Table S1. Preparation of binary mixtures of peanut in spelt wheat flours.

Mixture	%	mg/kg
S1	10	100,000
S2	1	10,000
S3	0.1	1,000
S4	0.05	500
S5	0.01	100
S6	0.005	50
S7	0.001	10
S8	0.0001	1
S9	0.00005	0.5
S10	0.00001	0.1

Table S2. Primers used for sequencing purposes.

Oligo	Sequence 5′→ 3′	Amplicon
trnH-psbA fw	ACATCCGCCCAAAGGAGAAAT	414
trnH-psbA rev	TCTGGTTTACCGCGTTAGGT	
rpl 16 fw	GCGATGGGAACGACGAAAAC	493
rpl 16 rev	ACGGCTCCTCGCGAATAAAA	
mat k fw	TGGACTCGCCTCTGGTCAT	392
mat k rev	CCAGATGGATAGGATAGGGTATTCG	
Ara h 6 fw	AGTACTCGATCCTCCGACCA	392
Ara h rev	AAGCCATAAGAGCACACCGAA	

Table S3. **Detection of mat K target by probe-based real-time PCR in untreated (control) and treated spiked samples.** DNA isolation protocol was DNeasy Plant Pro Kit (Qiagen, Protocol 1) for all samples.

Peanut quantity (mg/kg)	Control[1]	Boiling 60 min	DIC 7b 120s
100000	17.55 ± 0.17	18.69 ± 0.25 [ns]	24.15 ± 0.29
10000	21.52 ± 0.30	23.30 ± 0.30[ns]	28.27 ± 0.62
1000	24.17 ± 0.17	26.15 ± 0.28	31.51 ± 1.14
100	27.89 ± 0.14	28.33 ± 0.25 [ns]	34.62 ± 0.68
10	30.77 ± 0.25	33.36 ± 0.29	38.56 ± 0.33[†]
1	33.22 ± 0.20	34.17 ± 0.75	39.69 ± 0.25 (50%)[†]
0.5	32.74 ± 0.15[†]	36.47 ± 0.45	N.A.
0.1	33.87 ± 0.58[†]	N.A.	N.A.
Slope	-3.14	-3.17	-3.46
Efficiency (%)	108.30	106.73	94.43
R^2	0.995	0.982	0.995

Peanut quantity (mg/kg)	AU121[0]C 15 min	AU121[0]C 30 min	AU138[0]C 15 min	AU138[0]C 30 min
100000	20.93 ± 1.14	25.01 ± 0.28	29.50 ± 0.13	38.68 ± 0.89 (50%)
10000	24.84 ±1.20	29.55 ± 0.33	30.63 ± 0.05	38.92 ± 0.72 (50%)
1000	28.23 ± 0.67	33.28 ± 0.29	35.54 ± 0.06	39.41 ± 0.40 (50%)
100	30.87 ± 0.78	35.39 ± 0.43	37.09 ± 0.33[†]	39.70 ± 0.34 (25%)
10	32.75 ± 0.21[†]	37.93 ± 0.49[†]	39.6 ± 0.32 (75%)[†]	N.D.
1	34.79 ± 1.01[†]	39.87 ± 0.15[†] (25%)	N.D.	N.D.
Slope	-3.32	-3.48	-3.02	--
Efficiency (%)	100.05	93.65	114.23	--
R^2	0.993	0.975	0.885	--

[1]Ct±SE

[2]Percentage of positive amplification

N.A. Not assayed

N.D. Signal was not detected after 40 cycles of amplification

[†]Detection is possible but Ct is not in the calibration curve.

[ns] Not significant differences in mean Ct values compared to untreated control (t-student, p >0.05).

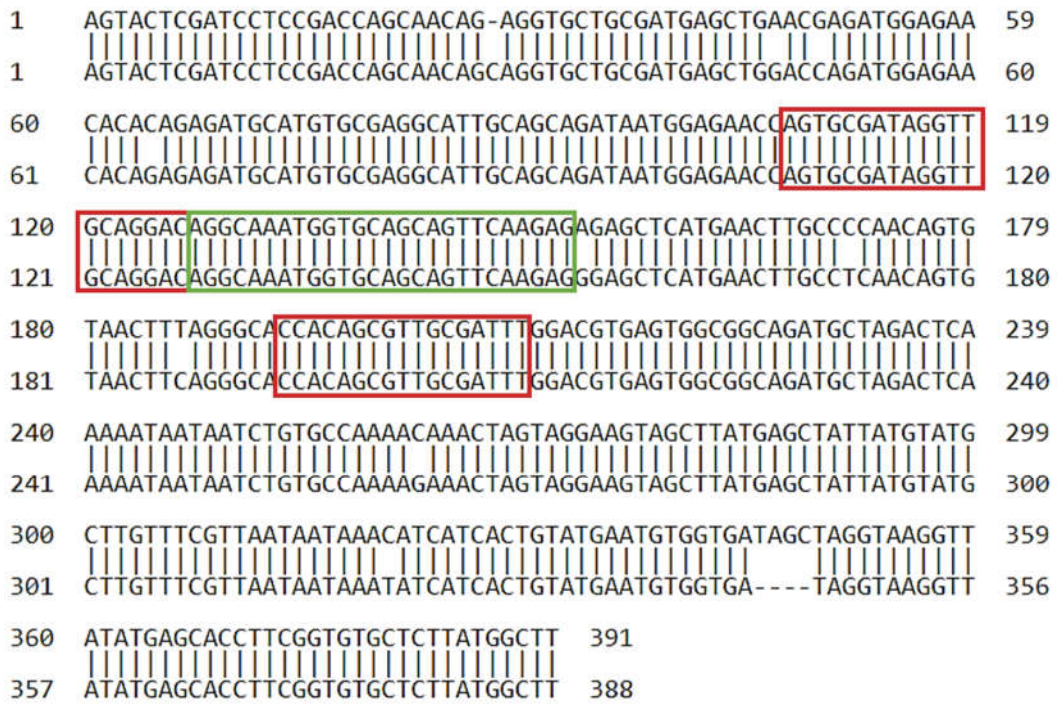

Figure S1. Sequence alignment of two clones of partial Ara h 6-allergen coding gene. Primers and probe designed for real-time PCR experiment are squared in red and green respectively.

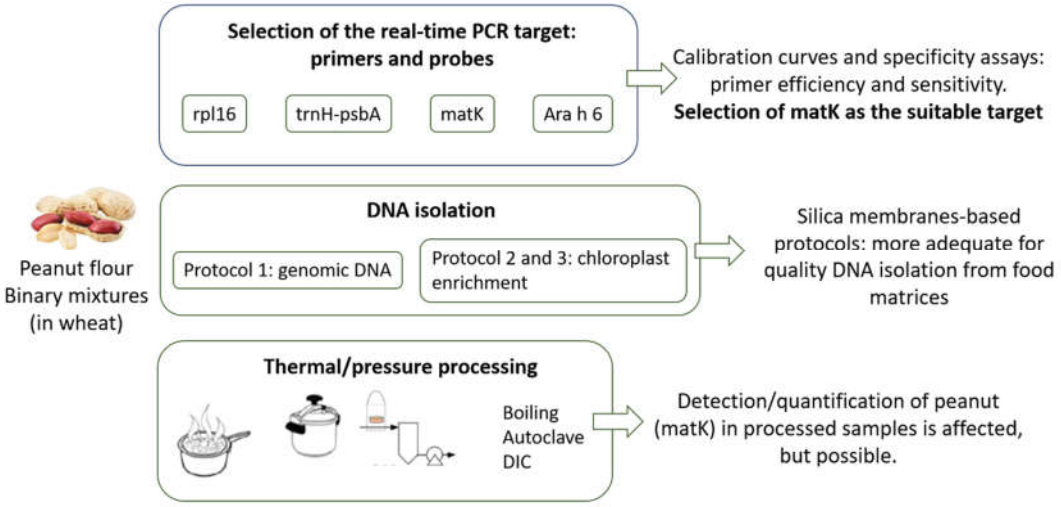

Figure S2. Workflow summarizing protocols, procedures, markers and the main findings of this study.