

**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.

<b>Oligo</b>	<b>Sequence 5' → 3'</b>	<b>Amplicon</b>
<b>trnH-psbA fw</b>	ACATCCGCCCAAAGGAGAAAT	414
<b>trnH-psbA rev</b>	TCTGGTTTACCGCGTTAGGT	
<b>rpl 16 fw</b>	GCGATGGGAACGACGAAAAC	493
<b>rpl 16 rev</b>	ACGGCTCCTCGCGAATAAAA	
<b>mat k fw</b>	TGGACTCGCCTCTGGTCAT	392
<b>mat k rev</b>	CCAGATGGATAGGATAGGGTATTCG	
<b>Ara h 6 fw</b>	AGTACTCGATCCTCCGACCA	392
<b>Ara h rev</b>	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 <sup>1</sup>	Boiling 60 min	DIC 7b 120s
100000	17.55 ± 0.17	18.69 ± 0.25 <sup>ns</sup>	24.15 ± 0.29
10000	21.52 ± 0.30	23.30 ± 0.30 <sup>ns</sup>	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 <sup>ns</sup>	34.62 ± 0.68
10	30.77 ± 0.25	33.36 ± 0.29	38.56 ± 0.33 <sup>†</sup>
1	33.22 ± 0.20	34.17 ± 0.75	39.69 ± 0.25 (50%) <sup>†</sup>
0.5	32.74 ± 0.15 <sup>†</sup>	36.47 ± 0.45	N.A.
0.1	33.87 ± 0.58 <sup>†</sup>	N.A.	N.A.
Slope	-3.14	-3.17	-3.46
Efficiency (%)	108.30	106.73	94.43
R <sup>2</sup>	0.995	0.982	0.995

Peanut quantity (mg/kg)	AU121°C 15 min	AU121°C 30 min	AU138°C 15 min	AU138°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 <sup>†</sup>	39.70 ± 0.34 (25%)
10	32.75 ± 0.21 <sup>†</sup>	37.93 ± 0.49 <sup>†</sup>	39.6 ± 0.32 (75%) <sup>†</sup>	N.D.
1	34.79 ± 1.01 <sup>†</sup>	39.87 ± 0.15 <sup>†</sup> (25%)	N.D.	N.D.
Slope	-3.32	-3.48	-3.02	--
Efficiency (%)	100.05	93.65	114.23	--
R <sup>2</sup>	0.993	0.975	0.885	--

<sup>1</sup>Ct±SE

<sup>2</sup>Percentage of positive amplification

N.A. Not assayed

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

<sup>†</sup>Detection is possible but Ct is not in the calibration curve.

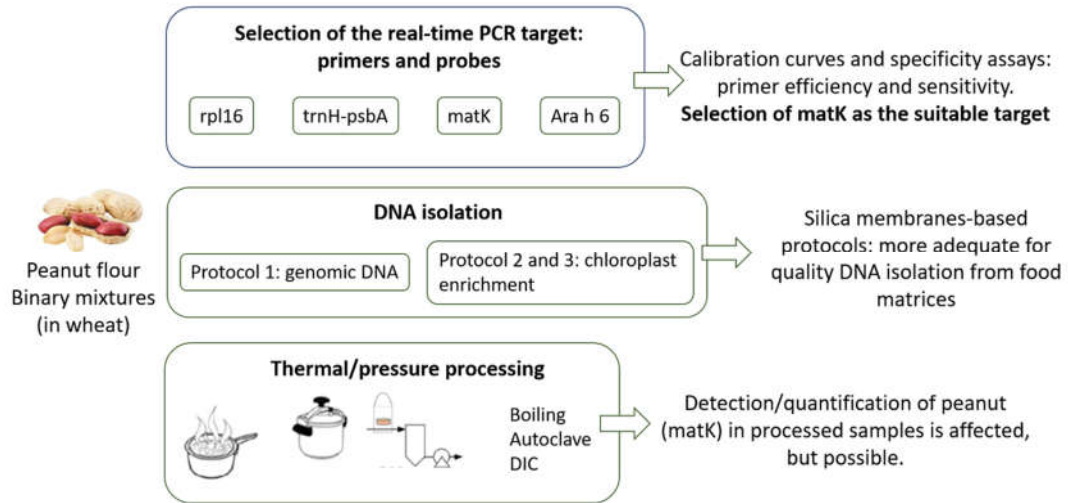
<sup>ns</sup> Not significant differences in mean Ct values compared to untreated control (t-student, p >0.05).

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1   AGTACTCGATCCTCCGACCAGCAACAG-AGGTGCTGCGATGAGCTGAACGAGATGGAGAA 59
1   AGTACTCGATCCTCCGACCAGCAACAGCAGGTGCTGCGATGAGCTGGACCAGATGGAGAA 60
60  CACACAGAGATGCATGTGCGAGGCATTGCAGCAGATAATGGAGAACCAGTGCGATAGGTT 119
61  CACAGAGAGATGCATGTGCGAGGCATTGCAGCAGATAATGGAGAACCAGTGCGATAGGTT 120
120 GCAGGACAGGCAAATGGTGCAGCAGTTCAAGAGAGAGCTCATGAACTTGCCCCAACAGTG 179
121 GCAGGACAGGCAAATGGTGCAGCAGTTCAAGAGGGAGCTCATGAACTTGCCTCAACAGTG 180
180 TAACTTTAGGGCAACACAGCGTTGCGATTGGACGTGAGTGGCGGCAGATGCTAGACTCA 239
181 TAACTTCAGGGCAACACAGCGTTGCGATTGGACGTGAGTGGCGGCAGATGCTAGACTCA 240
240 AAAATAATAATCTGTGCCAAAACAACTAGTAGGAAGTAGCTTATGAGCTATTATGTATG 299
241 AAAATAATAATCTGTGCCAAAAGAACTAGTAGGAAGTAGCTTATGAGCTATTATGTATG 300
300 CTTGTTTCGTTAATAATAAACATCATCACTGTATGAATGTGGTGATAGCTAGGTAAGGTT 359
301 CTTGTTTCGTTAATAATAAATATCATCACTGTATGAATGTGGTGA---TAGGTAAGGTT 356
360 ATATGAGCACCTTCGGTGTGCTCTTATGGCTT 391
357 ATATGAGCACCTTCGGTGTGCTCTTATGGCTT 388

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**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.