

Supporting Information for:

Comparison of protein precipitation ability of structurally diverse procyanidin-rich condensed tannins in two buffer systems

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Table of Contents	Page
Figure S1. ^1H - ^{13}C HSQC NMR spectrum of purified CTs from <i>Vitis vinifera</i> seed (grapeseed).....	3
Figure S2. ^1H - ^{13}C HSQC NMR spectrum of purified CTs from <i>Tilia inflourescentia</i> (lime tree) flowers.....	3
Figure S3. ^1H - ^{13}C HSQC NMR spectrum of purified CTs from <i>Vaccinium macrocarpon</i> (cranberry).	4
Figure S4. ^1H - ^{13}C HSQC NMR spectrum of purified CTs from <i>Trifolium pratense</i> (red clover) flowers	4
Figure S5. Precipitation studies using BSA, LYS, and ALF in MES buffer, pH 6.5 at 25 °C	5
Figure S6. Precipitation studies using BSA, LYS, and ALF in GVS buffer, pH 6.5. at 37 °C	6
Figure S7. Precipitation studies using BSA, LYS, and ALF in MES buffer, pH 6.5 at 25 °C and 37 °C.	7
Table S1. Summary of Tukey's HSD and connecting letters table for GVS buffer (37 °C) versus MES buffer (25 °C).....	8
Table S2. Summary of Tukey's HSD and connecting letters table for MES buffer (37 °C) versus MES buffer (25 °C).....	9
Table S3. Summary of Tukey's HSD and connecting letters table for GVS buffer (37 °C) versus MES buffer (37 °C).....	10

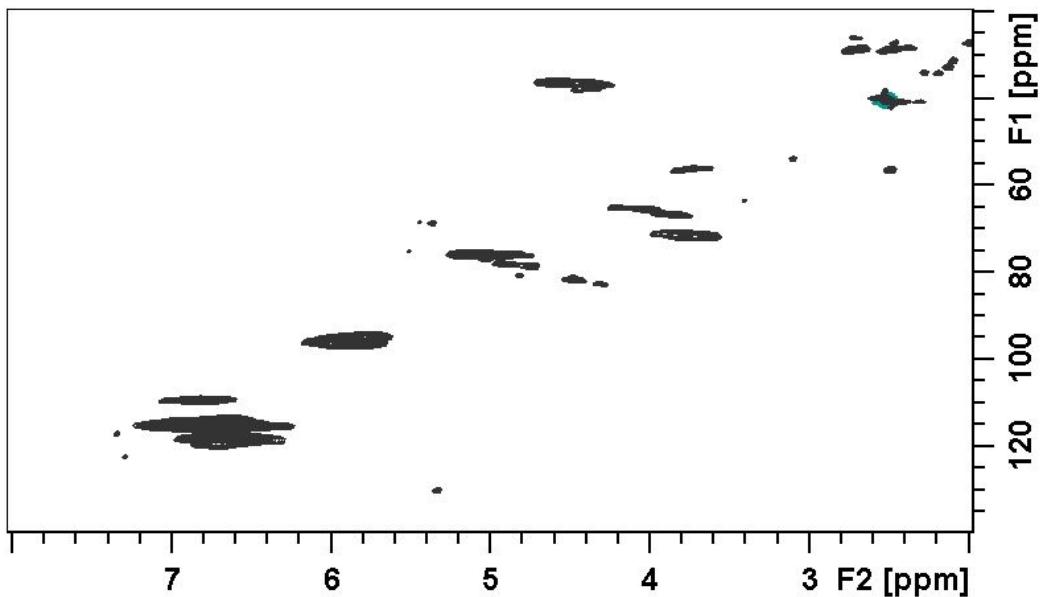


Figure S1. ¹H-¹³C HSQC NMR spectrum of purified CTs from *Vitis vinifera* seed (grapeseed).

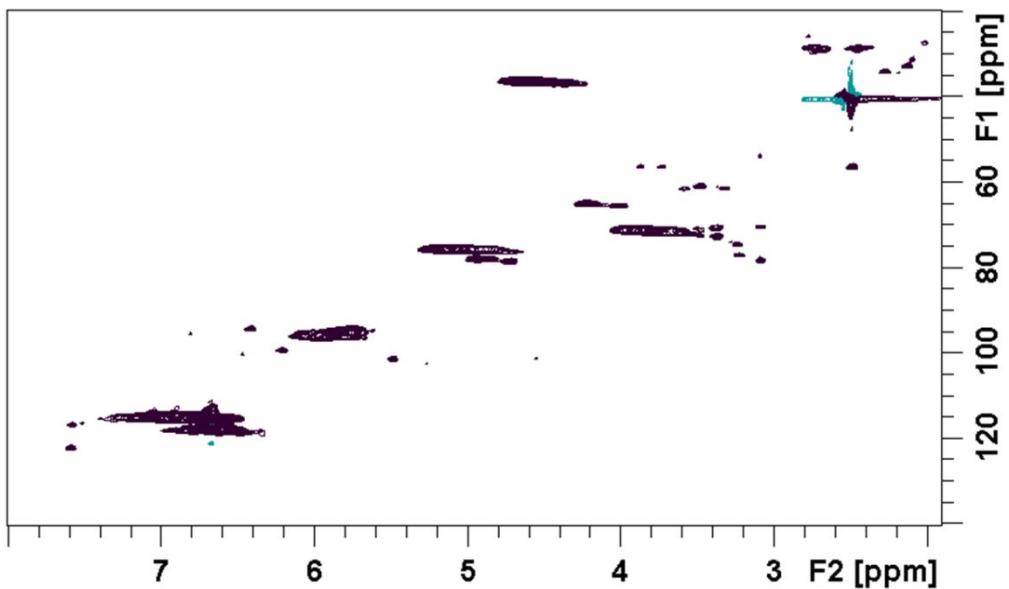


Figure S2. ¹H-¹³C HSQC NMR spectrum of purified CTs from *Tilia inflourescentia* (lime tree) flowers.

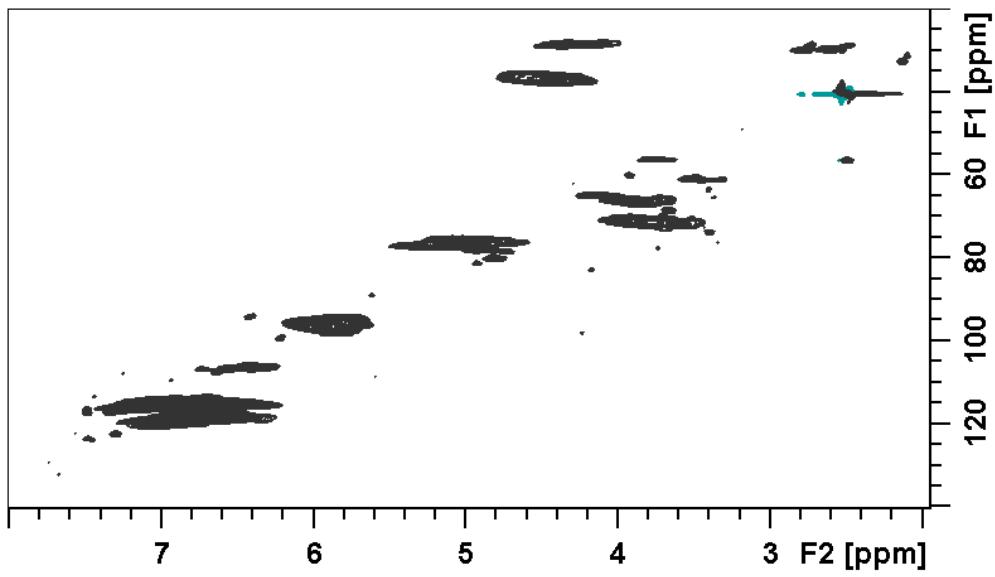


Figure S3. ¹H-¹³C HSQC NMR spectrum of purified CTs from *Vaccinium macrocarpon* (cranberry).

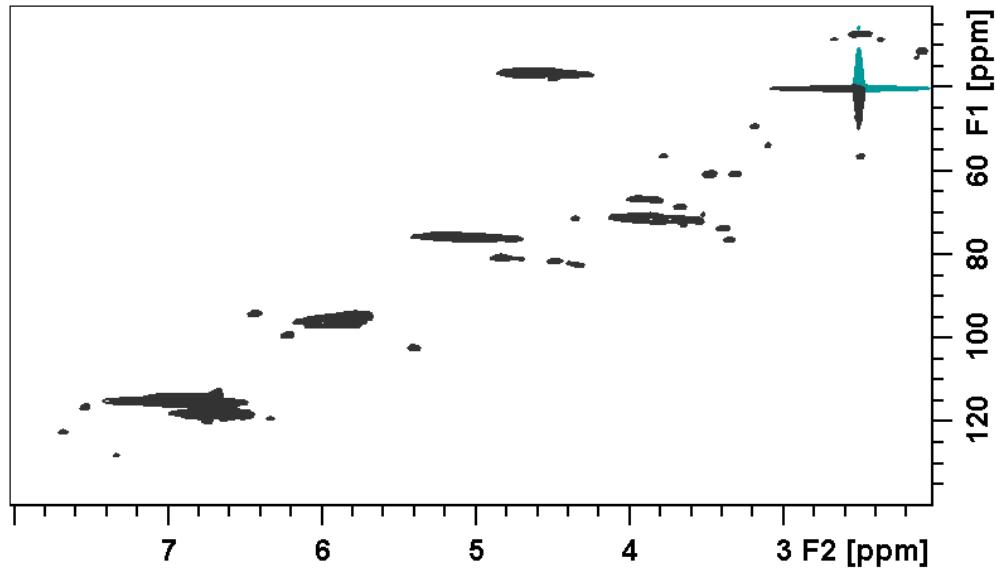
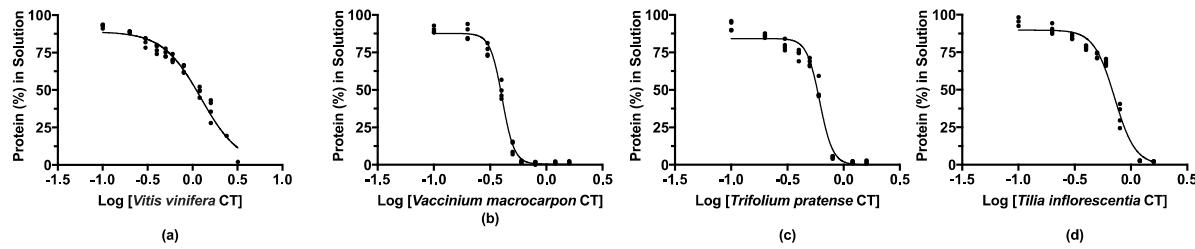
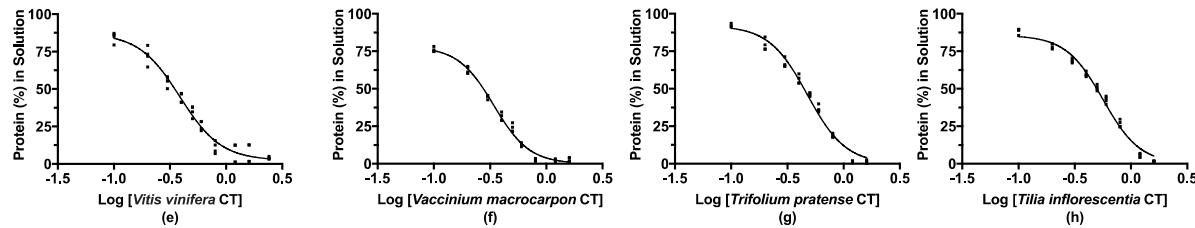


Figure S4. ¹H-¹³C HSQC NMR spectrum of purified CTs from *Trifolium pratense* (red clover) flowers.

BSA



LYS



ALF

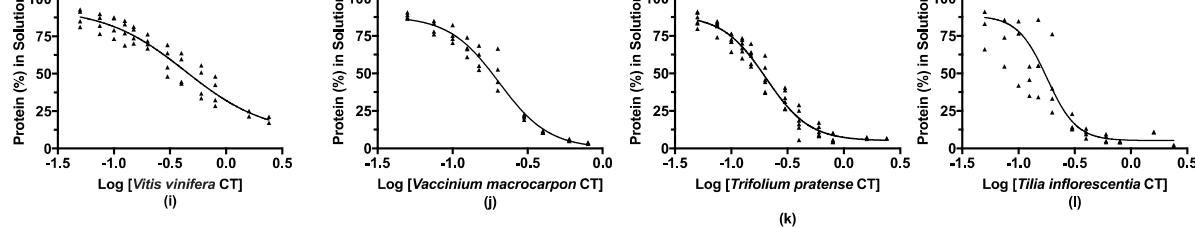
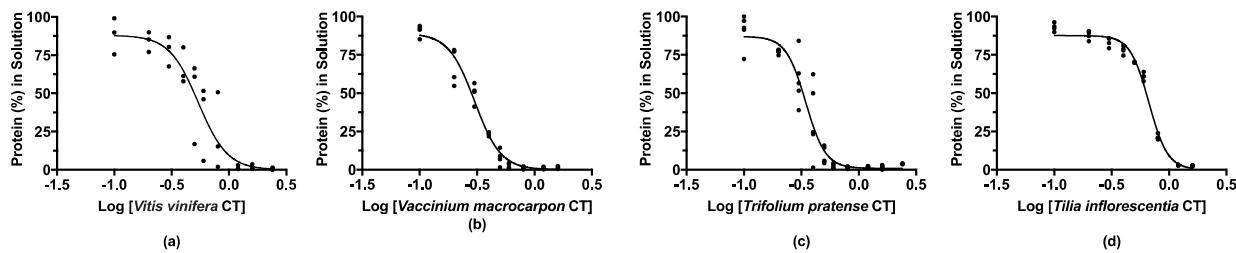
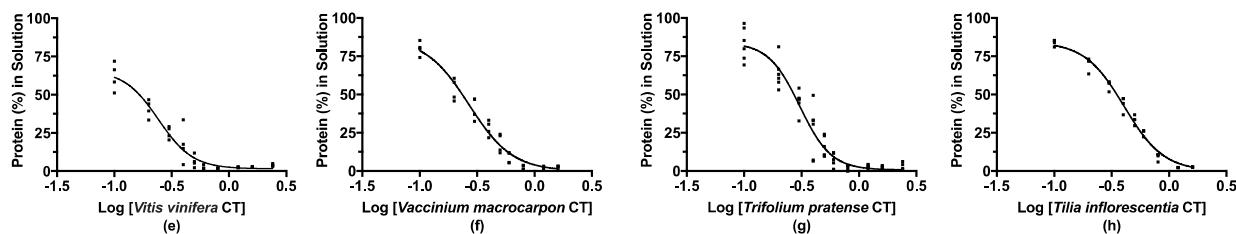


Figure S5. Precipitation studies were performed using BSA, LYS, and ALF in MES buffer, pH 6.5 at 25 °C. Experiments were performed in the presence of increasing CT (mg/mL) concentrations. Data were fit to a log (inhibitor) versus response model $\{\log y = A + [(B - A)/(1 + ((X^{\text{Hillslope}})/(PP_{50}^{\text{Hillslope}})))]\}$ and resulting curves are shown in BSA (●) (a)-(d), LYS (■) (e)-(h), and ALF (▲) (i)-(l) and determined PP₅₀ values are listed in **Table 2**. CTs were obtained from various plant materials: *Vitis vinifera* seed (a), (e), and (i), *Vaccinium macrocarpon* berries (b), (f), and (j), *Trifolium pratense* flowers (c), (f), and (k), *Tilia inflorescentia* flowers (d), (h), and (l). All data points are shown.

BSA



LYS



ALF

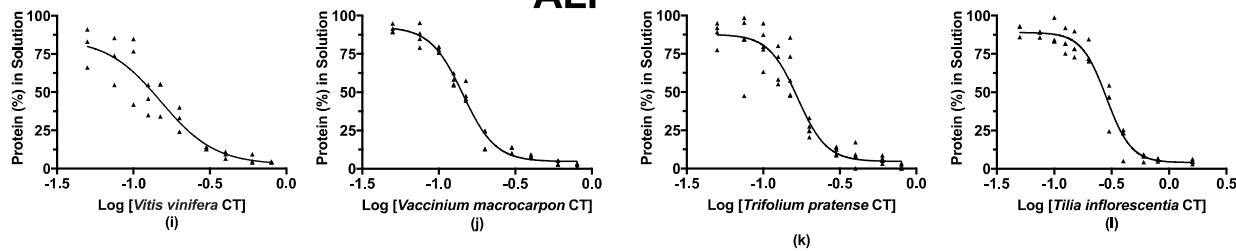
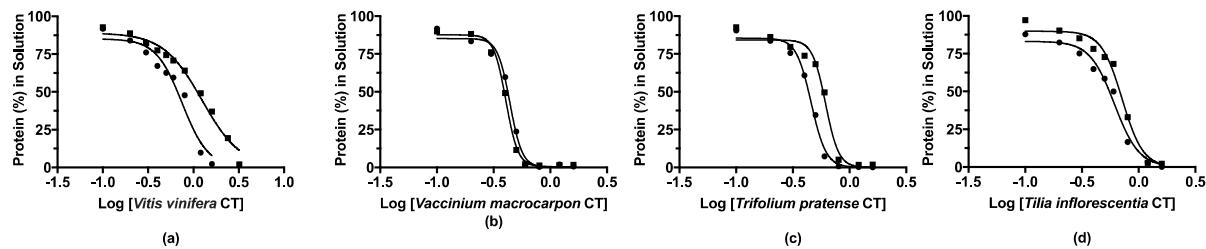
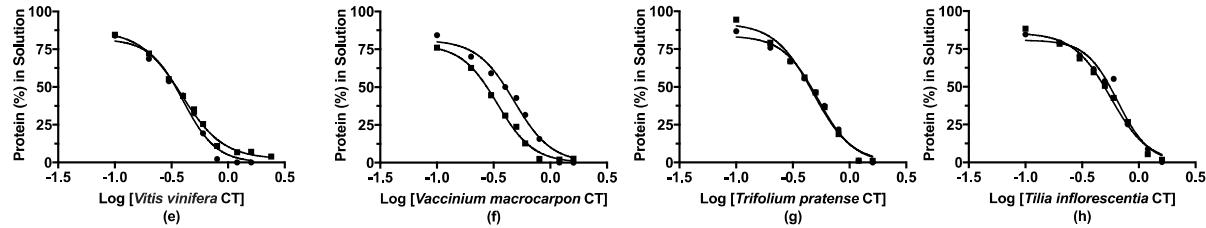


Figure S6. Precipitation studies were performed using BSA, LYS, and ALF in GVS buffer, pH 6.5 at 37 °C. Experiments were performed in the presence of increasing CT (mg/mL) concentrations. Data were fit to a log (inhibitor) versus response model $\{\log y = A + [(B - A)/(1 + ((X^{\text{Hillslope}})/(PP_{50}^{\text{Hillslope}})))]\}$ and resulting curves are shown in BSA (●) (a)-(d), LYS (■) (e)-(h), and ALF (▲) (i)-(l) and determined PP₅₀ values are listed in **Table 2**. CTs were obtained from various plant materials: *Vitis vinifera* seed (a), (e), and (i), *Vaccinium macrocarpon* berries (b), (f), and (j), *Trifolium pratense* flowers (c), (f), and (k), *Tilia influenta* flowers (d), (h), and (l). All data points are shown.

BSA



LYS



ALF

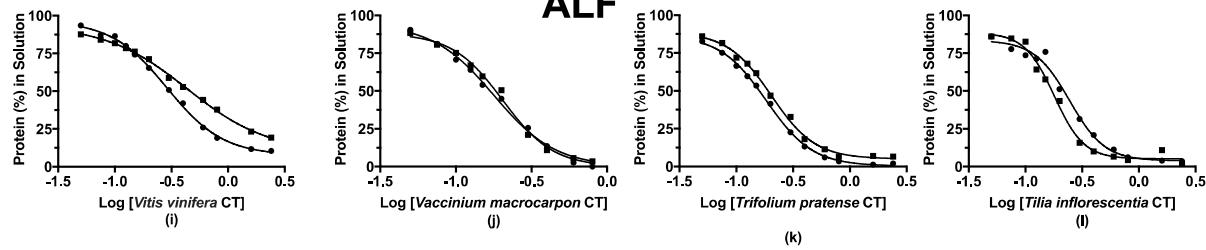


Figure S7. Precipitation studies were performed using BSA, LYS, and ALF in MES buffer, pH 6.5, at 25°C and 37°C. Experiments were performed in the presence of increasing CT (mg/mL) concentrations. Data were fit to a log (inhibitor) versus response model $\{\log y = A + [(B - A)/(1 + ((X^{\text{Hillslope}})/(PP_{50}^{\text{Hillslope}})))]\}$ and resulting curves are shown in BSA (a)-(d), LYS (e)-(h), and ALF (i)-(l) at 25°C (■) and 37°C (●). Determined PP₅₀ values are listed in **Table 2**. CTs were obtained from various plant materials: *Vitis vinifera* seed (a), (e), and (i), *Vaccinium macrocarpon* berries (b), (f), and (j), *Trifolium pratense* flowers (c), (f), and (k), *Tilia inflorescentia* flowers (d), (h), and (l). PP₅₀ values are significantly different ($p < 0.05$) only for curves (a) and (c) indicating precipitation at lower CT concentration for two of the four tannins studied with BSA. The only other PP₅₀ value that differed significantly ($p < 0.05$) with temperature is for curve (f).

Solvent	Protein	CT	Mean PP ₅₀	SD	CLD p<0.05
GVS	Alfalfa	Cranberry	0.144	0.0020	j
GVS	Alfalfa	Grapeseed	0.170275	0.0434	i j
GVS	Alfalfa	RCF	0.16546	0.0185	i j
GVS	Alfalfa	Tilia	0.31215	0.0586	f g h
GVS	BSA	Cranberry	0.295	0.0298	f g h i
GVS	BSA	Grapeseed	0.6066333	0.204	b c d
GVS	BSA	RCF	0.34252	0.0614	f g
GVS	BSA	Tilia	0.66785	0.0147	b c
GVS	Lysozyme	Cranberry	0.2689	0.049	f g h i j
GVS	Lysozyme	Grapeseed	0.236675	0.0553	g h i j
GVS	Lysozyme	RCF	0.3082167	0.0426	f g h
GVS	Lysozyme	Tilia	0.405675	0.026	e f
MES	Alfalfa	Cranberry	0.2015	0.034	h i j
MES	Alfalfa	Grapeseed	0.37457	0.0805	e f g
MES	Alfalfa	RCF	0.21365	0.039	h i j
MES	Alfalfa	Tilia	0.1645	0.0326	i j
MES	BSA	Cranberry	0.40675	0.0132	e f
MES	BSA	Grapeseed	1.24975	0.0792	a
MES	BSA	RCF	0.613225	0.0158	b c d
MES	BSA	Tilia	0.71665	0.0243	b
MES	Lysozyme	Cranberry	0.339975	0.0041	f g h
MES	Lysozyme	Grapeseed	0.38275	0.0312	e f
MES	Lysozyme	RCF	0.4758	0.0098	d e
MES	Lysozyme	Tilia	0.565325	0.0284	c d

Table S1. Summary of Tukey's HSD and connecting letters table for GVS buffer (37 °C) versus MES buffer (25 °C).

Multiple comparison-corrected significance table of group means from ANCOVA model fit. A lack of shared letters between groups indicates a significant difference at alpha = 0.05.

Solvent	Temp	Protein	CT	PP ₅₀	Mean														
					CLD p<0.05														
MES	25	BSA	grapeseed	1.2498	a														
MES	37	BSA	grapeseed	0.7512		b													
MES	25	BSA	tilia	0.7167		b	c												
MES	37	Lysosyme	tilia	0.6650		b	c	d											
MES	37	BSA	tilia	0.6456		b	c	d											
MES	25	BSA	RCF	0.6132			c	d	e										
MES	25	Lysosyme	tilia	0.5653				d	e	f									
MES	37	Lysosyme	RCF	0.5257					e	f	g								
MES	37	Lysosyme	cranberry	0.4807						f	g	h							
MES	25	Lysosyme	RCF	0.4758						f	g	h							
MES	37	BSA	RCF	0.4580							g	h							
MES	37	BSA	cranberry	0.4382							g	h	i						
MES	25	BSA	cranberry	0.4068							h	i							
MES	37	Lysosyme	grapeseed	0.4010							h	i							
MES	25	Lysosyme	grapeseed	0.3828							h	i	j						
MES	25	Alfalfa	grapeseed	0.3746							h	i	j						
MES	25	Lysosyme	cranberry	0.3400								i	j	k					
MES	37	Alfalfa	grapeseed	0.2864									j	k	l				
MES	37	Alfalfa	tilia	0.2442									k	l	m				
MES	25	Alfalfa	RCF	0.2137										l	m				
MES	25	Alfalfa	cranberry	0.2015										l	m				
MES	37	Alfalfa	RCF	0.1845											l	m			
MES	37	Alfalfa	cranberry	0.1799												l	m		
MES	25	Alfalfa	tilia	0.1645													m		

Table S2. Summary of Tukey's HSD and connecting letters table for MES buffer (37 °C) versus MES buffer (25 °C).

Multiple comparison-corrected significance table of group means from ANCOVA model fit. A lack of shared letters between groups indicates a significant difference at alpha = 0.05.

Solvent	Protein	CT	PP ₅₀	Mean						
				CLD p<0.05						
MES	BSA	grapeseed	0.7512	a						
GVS	BSA	tilia	0.6679	a						
MES	Lysosyme	tilia	0.6650	a						
MES	BSA	tilia	0.6456	a b						
GVS	BSA	grapeseed	0.6066	a b c						
MES	Lysosyme	RCF	0.5257	b c d						
MES	Lysosyme	cranberry	0.4807	c d						
MES	BSA	RCF	0.4580	c d e						
MES	BSA	cranberry	0.4382	d e f						
GVS	Lysosyme	tilia	0.4057	d e f g						
MES	Lysosyme	grapeseed	0.4010	d e f g						
GVS	BSA	RCF	0.3425	e f g h						
GVS	Alfalfa	tilia	0.3122	f g h i						
GVS	Lysosyme	RCF	0.3082	g h i						
GVS	BSA	cranberry	0.2950	g h i j						
MES	Alfalfa	grapeseed	0.2864	g h i j						
GVS	Lysosyme	cranberry	0.2689	g h i j k						
MES	Alfalfa	tilia	0.2442	h i j k						
GVS	Lysosyme	grapeseed	0.2367	h i j k						
MES	Alfalfa	RCF	0.1845	i j k						
MES	Alfalfa	cranberry	0.1799	i j k						
GVS	Alfalfa	grapeseed	0.1703	j k						
GVS	Alfalfa	RCF	0.1655	j k						
GVS	Alfalfa	cranberry	0.1440	k						

Table S3. Summary of Tukey's HSD and connecting letters table for GVS buffer (37 °C) versus MES buffer (37 °C).

Multiple comparison-corrected significance table of group means from ANCOVA model fit. A lack of shared letters between groups indicates a significant difference at alpha = 0.05.