Development of Non-Targeted Mass Spectrometry Method for Distinguishing Spelt and Wheat

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1. Supplementary Materials and Methods

1.1 List of spelt and wheat varieties

Table S1: List of spelt and wheat varieties used in the study

Spelt cultivars in the calibration set
Badekrone
Badensonne
Begrenzer Roter Spelz
Frankenkorn
Hohenloher
Holstenkorn
Lignee 24
Oberkulmer Rotkorn
Rottweiler Frühkorn
Schwabenspelz
Steiners Roter Tiroler
Wheat cultivars in the calibration set
Bernstein
Brilliant
Franz
JB Asano
Julius
Kerubino
Lear
Nordkap
Pionier
Produzent
RGT Reform

Untypical spelt cultivars in external validation set
Ardenne

Blauer Samtiger
Chinese Spring
Elsenegger Weisskorn
Meister
Waggershauser Hohenheimer Weisser Kolben
Zeiners Weisser Schlegeldinkel
Emiliano
Schwabenkorn
Voegelers Dinkel Weiss
Weisser Winter-Grannendinkel (Hohenheim)
Old wheat cultivars in external validation set
Criewener Nr 104
Ostpreussischer Eppweizen EPr
Roter Saechsischer Landweizen
Suckerts Sanddickkopf
Ackermanns Bayernkoenig
P.S.G. Sandweizen

2. Supplementary results

2.1 Internal validation: calibration set

The output of the convolutional neural networks (CNN) models are probability scores (p_i) which are transformed to log odds ($\ln(\frac{p_i}{1-p_i})$). The D scores are calculated using these log odds values. Both are listed below in Table S2 for the cultivars of the calibration set. An example calculation for the D score is described in the following.

The mean log odds are calculated for both the classes to be -7.66 and 7.303. The λ and θ values are then calculated according to Equations (S3) and (S4). The obtained λ and θ values are then used for the D score calculations for internal validation and external validation set.

$$\bar{\mu}_{spelt} = -7.660 \tag{S1}$$

$$\bar{\mu}_{wheat} = 7.303 \tag{S2}$$

$$\lambda = \frac{2}{\bar{\mu}_{spelt} - \bar{\mu}_{wheat}} = \frac{2}{-7.660 - 7.303} = -0.1336 \tag{S3}$$

$$\theta = 1 - \lambda \times \bar{\mu}_{spelt} = 1 - (-0.1336 \times -7.660) = -0.0238 \tag{S4}$$

For instance, the D score for measurement 1 of Badekrone spelt with log odds of -6.980 can be calculated as follows to give 0.909.

$$D_{Badekrone,M1} = (-6.980 \times -0.1336) - 0.0238 = 0.909 \tag{S5}$$

This way the D scores for all measurements are obtained.

Table S2A: Log odds and D scores for spelt cultivars in the calibration set.

Spelt cultivars in the calibration set	Log odds		D score	
	Measurement 1	Measurement 2	Measurement 1	Measurement 2
Badekrone	−6.980	−6.870	0.909	0.894
Badensonne	−4.758	−6.384	0.612	0.829
Begrenzer Roter Spelz	−8.958	−10.079	1.173	1.323
Frankenkorn	−7.178	−7.867	0.936	1.028
Hohenloher	−7.187	−7.715	0.937	1.007
Holstenkorn	−8.682	−8.476	1.137	1.109
Lignee 24	−7.403	−7.260	0.966	0.946
Oberkulmer Rotkorn	−8.877	−7.619	1.163	0.994
Rottweiler Frühkorn	−8.898	−7.029	1.165	0.916
Schwabenspelz	−8.409	−6.739	1.100	0.877
Steiners Roter Tiroler	−8.127	−7.029	1.062	0.916
Measurement mean value	−7.769	−7.551	1.015	0.985
Class mean value	−7.660		1	

Table S2B: Log odds and D scores for wheat cultivars in the calibration set

Wheat cultivars in the calibration set	Log odds		D score	
	Measurement 1	Measurement 2	Measurement 1	Measurement 2
Bernstein	7.229	7.745	−0.990	−1.059
Brillant	5.844	6.289	−0.805	−0.864
Franz	8.409	8.702	−1.148	−1.187
JB Asano	6.451	5.683	−0.886	−0.783
Julius	7.422	7.846	−1.016	−1.073
Kerubino	7.118	6.990	−0.975	−0.958
Lear	6.970	6.999	−0.955	−0.959
Nordkap	7.757	10.229	−1.061	−1.391
Pionier	7.044	6.852	−0.965	−0.940
Produzent	5.149	7.579	−0.712	−1.037
RGT Reform	8.101	8.264	−1.107	−1.128
Measurement mean value	7.045	7.562	−0.965	−1.035
Class mean value	7.303		−1	

2.2 Precision parameters

Table S3: Summary of precision parameters for spelt and wheat

	Spelt	Wheat
Class mean D score value	1	−1
Intermediate SD	0.075	0.074
Classification SD	0.393	0.391

2.2 External validation: processed goods and artificial mixes

Table S4 shows the average log odds and D scores obtained from predictions of the eleven NCV models, for the duplicate measurements of processed goods and artificial mix. The mean D score for artificial mixes is around 0.9.

Table S4: Log odds and D scores for processed goods and artificial mixes

Processed goods and artificial mixes		**Log odds**		**D score**	
		Measurement 1	**Measurement 2**	**Measurement 1**	**Measurement 2**
Processed goods					
Spelt flour mix		−6.133	−6.128	0.796	0.795
Spelt bread		−6.087	−5.830	0.790	0.755
Measurement mean value		−6.110	−5.979	0.793	0.775
Processed goods mean		−6.045		0.784	
Artificial mixes					
90% Oberkulmer Rotkorn +	10% Bernstein	−7.437	−6.701	0.970	0.872
	10% Brillant	−7.059	−6.298	0.920	0.818
	10% Franz	−7.011	−6.201	0.913	0.805
	10% JB Asano	−7.120	−6.589	0.928	0.857
	10% Julius	−7.149	−6.595	0.932	0.858
	10% Kerubino	−7.238	−6.619	0.944	0.861
	10% Lear	−7.734	−6.991	1.010	0.911
	10% Nordkap	−7.349	−6.372	0.958	0.828
	10% Pionier	−7.199	−6.530	0.938	0.849
	10% Produzent	−7.688	−6.817	1.004	0.887
	10% RGT Reform	−7.043	−6.283	0.917	0.816
Measurement mean value		−7.275	−6.545	0.949	0.851
Artificial mix mean value		−6.910		0.9	

2.3 External validation: untypical spelt

Table S5 shows the average log odds and D scores obtained from predictions of the eleven NCV models, for the duplicate measurements of untypical spelt cultivars. The mean D score is 0.572.

Table S5: Log odds and D scores for untypical spelt cultivars

Untypical spelt cultivars	Log odds		D score	
	Measurement 1	Measurement 2	Measurement 1	Measurement 2
Ardenne	−1.726	−4.539	0.207	0.583
Blauer Samtiger	−3.114	−5.365	0.392	0.693
Chinese Spring	−2.919	−4.730	0.366	0.608
Elsenegger Weisskorn	−4.758	−4.596	0.612	0.590
Emiliano	−4.744	−4.142	0.610	0.530
Meister	−4.182	−4.604	0.535	0.592
Schwabenkorn	−5.012	−3.731	0.646	0.475
Voegelers Dinkelweiss	−5.325	−4.628	0.688	0.595
Waggershauser Hohenheimer Weisser Kolben	−5.919	−4.018	0.767	0.513
Weißer Winter-Grannendinkel aus Hohenheim	−5.341	−4.689	0.690	0.603
Zeiners weisser Schlegeldinkel	−5.240	−4.810	0.676	0.619
Measurement mean value	−4.389	−4.532	0.563	0.582
Untypical spelt mean	−4.460		0.572	

2.4 Extenal validation: old wheat

Table S6 shows the average log odds and D scores obtained from predictions of the eleven NCV models, for the duplicate measurements of old wheat cultivars. The mean D score for Ackermanns Bayernkönig is 0.11. And the mean D score for the duplicate measurements of Criewener Nr. 104, Ostpreußischer Eppweizen, P.S.G. Sandweizen, Roter Sächsischer Landweizen and Suckers Sanddickkopf is −0.1.

Table S6: Log odds and D scores for old wheat cultivars

Old wheat cultivars	**Log odds**		**D score**	
	Measurement 1	**Measurement 2**	**Measurement 1**	**Measurement 2**
Ackermanns Bayernkönig	−1.198	−0.824	0.136	0.086
Criewener Nr. 104	0.111	0.332	−0.039	−0.068
Ostpreußischer Eppweizen	0.521	0.424	−0.094	−0.081
P.S.G. Sandweizen	0.678	0.592	−0.114	−0.103
Roter Sächsischer Land-weizen	0.558	1.735	−0.098	−0.256
Suckers Sanddickkopf	0.704	0.240	−0.118	−0.056
Measurement mean value	0.229	0.417	−0.054	−0.080
Old wheat mean value	0.323		−0.067	