# Antioxidant Activity and Hypoallergenicity of Egg Protein Matrices Containing Polyphenols from Citrus Waste

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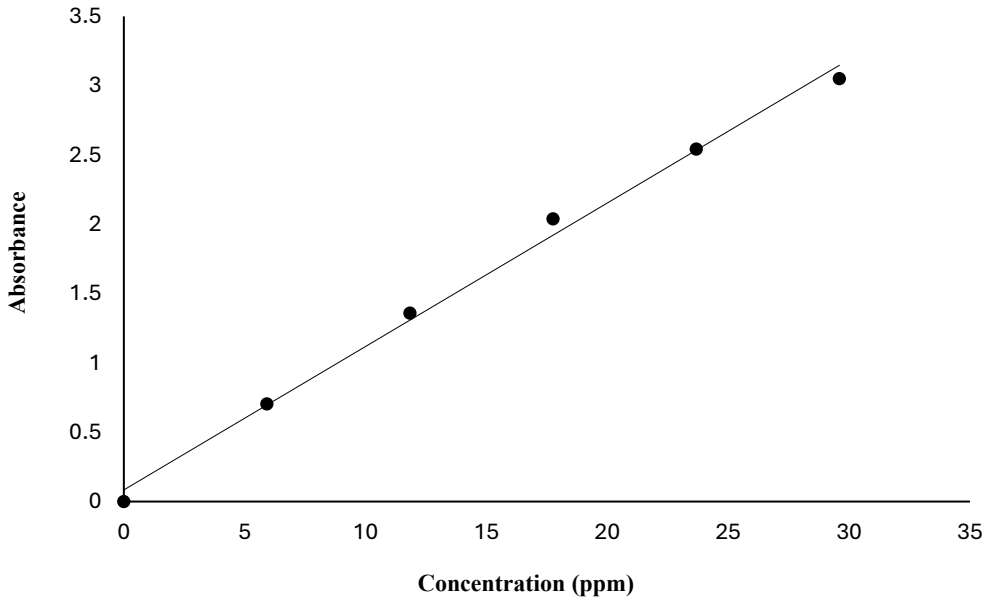


**Figure S1.** Linear relationship for determining the concentration of total polyphenolics in orange peels extracts.

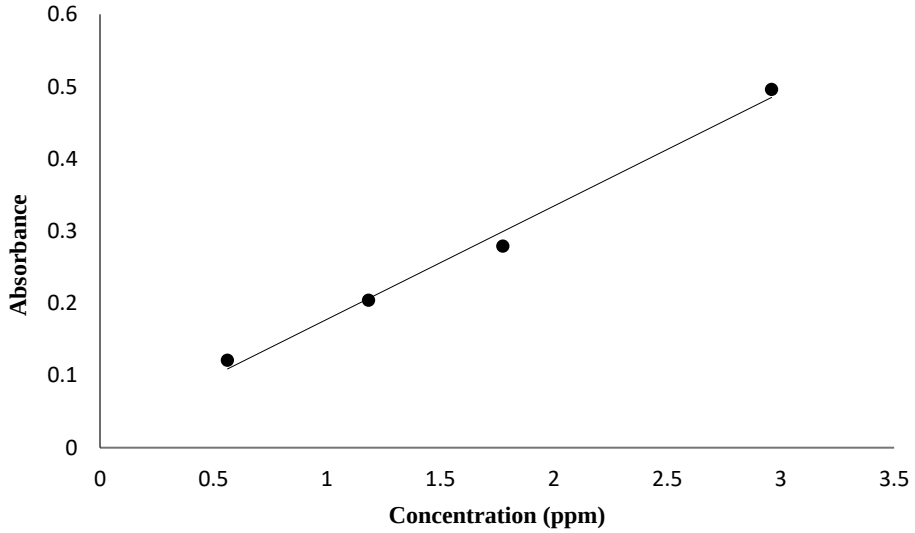


**Figure S2.** Linear regression for calculating the concentration of total polyphenolics in orange juice extracts.

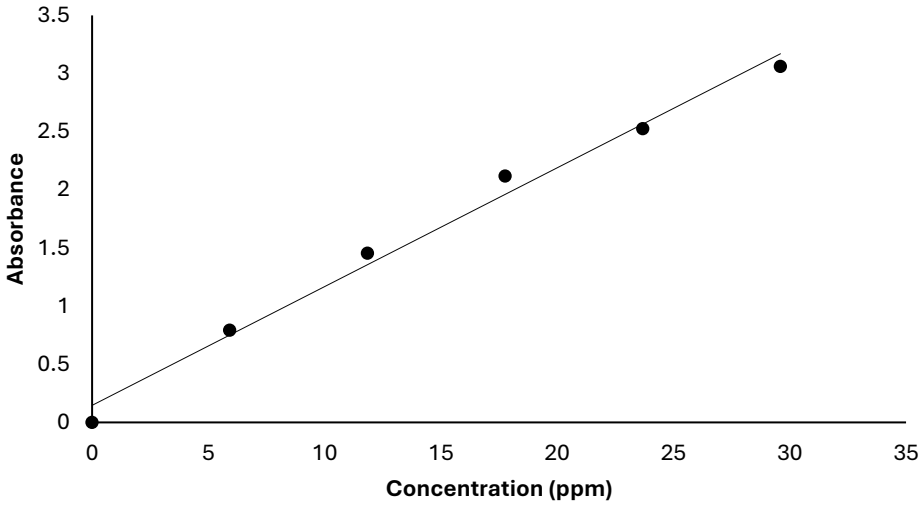


**Figure S3.** Linear relationship for determining the concentration of total polyphenolics in lemon peels extracts.

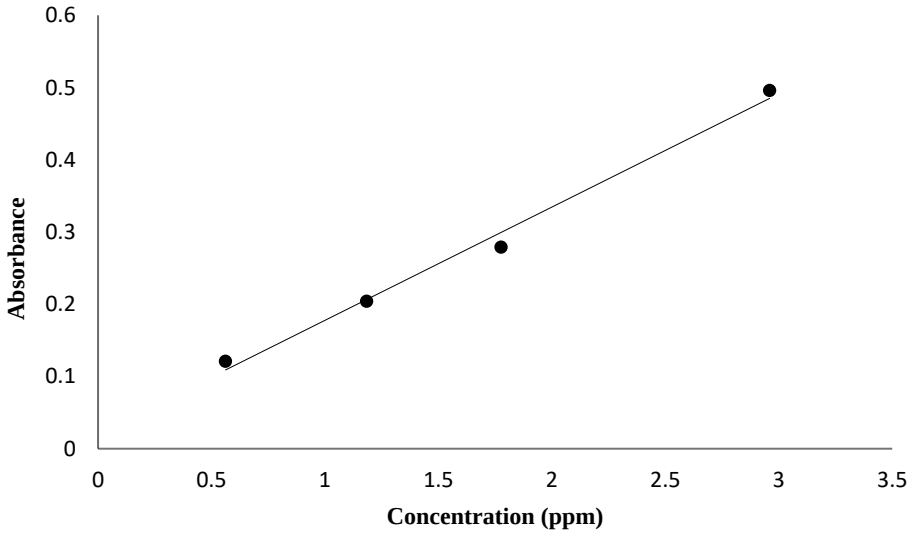


**Figure S4.** Linear regression for calculating the concentration of total polyphenolics in lemon juice extracts.

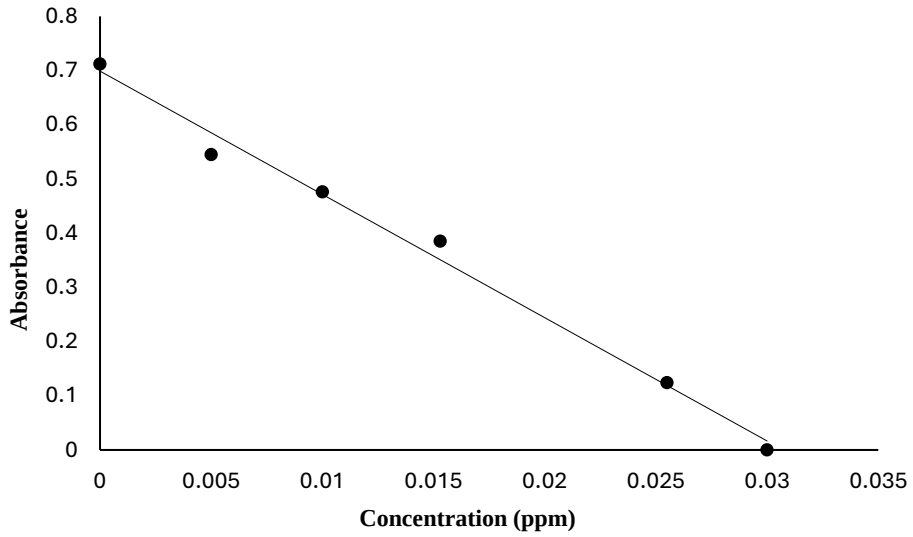


**Figure S5.** Linear plot obtained for antioxidant capacity reaction of orange peels extracts. The vitamin E analog Trolox was employed as external standard.

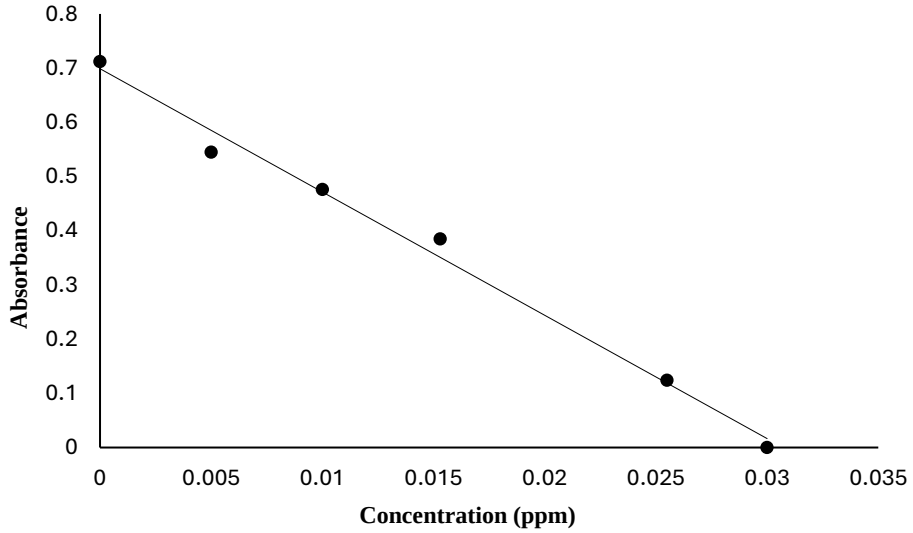


**Figure S6.** Linear plot obtained for antioxidant capacity reaction of lemon peels extracts. The vitamin E analog Trolox was employed as external standard.

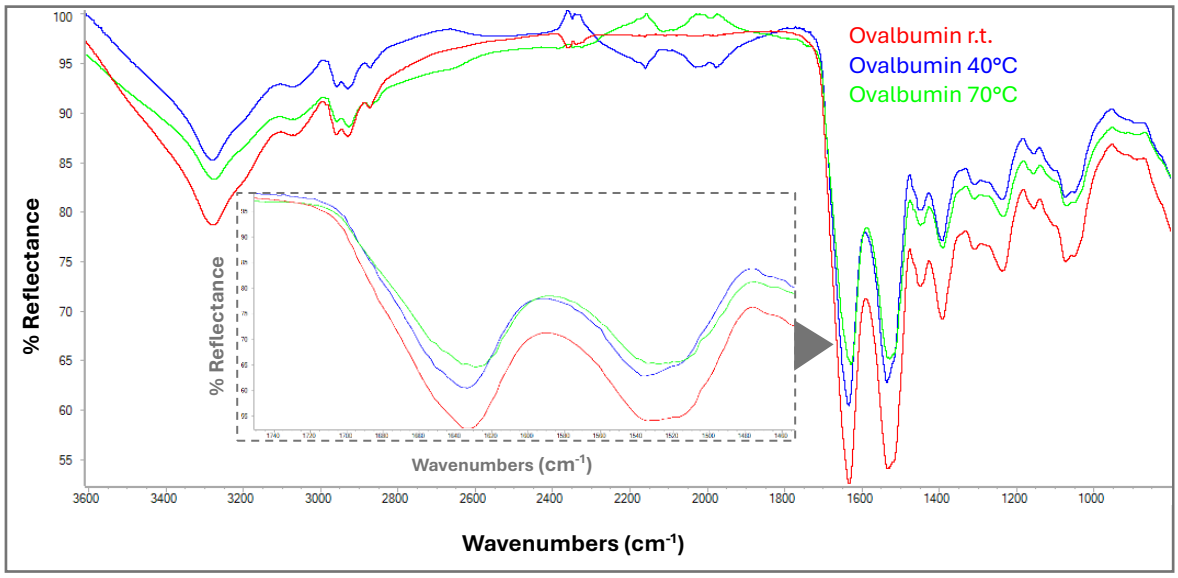


**Figura S7.** ATR-FTIR spectra recorded for ovalbumin at room temperature, 40 ºC, and 70 ºC. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

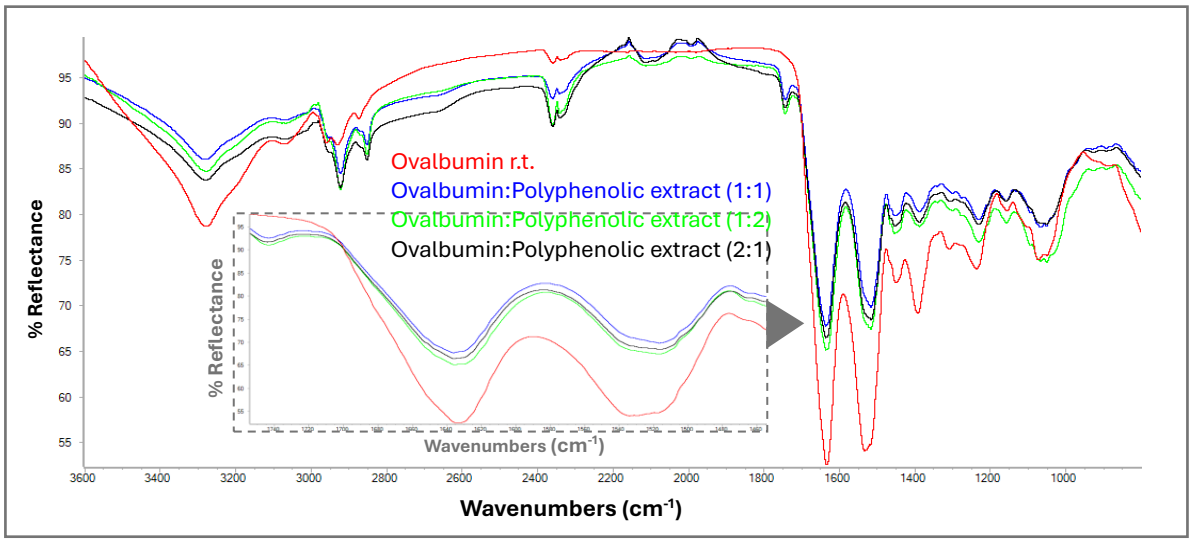


**Figura S8.** ATR-FTIR spectra of unmodified ovalbumin and its matrices with polyphenols from orange peels at room temperature. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

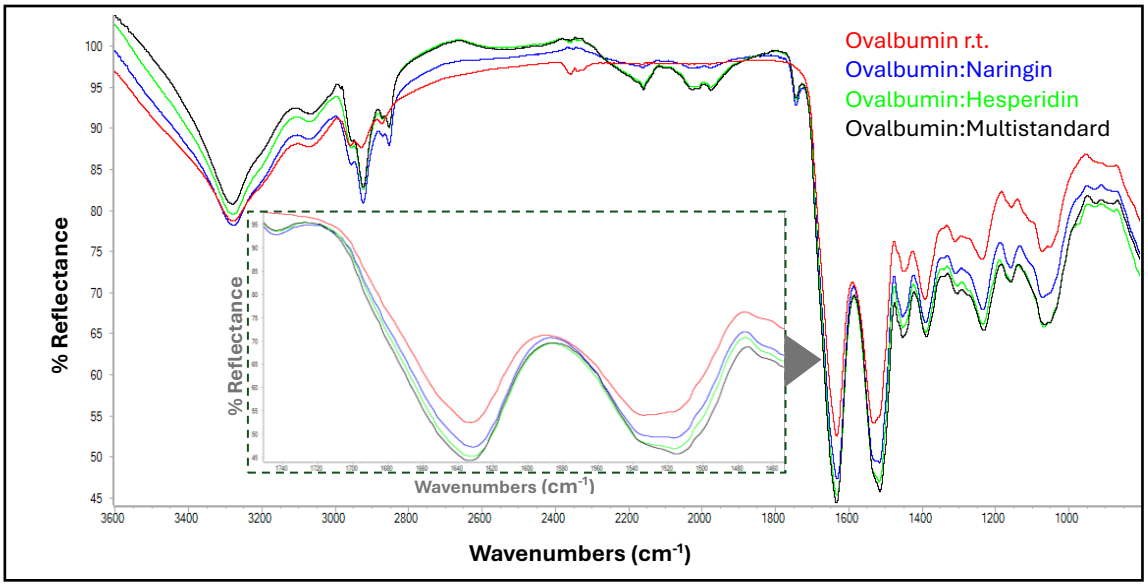


**Figura S9.** ATR-FTIR spectra of ovalbumin and its matrices with pure polyphenols and a multistandard solution. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

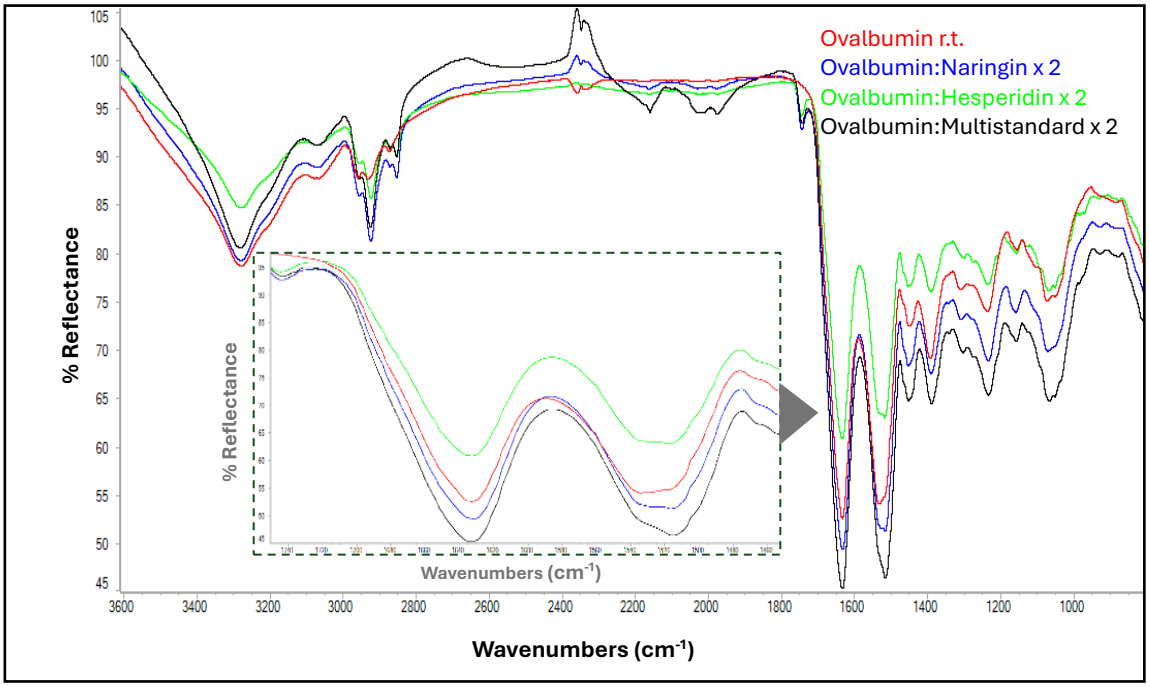


**Figura S10.** ATR-FTIR spectra of ovalbumin and its matrices with pure polyphenols and a multistandard solution with double concentrations of phenolics. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

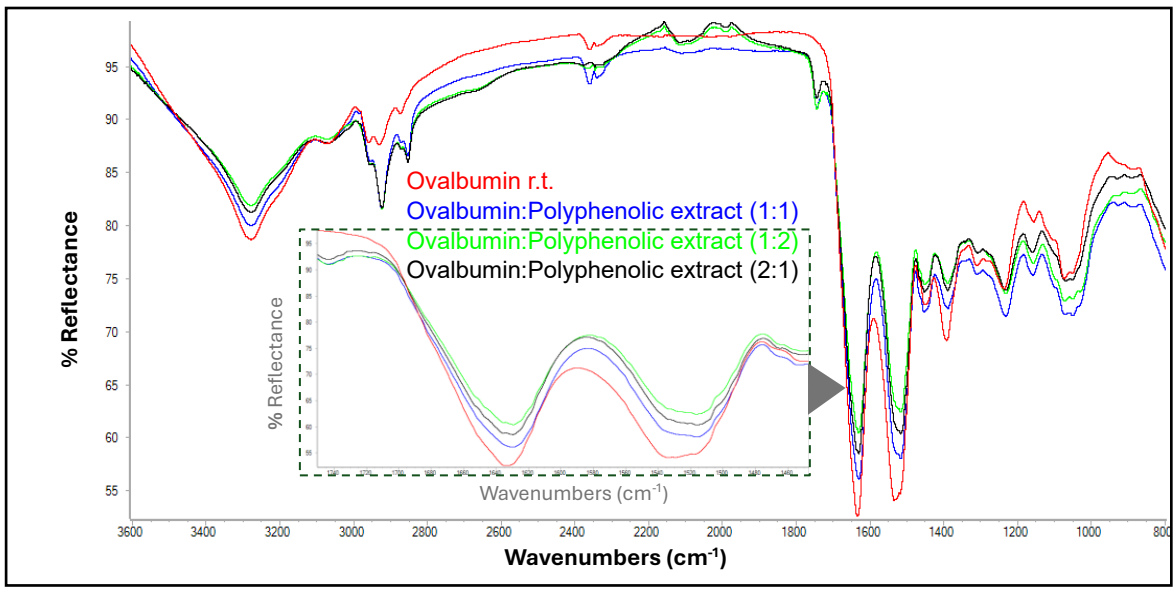


**Figure S11**. ATR-FTIR spectra of unmodified ovalbumin and its matrices with polyphenols from lemon peels at room temperature. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

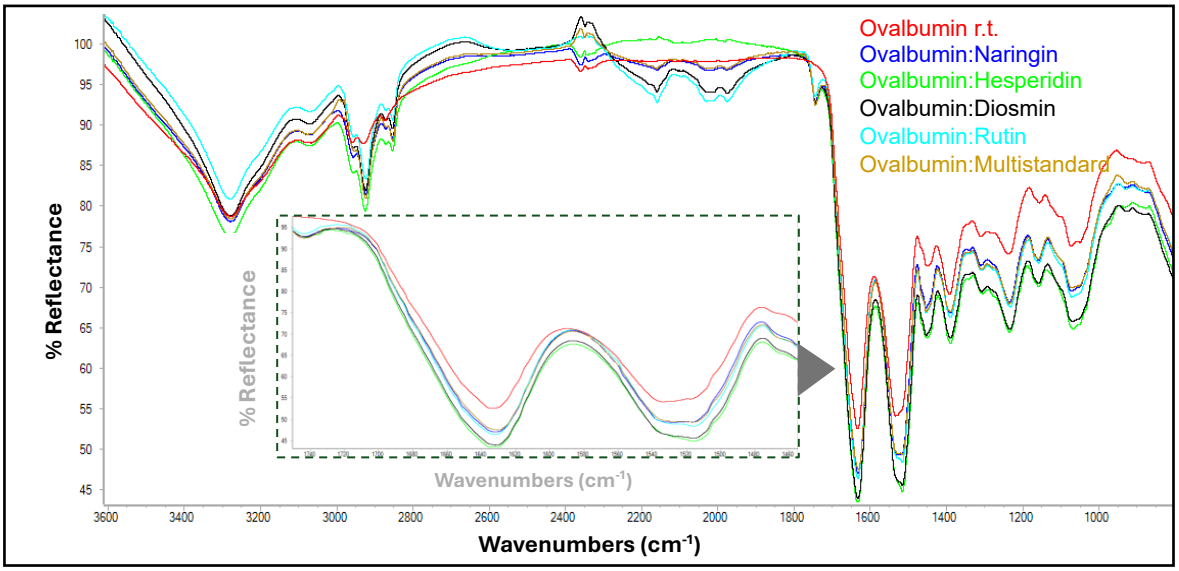


**Figure S12.** ATR-FTIR spectra of ovalbumin and its matrices with pure polyphenols and a multistandard solution. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

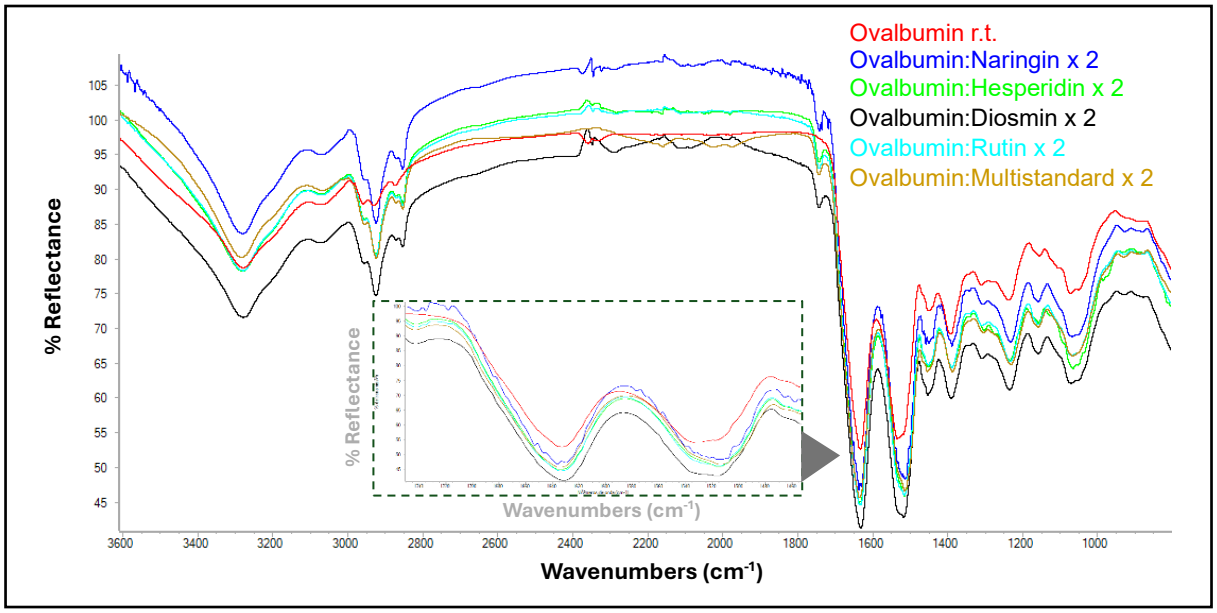


**Figure S13**. ATR-FTIR spectra of lysozyme and its matrices with pure polyphenols and a multistandard solution with double concentrations of phenolics. The inner inset shows an enlargement of the absorptions corresponding to amide I and II bands (1200-1800 $cm^{-1}$).

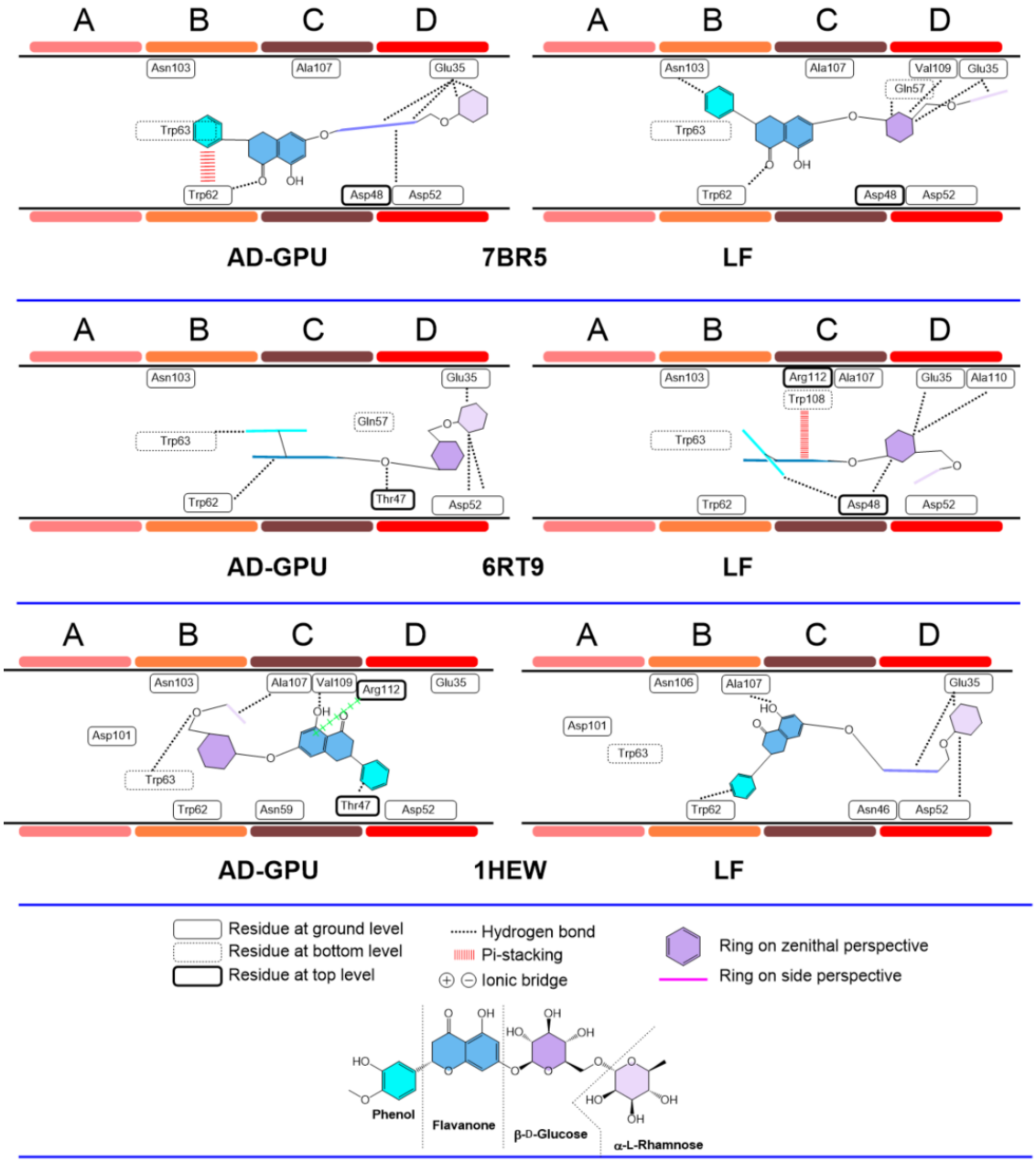


**Figure S14**. Hesperidin-lysozyme binding topologies (acidic pH) with 7BR5, 6RT9 and 1HEW, showing the relative position of the ligand to the ABCD pocket clefts.

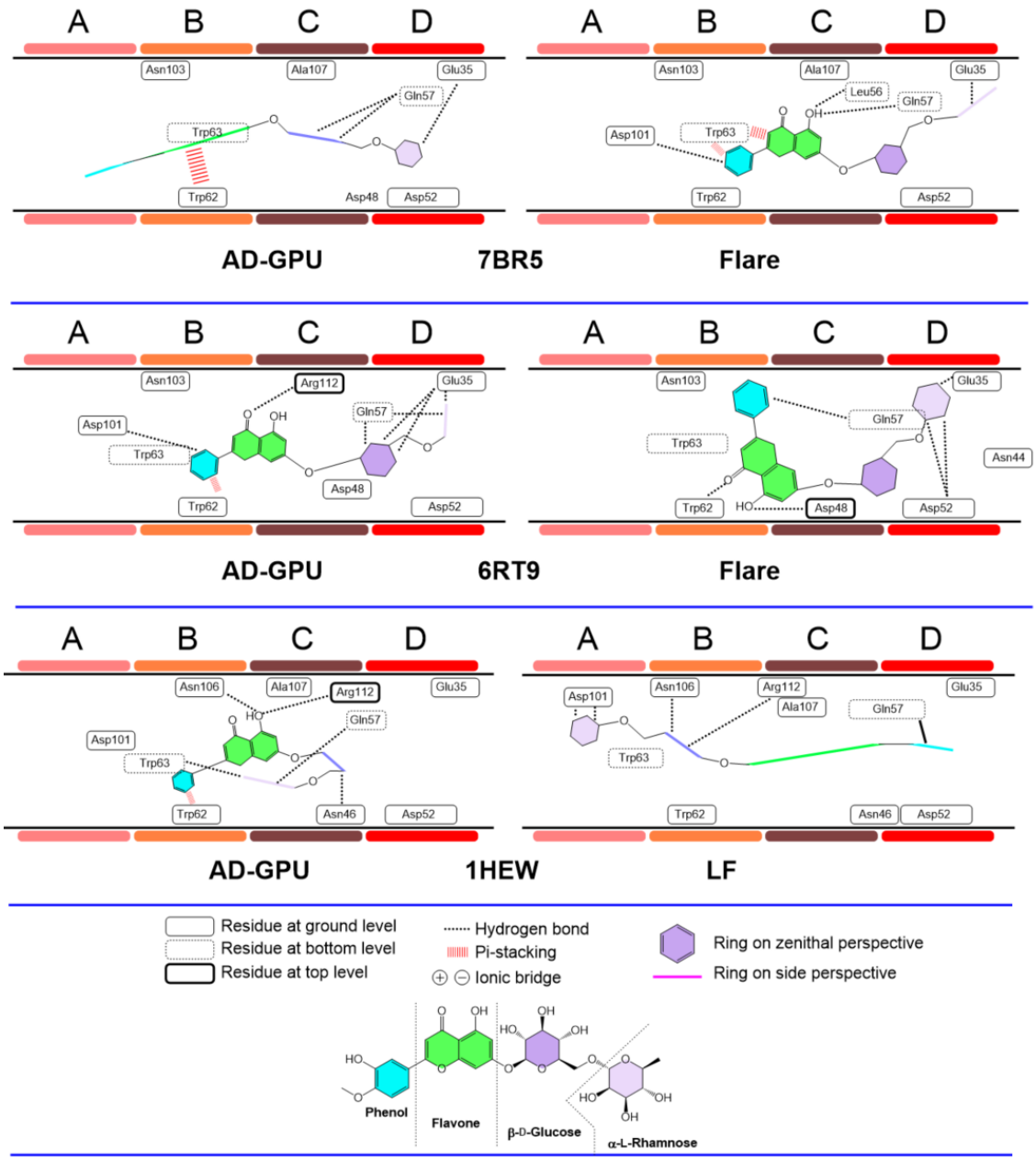


**Figure S15**. Diosmin-lysozyme binding topologies (acidic pH) with 7BR5, 6RT9 and 1HEW, showing the relative position of the ligand to the ABCD pocket clefts.

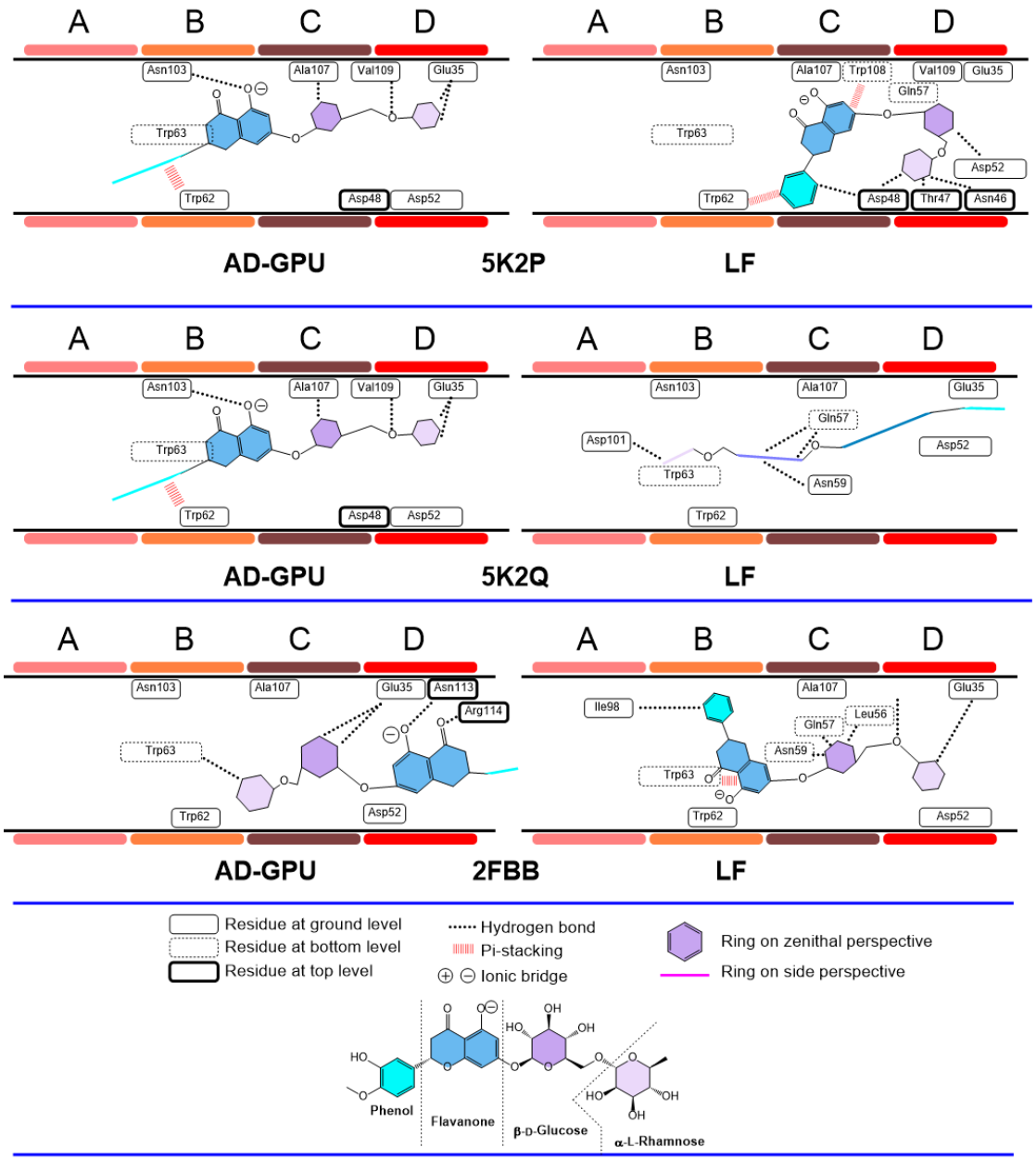


**Figure S16**. Hesperidin-lysozyme binding topologies (basic pH) with 5K2P, 5K2Q and 2FBB, showing the relative position of the ligand to the ABCD pocket clefts.

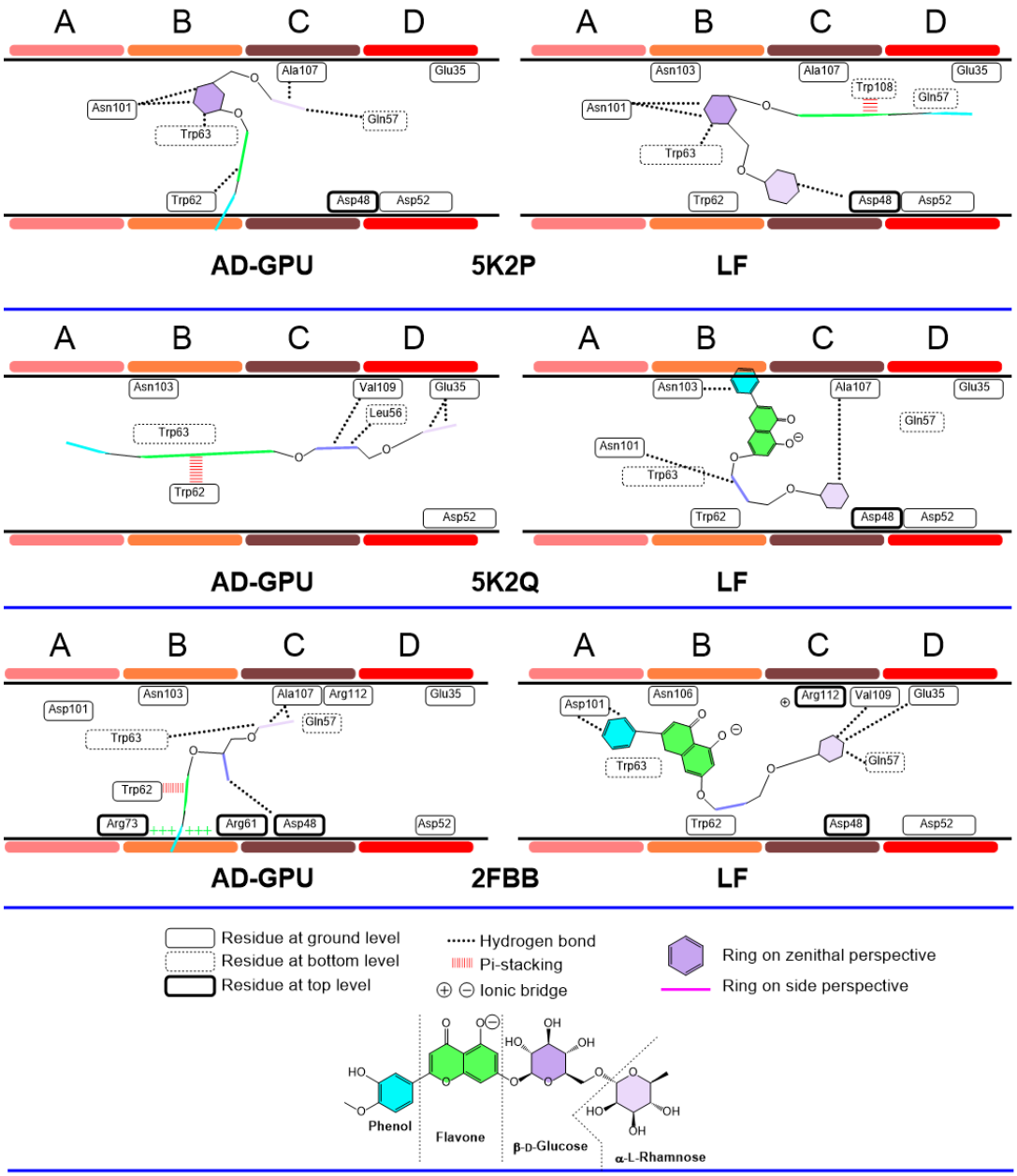


**Figure S17**. Diosmin-lysozyme binding topologies (basic pH) with 5K2P, 5K2Q and 2FBB, showing the relative position of the ligand to the ABCD pocket clefts.

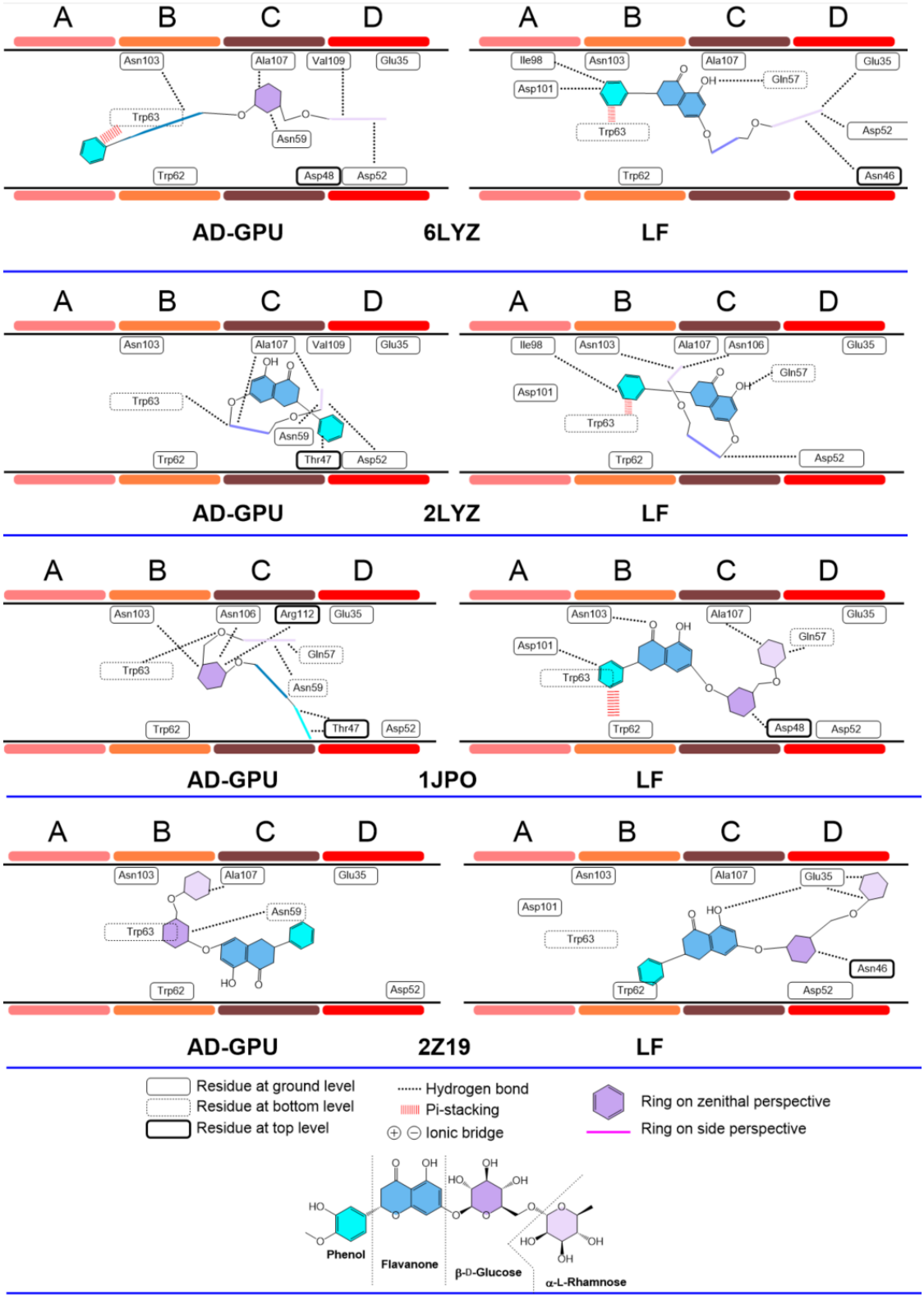


**Figure S18**. Hesperidin-lysozyme binding topologies (neutral pH, non-ionized ligand) with 2LYZ, 6LYZ, 1JPO and 2Z19, showing the relative position of the ligand to the ABCD pocket clefts.

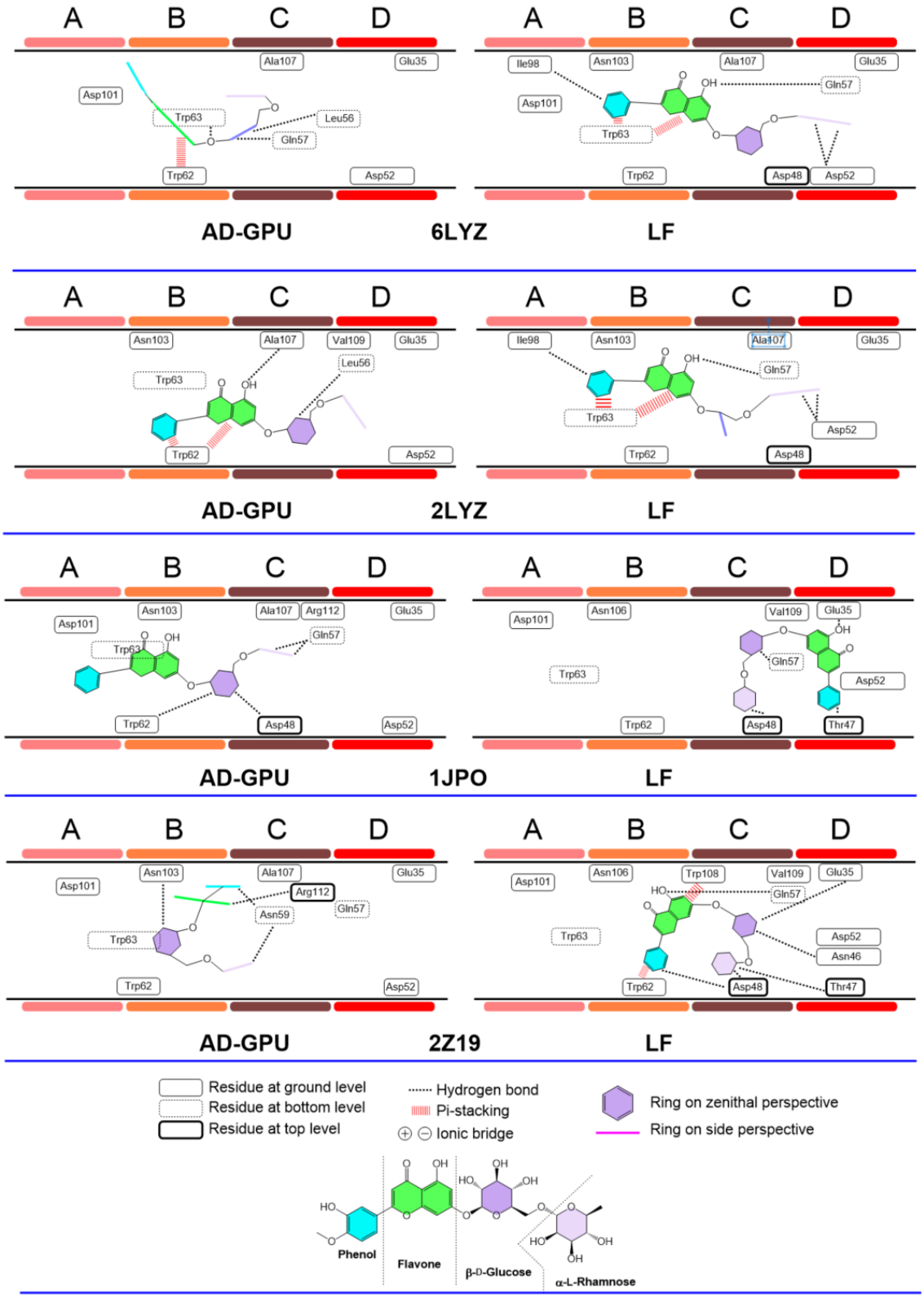


**Figure S19**. Diosmin-lysozyme binding topologies (neutral pH, non-ionized ligand) 2LYZ, 6LYZ, 1JPO and 2Z19, showing the relative position of the ligand to the ABCD pocket clefts.

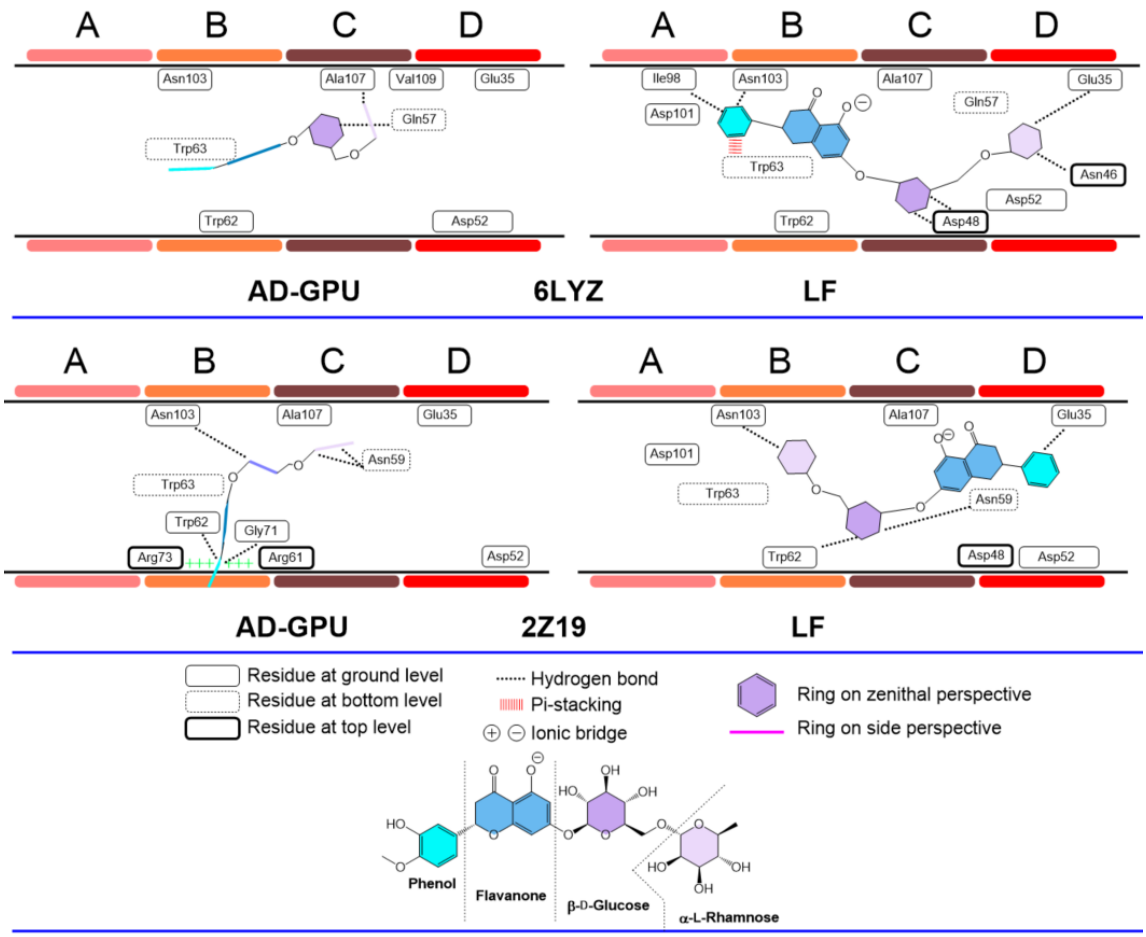


**Figure S20**. Hesperidin-lysozyme binding topologies (neutral pH, ionized ligand) with 6LYZ and Z19, showing the relative position of the ligand to the ABCD pocket clefts.

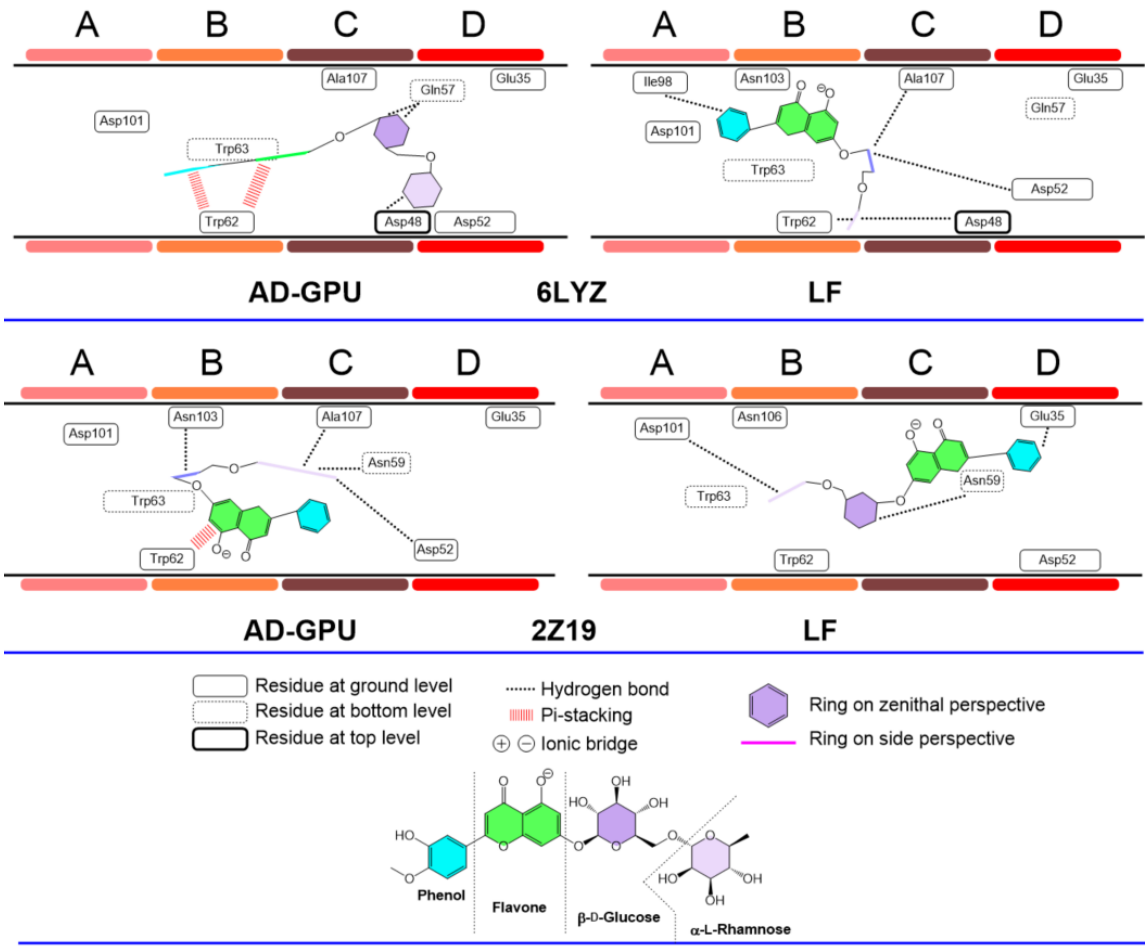


**Figure S21**. Diosmin-lysozyme binding topologies (neutral pH, non-ionized ligand) 6LYZ and 2Z19, showing the relative position of the ligand to the ABCD pocket clefts.

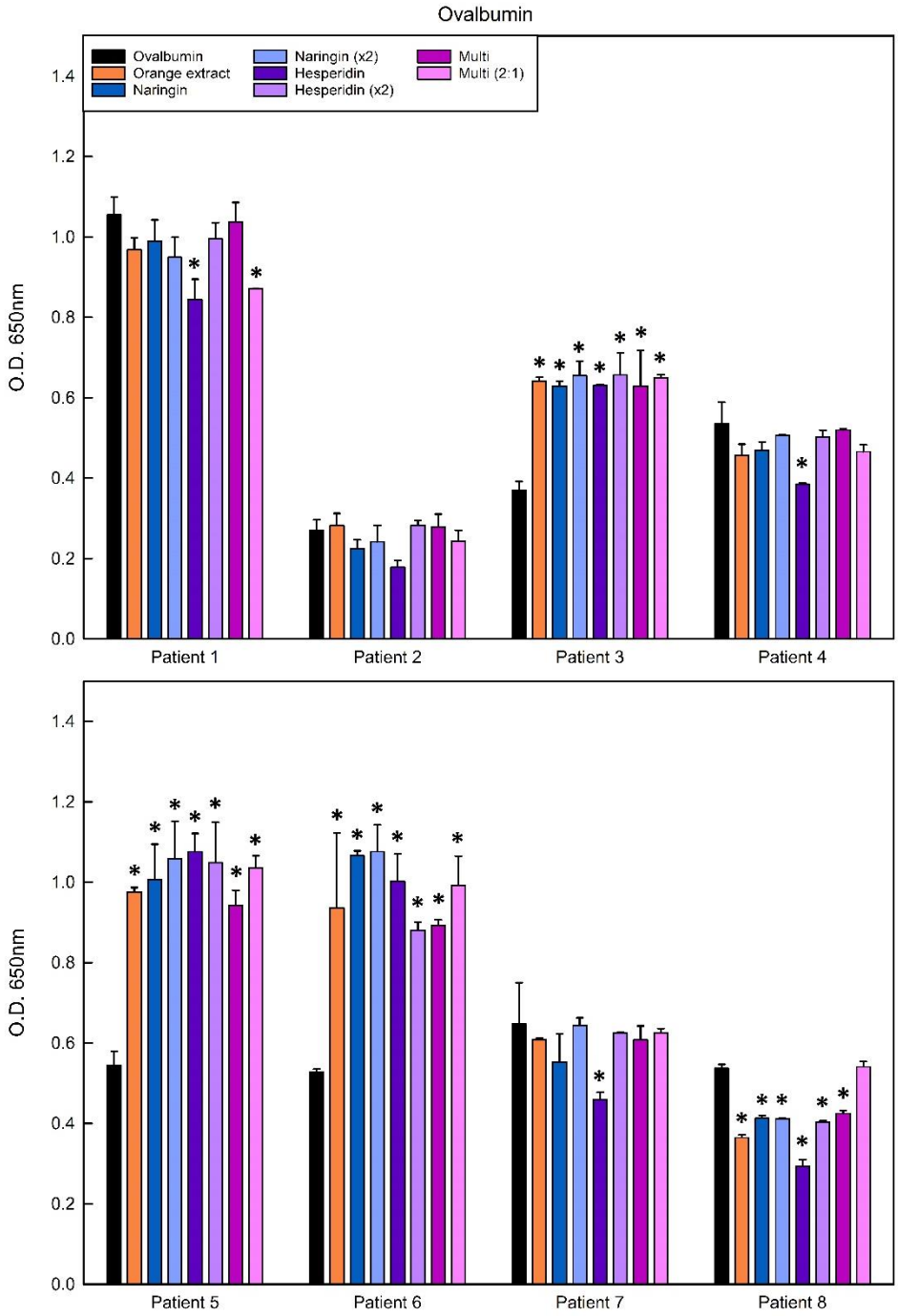


**Figure S22.** Values observed from indirect ELISA with diluted sera from eight patients allergic to hen egg ovalbumin, using the native protein as positive control and different protein-polyphenol present in orange peel matrices. Statistically significant differences between the matrices and the native protein were analysed by one-way ANOVA and are indicated by an asterisk ($p < 0.05$).

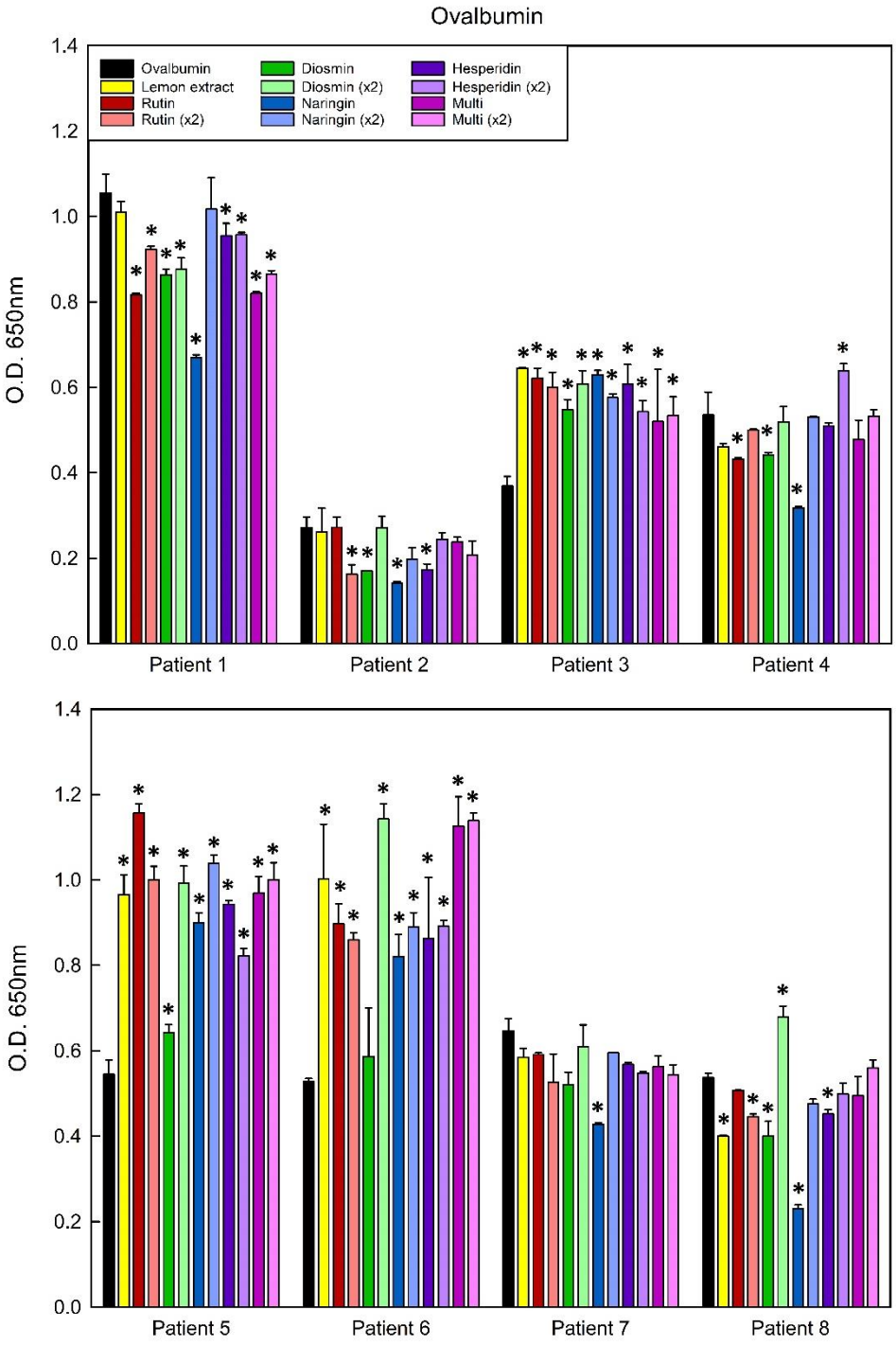


**Figure S23.** Resulting indirect ELISA plot with diluted sera from eight patients allergic to hen egg lysozyme, using the native protein as a positive control and different protein-polyphenol matrices from lemon peel. Statistically significant differences between the matrices and the native protein were analysed by one-way ANOVA and are indicated by an asterisk ($p < 0.005$).

**Table S1.** Identification and quantitative analysis of phenolics from orange peel extract in the supernatant after matrix formation with ovoalbumin at r.t.[a]

| Polyphenol | Concentration in extract (mg/g extract)[b] | Concentration in supernatant (mg/g extract)[b] | % Interaction |
|---|---|---|---|
| **Ratio 1:1** | | | |
| **Naringin** | 8.079 ± 0.029 | 3.376[c] ± 0.018 | 58.2 |
| **Hesperidin** | 17.442 ± 0.039 | 6.668[c] ± 0.022 | 61.8 |
| **Ratio 1:2** | | | |
| **Naringin** | 8.079 ± 0.029 | 5.084[c] ± 0.018 | 37.0 |
| **Hesperidin** | 17.442 ± 0.039 | 10.133[c] ± 0.006 | 41.9 |
| **Ratio 2:1** | | | |
| **Naringin** | 8.079 ± 0.029 | 3.041[c] ± 0.019 | 62.4 |
| **Hesperidin** | 17.442 ± 0.039 | 6.114[c] ± 0.020 | 64.9 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentration in extract.

**Table S2.** Identification and quantitative analysis of phenolics from lemon peel extract in the supernatant after matrix formation with ovoalbumin at r.t.[a]

| Polyphenol | Concentration in extract (mg/g extract)[b] | Concentration in supernatant (mg/g extract)[b] | % Interaction |
|---|---|---|---|
| **Ratio 1:1** | | | |
| **Naringin** | 0.563 ± 0.038 | 0.345[c] ± 0.044 | 38.7 |
| **Hesperidin** | 9.662 ± 0.087 | 5.561[c] ± 0.037 | 42.4 |
| **Eriocitrin** | 2.627 ± 0.071 | 1.487[c] ± 0.016 | 43.4 |
| **Rutin** | 0.130 ± 0.023 | 0.076[c] ± 0.007 | 41.5 |
| **Diosmin** | 0.115 ± 0.011 | 0.051[c] ± 0.002 | 55.6 |
| **Ratio 1:2** | | | |
| **Naringin** | 0.563 ± 0.038 | 0.349[c] ± 0.017 | 38.0 |
| **Hesperidin** | 9.662 ± 0.087 | 5.826[c] ± 0.024 | 39.7 |
| **Eriocitrin** | 2.627 ± 0.071 | 1.557[c] ± 0.029 | 40.7 |
| **Rutin** | 0.130 ± 0.023 | 0.0727[c] ± 0.007 | 44.1 |
| **Diosmin** | 0.115 ± 0.011 | 0.058[c] ± 0.007 | 49.6 |
| **Ratio 2:1** | | | |
| **Naringin** | 0.563 ± 0.038 | 0.265[c] ± 0.022 | 52.9 |
| **Hesperidin** | 9.662 ± 0.087 | 3.078[c] ± 0.037 | 68.1 |
| **Eriocitrin** | 2.627 ± 0.071 | 0.815[c] ± 0.012 | 69.0 |
| **Rutin** | 0.130 ± 0.023 | 0[c] | 100 |
| **Diosmin** | 0.115 ± 0.011 | 0[c] | 100 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentration in extract.

**Table S3.** Quantitative analysis of phenolics in supernatants after matrix formation.[a]

| Polyphenol | | Concentration in starting solution (mg/L)[b] | Concentration in the supernatant (mg/L)[b] | % Interaction |
|---|---|---|---|---|
| **Naringin** | | 36.04 ± 0.073 | 39.094 ± 0.011 | - |
| **Hesperidin** | | 78.367 ± 0.478 | 13.930[c] ± 0.057 | 82.2 |
| **Multistandard solution** | **Naringin** | 36.233 ± 0.597 | 28.705[c] ± 0.018 | 20.8 |
| | **Hesperidin** | 78.447 ± 0.048 | 8.925[c] ± 0.074 | 88.6 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentrations.

**Table S4.** Quantitative analysis of phenolics (double concentration) in supernatants after matrix formation.[a]

| Polyphenol | | Concentration in starting solution (mg/L)[b] | Concentration in the supernatant (mg/L)[b] | % Interaction |
|---|---|---|---|---|
| **Naringin** | | 72.735 ± 0.352 | 96.372 ± 0.008 | - |
| **Hesperidin** | | 157.104 ± 0.006 | 16.753[c] ± 0.026 | 89.3 |
| **Multistandard solution** | **Naringin** | 71.922 ± 0.080 | 70.119[c] ± 0.034 | 2.5 |
| | **Hesperidin** | 156.011 ± 0.150 | 13.659[c] ± 0.054 | 91.2 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentrations.

**Table S5.** Quantitative analysis of phenolics in supernatants after matrix formation.[a]

| Polyphenol | | Concentration in starting solution (mg/L)[b] | Concentration in the supernatant (mg/L)[b] | % Interaction |
|---|---|---|---|---|
| **Naringin** | | 2.502 ± 0.067 | 2.711 ± 0.022 | - |
| **Hesperidin** | | 43.852 ± 0.043 | 4.754[c] ± 0.010 | 89.2 |
| **Diosmin** | | 0.463 ± 0.006 | 0[c] | 100 |
| **Rutin** | | 0.548 ± 0.017 | 0.052[c] ± 0.005 | 90.5 |
| **Multistandard solution** | **Naringin** | 2.599 ± 0.081 | 3.071[c] ± 0.003 | - |
| | **Hesperidin** | 43.825 ± 0.034 | 1.748[c] ± 0.004 | 96.0 |
| | **Diosmin** | 0.474 ± 0.012 | 0[c] | 87.8 |
| | **Rutin** | 0.570 ± 0.017 | 0.058[c] ± 0.002 | 100 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentrations.

**Table S6.** Quantitative analysis of phenolics (double concentration) in supernatants after matrix formation.[a]

| Polyphenol | | Concentration in starting solution (mg/L)[b] | Concentration in the supernatant (mg/L)[b] | % Interaction |
|---|---|---|---|---|
| **Naringin** | | 5.159 ± 0.023 | 5.167 ± 0.004 | - |
| **Hesperidin** | | 87.910 ± 0.130 | 11.719[c] ± 0.026 | 86.7 |
| **Diosmin** | | 0.920 ± 0.010 | 0.032[c] ± 0.002 | 88.5 |
| **Rutin** | | 1.129 ± 0.028 | 0.142[c] ± 0.003 | 87.4 |
| **Multistandard solution** | **Naringin** | 5.143 ± 0.018 | 5.323 ± 0.006 | - |
| | **Hesperidin** | 87.831 ± 0.028 | 2.773[c] ± 0.021 | 96.8 |
| | **Diosmin** | 0.918 ± 0.012 | 0.042[c] ± 0.003 | 95.4 |
| | **Rutin** | 1.135 ± 0.009 | 0.113[c] ± 0.013 | 90.0 |

[a]Determined by HPLC analyses. [b]Data expressed as mean ± standard deviation. [c]Significant differences ($p < 0.05$) relative to the initial concentrations.