**Table 1 SM.** 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 2 SM. Primers used for sequencing purposes.

|  |  |  |
| --- | --- | --- |
| Oligo | Sequence 5’🡪 3’ | Amplicon size |
| 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 3SM. 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)** | **Control1** | **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 ± 0.30ns | | 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 | |
| R2 | | 0.995 | | 0.982 | | 0.995 | |
| **Peanut quantity (mg/kg)** | **AU121⁰C 15 min** | | **AU121⁰C 30 min** | | **AU138⁰C 15 min** | | **AU138⁰C 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 | | -- |
| R2 | 0.993 | | 0.975 | | 0.885 | | -- |

1Ct±SE

2Percentage 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 1SM. 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 2SM.** Workflow summarizing protocols, procedures, markers and the main findings of this study.