Poster # MP 188

Towards Development of a Matrix-independent Calibration Strategy for Targeted Quantification of Milk Allergens

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Background

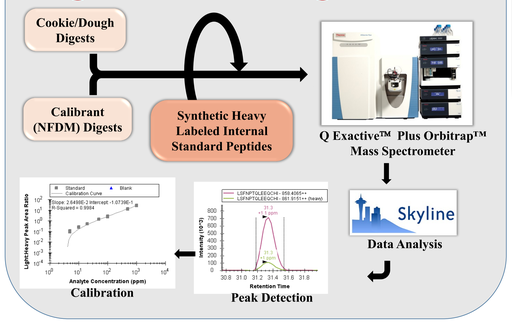
- Accurate quantification of food allergens is needed
- Calibration curves generated in matrix background will alleviate matrix interferences
- Matrix-matched calibration is impractical in food allergen analysis as the variety of products to be analyzed can be infinite
- A matrix-independent calibration approach may be useful where true matrix blanks are unavailable

Goal

Develop a matrix independent calibration using two inert proteins, porcine gelatin and egg white, as background proteomes for quantification of milk allergens in cookie/dough matrix

Workflow

High resolution targeted PRM



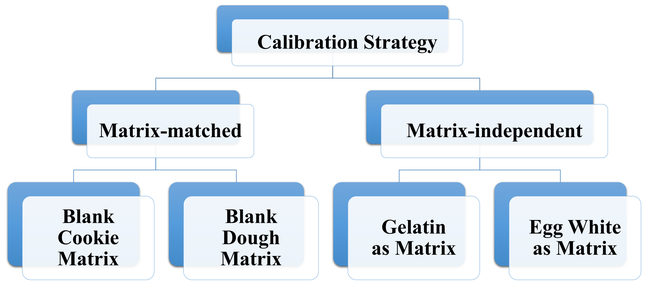
Milk Allergens: From detection to quantification

Targets

	Protein	Peptide
Whey	BLG	-.LIVTQTMK.G
		K.VLVLDTDYK.K
		R.LSFNPTQLEEQCHI.-
Casein	AS1-CN	R.FFVAPFPEVFGK.E
		R.YLGYLEQLLR.L
	AS2-CN	R.NAVPITPTLNR.E
	B-CN	K.VLPVPQK.A
		K.AVPYPQR.D
		R.GPFPIIV.-
	K-CN	K.YIPIQYVLSR.Y

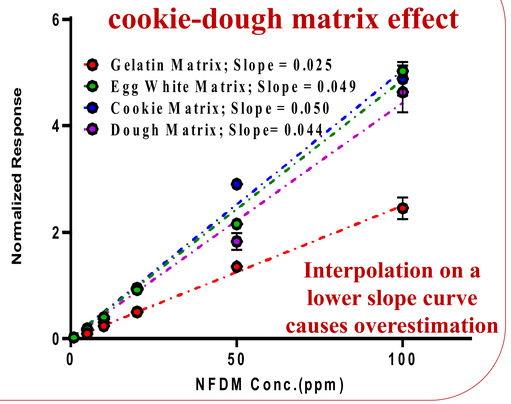
Targets both casein and whey

Calibration strategy



A matrix-independent strategy alleviates requirements for blank food matrices

Egg white as matrix replicates cookie-dough matrix effect



Results I

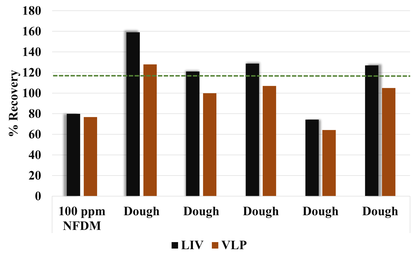
Quantitative recovery from 100 ppm cookie and dough

Peptides	Matrix-matched		Matrix-independent			
			Gelatin		Egg white	
	Cookie*	Dough	Cookie*	Dough	Cookie*	Dough
VLV	19.0%	104.6%	17.4%	115.7%	12.2%	98.6%
LSF	30.6%	125.0%	41.6%	113.5%	38.7%	129.0%
LIV	17.7%	97.7%	20.9%	165.4%	17.3%	101.7%
FFV	12.9%	114.2%	235.3%	2017.4%	13.2%	119.8%
YLG	12.7%	121.3%	11307.9%	77751.6%	13.3%	101.2%
YIP	22.5%	108.7%	264.5%	1260.3%	22.1%	142.1%
NAV	12.9%	95.7%	24.5%	159.1%	14.8%	101.4%
VLP	12.3%	101.8%	13.6%	104.6%	12.2%	108.6%
AVP	16.2%	95.1%	18.0%	103.7%	13.6%	103.2%
GPF	22.9%	104.8%	35.5%	137.9%	25.5%	110.3%

* Lower recovery in cookie is attributed to the thermal processing effect on milk allergen proteins

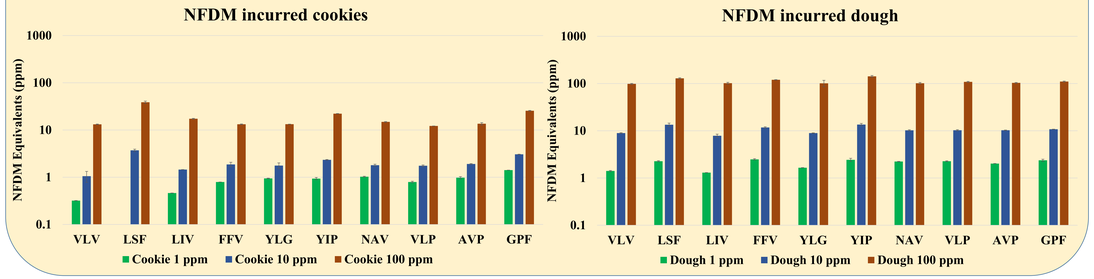
Calibration curves with egg white as background yield comparable recoveries as matrix-matched calibration curves

Presence of matrix during sample processing improves recovery of analytes



Results II

Quantification using Matrix-independent Calibration with Egg White



Conclusions

- Quantified casein and whey peptides with 98-129% accuracy by matrix-independent strategy using egg white as background
- Testing with other food matrices such as chocolates, fruit juices in future

Acknowledgements

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