**SUPPORTING INFORMATION**

**Development and validation of a GC-MS method for soybean organ-specific metabolomics**

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**Appendix A. Supplementary data**

Table S1 Variation of peak area of same metabolites exacted by different solvent system in seed

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Figure S1 Total ion chromatograms of 10 different solvent systems for soybean seed (a) and pod (b).

Figure S2 Numbers of extracted peaks (a and c) and sum of relative peak area of common substances (b and d) of 10 different solvent systems for soybean seed and pod, respectively.

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Table S1 Variation of peak area of same metabolites exacted by different solvent system in seed

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Solvent System | | | | | | | | | | | | |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | CV(%) |
| Phenol | Total phenol | 0.261±0.003 | 0.240±0.008 | 0.183±0.002 | 0.143±0.003 | 0.274±0.015 | 0.154±0.003 | 0.317±0.013 | 0.375±0.013 | 0.210±0.006 | 0.223±0.008 | 28.798 |
| Catechin | 0.012±0.001 | 0.015±0.000 | 0.01±0.000 | 0.008±0.000 | 0.012±0.000 | 0.009±0.000 | 0.012±0.000 | 0.013±0.001 | 0.008±0.000 | 0.01±0.000 | 19.757 |
| Epicatechin | 0.249±0.003 | 0.225±0.008 | 0.173±0.002 | 0.135±0.003 | 0.262±0.014 | 0.145±0.003 | 0.306±0.013 | 0.361±0.013 | 0.202±0.006 | 0.213±0.009 | 29.519 |
| Alcohol | Total alcohol | 0.956±0.028 | 1.104±0.027 | 0.505±0.014 | 1.097±0.053 | 0.911±0.014 | 0.978±0.038 | 0.990±0.056 | 0.896±0.019 | 0.810±0.010 | 1.072±0.027 | 18.009 |
| myo-Inositol | 0.226±0.025 | 0.343±0.009 | 0.055±0.004 | 0.406±0.039 | 0.387±0.032 | 0.412±0.033 | 0.352±0.022 | 0.366±0.041 | 0.120±0.023 | 0.291±0.005 | 39.722 |
| D-Pinitol | 0.665±0.013 | 0.604±0.015 | 0.436±0.011 | 0.596±0.011 | 0.408±0.013 | 0.467±0.005 | 0.545±0.039 | 0.414±0.026 | 0.641±0.013 | 0.709±0.027 | 19.151 |
| allo-Inositol | 0.065±0.003 | 0.157±0.004 | 0.013±0.002 | 0.095±0.003 | 0.117±0.004 | 0.099±0.001 | 0.093±0.006 | 0.116±0.004 | 0.049±0.001 | 0.072±0.003 | 43.340 |
| Suger | Total suger | 6.599±0.080 | 5.880±0.072 | 1.832±0.036 | 6.907±0.069 | 6.038±0.127 | 6.108±0.036 | 6.691±0.250 | 7.948±0.159 | 6.546±0.071 | 4.084±0.054 | 27.833 |
| Galactose | 0.052±0.004 | 0.042±0.001 | 0.218±0.009 | 0.058±0.002 | 0.033±0.005 | 0.022±0.004 | 0.170±0.004 | 0.163±0.005 | 0.184±0.014 | 0.182±0.015 | 64.691 |
| Sucrose | 6.548±0.078 | 5.838±0.072 | 1.614±0.027 | 6.849±0.068 | 6.005±0.127 | 6.085±0.035 | 6.520±0.247 | 7.786±0.164 | 6.362±0.085 | 3.902±0.069 | 28.920 |
| Fatty acid | Total fatty acid | 0.211±0.011 | 0.369±0.006 | 0.259±0.005 | 0.206±0.008 | 0.263±0.006 | 0.117±0.002 | 0.108±0.005 | 0.079±0.003 | 0.159±0.007 | 0.126±0.003 | 44.775 |
| Palmitic acid | 0.139±0.004 | 0.271±0.006 | 0.136±0.003 | 0.158±0.009 | 0.194±0.005 | 0.072±0.001 | 0.048±0.003 | 0.040±0.003 | 0.085±0.005 | 0.056±0.002 | 58.719 |
| Octadecanoic acid | 0.043±0.005 | 0.033±0.001 | 0.045±0.001 | 0.036±0.002 | 0.038±0.002 | 0.026±0.001 | 0.025±0.002 | 0.024±0.002 | 0.032±0.002 | 0.030±0.001 | 20.972 |
| Linoleic acid | 0.029±0.005 | 0.065±0.000 | 0.078±0.004 | 0.012±0.001 | 0.032±0.002 | 0.019±0.001 | 0.036±0.000 | 0.016±0.000 | 0.041±0.003 | 0.040±0.002 | 54.218 |

Note: The table shows the relative peak area ± SE.

Table S2 Variation of peak area of same metabolites exacted by different solvent system in pod

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Solvent System | | | | | | | | | | | | |
|  |  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | CV(%) | |
| Acid | Total acid | 5.308±0.030 | 4.706±0.044 | 0.088±0.002 | 6.852±0.124 | 4.336±0.282 | 4.364±0.045 | 3.116±0.040 | 4.146±0.137 | 0.527±0.010 | 1.644±0.018 | 61.492 | |
| Malic acid | 3.207±0.023 | 2.751±0.028 | 0.058±0.002 | 2.996±0.04 | 2.957±0.101 | 2.765±0.011 | 2.225±0.032 | 2.717±0.006 | 0.408±0.006 | 1.191±0.001 | 53.998 | |
| Fumaric acid | 0.092±0.002 | 0.079±0.001 | 0.009±0.000 | 0.089±0.003 | 0.095±0.008 | 0.082±0.002 | 0.071±0.001 | 0.066±0.006 | 0.043±0.002 | 0.044±0.004 | 41.024 | |
| Phosphoric acid | 1.918±0.004 | 1.800±0.014 | 0.017±0.001 | 3.688±0.099 | 1.205±0.187 | 1.443±0.060 | 0.756±0.008 | 1.298±0.114 | 0.040±0.001 | 0.348±0.022 | 87.454 | |
| Glyoxylic acid | 0.092±0.002 | 0.075±0.001 | 0.005±0.000 | 0.078±0.002 | 0.078±0.003 | 0.074±0.001 | 0.063±0.003 | 0.065±0.002 | 0.036±0.001 | 0.061±0.001 | 40.053 | |
| Alcohol | Total alcohol | 10.642±0.243 | 9.252±0.285 | 7.805±0.017 | 7.920±0.119 | 9.172±0.181 | 6.831±0.125 | 9.281±0.072 | 8.650±0.117 | 6.569±0.069 | 7.382±0.029 | 15.251 | |
| Glycerol | 0.416±0.053 | 0.254±0.002 | 0.158±0.002 | 1.669±0.049 | 0.235±0.009 | 1.197±0.004 | 0.146±0.012 | 0.181±0.015 | 0.212±0.019 | 0.172±0.009 | 113.853 | |
| myo-Inositol | 0.528±0.062 | 0.324±0.006 | 0.058±0.004 | 0.422±0.021 | 0.296±0.010 | 0.356±0.004 | 0.276±0.002 | 0.544±0.048 | 0.155±0.003 | 0.123±0.006 | 53.056 | |
| D-Pinitol | 9.585±0.335 | 8.572±0.280 | 7.519±0.014 | 5.756±0.116 | 8.526±0.175 | 5.178±0.124 | 8.761±0.059 | 7.821±0.061 | 6.112±0.082 | 7.000±0.035 | 19.347 | |
| Arabitol | 0.016±0.001 | 0.015±0.002 | 0.012±0.000 | 0.013±0.000 | 0.024±0.003 | 0.012±0.000 | 0.012±0.000 | 0.017±0.001 | 0.012±0.000 | 0.009±0.000 | 28.745 | |
| Mannitol | 0.097±0.003 | 0.087±0.000 | 0.058±0.000 | 0.061±0.015 | 0.092±0.003 | 0.088±0.000 | 0.086±0.001 | 0.087±0.002 | 0.078±0.002 | 0.089±0.003 | 15.730 | |
| Suger | Total suger | 7.102±0.082 | 6.700±0.206 | 2.560±0.011 | 9.138±0.184 | 5.296±0.068 | 5.985±0.129 | 5.291±0.069 | 5.343±0.042 | 4.640±0.081 | 6.134±0.186 | 29.405 | |
| Galactose | 6.954±0.081 | 6.619±0.203 | 2.508±0.008 | 8.303±0.141 | 5.183±0.062 | 5.867±0.129 | 5.207±0.076 | 5.235±0.043 | 4.547±0.080 | 6.040±0.186 | 27.407 | |
| Lyxose | 0.148±0.002 | 0.081±0.003 | 0.052±0.004 | 0.834±0.050 | 0.113±0.006 | 0.118±0.001 | 0.084±0.007 | 0.108±0.001 | 0.093±0.001 | 0.094±0.004 | 135.479 | |
| Fatty acid | Total fatty acid | 1.094±0.068 | 1.154±0.017 | 0.561±0.023 | 0.839±0.013 | 0.996±0.034 | 0.784±0.003 | 0.956±0.022 | 0.848±0.008 | 1.165±0.047 | 0.523±0.012 | 25.379 | |
| Palmitic acid | 0.630±0.063 | 0.839±0.010 | 0.281±0.006 | 0.525±0.009 | 0.621±0.021 | 0.492±0.002 | 0.604±0.017 | 0.513±0.015 | 0.758±0.019 | 0.284±0.005 | 32.321 | |
| Octadecanoic acid | 0.463±0.013 | 0.315±0.008 | 0.280±0.017 | 0.315±0.005 | 0.375±0.013 | 0.292±0.002 | 0.353±0.005 | 0.335±0.009 | 0.407±0.031 | 0.239±0.007 | 19.404 | |

Note: The table shows the relative peak area ± SE

Table S3 Identified metabolites in soybean seed

|  |  |  |
| --- | --- | --- |
| Compound Name | RT(min) | Characteristic Ions |
| Butyro-1,4-lactam(1TMS) | 8.87 | 157 142 100 |
| Phosphoric acid monomethyl ester(2TMS) | 10.20 | 241 170 163 |
| Ethanolamine(3TMS) | 13.23 | 174 100 86 |
| Phosphoric acid(3TMS) | 13.85 | 299 211 133 |
| Proline(2TMS) | 14.45 | 143 142 216 |
| Glycine(3TMS) | 14.82 | 248 174 86 |
| 2-Piperidinecarboxylic acid(2TMS) | 17.04 | 230 156 84 |
| Serine(3TMS) | 17.28 | 218 204 100 |
| Malic acid(3TMS) | 22.53 | 245 233 133 |
| Asparagine[-H2O](2TMS) | 22.63 | 128 115 100 |
| Pyroglutamic acid(2TMS) | 23.54 | 258 230 156 |
| 4-amino-Butanoic acid(3TMS) | 23.70 | 304 174 100 |
| Glutamic acid(2TMS) | 23.89 | 174 158 84 |
| Citric acid(4TMS) | 34.83 | 347 273 211 |
| D-Pinitol(5TMS) | 35.29 | 260 217 191 |
| Fructose(1MEOX)(5TMS) | 37.17 | 307 217 103 |
| Galactose(1MEOX)(5TMS) | 37.62 | 319 217 205 |
| allo-Inositol(6TMS) | 39.24 | 318 217 191 |
| myo-Inositol(6TMS) | 43.32 | 305 217 191 |
| Linoleic acid(1TMS) | 46.04 | 129 95 81 |
| Octadecanoic acid(1TMS) | 46.92 | 145 129 117 |
| Sucrose(8TMS) | 58.67 | 361 217 103 |
| Epicatechin(5TMS) | 63.27 | 368 355 179 |
| Catechin(5TMS) | 63.85 | 368 355 179 |
| Kestose(11TMS) | 75.26 | 361 355 217 |

Note: TMS: Tetramethyl silane; MEOX: Methoxy; cell with colors indicate the key metabolites with a VIP score greater than 1 in PLS-DA

Table S4 Identified metabolites in soybean pod

|  |  |  |
| --- | --- | --- |
| Compound Name | RT(min) | Characteristic Ions |
| Glyoxylic acid(1MEOX)(1TMS) | 4.60 | 116 89 85 |
| Phosphoric acid monomethyl ester(2TMS) | 10.20 | 241 170 71 |
| Phosphoric acid(3TMS) | 13.83 | 299 211 133 |
| Fumaric acid(2TMS) | 16.46 | 143 133 83 |
| Malic acid(3TMS) | 22.50 | 245 233 133 |
| 1-Pyrroline-3-hydroxy-5-carboxylic-acid(2TMS) | 23.50 | 258 230 156 |
| Lyxose(1MEOX)(4TMS) | 30.04 | 307 217 103 |
| Lyxonic acid(5TMS) | 33.09 | 292 217 103 |
| Citric acid(4TMS) | 34.76 | 217 273 211 |
| D-Pinitol(5TMS) | 35.27 | 260 217 191 |
| Sorbose(1MEOX)(5TMS) | 36.81 | 307 217 103 |
| Galactose(1MEOX)(5TMS) | 37.63 | 319 217 205 |
| Mannitol(6TMS) | 38.47 | 319 217 103 |
| allo-Inositol(6TMS) | 39.22 | 318 217 191 |
| Hexadecanoic acid(1TMS) | 41.12 | 145 129 117 |
| myo-Inositol(6TMS) | 43.26 | 305 191 129 |
| Octadecanoic acid(1TMS) | 46.88 | 132 129 117 |

Note: TMS: Tetramethyl silane; MEOX: Methoxy; cell with colors indicate the key metabolites with a VIP score greater than 1 in PLS-DA



Figure S1 Total ion chromatograms of 10 different solvent systems for soybean seed (a) and pod (b). The X represents time and its unit is min. The Y axis represents 10 different solvents. The Z axis represents peak intensity and its unit is PA.

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Figure S2 Numbers of extracted peaks (a and c) and sum of relative peak area of common substances (b and d) of 10 different solvent systems (X axis) for soybean seed and pod, respectively. Different lowercase letters indicate the significant differences among 10 different solvents by Tukey’s test at P ＜ 0.05. Error bar show SE of the mean (three biological replicates).