



Insights into the total antioxidant capacities of different cultivars of gluten-free grains using benchtop and handheld NIR spectroscopy

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Content

- » Gluten-free grains
- » Total antioxidant capacity
- » Near-infrared spectroscopy
- » Results
- » Conclusion and outlook

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Gluten-free grains – What is that?

- » Gluten-free-crops: corn, millet, rice, some oats
- » Pseudocereals: buckwheat, quinoa, amaranth



Gluten-free grains – Why analyse

- » Little is known about the chemical profiles of different cultivars
- » Often inferior nutraceutical properties than wheat
- » Better the diet of coeliac patients

Gluten-free grains – RE-Cereal Project

» Aims

- Higher quality of gluten-free products (mainly millet and buckwheat)
- Breed new, better cultivars of millet and buckwheat
- Optimize quality control and quality analysis

» Project partners

- Breeders
- Universities
- Producers



Gluten-free grains – Samples

» 77 samples

- 40 buckwheat
- 31 millet
- 6 oat

Millet



Buckwheat



Oat



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Total antioxidant capacity (TAC)

» Sum parameter

» Antioxidants

- Molecules which inhibit oxidation or reactions promoted by oxygen.
- Prevent or reduce oxidative stress (reactions with lipids, proteins, DNA)
- Help reduce risk of cardiovascular diseases, chronic inflammations, cancer
- Vitamins, polyphenols, phenolic acids

» Oxidative stability

Total antioxidant capacity (TAC) – Analysis (I)

- » Samples were milled before analysis
- » Extraction
 - 1g of milled sample
 - Acidified methanol [95% methanol, 5% hydrochloric acid (37%)]
- » Extraction cycle (6/9 times)
 - Ultrasonic bath at 60°C for 20 minutes
 - Centrifuge (3500 rpm, 5 min)

Total antioxidant capacity (TAC) – Analysis (II)

- » Various assays, all use redox reactions
- » Folin-Ciocalteu
 - Mixture of phosphomolybdate and phosphotungstate
 - Reduction of Mo(VI) to Mo(V) under basic conditions
 - Blue coloured complex: 750 nm
- » Reference: gallic acid



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Near-infrared spectroscopy – Advantages

- » Fast
- » Rather cheap
- » No chemicals
- » Miniaturized technology available

Near-infrared spectroscopy – Measurements

- » 4 devices
 - 1 benchtop
 - 3 handheld
- » Measurements of milled and intact samples
- » Cylindrical quartz cuvette (h=25 mm, $\varnothing$ =31.6 mm)

Near-infrared spectroscopy – Instruments (I)



Near-infrared spectroscopy – Instruments (II)

Instrument	Wavenumber range / cm^{-1}	Wavelength range / nm	(Average) Resolution / cm^{-1}	Number of data points
NIRFlex N-500 (Büchi)	10.000–4.000	1.000–2.500	8	1501
microPhazir RX (Thermo Fischer)	6.270–4.180	1.600–2.400	21	100
MicroNIR 2200 (Viavi)	8.870–4.630	1.130–2.160	33	128
SCiO (Consumer Physics)	13.514–9.346	740–1.070	13	331

Near-infrared spectroscopy – Spectral ranges

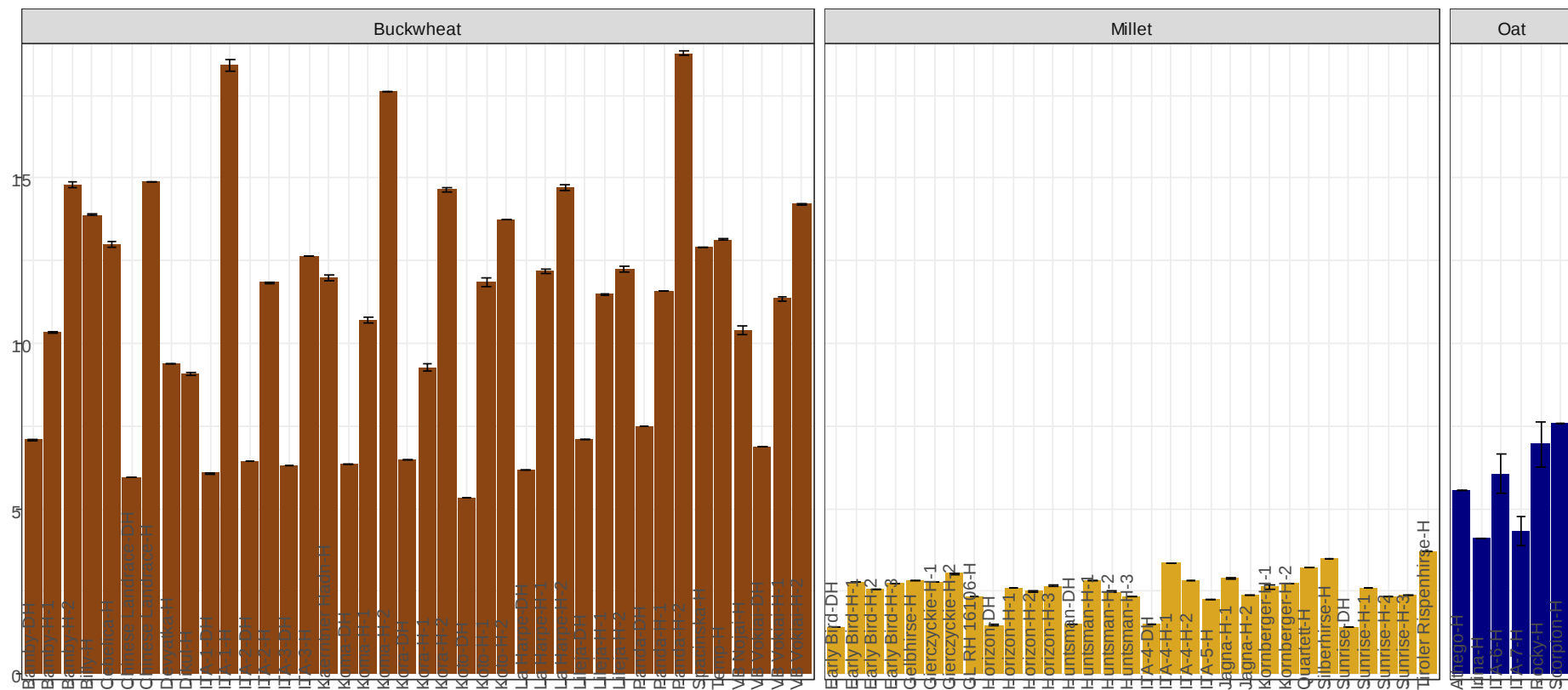


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Results – TAC analysis

Total antioxidant capacity / mgGAE/ç



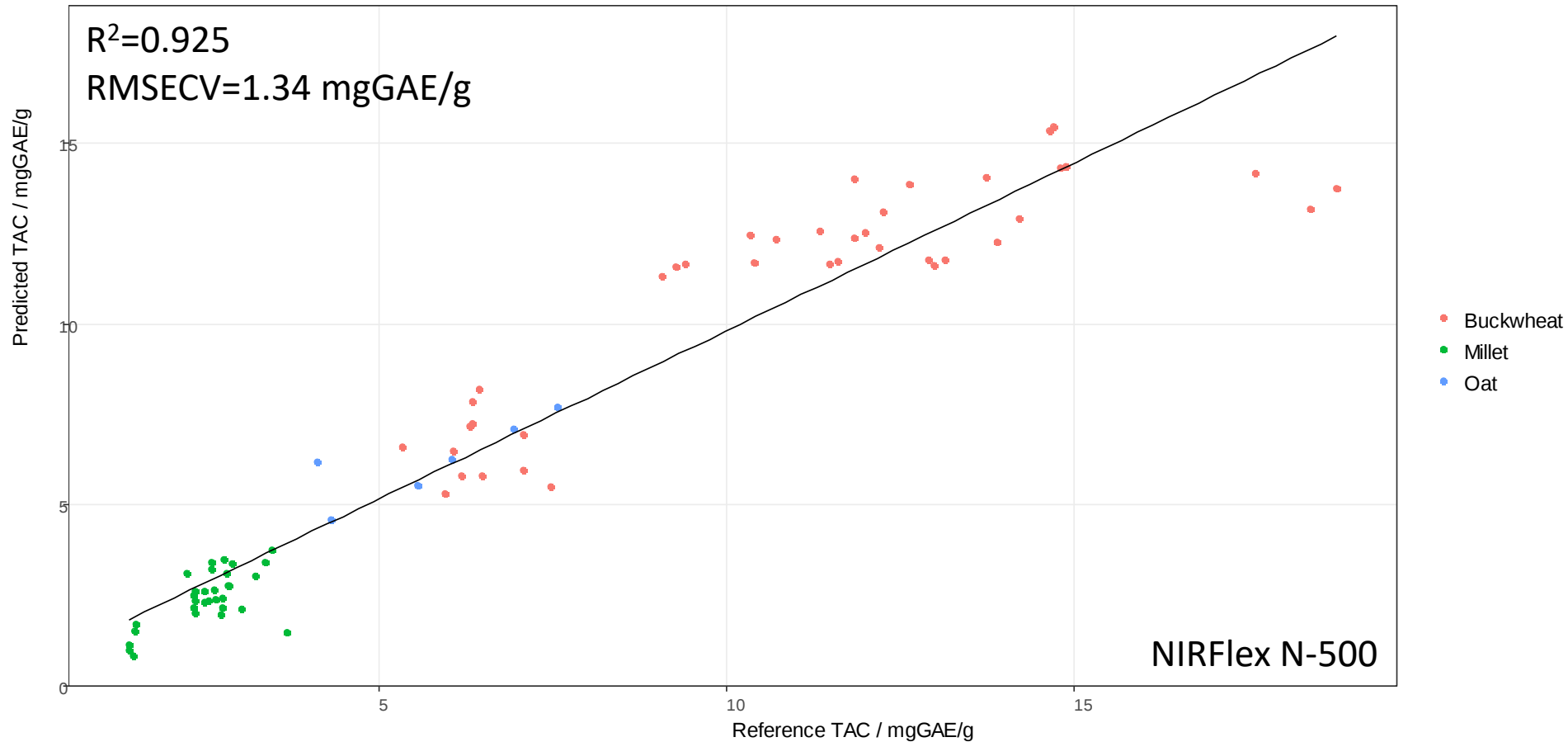
Results – Near-infrared spectroscopy (I)

Vibration	Wavenumber / cm^{-1}		Wavelength / nm
C-H str. & C-H def. comb.	microPhazir RX	4280	2336
N-H def. & C-H str.		4320	2315
N-H str.		4748	2106
2x O-H def. & 2x C-O str.		4807–4766	2080–2098
O-H str. (water)		5186	1928
C-H str. 1 st overtone	MicorNIR 2200	5788–5646	1728–2080
N-H str. 1 st overtone		6805	1470
C-H str. 2 nd overtone		8318	1202
2x C-H str. & 2x C-H def + $(\text{CH}_2)_n$	NIRFlex N-500	9506	1052
N-H 2 nd overtone		9747	1026
O-H str. 2 nd overtone		10549	948
C-H str. 3 rd overtone	SCiO	11111	900

Results – Near-infrared spectroscopy (II)

Device	State of grain	Spectral pre-treatments
NIRFlex N-500	Intact	Savitzky Golay 2 nd derivation 13 SM, SNV (8948–4032 cm ⁻¹ , 1118–2480 nm), OSC
	Milled	Savitzky Golay 2 nd derivation 17 SM, SNV (8948–4032 cm ⁻¹ , 1118–2480 nm), OSC
MicroPhazir RX	Intact	Savitzky Golay 2 nd derivation 7 SM, SNV, OSC
	Milled	Savitzky Golay 2 nd derivation 7 SM, SNV, OSC
MicroNIR 2200	Intact	Moving Average smoothing, Savitzky Golay 2 nd derivation 11 SM, SNV, OSC
	Milled	Moving Average smoothing, Savitzky Golay 2 nd derivation 11 SM, SNV, OSC
SCiO	Intact	Savitzky Golay 2 nd derivation, 17 SM, SNV (11364–10000 cm ⁻¹ , 880–1000 nm), OSC
	Milled	Savitzky Golay 2 nd derivation, 17 SM, SNV (11364–10000 cm ⁻¹ , 880–1000 nm), OSC

Results – Partial least squares regressions



Results – Correlation statistics

Device	State of the grains	Factors	R ² (CV)	RMSECV / mgGAE/g	Bias (CV)	R ² (TV)	RMSEP / mgGAE/g	Bias (TV)
NIRFlex N-500	Intact	2	0.925	1.34	0.0150	0.912	1.58	-0.0555
	Milled	4	0.892	1.60	0.0206	0.883	1.66	0.0592
microPhazir RX	Intact	3	0.921	1.46	0.0084	0.893	1.37	0.8504
	Milled	3	0.913	1.35	0.0177	0.891	1.78	-0.4993
MicroNIR 2200	Intact	3	0.951	1.11	-0.0012	0.952	1.02	-0.0343
	Milled	4	0.910	1.46	0.0468	0.919	1.44	-0.2304
SCiO	Intact	2	0.823	1.98	0.0116	0.895	1.24	-0.2890
	Milled	2	0.824	2.08	0.0334	0.849	1.86	-0.3235

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Results – multivariate LOD/LOQ (I)

- » The matrix might differ from sample to sample
- » Not all changes in the spectrum can be attributed to changes in the analyte
- » Univariate $\leftrightarrow$ Multivariate
- » LOD and LOQ range (Allegrini & Olivieri)

Results – multivariate LOD/LOQ (II)

Device	State of the grains	LOD _{min} / mgGAE/g	LOD _{max} / mgGAE/g	LOQ _{min} / mgGAE/g	LOQ _{max} / mgGAE/g
NIRFlex N-500	Intact	0.867	1.716	2.600	5.148
	Milled	1.238	2.797	3.714	8.391
microPhazir RX	Intact	1.396	2.386	4.188	7.159
	Milled	1.477	2.549	4.432	7.646
MicroNIR 2200	Intact	1.237	1.834	3.701	5.502
	Milled	1.655	3.229	4.964	9.688
SCiO	Intact	1.783	3.031	5.348	9.094
	Milled	1.703	2.617	5.109	7.852

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Conclusion (I)

» NIR can be used for TAC estimation with limitation

- Millet samples range: 1.4–3.7 mgGAE/g
- Oat samples range: 4.1–7.6 mgGAE/g
- Buckwheat samples range: 5.3–18.8 mgGAE/g

- RMSEP range: 1.02–1.86 mgGAE/g
- LOD_{max} range: 1.72–3.23 mgGAE/g
- LOQ_{max} range: 5.15–9.69 mgGAE/g

Conclusion (II)

- » Resolution is not all
 - MicroNIR 2200 worst resolution, but good results

- » Handheld spectrometer perform similarly to benchtop
 - Benchtop results are more stable in regards to milled/intact
 - Benchtop performs best at multivariate LOD/LOQ

Outlook

» Publication

- Verena, Wiedemair; Huck, Christian W. (2018): Evaluation of the performance of three hand-held near-infrared spectrometer through investigation of total antioxidant capacity in gluten-free grains. In: *Talanta*. DOI: 10.1016/j.talanta.2018.06.056.

» Look at other parameters of gluten-free grains

- Protein content
- Amino acids

Thank you for your attention

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Thank you for your attention – Questions?

Thank
you 😊



