Supplementary Material

A new approach of extraction of α-amylase/trypsin inhibitors from wheat based on optimization using Plackett–Burman and Box–Behnken designs

Sorel Tchewonpi Sagu [1], Gerd Huschek [2], Josephine Bönick[1,2], Thomas Homann[1] and Harshadrai M. Rawel[1,*]

1. Institute of Nutritional Science, University of Potsdam, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Potsdam, Germany; sorelsagu@uni-potsdam.de (S.S.) ; homann@uni-potsdam.de (T.H.)

2. IGV-Institut für Getreideverarbeitung GmbH, Arthur-Scheunert-Allee 40/41, D-14558, Nuthetal OT Bergholz-Rehbrücke, Germany; gerd.huschek@igv-gmbh.de (G.H.)

* Corresponding author.

Tel: +49-33200-88-5525/5578 (H.R.)

E-mail: rawel@uni-potsdam.de

Content

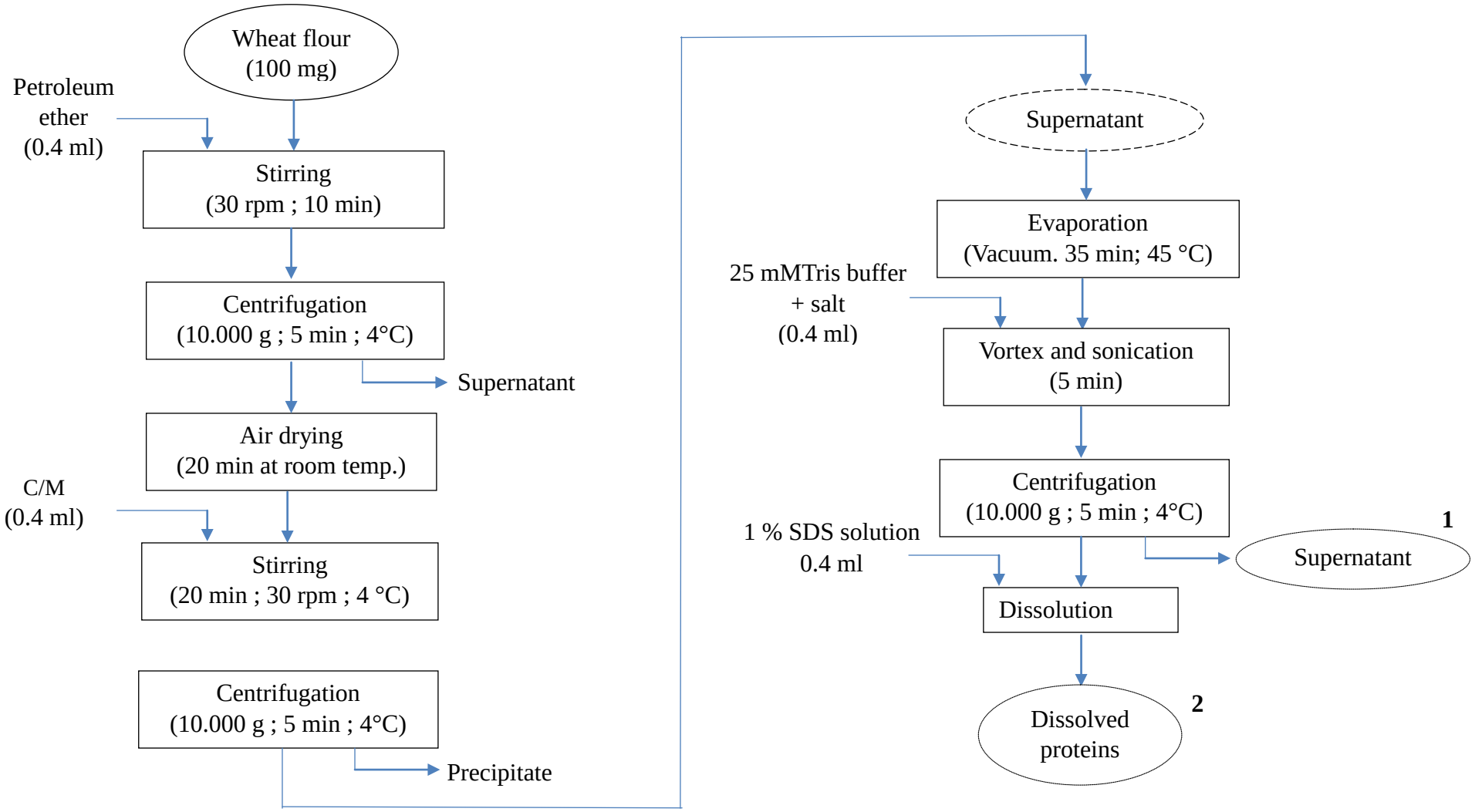

Figure S1. Process flow chartof wheat amylase/trypsin extraction using mixtures of chloroform/methanol (C/M) or dichloromethane/methanol (D/M) as extraction solvents. Sample 1 contains the ATIs and Sample 2 contains other proteins of higher molecular weights.

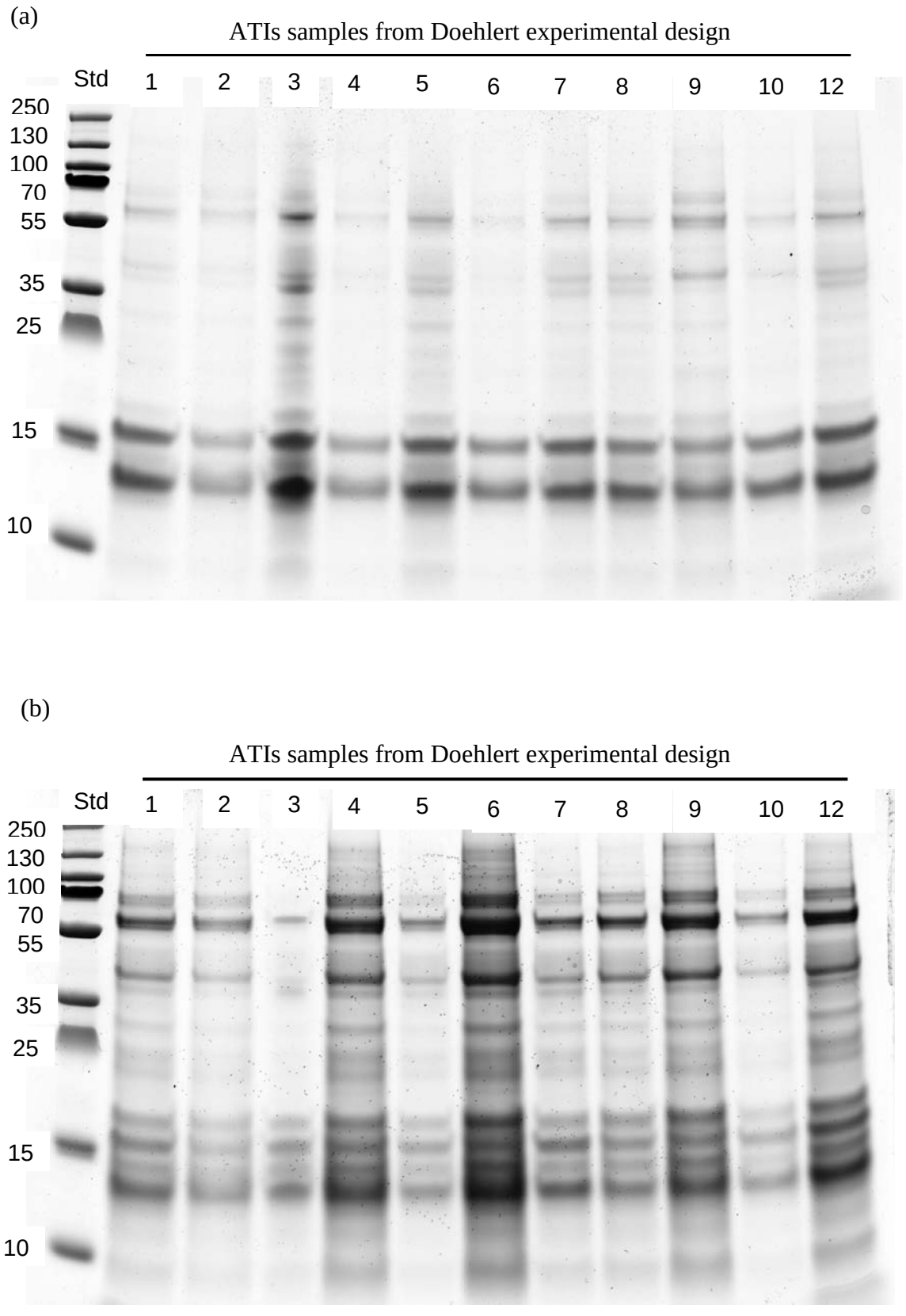

Figure S2. Protein profiles of selected (a) *Julius* extract and (b) *Ponticus* extract for different experimental conditions of the Doehlert design determined by SDS-PAGE

Table S1. Experimental factors and their level in Doehlert design

Factor	**Factor significance**	**Low level**	**High level**
X_6	Extraction time (min)	30	180
X_8	pH (/)	7	9
X_{10}	Salt concentration (molarity)	0.2	1.8

Table S2. Solvent gradient during HPLC fractionation

Time (min)	**Command**	**Value (%)**
0.1	Pump B concentration	0
3.0	Pump B concentration	5
10.0	Pump B concentration	10
20.0	Pump B concentration	50
23.0	Pump B concentration	100
26.0	Pump B concentration	100
28.0	Pump B concentration	0
33.0	Pump B concentration	0
33.0	Stop controller	

Solutions of 0.1% trifluoroacetic acid (TFA) and 70% acetonitrile (ACN) were used as eluent A and B, respectively. Elution was carried out at a flow rate of 1 ml/min.

Table S3. Weighted effects of extraction time sodium chloride concentration and pH on experimental responses expressed in percent

		Julius						***Ponticus***					
Factors	Coeff.	Coeff. Protein	Effect (%)	Coeff. IAA	Effect (%)	Sum of effects (%)		Coeff. Protein	Effect (%)	Coeff. IAA	Effect (%)	Sum of effects (%)	
Constance													
	b_0	0.472		71.5				0.406		60.62			
Linear													
X_{10}	b_1	-0.071	13.9	-1.0	2.1	8.0	**29.6**	-0.213	14.7	-13.8	22.7	18.7	**44.1**
X_6	b_2	0.086	16.7	-1.7	3.6	10.2		-0.115	8.0	5.1	8.4	8.2	
X_8	b_3	0.102	19.9	-1.4	3.1	11.5		-0.149	10.3	-14.6	24.1	17.2	
Quadratic													
X_{10}*X_{10}	b_{11}	0.023	4.5	11.4	24.8	14.6	**49.0**	0.089	6.2	-0.2	0.4	3.3	**25.0**
X_6*X_6	b_{22}	-0.047	9.1	11.5	25.1	17.1		0.156	10.8	-14.4	23.7	17.2	
X_8*X_8	b_{33}	0.098	19.1	7.1	15.4	17.2		0.112	7.8	-0.8	1.3	4.5	
Interaction													
X_{10}*X_6	b_{12}	-0.017	3.4	-1.6	3.5	3.4	**21.4**	-0.208	14.4	-1.9	3.1	8.7	**30.9**
X_{10}* X_8	b_{13}	0.031	6.0	-6.7	14.6	10.3		0.049	3.4	-8.8	14.5	9.0	
X_6* X_8	b_{23}	-0.039	7.6	3.6	7.8	7.7		0.356	24.6	-1.1	1.9	13.2	
Total		0.514	100.0	46.0	100.0	100.0	**100.0**	1.447	100.0	60.8	100.0	100.0	100.0

X_6 is extraction time.X_8 the pH and X_{10} sodium chloride concentrations.

Table S4. Regression coefficients and analysis of variance for protein concentration and IAA from Plackett–Burman design

	Julius				***Poncticus***			
	Protein		*IAA*		Protein		*IAA*	
	Coeff.	*p*-value	Coeff.	*p*-value	Coeff.	*p*-value	Coeff	*p*-value
Constant	0.404		63.79		0.387		59.99	
Type of solvent, X_1	0.010	0.1128	-1.10	0.5492	0.015	0.0659	-0.21	0.7529
Composition of solvent, X_2	-0.003	0.6749	-4.20	0.0376*	0.005	0.4777	-2.31	0.0052*
Ratio samples/solvent, X_3	-0.012	0.0695	4.68	0.0234*	-0.021	0.0172*	1.40	0.0580
Concentration Urea, X_4	0.008	0.2226	3.35	0.0866	-0.001	0.8942	-5.69	0.0000*
Temperature, X_5	0.016	0.0196*	-4.33	0.0332*	-0.008	0.2882	1.07	0.1334
Time, X_6	-0.024	0.0016*	4.72	0.0224*	0.022	0.0128*	-3.76	0.0001*
Stirringspeed, X_7	-0.004	0.4877	3.83	0.0543	-0.021	0.0189*	3.16	0.0006*
pH, X_8	0.015	0.0254*	4.80	0.0206*	0.018	0.0338*	5.35	0.0000*
Type ofsalt, X_9	-0.175	0.0000*	-20.78	0.0000*	-0.137	0.0000*	-	0.0000*
Concentrationofsalt, X_{10}	-0.055	0.0000*	-9.79	0.0089*	-0.039	0.0014*	-5.46	0.0000*
Centrifugationspeed, X_{11}	0.008	0.1788	0.86	0.6392	0.008	0.3112	1.08	0.1308

* Significant values