

# **Supplementary Material**

## **Optimization of the Osborne extraction method for fractionation and characterization of oat proteins**

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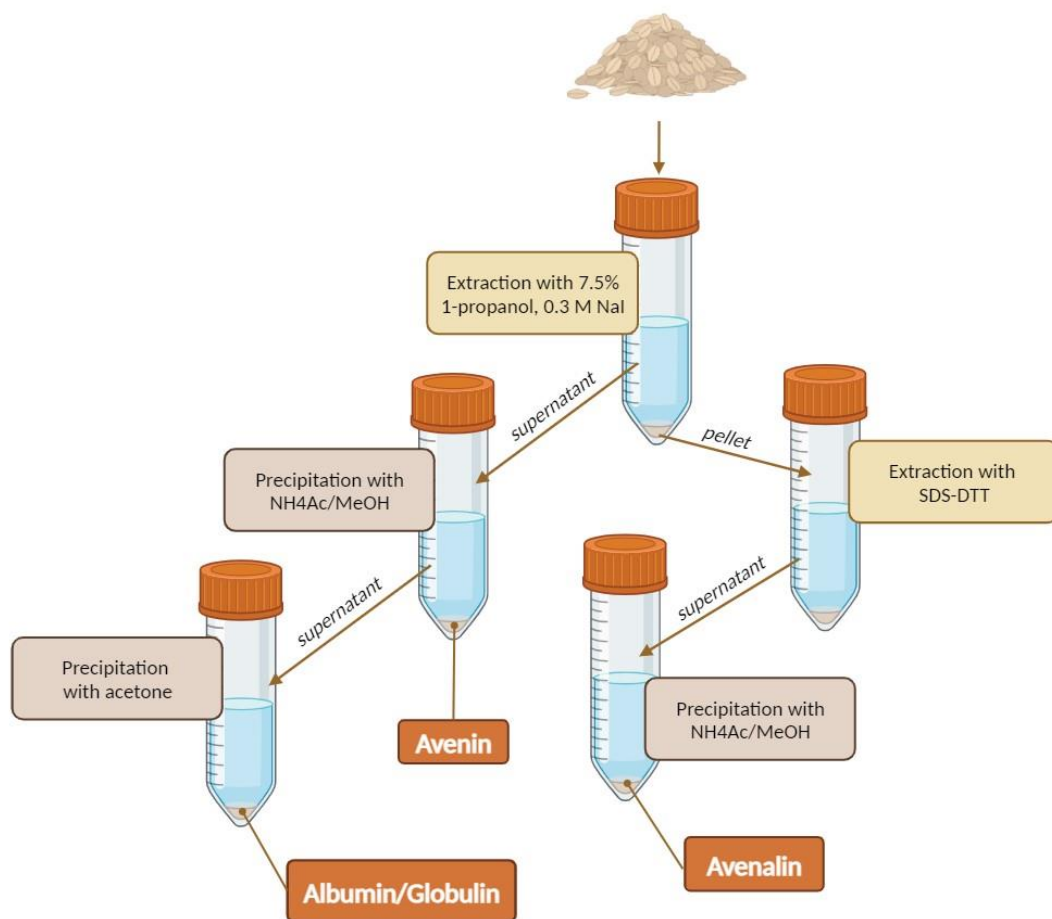
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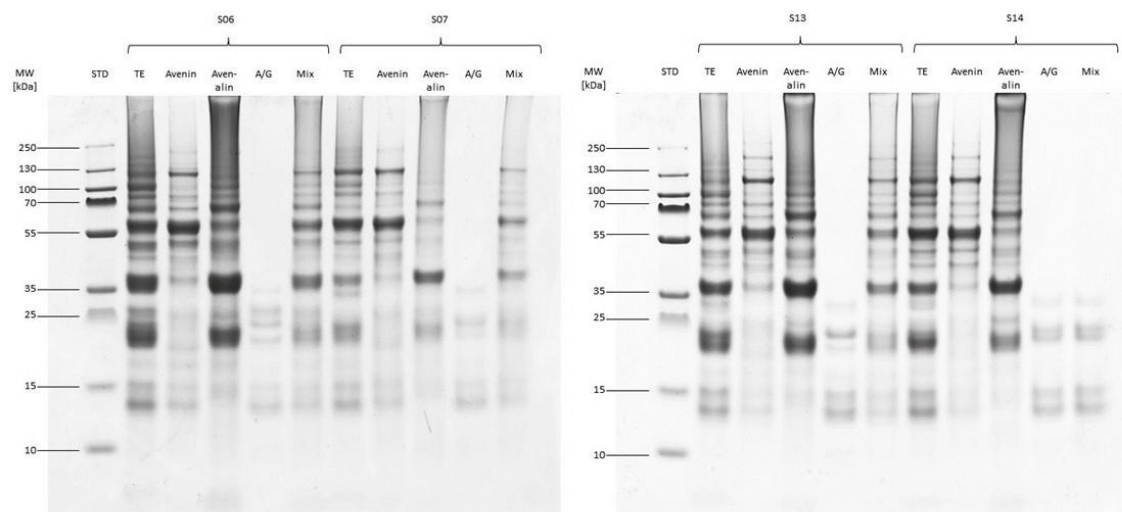
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**Figure S1.** Workflow of the sequential extraction of oat proteins based on their solubility. The workflow was adapted according to the methodology described by DuPont et al. (2005) [1] with some modifications



**Figure S2.** SDS-PAGE of total extract as well as avenin, avenalin and albumin/globulin fractions of selected oat samples. *Protein mix consists of equal parts of avenin, avenalin, and albumin/globulin obtained by sequential protein extraction process.* MW: Molecular Weight; STD: Standard; TE: Total Extract from ammonium bicarbonate/urea buffer; A/G: Albumin/Globulin.

**Table S1.** Overview of the analyzed commercial whole grain oat and oat product samples.

Sample No.	Designation	Producer	Contact information
S01	Oat	Alnatura	Alnatura GmbH, 64295 Darmstadt, Germany
S02	Oat, dehulled	Bio Company	Bio Company GmbH, 12161 Berlin, Germany
S03	Oat Flakes, <i>Feinblatt</i>	Alnatura	Alnatura GmbH, 64295 Darmstadt, Germany
S04	Oat Flakes, <i>Feinblatt</i>	Alnavit	Alnavit GmbH, 64295 Darmstadt, Germany

**Table S2.** Overview of the analyzed homogenous oat grain samples provided by the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben

Nr.	Accession Number	Oat Species	Genotype	Origin	Date of Access
S05	AVE 16	<i>Avena sativa</i> L. var. Aristata Schltdl.	Beseler II	Germany	1947
S06	AVE 255	<i>Avena sativa</i> L. var. Aurea Körn.	Lüneburger Heidegold	Germany	1947
S07	AVE 401	<i>Avena sativa</i> L. var. Pugnax Alef.	Schwarzer Begrannter	Germany	1950
S08	AVE 612	<i>Avena sativa</i> L. var. Mutica Schltdl.	Gelber August	Germany	1950
S09	AVE 1279	<i>Avena sativa</i> L. var. Mutica Schltdl.	Silva	Germany	1969
S10	AVE 1291	<i>Avena sativa</i> L. var. Aurea Körn.	Delphin	Germany	1969
S11	AVE 3478	<i>Avena sativa</i> L. var. Pugnax Alef.	Schottischer Fahnen	Germany	2003
S12	AVE 4903	<i>Avena sativa</i> L. var. Aristata Schltdl.	Beseler II	Germany	2003
S13	AVE 1464	<i>Avena sativa</i> L. var. Aurea Körn.	n.s.	Czech Republic	1975
S14	AVE 45	<i>Avena sativa</i> L. var. Aurea Körn.	Niermanski	Poland	1947
S15	AVE 321	<i>Avena sativa</i> L. var. Aristata Schltdl.	n.s.	France	1948
S16	AVE 877	<i>Avena sativa</i> L. var. Aurea Körn.	Belar	Australia	1964
S17	AVE 349	<i>Avena sativa</i> L. var. Mutica Schltdl.	n.s.	Australia	1947
S18	AVE 3357	<i>Avena sativa</i> L.	Santon	South Africa	2003
S19	AVE 4663	<i>Avena sativa</i> L. var. Mutica Schltdl.	Amor	Kenia	2003
S20	AVE 1581	<i>Avena sativa</i> L. var. Aurea Körn.	Oxford	Canada	1978
S21	AVE 3469	<i>Avena sativa</i> L. var. Mutica Schltdl.	n.s.	USA	2003
S22	AVE 381	<i>Avena sativa</i> L. var. Aristata Schltdl.	Mestiza	Uruguay	1949
S23	AVE 3535	<i>Avena sativa</i> L. var. Mutica Schltdl.	Huatasani	Peru	2003
S24	AVE 869	<i>Avena sativa</i> L. var. Grisea Körn.	Pin-Lan-Che-U-Sao	China	1962

**Table S3.** Overview of the analyzed commercial wheat flour and whole-grain rice flour.

Designation	Brand	Producer Contact
Wheat Flour, Type 405	Ja!	Scheller Mühle GmbH, 85276 Pfaffenhofen-Reisgang, Germany
Whole-Grain Rice Flour	Bauckhof	Bauck GmbH, 29571 Rosche, Germany

**Table S4.** Box behnken design presenting the experimental design with the coded and the real values

Exp. No.	Coded values			Real values		
	x1	x2	x3	X1	X2	X3
1	-1	-1	0	1	0.8	27.5
2	1	-1	0	10	0.8	27.5
3	-1	1	0	1	6	27.5
4	1	1	0	10	6	27.5
5	-1	0	-1	1	3.4	5
6	1	0	-1	10	3.4	5
7	-1	0	1	1	3.4	50
8	1	0	1	10	3.4	50
9	0	-1	-1	5.5	0.8	5
10	0	1	-1	5.5	6	5
11	0	-1	1	5.5	0.8	50
12	0	1	1	5.5	6	50
13	0	0	0	5.5	3.4	27.5
14	0	0	0	5.5	3.4	27.5
15	0	0	0	5.5	3.4	27.5

x1, x2 and x3 are the coded values while X1, X2 and X3 are the real values of the extraction time, extraction speed and sample/solvent ratio, respectively.

**Table S5.** Parameters of the applied MRM method for the analyzed oat proteins

Protein	Quantifier	Q1 Mass	Q3 Masses	Retention Time [min]
P12615	R.FAQGQSK.S	383.19	618.3	3.5
			547.3	
			418.2	
P14812	K.TNPNSMVSQIAGK.T	673.84	702.4	8.6
			388.3	
			275.2	
P27919	R.EYVAER.C	383.69	637.3	6.7
			474.3	
			375.2	
P80356	R.QLEQIPEQLR.C	627.35	883.5	8.9
			755.4	
			545.3	
Q09114	R.QLAQIPEQLR.C	598.34	755.4	9.0
			642.4	
			441.2	
A0A1B2LQD8	K.TAILSSMLQR.C	560.31	721.4	9.9
			634.3	
			573.3	
A0A1B2LQC9	R.DFAR.I	254.63	393.3	6.6
			263.1	

*Q1 Mass indicates masses of the precursor ions and Q3 the masses of the selected fragment ions*

**Table S6.** Analysis of Variance for yield of the avenin fraction

Source	Sum of squares	Df	Mean square	F-Ratio	P-Value
A: Extraction time	0.32	1	0.32	0.41	0.5483
B: Speed	153.125	1	153.125	1.98	0.2183
C: Sample/solvent ratio	864.613	1	864.613	111.88	0.0001
AA	0.262564	1	0.262564	0.34	0.5853
AB	0.9025	1	0.9025	1.17	0.3292
AC	11.025	1	11.025	1.43	0.2859
BB	440.026	1	440.026	5.69	0.0627
BC	1.69	1	1.69	2.19	0.1993
CC	539.103	1	539.103	6.98	0.0459
Total error	386.417	5	0.772833		
Total (corr.)	106.757	14			



**Table S7.** Analysis of Variance for yield of the avenalin fraction

Source	Sum of squares	Df	Mean square	F-Ratio	P-Value
A: Extraction time	0.845	1	0.845	4.19	0.0961
B: Speed	0.10125	1	0.10125	0.50	0.5104
C: Sample/solvent ratio	780.125	1	780.125	38.65	0.0016
AA	0.862564	1	0.862564	4.27	0.0936
AB	0.0025	1	0.0025	0.01	0.9157
AC	0.4225	1	0.4225	2.09	0.2076
BB	115.103	1	115.103	5.70	0.0625
BC	0.04	1	0.04	0.20	0.6748
CC	304.641	1	304.641	15.09	0.0116
Total error	100.917	5	0.201833		
Total (corr.)	146.893	14			

**Table S8.** Analysis of Variance for yield of the albumin/globulin fraction

Source	Sum of squares	Df	Mean square	F-Ratio	P-Value
A: Extraction time	0.03125	1	0.03125	0.10	0.7695
B: Speed	0.15125	1	0.15125	0.46	0.5263
C: Sample/solvent ratio	13.005	1	13.005	39.83	0.0015
AA	168.231	1	168.231	5.15	0.0724
AB	0.16	1	0.16	0.49	0.5151
AC	0.0025	1	0.0025	0.01	0.9337
BB	0.00230769	1	0.00230769	0.01	0.9363
BC	0.5625	1	0.5625	1.72	0.2463
CC	113.077	1	113.077	34.63	0.0020
Total error	16.325	5	0.3265		
Total (corr.)	292.973	14			

**Table S9:** Relative protein content of the 6 targeted proteins in the total extract of the 24 investigated oat samples. The results are expressed as peak area per  $\mu\text{g}$  proteins (PA/ $\mu\text{g}$  proteins)

Sample Nr.	Avenin Content (PA/ $\mu\text{g}$ Protein)			Avenin-3 Content (PA/ $\mu\text{g}$ Protein)			Avenin-E Content (PA/ $\mu\text{g}$ Protein)			SSG1 Content (PA/ $\mu\text{g}$ Protein)			SSG2 Content (PA/ $\mu\text{g}$ Protein)			ATI-2 Content (PA/ $\mu\text{g}$ Protein)		
<b>S01</b>	1311	$\pm$	30	11617	$\pm$	108	36367	$\pm$	109	4162	$\pm$	827	2691	$\pm$	1426	798	$\pm$	72
<b>S02</b>	21040	$\pm$	7583	14653	$\pm$	4930	44021	$\pm$	15994	1604	$\pm$	334	2234	$\pm$	1060	28029	$\pm$	7923
<b>S03</b>	5994	$\pm$	454	34807	$\pm$	402	108900	$\pm$	1677	15325	$\pm$	842	7610	$\pm$	485	4155	$\pm$	243
<b>S04</b>	5608	$\pm$	247	18183	$\pm$	360	57989	$\pm$	331	7550	$\pm$	168	4687	$\pm$	148	2833	$\pm$	41
<b>S05</b>	2254	$\pm$	3	7150	$\pm$	21	23787	$\pm$	313	924	$\pm$	270	2115	$\pm$	37	2743	$\pm$	85
<b>S06</b>	2583	$\pm$	121	7319	$\pm$	17	25707	$\pm$	51	n. d.			3063	$\pm$	118	2621	$\pm$	11
<b>S07</b>	2069	$\pm$	197	8017	$\pm$	28	30674	$\pm$	241	337	$\pm$	9	1965	$\pm$	93	3017	$\pm$	176
<b>S08</b>	2415	$\pm$	179	5946	$\pm$	15	18176	$\pm$	150	936	$\pm$	54	1940	$\pm$	95	4596	$\pm$	145
<b>S09</b>	2486	$\pm$	28	4527	$\pm$	158	24644	$\pm$	63	599	$\pm$	29	2448	$\pm$	10	3487	$\pm$	74
<b>S10</b>	2264	$\pm$	15	2260	$\pm$	23	9169	$\pm$	114	313	$\pm$	18	965	$\pm$	4	3520	$\pm$	33
<b>S11</b>	2127	$\pm$	41	6376	$\pm$	51	24055	$\pm$	170	391	$\pm$	12	1778	$\pm$	221	3503	$\pm$	48
<b>S12</b>	1761	$\pm$	48	8917	$\pm$	208	26710	$\pm$	134	448	$\pm$	4	403	$\pm$	6	2373	$\pm$	52
<b>S13</b>	3012	$\pm$	79	3351	$\pm$	110	14852	$\pm$	94	589	$\pm$	19	607	$\pm$	74	3559	$\pm$	349
<b>S14</b>	1973	$\pm$	20	5226	$\pm$	27	17176	$\pm$	152	317	$\pm$	22	1401	$\pm$	168	3332	$\pm$	30
<b>S15</b>	2556	$\pm$	127	4167	$\pm$	65	25053	$\pm$	150	661	$\pm$	25	4415	$\pm$	150	5745	$\pm$	124
<b>S16</b>	2262	$\pm$	35	5109	$\pm$	16	19927	$\pm$	159	595	$\pm$	18	2201	$\pm$	109	3421	$\pm$	296
<b>S17</b>	3565	$\pm$	273	7868	$\pm$	97	35024	$\pm$	196	794	$\pm$	7	1563	$\pm$	20	4853	$\pm$	7
<b>S18</b>	4089	$\pm$	71	5732	$\pm$	160	43301	$\pm$	145	791	$\pm$	11	2838	$\pm$	39	5165	$\pm$	248
<b>S19</b>	2175	$\pm$	80	7591	$\pm$	23	31501	$\pm$	76	938	$\pm$	61	2537	$\pm$	68	3139	$\pm$	202
<b>S20</b>	1882	$\pm$	52	3099	$\pm$	43	13041	$\pm$	56	439	$\pm$	14	1428	$\pm$	16	3826	$\pm$	105
<b>S21</b>	4032	$\pm$	169	13175	$\pm$	85	37506	$\pm$	357	1032	$\pm$	6	2324	$\pm$	111	5451	$\pm$	143
<b>S22</b>	3434	$\pm$	61	10221	$\pm$	111	39966	$\pm$	221	n. d.			1866	$\pm$	50	5011	$\pm$	132
<b>S23</b>	2833	$\pm$	8	16820	$\pm$	197	67082	$\pm$	869	403	$\pm$	34	3468	$\pm$	77	2618	$\pm$	39
<b>S24</b>	2899	$\pm$	223	14833	$\pm$	182	36846	$\pm$	874	538	$\pm$	36	3509	$\pm$	616	4207	$\pm$	1414

PA: Peak Area; SSG: Seed Storage Globulin; ATI-2: Amylase Trypsin Inhibitor-2; n. d.: non-detectable

**Table S10:** Relative protein content of the 6 targeted proteins in the avenin fractions of the 24 investigated oat samples. The results are expressed as peak area per  $\mu\text{g}$  proteins (PA/ $\mu\text{g}$  proteins)

Sample Nr.	Avenin Content (PA/ $\mu\text{g}$ Protein)			Avenin-3 Content (PA/ $\mu\text{g}$ Protein)			Avenin-E Content (PA/ $\mu\text{g}$ Protein)			SSG1 Content (PA/ $\mu\text{g}$ Protein)			SSG2 Content (PA/ $\mu\text{g}$ Protein)			ATI-2 Content (PA/ $\mu\text{g}$ Protein)		
<b>S01</b>	1987	$\pm$	212	1594	$\pm$	28	5448	$\pm$	280	3945	$\pm$	400	1356	$\pm$	93	512	$\pm$	75
<b>S02</b>	3152	$\pm$	63	2304	$\pm$	152	9419	$\pm$	358	606	$\pm$	278	485	$\pm$	245	650	$\pm$	48
<b>S03</b>	3537	$\pm$	609	6317	$\pm$	210	23194	$\pm$	240	5050	$\pm$	1303	1000	$\pm$	176	627	$\pm$	56
<b>S04</b>	3520	$\pm$	0	2546	$\pm$	9	6432	$\pm$	452	5052	$\pm$	539	1275	$\pm$	40	864	$\pm$	135
<b>S05</b>	3027	$\pm$	8	469	$\pm$	32	1226	$\pm$	41	769	$\pm$	33	1118	$\pm$	136	663	$\pm$	10
<b>S06</b>	3469	$\pm$	15	475	$\pm$	42	1889	$\pm$	81	n. d.			1986	$\pm$	198	412	$\pm$	18
<b>S07</b>	1640	$\pm$	0	664	$\pm$	104	2233	$\pm$	54	443	$\pm$	43	1578	$\pm$	113	339	$\pm$	33
<b>S08</b>	2615	$\pm$	75	713	$\pm$	39	1834	$\pm$	64	671	$\pm$	28	1551	$\pm$	144	808	$\pm$	5
<b>S09</b>	1287	$\pm$	79	202	$\pm$	4	687	$\pm$	3	1232	$\pm$	51	400	$\pm$	112	693	$\pm$	23
<b>S10</b>	1458	$\pm$	94	232	$\pm$	31	646	$\pm$	47	1269	$\pm$	77	1032	$\pm$	15	503	$\pm$	4
<b>S11</b>	1556	$\pm$	110	250	$\pm$	15	485	$\pm$	66	712	$\pm$	48	590	$\pm$	104	428	$\pm$	11
<b>S12</b>	2270	$\pm$	59	842	$\pm$	17	1411	$\pm$	62	1178	$\pm$	155	873	$\pm$	89	345	$\pm$	6
<b>S13</b>	2317	$\pm$	164	423	$\pm$	35	1584	$\pm$	56	514	$\pm$	18	152	$\pm$	20	492	$\pm$	17
<b>S14</b>	2070	$\pm$	28	1113	$\pm$	51	2247	$\pm$	73	775	$\pm$	109	511	$\pm$	148	760	$\pm$	3
<b>S15</b>	2802	$\pm$	29	528	$\pm$	60	1880	$\pm$	42	508	$\pm$	89	3369	$\pm$	3258	653	$\pm$	36
<b>S16</b>	2365	$\pm$	17	630	$\pm$	8	1940	$\pm$	8	680	$\pm$	249	1928	$\pm$	2028	788	$\pm$	66
<b>S17</b>	5763	$\pm$	53	1542	$\pm$	16	7038	$\pm$	104	389	$\pm$	84	1892	$\pm$	1944	1061	$\pm$	4
<b>S18</b>	3462	$\pm$	1	1439	$\pm$	9	11028	$\pm$	93	379	$\pm$	107	1466	$\pm$	1521	1131	$\pm$	44
<b>S19</b>	5679	$\pm$	14	1392	$\pm$	98	9225	$\pm$	11	380	$\pm$	60	1640	$\pm$	1630	1154	$\pm$	13
<b>S20</b>	3709	$\pm$	53	1051	$\pm$	52	3276	$\pm$	25	574	$\pm$	69	666	$\pm$	21	1306	$\pm$	57
<b>S21</b>	5362	$\pm$	93	1979	$\pm$	77	8637	$\pm$	232	459	$\pm$	119	1546	$\pm$	138	1188	$\pm$	17
<b>S22</b>	4617	$\pm$	232	1565	$\pm$	16	6880	$\pm$	265	n. d.			634	$\pm$	118	1155	$\pm$	77
<b>S23</b>	4450	$\pm$	184	1622	$\pm$	26	6753	$\pm$	137	419	$\pm$	46	688	$\pm$	98	780	$\pm$	108
<b>S24</b>	7783	$\pm$	317	1747	$\pm$	49	3054	$\pm$	43	437	$\pm$	50	1059	$\pm$	127	3210	$\pm$	61

PA: Peak Area; SSG: Seed Storage Globulin; ATI-2: Amylase Trypsin Inhibitor-2; n. d.: non-detectable

**Table S11:** Relative protein content of the 6 targeted proteins in the avenalin fractions of the 24 investigated oat samples. The results are expressed as peak area per µg proteins (PA/µg proteins)

Sample Nr.	Avenin Content (PA/µg Protein)			Avenin-3 Content (PA/µg Protein)			Avenin-E Content (PA/µg Protein)			SSG1 Content (PA/µg Protein)			SSG2 Content (PA/µg Protein)			ATI-2 Content (PA/µg Protein)		
S01	2760	±	2	3234	±	72	10928	±	138	643	±	165	211	±	42	2781	±	45
S02	464	±	95	1491	±	41	4807	±	283	571	±	208	88	±	41	79	±	56
S03	1256	±	46	2158	±	68	7944	±	71	237	±	50	162	±	48	1092	±	21
S04	2086	±	150	5041	±	117	14254	±	550	1304	±	81	957	±	22	2839	±	55
S05	761	±	15	1150	±	17	4692	±	84	401	±	18	412	±	54	291	±	63
S06	626	±	48	314	±	4	1998	±	58	n. d.			462	±	37	123	±	23
S07	517	±	9	1167	±	76	4858	±	135	181	±	5	359	±	55	207	±	24
S08	1055	±	65	1439	±	9	7773	±	16	1183	±	98	384	±	57	464	±	61
S09	745	±	25	720	±	40	2673	±	50	1956	±	108	557	±	104	100	±	19
S10	952	±	17	1747	±	81	8005	±	202	1932	±	136	539	±	19	223	±	20
S11	682	±	5	1587	±	149	4645	±	166	1424	±	100	580	±	49	162	±	33
S12	558	±	4	1482	±	12	3571	±	35	1034	±	142	207	±	18	128	±	25
S13	648	±	6	352	±	21	2358	±	59	267	±	38	66	±	21	70	±	2
S14	963	±	12	987	±	53	3157	±	51	246	±	13	251	±	33	223	±	4
S15	810	±	50	510	±	11	2397	±	19	1121	±	212	1041	±	819	142	±	27
S16	819	±	69	694	±	17	3692	±	121	523	±	105	924	±	866	205	±	5
S17	1379	±	97	5511	±	65	16865	±	257	469	±	112	638	±	498	306	±	9
S18	929	±	4	1330	±	40	8442	±	63	284	±	71	380	±	295	193	±	9
S19	790	±	13	1895	±	68	7643	±	8	511	±	94	445	±	288	171	±	5
S20	1976	±	38	1417	±	68	7032	±	148	656	±	278	549	±	75	612	±	42
S21	1253	±	46	2845	±	133	8737	±	64	228	±	8	402	±	35	173	±	5
S22	1531	±	34	2738	±	21	11047	±	256	n. d.			518	±	111	249	±	5
S23	1096	±	38	1183	±	38	5131	±	113	409	±	11	239	±	2	119	±	21
S24	2562	±	48	3508	±	221	5377	±	43	938	±	43	364	±	17	809	±	2

PA: Peak Area; SSG: Seed Storage Globulin; ATI-2: Amylase Trypsin Inhibitor-2; n. d.: non-detectable

**Table S12:** Relative protein content of the 6 targeted proteins in the albumin/globulin fractions of the 24 investigated oat samples. The results are expressed as peak area per  $\mu\text{g}$  proteins (PA/ $\mu\text{g}$  proteins)

Sample Nr.	Avenin Content (PA/ $\mu\text{g}$ Protein)	Avenin-3 Content (PA/ $\mu\text{g}$ Protein)	Avenin-E Content (PA/ $\mu\text{g}$ Protein)	SSG1 Content (PA/ $\mu\text{g}$ Protein)	SSG2 Content (PA/ $\mu\text{g}$ Protein)	ATI-2 Content (PA/ $\mu\text{g}$ Protein)
<b>S01</b>	579 $\pm$ 112	2166 $\pm$ 317	3387 $\pm$ 68	n. d.	n. d.	n. d.
<b>S02</b>	2849 $\pm$ 101	32286 $\pm$ 285	94092 $\pm$ 1139	n. d.	n. d.	8772 $\pm$ 544
<b>S03</b>	606 $\pm$ 235	7603 $\pm$ 332	25923 $\pm$ 1257	n. d.	n. d.	n. d.
<b>S04</b>	1224 $\pm$ 384	5808 $\pm$ 56	14686 $\pm$ 2226	n. d.	n. d.	n. d.
<b>S05</b>	2239 $\pm$ 95	5684 $\pm$ 211	14511 $\pm$ 20	n. d.	n. d.	4529 $\pm$ 219
<b>S06</b>	2070 $\pm$ 26	11364 $\pm$ 225	41068 $\pm$ 239	n. d.	n. d.	4391 $\pm$ 44
<b>S07</b>	769 $\pm$ 27	3466 $\pm$ 71	9480 $\pm$ 35	n. d.	n. d.	3068 $\pm$ 57
<b>S08</b>	1351 $\pm$ 27	15217 $\pm$ 196	34732 $\pm$ 943	n. d.	n. d.	5896 $\pm$ 13
<b>S09</b>	2565 $\pm$ 112	4363 $\pm$ 189	21600 $\pm$ 219	n. d.	n. d.	6994 $\pm$ 283
<b>S10</b>	2862 $\pm$ 26	6097 $\pm$ 162	22951 $\pm$ 115	n. d.	n. d.	5471 $\pm$ 101
<b>S11</b>	2987 $\pm$ 17	8011 $\pm$ 404	24461 $\pm$ 779	n. d.	n. d.	8596 $\pm$ 381
<b>S12</b>	3130 $\pm$ 9	12738 $\pm$ 134	35032 $\pm$ 334	n. d.	n. d.	5716 $\pm$ 86
<b>S13</b>	3933 $\pm$ 327	10666 $\pm$ 577	45121 $\pm$ 99	n. d.	n. d.	8678 $\pm$ 200
<b>S14</b>	3249 $\pm$ 30	21434 $\pm$ 500	62553 $\pm$ 219	n. d.	n. d.	9270 $\pm$ 149
<b>S15</b>	4147 $\pm$ 297	8741 $\pm$ 461	46532 $\pm$ 286	n. d.	n. d.	11571 $\pm$ 584
<b>S16</b>	4052 $\pm$ 26	22098 $\pm$ 307	80304 $\pm$ 1987	n. d.	n. d.	9041 $\pm$ 49
<b>S17</b>	2293 $\pm$ 26	17235 $\pm$ 137	65373 $\pm$ 1047	n. d.	n. d.	8582 $\pm$ 362
<b>S18</b>	1989 $\pm$ 14	7320 $\pm$ 347	35147 $\pm$ 204	n. d.	n. d.	9758 $\pm$ 121
<b>S19</b>	2370 $\pm$ 72	9799 $\pm$ 80	25185 $\pm$ 11	n. d.	n. d.	9793 $\pm$ 313
<b>S20</b>	2811 $\pm$ 200	14252 $\pm$ 241	35317 $\pm$ 136	n. d.	n. d.	12883 $\pm$ 66
<b>S21</b>	5381 $\pm$ 303	12709 $\pm$ 39	26442 $\pm$ 902	n. d.	n. d.	34041 $\pm$ 347
<b>S22</b>	2687 $\pm$ 54	7149 $\pm$ 66	24046 $\pm$ 41	n. d.	n. d.	15800 $\pm$ 846
<b>S23</b>	5928 $\pm$ 1710	17056 $\pm$ 112	43684 $\pm$ 573	n. d.	n. d.	25263 $\pm$ 770
<b>S24</b>	1304 $\pm$ 17	7061 $\pm$ 167	17564 $\pm$ 271	n. d.	n. d.	8385 $\pm$ 282

PA: Peak Area; SSG: Seed Storage Globulin; ATI-2: Amylase Trypsin Inhibitor-2; n. d.: non-detectable

## References

1. DuPont, F.M.; Chan, R.; Lopez, R.; Vensel, W.H. Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. *J Agric Food Chem* **2005**, *53*, 1575-1584.