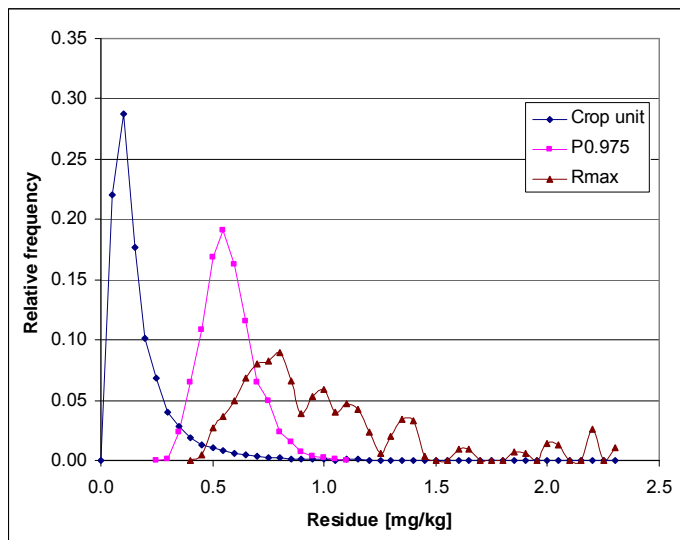


Consumer exposure: TMDI, EDI

- $TMDI = \sum MRL_i * F_i$
- $EDI = \sum STMR_i * E_i * P_i * F_i$ 
  - $STMR_i$ : median of residues (contaminants)
  - $E_i$ : edible portion
  - $P_i$ : processing factor
  - $F_i$ : food intake
  - MRL: maximum residue limit



Distribution of  $R_i$ ,  $P_{0.975}$ , and  $R_{max}$ ,  $n=100$ ,  
 in a log-normal parent population ( $k=10000$ )



## Short term intake: ESTI

- Relation of average residue in composite sample and in individual crop unit.
- **Variability factor:** the 97.5<sup>th</sup> percentile of residues present in individual crop units divided by the average residue in the lot:

$$R_{P0.975}/\mu \rightarrow R_{0.975}/R_j$$



## IESTI

Case 1: unit weight < 25 g

$$ESTI = \frac{LP * (HRorHR - P)}{bw}$$

Case 2: Weight of crop unit is smaller than the large portion size:  $U < LP$

$$IESTI = \frac{[U * (HRorHR - P) * v] + [LP - U] * (HRorHR - P)}{bw}$$



## Cumulative exposure

- Combined toxicological effect of multi residues of similar toxicological endpoints present in a food item:

$$C_{Cum1} = C_1 + C_2 \frac{ARfD_1}{ARfD_2} + C_3 \frac{ARfD_1}{ARfD_3} + \dots + C_n \frac{ARfD_1}{ARfD_n}$$



## Dioxins and dioxin-like PCBs: TEF - TEQ

- Sum of polychlorinated dibenzo-para-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), expressed in WHO toxic equivalents using the WHO-TEFs
- **TEF toxic equivalency factor** expresses the toxicity of congeners relative to the most toxic 2,3,7,8-TCDD
- **TEQ** toxicologically equivalent quantity:
- 

$$\text{TEQ} = \text{TEF}_i \times C_i$$
$$\text{MPL} \geq \sum_{i=1}^n \text{TEQ} = \sum_{i=1}^n (\text{TEF}_i \times C_i)$$



## Analytical requirements of food safety risk assessment

**Analytical methods** should be:

- capable to quantify all, but not more, residue components included in the **residue definition for risk assessment**;
- accurate, specific, sufficiently sensitive;

**Analytical results** should represent the concentration of the contamination in sampled food with known uncertainty.



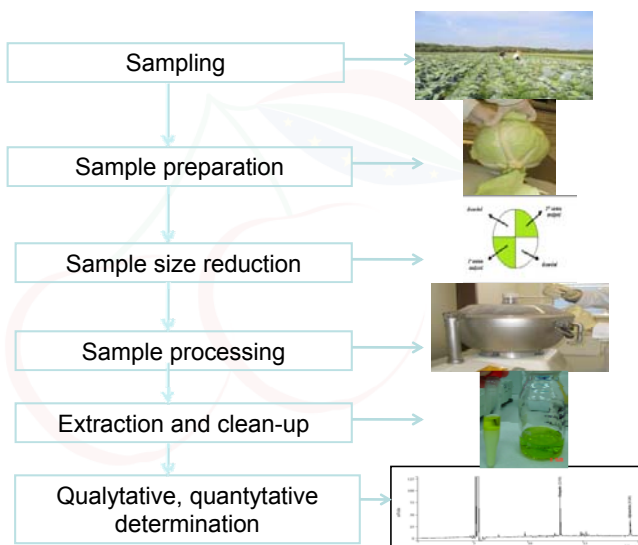
Do the current food control and analytical practices fulfill the food safety requirements?

Do we detect all pesticide residues, contaminants present?

Do we know the uncertainty of the reported results?



### Steps in pesticide residue analysis



## Combined uncertainty of analytical results ( $S_{Res}$ )

$$S_{Res} = \sqrt{S_S^2 + S_L^2}$$

$$CV_{Res} = \sqrt{CV_S^2 + CV_L^2}$$

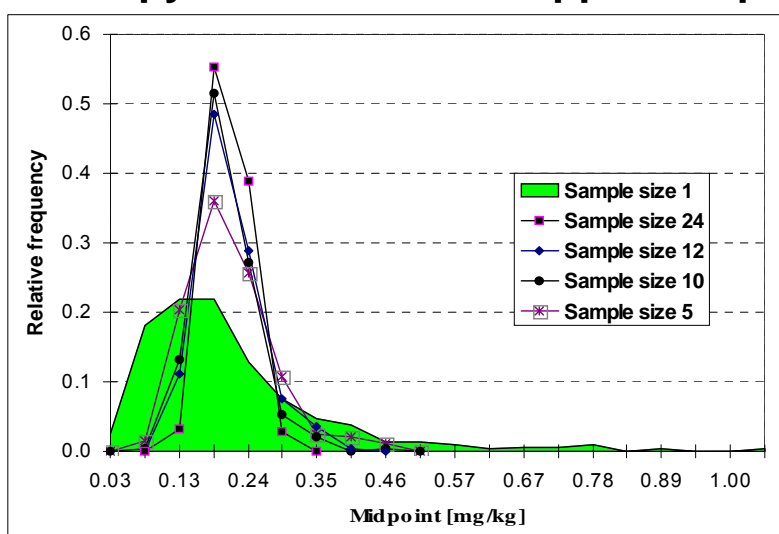
$$CV_{Lc} = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2}$$

Proficiency tests and most internal quality control procedures provide information only for  $CV_A$ !

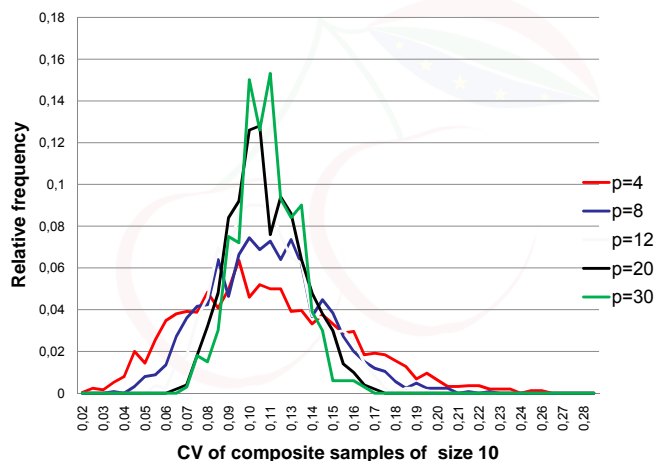
What is about  $CV_S$ ,  $CV_{SS}$ ,  $CV_{Sp}$  ??



## Relative frequency distribution of chlorpyrifos residues in apple samples



Distribution of estimated CV values based on p duplicate samples taken from a known population with CV = 0.157

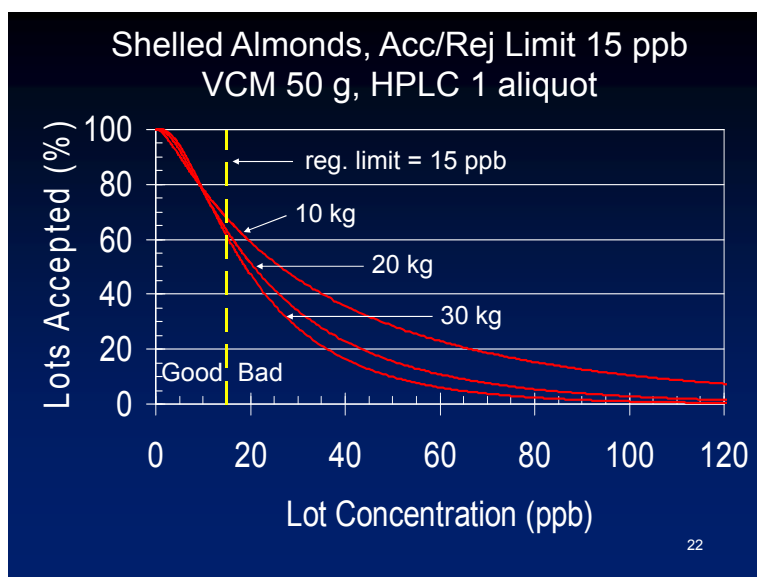


### Typical sampling uncertainties

Crop	CV <sub>typ,n=1</sub> %	CV <sub>typ,n</sub> %	CV <sub>typ,n,UCL</sub> 0.99%
Small fruits (unit weight < 25 g, sample size ≥ 10)	74	23	26
Medium size crops (unit weight between 25-250 g, sample size ≥ 10)	81	25	28
Large crops (unit weight >250 g, sample size ≥ 5)	74	33	37
Cabbage, kale, chicory (unit weight >250 g, sample size ≥ 5)	45	20	25

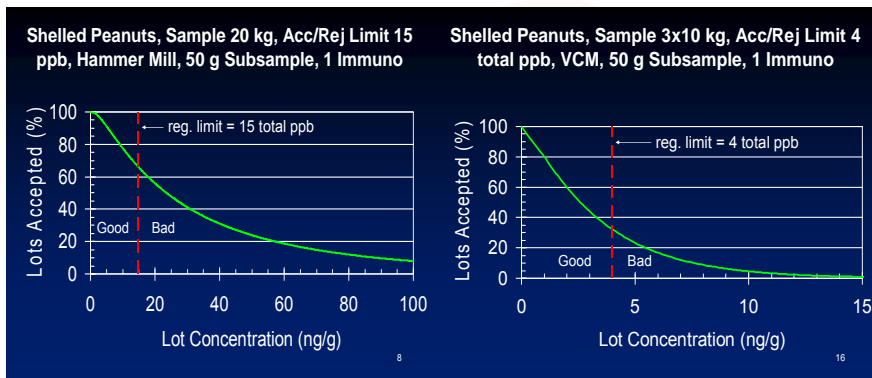
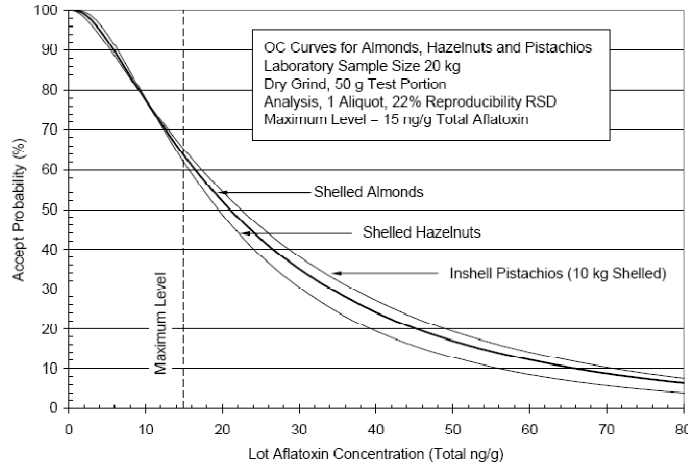


n	$\beta_p = 0.95, \beta_t = 0.95$		$\beta_p = 0.99, \beta_t = 0.99$		
	CV values				
	Average	Min	Max	Min	Max
1	0.81	0.24	1.38	0.01	1.61
5	0.37	0.11	0.62	0.01	0.72
10	0.25	0.07	0.43	0.00	0.61
25	0.16	0.05	0.28	0.00	0.52



Courtesy of Prof. Whitaker

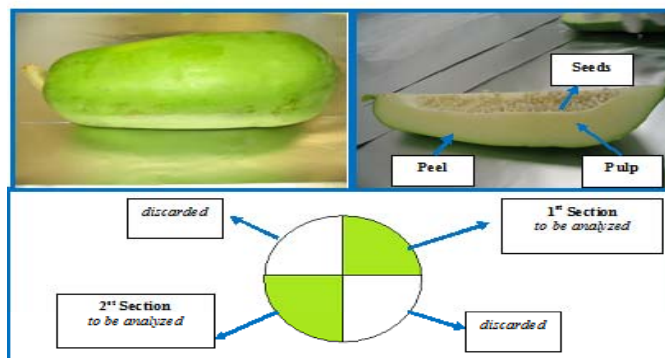




Courtesy of Prof. Whitaker



## Sample size reduction

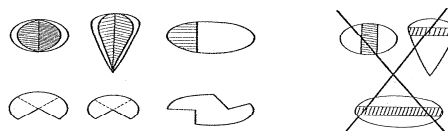


Courtesy of Perihan Yolci



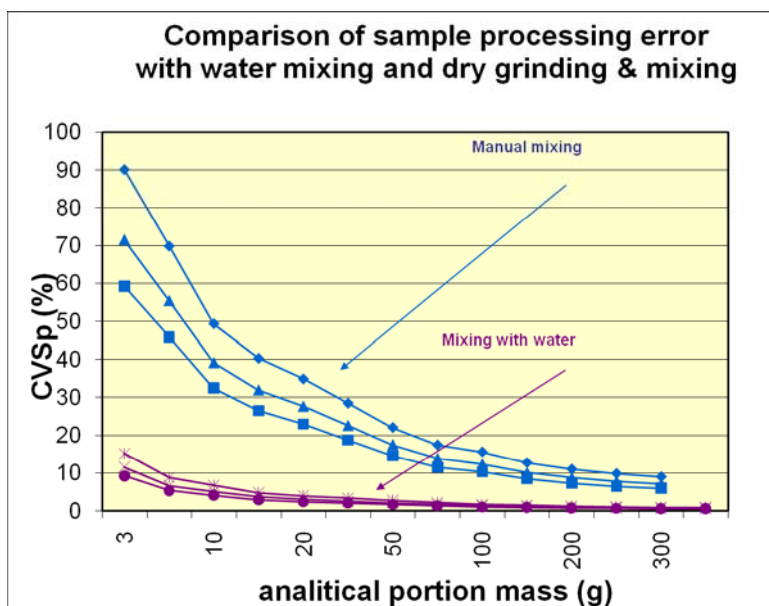
## Sample preparation and processing

PREPARATION, HANDLING AND STORAGE OF SAMPLES



Cutting representative portions of large crops





## Efficiency of sample processing

- Independent from analyte
- Depends on equipment, sample matrix;
- May significantly affect  $CV_L$ , therefore it should be regularly checked within IQ;
- May affect stability of analyte, which should be studied as part of the method validation, and at the time of analysis of labile compounds.



## Establishing within lab reproducibility of results, $CV_L$

- Collect results of replicate test portion analysis with differences within 99% probability critical difference range.
- Calculate the relative difference of the residues measured in replicate portions:

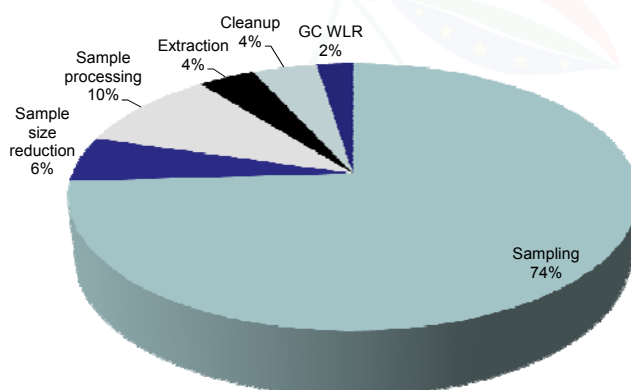
$$R_{\Delta i} = 2(R_{i1} - R_{i2}) / (R_{i1} + R_{i2})$$

$$CV_{Lab} = \sqrt{\frac{\sum_{i=1}^n R_{\Delta i}^2}{2n}}$$

v = n  
(# of replicate tests)



## Contribution of individual steps of pesticide residue analysis to the combined uncertainty (CV=0.38)



**The contribution of analysis of test portion is only 11%**



## Conclusions

- The analysis of residues in test portions of size 30 g contributes typically only with 11% to the combined uncertainty of residue data in large crops.
- When smaller test portions are analysed (10-15 g) the relative contribution is even smaller.
- The combined uncertainty of aflatoxin, ochratoxin data is larger than that of pesticides.
- **Based on current practice the major proportion of uncertainty remains unnoticed!!!**
- **Much more attention and systematic actions are required to provide accurate and reliable data for assessing consumer exposure and verifying safety of our food.**



Thank you for your attention



# Slides for discussion



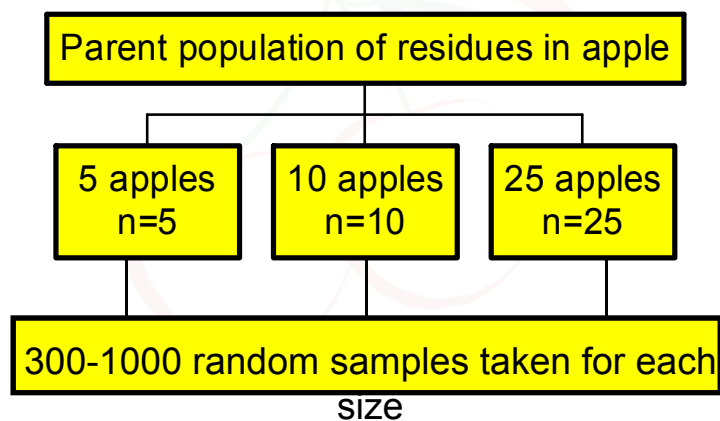
## Experimental

Crop	Parts that was analyzed	
11 papaya sample x 2 section = <b>22 sample</b>		Benomyl (as carbendazim) with HPLC-UV
5 Jackfruit sample x 5 section = <b>25 sample</b>		Thiophanate-methyl (as carbendazim) with HPLC-UV
3 cucumber sample x 2 section = <b>6 sample</b>		Iprodione and Primiphos-methyl with GC-NPD
3 cucumber sample x 3 section = <b>9 sample</b>		

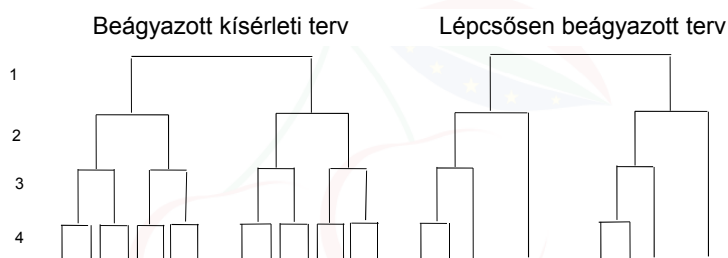
68 sample+ 70 QC = **138 analysis**



## Schematic illustration of drawing random samples from apples



## Kísérleti tervek a mintavétel és mintafeldolgozás véletlen hibájának meghatározására.



1: Tételek; 2: Tétel mintázása; 3: Minta tömeg csökkentés; 4: mérés

ISO 11648-1 az ömlesztett anyagok mintázására javasolja, hogy a mérendő komponens variabilitásának meghatározására legalább 20 tételből tételenként lehetőleg több mintapárt kell venni.



## Determination of $K_{Sp}$

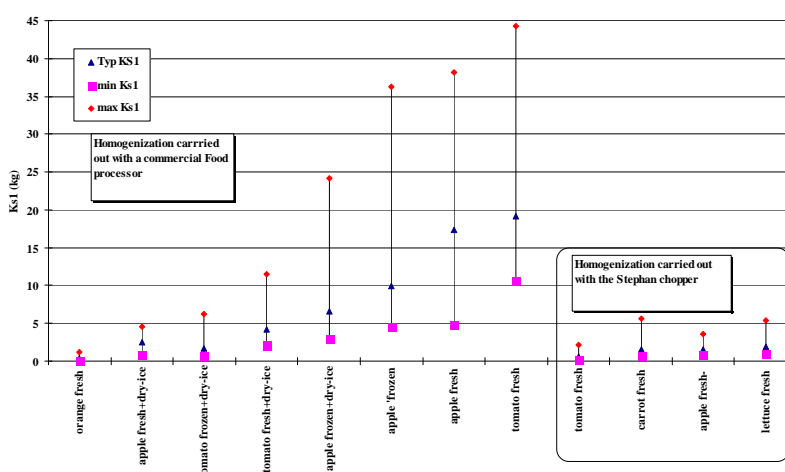
- Analyze  $\geq 5$  small and large test portions in minimum 3 replicates each
- If sample well mixed:  $K_{Sp,large} \equiv K_{Sp,small}$
- Test if  $F_{calc} \leq F_{crit}(0.95, \nu)$

$$F_{calc} = \frac{V_{small} \times m_{small} / m_{large}}{V_{large}}$$

- Test if  $V_T \gg V_A \rightarrow V_{Sp} = V_T - V_A$



Typical values and calculated ranges of  $K_{s1}$



$$vs^2/\chi^2_{0.025} \leq \sigma^2 \leq vs^2/\chi^2_{0.975}$$

## Ranges of the sampling constant [kg]

Matrix	Ks (Stephan)	Ks (P-ch)	Ks (WB)
Lettuce	1.7-3.0		0.1-0.6
Tomato	2.7-9.0	0.7-9.0	0.3-1.3
Carrot	0.3-3.0	5.0	0.3
Apple	0.7-11.0	1.5-20	0.2-0.3



## Advantage of Ks versus ANOVA

- Where the material is well mixed, one can calculate the expectable uncertainty of sample processing for any test portion size above the small portion tested.
- One ANOVA test gives limited information
- Estimation of uncertainty based on small number of measurements is not precise.
- No prediction of  $CV_{SP}$  for other test sample size.

