# Supporting Information

*Article*

# Precise and rapid authenticity of functional food components using optimized TaqMan real-time quantitative PCR

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**Figure S1.** Specific analysis of primer and probe. (A) Specificity comparison of primer and probe sequence for the ITS gene region developed using MEGA 4.0 for the specific qPCR detection of wild rice source component. (B) Specificity comparison of primer and probe sequences for the CIA-2 gene region developed for specific qPCR detection of chickpea source component using MEGA 4.0.

**Table S1.** Conditions of robustness test as an orthogonal design [a]

| Factors | Combination | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **Control** |
| qPCR instrument | A | A | A | A | B | B | B | B | B |
| qPCR reagent | X | X | Y | Y | X | X | Y | Y | X |
| Primer concentration (pmol/μL) | Unchanged | -20% | Unchanged | -20% | Unchanged | -20% | Unchanged | -20% | Unchanged |
| Probe concentration (pmol/μL) | Unchanged | -20% | -20% | Unchanged | -20% | Unchanged | Unchanged | -20% | Unchanged |
| Volume of reaction mixture (2×) | 13.5 μL | 13.5μL | 11.5 μL | 11.5μL | 13.5 μL | 13.5μL | 11.5 μL | 11.5μL | Unchanged |
| Annealing temperature | +1°C | - 1°C | +1°C | - 1°C | - 1°C | +1°C | - 1°C | +1°C | Unchanged |

[a] A : LightCycler® 480 II Real-Time Fluorescence PCR (Roche, Germany). B: QuantStudio6 Flex Real-Time Fluorescence PCR (ABI, USA). X: Premix Ex TaqTM Probe qPCR, RR390 A, TAKARA, China. Y: GoTaq qPCR Master Mix, A6101, Promega, USA.

**Table S2.** qPCR specific analysis results of quinoa, coix seed, wild rice and chickpea

| Samples | | Average Ct value | | | | |
|---|---|---|---|---|---|---|
| **Common name** | **Latin name** | **wild rice system** | **quinoa system** | **coix seed system** | **chickpea system** | **18S rRNA** |
| coix seed | *Coix lacryma-jobi* | negative | negative | 22.2 ± 1.02 | negative | 18.8 ± 0.08 |
| wild rice | *Zizania latifolia* | 22.9 ± 0.10 | negative | negative | negative | 17.4 ± 0.07 |
| wild rice | *Zizania palustris* | 23.7 ± 0.69 | negative | negative | negative | 17.8 ± 0.06 |
| wild rice | *Zizania aquatica* | 25.4 ± 0.60 | negative | negative | negative | 17.3 ± 0.04 |
| wild rice | *Zizania texana* | 21.9 ± 0.12 | negative | negative | negative | 17. 7 ± 0.07 |
| quinoa (white) | *Chenopodium quinoa Willd.* | negative | 26.2 ± 1.13 | negative | negative | 13.6 ± 0.13 |
| quinoa (red) | *Chenopodium quinoa Willd.* | negative | 26.1 ± 1.12 | negative | negative | 15.7 ± 0.07 |
| quinoa (black) | *Chenopodium quinoa Willd.* | negative | 26.4 ± 1.14 | negative | negative | 14.1 ± 0.11 |
| chickpea | *Cicer arietinum L.* | negative | negative | negative | 24.3 ± 0.79 | 16.3 ± 1.98 |
| sorghum rice | *Sorghum* | negative | negative | negative | negative | 16.5 ± 0.22 |
| sugarcane | *Saccharum officin* | negative | negative | negative | negative | 18.4 ± 0.14 |
| pseudo sorghum rice | *Sorghum halepense (L.) Pers* | negative | negative | negative | negative | 18.4 ± 0.15 |
| corn | *Zea mays L.* | negative | negative | negative | negative | 15.8 ± 0.11 |
| Chia seed | *Salvia Hispanica L.* | negative | negative | negative | negative | 13.9 ± 0.21 |
| red rice | *Lpomoea batatas (L.)* | negative | negative | negative | negative | 17.7 ± 0.08 |
| black kerneled rice | *Semen Trigone* | negative | negative | negative | negative | 14.8 ± 1.06 |
| rice | *Oryza sativa* | negative | negative | negative | negative | 14.5 ± 0.11 |
| coarse rice | *Panicum miliaceum* | negative | negative | negative | negative | 14.8 ± 0.07 |
| Rye | *Secale cereale L.* | negative | negative | negative | negative | 13.9 ± 2.33 |
| buckwheat | *Fagopyrum esculentum Moench.* | negative | negative | negative | negative | 15.0 ± 0.21 |
| tartary buckwheat | *Fagopyrum tataricum (L.) Gaertn.* | negative | negative | negative | negative | 16.6 ± 0.14 |
| oats | *Avena sativa L.* | negative | negative | negative | negative | 15.1 ± 0.37 |
| barley | *Hordeum vulgare L.* | negative | negative | negative | negative | 16.4 ± 0.10 |
| wheat | *Triticum aestivum L.* | negative | negative | negative | negative | 15.1 ± 0.05 |
| Semen sesami nigrum | *Sesamum radiatum* | negative | negative | negative | negative | 16.6 ± 0.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| black soya bean | *Glycine max (L.) merr.* | negative | negative | negative | negative | 14.1 ± 0.14 |
| kidney bean | *Phaseolus vulgaris* | negative | negative | negative | negative | 14.7 ± 0.08 |
| soybean | *Glycine max (Linn.) Merr.* | negative | negative | negative | negative | 14.8 ± 0.15 |
| Mung bean | *Vigna radiata (Linn.) Wilczek.* | negative | negative | negative | negative | 14.4 ± 0.07 |
| hyacinth bean | *Lablab purpureus (Linn.) Sweet* | negative | negative | negative | negative | 12.8 ± 0.02 |
| pea | *Pisum sativum L.* | negative | negative | negative | negative | 15.7 ± 0.05 |
| red bean | *Vigna angularis* | negative | negative | negative | negative | 12.5 ± 0.17 |
| agaric | *Auricularia auricula (L.ex Hook.)Unegativeerwood* | negative | negative | negative | negative | 14.8 ± 0.19 |
| Xianggu mushroom | *Lentinus edodes (Berkeley) Singer* | negative | negative | negative | negative | 14.6 ± 0.15 |

**Table S3.** linear equation of qPCR assay of quinoa, coix seed, wild rice anegative chickpea

| Event | Linear equation | $R^2$ | Efficiency (E)% |
|---|---|---|---|
| quinoa source component | y = 27.16 - 3.27 Log10 X | 0.9923 | 102.22% |
| coix seed source component | y = 26.59 - 3.35 Log10 X | 0.9984 | 98.74% |
| wild rice source component | y = 23.67 - 3.40 Log10 X | 0.9965 | 96.97% |
| chickpea source component | y = 26.82 - 3.38 Log10 X | 0.9989 | 97.80% |

**Table S4.** Robustness results of quinoa, coix seed, wild rice and chickpea systems

| Methods | Ct value of combination | | | | | | | | | | RSD (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Mean | Control | |
| Quinoa | 35.32 ± 0.11 | 35.43 ± 0.15 | 35.45 ± 0.11 | 35.38 ± 0.15 | 35.34 ± 0.04 | 35.36 ± 0.12 | 35.42 ± 0.15 | 35.47 ± 0.14 | 35.40 ± 0.05 | 35.27 ± 0.05 | 0.14 |
| Coix seed | 35.89 ± 0.14 | 35.92 ± 0.12 | 35.99 ± 0.11 | 35.81 ± 0.16 | 35.94 ± 0.16 | 35.92 ± 0.14 | 36.02 ± 0.14 | 35.96 ± 0.13 | 35.93 ± 0.06 | 35.93 ± 0.08 | 0.17 |
| Wild rice | 32.73 ± 0.14 | 32.72 ± 0.22 | 32.66 ± 0.15 | 32.74 ± 0.20 | 33.05 ± 0.18 | 33.01 ± 0.20 | 32.97 ± 0.11 | 32.99 ± 0.11 | 32.86 ± 0.16 | 32.82 ± 0.07 | 0.49 |
| Chickpea | 35.67 ± 0.10 | 35.67 ± 0.08 | 35.80 ± 0.12 | 35.81 ± 0.11 | 36.27 ± 0.52 | 36.00 ± 0.14 | 36.01 ± 0.19 | 36.05 ± 0.17 | 35.91 ± 0.21 | 35.77 ± 0.05 | 0.58 |

**Table S5.** Detection of commercial samples containing quinoa, coix seed, wild rice and chickpea

| Sample No. | Sample name | qPCR analysis results (Ct value) | | | |
|---|---|---|---|---|---|
| | | Quinoa | Coix seed | Wild rice | Chickpea |
| 1 | Coix seed (100%) | negative | 23.2 ± 0.08 | negative | negative |
| 2 | Red bean coix seed Yam powder | negative | 32.3 ± 0.12 | negative | negative |
| 3 | Coix seed red bean Meal replacement cake | negative | negative | negative | negative |
| 4 | Red bean coix seed pellet | negative | negative | negative | negative |
| 5 | Red bean coix seed Poria cocos cream | negative | 36.2 ± 0.11 | negative | negative |
| 6 | Red bean coix seed steamed bun | negative | 36.0 ± 0.04 | negative | negative |
| 7 | Red bean coix seed tea | negative | 33.4 ± 0.14 | negative | negative |
| 8 | Red bean coix seed Soft Canegativey | negative | 35.8 ± 0.21 | negative | negative |
| 9 | Coix seed noodle | negative | 37.5 ± 0.12 | negative | negative |
| 10 | Red bean coix seed Poria cocos tea | negative | 32.7 ± 0.10 | negative | negative |
| 11 | Chickpea (100%) | negative | negative | negative | 24.6 ± 0.09 |
| 12 | Chickpea noodle (30%) | negative | negative | negative | 27.3 ± 0.30 |
| 13 | Steamed and ready to eat chickpea chips (100%) | negative | negative | negative | 24.2 ± 0.08 |
| 14 | Refined pure raw chickpea leather (100%) | negative | negative | negative | 30.6 ± 0.19 |
| 15 | Canned Turkish chickpea (100%) | negative | negative | negative | 37.8 ± 0.07 |
| 16 | Canned chickpea (100%) | negative | negative | negative | 31.3 ± 0.15 |
| 17 | Fried chickpea (100%) | negative | negative | negative | 36.9 ± 0.21 |
| 18 | Quinoa Bolivia imports quinoa (100%) | 26.0 ± 0.17 | negative | negative | negative |
| 19 | Qinghai 3 colors quinoa (100%) | 26.8 ± 0.22 | negative | negative | negative |
| 20 | Quinoa soda biscuit (3.2%) | negative | negative | negative | negative |
| 21 | Quinoa wheat Germ Mixed Cereal (2%) | 36.7 ± 0.57 | negative | negative | negative |
| 22 | Quinoa powder (100%) | 26.2 ± 0.08 | negative | negative | negative |
| 23 | Canada No.1 Ice lake wild rice (100%) | negative | negative | 20.7 ± 0.14 | negative |
| 24 | Wild rice powder (100%) | negative | negative | 22.1 ± 0.04 | negative |