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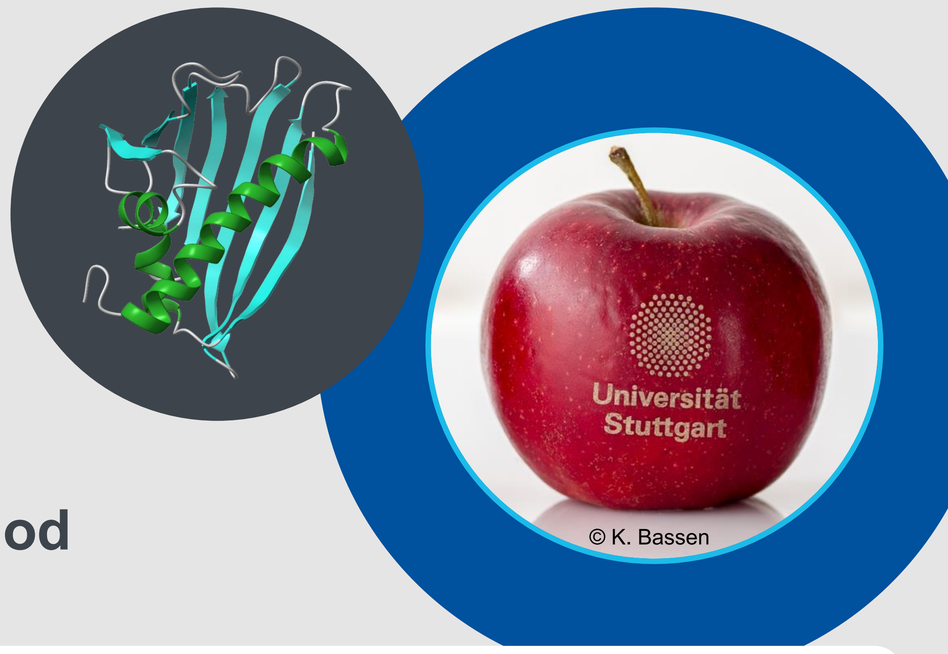
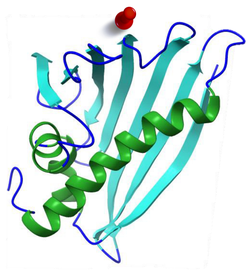


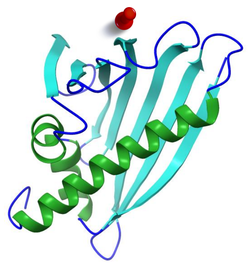
J. Kaeswurm, L. Straub, M. Buchweitz

Development of an isoallergen-specific quantification method for the apple allergen Mal d 1

A very brief introduction about apples and apple allergy



Mal d 1



Bet v 1

- Apples are an important source of vitamins, secondary plant metabolites and fibers in a Western diet [1]
- Due to the structural homology of the apple allergen Mal d 1 to Bet v 1 in birch, 70% of the birch pollinosis patients in Northern and Central Europe develop an apple allergy [2]
- Variety specific allergenic potentials are observed [3;4]

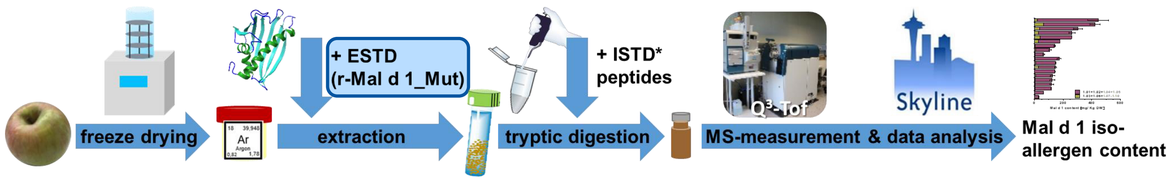
  → No correlation between allergenicity and Mal d 1 content (determined by ELISA) could be identified, [4] possible reasons:

  1. Different allergenicity of Mal d 1 isoallergens
  2. Protein extraction might be influenced by polysaccharides

- ELISA is insufficient to monitor protein extraction and does not allow isoallergen specific quantification

  → Therefore, a mass spectrometric based quantification method was developed

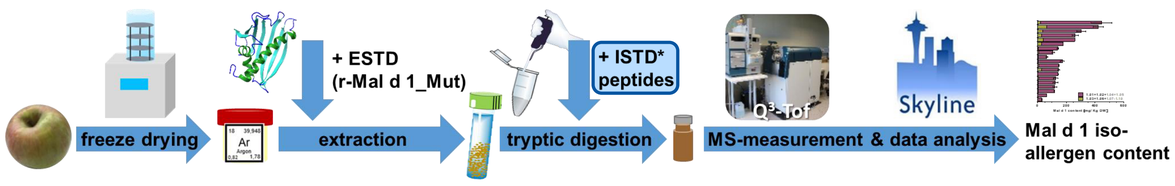
Importance of the extraction standard (ESTD)



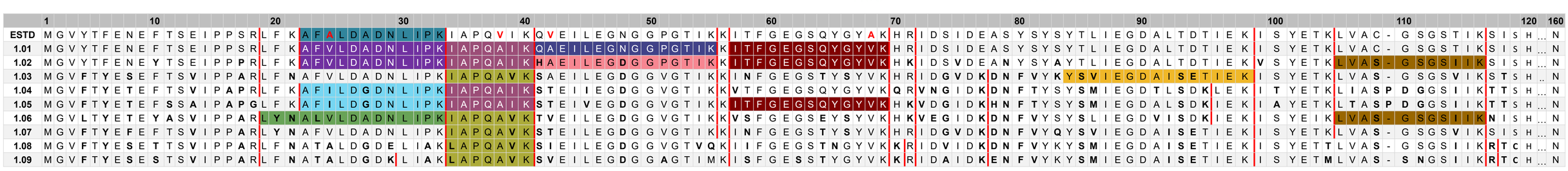
- Mal d 1.0101 was recombinant expressed [5] and added during extraction for compensating differences in the extractability of the samples
- Extractability is higher from the peel than from the flesh
- Extractability differed between the varieties and harvest years
- No similarity in the extractability of the same varieties in the different harvest years

Harvest year	Recovery of ESTD from the flesh	Recovery of ESTD from the peel
2019	Ø 42% (36-69%)	Ø 80% (62-120%)
2020	Ø 63% (40-100%)	Ø 83% (65-96%)

Selected isotope labeled marker peptides (ISTD*) for quantification

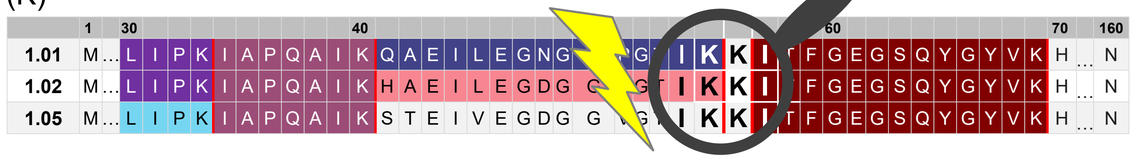


Isoallergen specific markers	Combination markers	Global markers	Marker for ESTD
1.01	1.01+1.02	1.01+1.02+1.04+1.05	ESTD
1.02	1.01+1.02+1.05	1.03+1.06+1.07+1.08+1.09	
1.03	1.02+1.06		
1.06	1.04+1.05		



Suitability of the marker peptides selected [6]

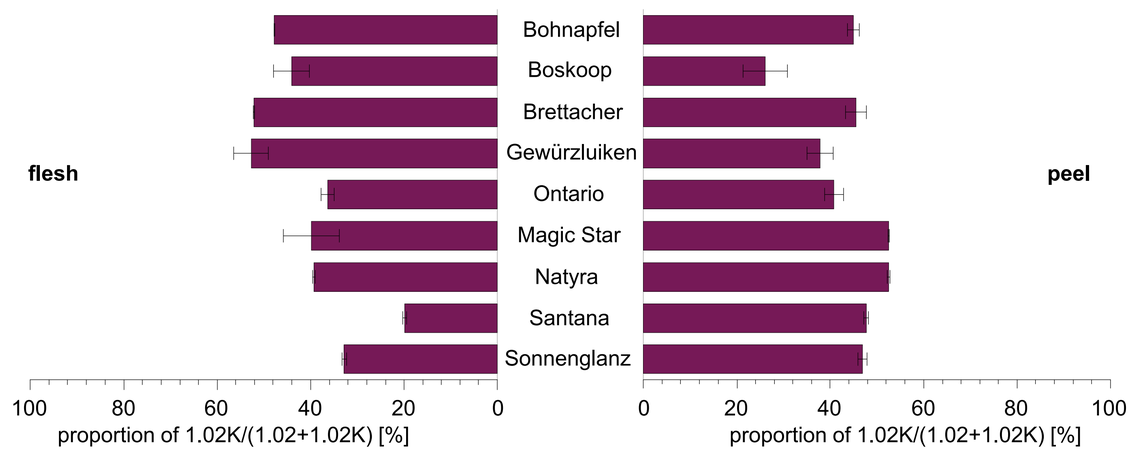
- Individual markers for isoallergens 1.01 and 1.02 and the combination marker for 1.01+1.02+1.05 suffer from a missed cleavage site, due to two successive lysines (K)



- Similar precursor charge and fragmentation patterns of isoallergene specific markers 1.02 and 1.02 with a second lysine at the end (1.02K)

  → This allows 1.02K quantification using isotopically labeled marker peptide 1.02*

  → Ratio of 1.02K/(1.02+1.02K) differs between varieties and tissues



- Precursor charges and fragmentation patterns differ between 1.01/1.01K and (K)1.01+1.02+1.05/1.01+1.02+1.05
- It is assumed that the ratio of missed cleavages is similar in the different isoallergens

  → Therefore, estimation of the total content of markers 1.01 and 1.01+1.02+1.05 is possible based on ratio of 1.02/1.02K

- Good agreement between allergen content quantified *via* global, combination and isoallergen specific markers (exception: 1.01+1.02+1.05) underlines suitability of the selected marker peptides
- Combination marker 1.04+1.05 was not detected in any of the samples, even through commercial peptides showed good flying properties

Ratio of the summed up isoallergene specific marker peptides to quantified combination and global markers [6]

Isoallergen(s)	Flesh (%)	Peel (%)
1.04+1.05	Not detected	Not detected
1.01+1.02	79.8 ± 9.4	71.5 ± 6.4
1.02+1.06	108.7 ± 14.3	92.7 ± 16.4
(1.01+1.02+1.05	*123.8 ± 37.8*	*111.3 ± 35.9)*
1.01+1.02+1.04+1.05	88.7 ± 9.3	79,6 ± 7.5
1.03+1.06.1.07+1.08+1.09	90.0 ± 9.9	88.2 ± 15.2

Summary

- Differences in the extractability between the varieties, harvest year, peel and flesh highlight the importance of determining the ESTD recovery for each sample individually
- Protein extractability from the samples needs to be considered in quantification
- Ratio of missed cleavages can be determined for 1.02 and 1.02K due to similarities in fragmentation pattern of precursor ions and transferred to ratio of 1.01K/1.01
- Good agreement between combination, global and isoallergene specific markers (exception: 1.01+1.02+1.05), demonstrates suitability of selected ISTD*s

References

[1] Wolfe et al. J Agric Food Chem (2003) 29;51(3):609. [2] Gao et al. BMC Plant Biol 2008;8:116. [3] Romer et al. Sci Rep (2020) 4;10(1):9144. [4] Siekierzynska et al. (2021) Int J Mol Sci. 29;22(7):3527. [5] Kaeswurm et al. (2020) Methods Protoc 27;4(1):3. [6] Kaeswurm et al. J Agric Food Chem (2022) 21;70(37):11813.

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