Supplement

Agronomy

The Energy Efficiency of the Production and Conversion of Spring Triticale Grain into Bioethanol

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Part I. Information about starch analysis

The content of starch will be determined in 24 average grain samples collected under the presented experiment by polarimetric methods at the Central Agroecological Laboratory of the University of Life Sciences in Lublin (CLA/PLC/30).
Standard operating procedure:
Method no.: CLA/PLC/30/2011. Version 2, 01/02/2011.
Title: Determination of starch content by the polarimetric method.
The method developed on the basis of PN-EN ISO 10520: 2002. Natural starch. Determination of starch content. Polarimetric method of Ewers.

The description of the method presented below is an own study. It was made based on the most important issues described in the method CLA/PLC/30.

1. Purpose

The procedure describes the quantitative determination of starch content in samples of various food products and plant materials using the polarimetric method.

2. The principle of the methods

The determination consists in extraction of starch from the sample with hydrochloric acid, and then measuring the optical rotation of the solution is measured by polarimetry.

3. Apparatus and laboratory glass.

3.1. Polarimetr (Circular polarimeter P1000 WXG4, Producent Mikrolab PL.. Data: rotation range ± 180°, reading accuracy ± 0,05°, sensitivity 0,05°, monochromatic lamp 589nm, power 230V.

3.2. Automatic analytical scales WAA 160/C/2 (Producent Radwag).

3.3. 400 cm^3 flask.

3.4. Graduated flask 100 cm^3.

3.5. Volumetric flask 1 dm^3.

3.6. 250 cm^3 erlenmeyer flask.

3.7. Glass funnel.

3.8. Porcelain mortar with pestle.

3.9. 50 cm^3 measuring flask.

4. Reagents

4.1. Demineralized water.

1.1. Hydrochloric acid 0,31 mol/dm^3 (25,6 cm^3 HCl about d=1,19 g/cm^3 fill with deionized water in 1 dm^3 volumetric flask).

1.2. Hydrochloric acid 25% (640 cm^3 HCl about d=1,19 g/cm^3 fill with deionized water in 1 dm^3 volumetric flask).

1.3. Carrez solution I (Add to the 100 cm^3 graduated flask of weighed 10,6 g $K_4[Fe(CN)_6]\cdot 3H_2O$ (*Standard*) and make up of deionized water to the mark).

1.4. Carrez solution II (Add to the 100 cm3 graduated flask of weighed 21,9 g $Zn(CH_3COO)_2]\cdot 2H_2O$ (*Standard*) and add 3 cm^3 glacial acetic acid (*Standard*) and make up of deionized water to the mark).

1.5. Paper filters.

5. Procedure

5.1. Correct sample

5.1.1. Measure 50 cm^3 of hydrochloric acid 0.31 mol/dm^3 in a measuring flask.

5.1.2. Weigh accurately approximately 5 g of the sample (*m*) and grate in a mortar with the addition of several cm^3 of hydrochloric acid taken from the graduated flask.

5.1.3. Transfer the contents of the mortar into a 100 cm^3 volumetric flask through a glass funnel, rinsing with the remaining hydrochloric acid 0.31 mol/dm^3.

5.1.4. Insert the flask into a beaker with boiling water set on the electric stove, close the flask with a cap slightly smaller than its neck and cook for 15 minutes, shaking the contents of the flask every few minutes.

5.1.5. Remove the flask from the oven and let it reach room temperature.

5.1.6. Add 1 cm^3 of Carrez I and II solutions to the flask, make up to the mark with deionized water and shake the contents.

5.1.7. The solution is filtered into a polarimetric tube through a pleated filter paper.

5.1.8. Measure the value of the plane angle of polarized light (α_w).

5.2. Blind sample

5.2.1. Weigh accurately twice the number of samples as in the correct sample (*2m*), transfer to a 100 cm^3 volumetric flask through a glass funnel by rinsing with deionized water and add 75 cm^3 of this water.

5.2.2. Insert the flask into a beaker with boiling water set on the electric stove, close the flask with a stopper slightly smaller than its neck and cook for 15 minutes, shaking the contents of the flask every few minutes.

5.2.3. Remove the flask from the oven and let it reach room temperature.

5.2.4. Add 1 cm^3 of Carrez I and II solutions to the flask, make up to the mark with deionised water and shake the contents.

5.2.5. Filter the solution through a pleated paper filter into a 250 cm^3 erlenmeyer flask.

5.2.6. Take 50 cm^3 of the filtrate, transfer to a 100 cm^3 graduated volumetric flask. and add 3 cm^3 of 25% hydrochloric acid.

5.2.7. Insert the flask into a beaker with boiling water set on the electric stove, close the flask with a cap slightly smaller than its neck and cook for 15 minutes, shaking the contents of the flask every few minutes.

5.2.8. Remove the flask from the oven and let it reach room temperature.

5.2.9. Make up the contents of the flask to the mark with deionized water.

5.2.10. The solution is filtered into a polarimetric tube through a pleated filter paper.

5.2.11. Measure the value of the plane angle of polarized light (α_s).

Attention!!!

Measure the sample in triplicate and perform calculations separately.

6. Calculations

$$x = \frac{100 \cdot 100 \cdot (\alpha_w - \alpha_s)}{m \cdot l \cdot [\alpha]_D^{20}} \qquad \text{[g/100g fresk sample]}$$

where:

α_w – value of the optical rotation in angle degrees for the correct sample [°];

α_s – value of the optical rotation in angle degrees for the blind sample [°];

m – weight of the sample [g];

l – length of the polarimetric tube [dm];

$[\alpha]_D^{20}$ – specific optical rotation of pure starch [see table below].

Table S1. Numerical values for various types of starch

Starch	Specific optical rotation $[\alpha]_D^{20}$
Rice	+ 185.9
Potato	+ 185.7
Maize	+ 184.6
Wheat	+ 182.7
Barley	+ 181.5
Oat	+ 181.3
Other types of starch and starch mixtures	+ 184.0

7. Literature

1. Food analysis, Script for exercises. Part. I. Red. M. Klepacka. Fundacja Rozwój SGGW. Warszawa 1998. (in Polish).
2. Consolidated TEXT produced by the CONSLEG system of the Office for Official Publications of the European Communities. CONSLEG: 1972L0199 — 27/08/1999. Office for Official Publications of the European Communities. Commission Directive 1999/79/EC of 27 July 1999. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF. (accessed 20.03.2019)

Part II. Basic data for the methods and preparation of tables in the mansuscript

Table S2. Chemicals used in the protection of spring wheat against pests.

Pests	The pesticide	Active substance	Dose	BBCH stage
Grain	Vitavax 200FS	Carboxin + tiuram	0.3 L/100 kg of grain	Grain dressing
Weeds	Granstar 75 WG	tribenuron-methyl	20 g/ha	BBCH 28
	Puma Super 069	fenoxaprop-P-ethyl	1 L/ha	BBCH 28
Fungi	Alert 375 SC	flusilazole + carbendazim	1 L/ha	BBCH 30–32
	Tilt CB 39.5	propiconazole + carbendazim	1 L/ha	BBCH 58–59
Retardant	Terpal C 460 SL	ethephon + mepiquat	0.25 L/ha	BBCH 58–59

Table S3. Basic data for the preparation of the Table 3: Grain yield and bioethanol yield of spring triticale as affected by N fertilization

Tillage systems	Nitrogen dosage	Year	Grain yield	Starch content	Starch yield	Efficiency of ethanol	Bioethanol yield	Agronomic efficiency of N fertilizers
			[t ha-1]	[%]	[t ha-1]	[L t-1]	[L ha-1]	[L 1 kg N-1 applied]
TRD	0	2012	41.6	66.70	27.75	479.18	1993.40	-
		2013	38.2	66.70	25.48	479.18	1860.48	-
		2014	33.5	66.60	22.31	478.46	1602.86	-
	40	2012	52.2	66.70	34.82	478.18	2501.33	12.70
		2013	55.4	66.90	37.06	480.62	2662.63	20.80
		2014	48.1	66.50	31.99	477.75	2297.96	17.38
	80	2012	58.7	66.40	38.98	477.03	2800.15	10.08
		2013	58.8	67.00	39.40	481.34	2830.27	12.50
		2014	56.6	66.70	37.75	479.18	2712.17	13.87
	120	2012	59.4	66.30	39.38	476.31	2829.28	6.97
		2013	60.7	67.50	40.97	484.93	2943.53	9.28
		2014	58.9	66.90	39.40	480.62	2830.85	10.23
RED	0	2012	34.4	66.80	22.98	479.90	1650.86	-
		2013	36.4	66.80	24.32	479.90	1746.84	-
		2014	31.6	67.50	21.33	484.93	1532.38	-
	40	2012	41.5	66.70	27.68	479.18	1988.61	8.44
		2013	46.7	66.90	31.24	480.62	2244.49	12.44
		2014	34.9	67.30	23.49	483.49	1687.39	3.88
	80	2012	44.2	66.50	29.39	477.75	2111.64	5.76
		2013	54.7	66.20	36.21	475.59	2601.48	10.68
		2014	44.4	67.40	29.93	484.21	2149.90	7.72
	120	2012	47.8	66.60	31.83	478.46	2287.06	5.30
		2013	57.7	66.50	38.37	477.75	2756.59	8.41
		2014	45.1	67.50	30.44	484.93	2187.04	5.46

Explanations: TRD – traditional tillage system, RED – reduced tillage system

Table S4. Results of statistical calculations for the studied technological features of spring triticale grains and bioethanol - for the preparation of the Table S2.

Variable		Grain yield	Starch content	Starch yield	Efficiency of bioethano	Bioethanol yield	Agronomic efficiency of N fertilizers
		[t ha^{-1}]	[%]	[t ha^{-1}]	[L t^{-1}]	[L ha^{-1}]	[L 1 kg N^{-1} applied]
CV%	T	8.99	0.11	8.93	0.11	8.93	27.89
	N	15.55	0.10	15.53	0.10	15.53	19.32
	Y	5.96	0.28	5.86	0.28	5.86	18.27
	T × N	18.22	0.20	18.18	0.20	18.18	36.32
	T × Y	11.23	0.47	11.04	0.47	11.04	35.35
	N × Y	16.86	0.35	16.83	0.35	16.83	28.36
Estimantion F	T	107.00***	2.40	121.92***	2.40	121.92***	93.81***
	N	106.58***	0.69	123.01***	0.69	123.01***	15.00**
	Y	23.45**	7.61*	26.27**	7.61*	26.27**	20.13**
	T × N	4.06	1.04	4.87*	1.04	4.87*	6.76*
	T × Y	6.38*	12.29**	5.98*	12.29**	5.98*	8.33*
	N × Y	1.49	0.92	1.95	0.92	1.95	1.95
p-value	T	0.0000	0.1723	0.0000	0.1723	0.0000	0.0001
	N	0.0000	0.5899	0.0000	0.5889	0.0000	0.0034
	Y	0.0015	0.0226	0.0011	0.0226	0.0011	0.0022
	T × N	0.0682	0.4411	0.0476	0.4411	0.0476	0.0237
	T × Y	0.0327	0.0076	0.0375	0.0076	0.0373	0.0186
	N × Y	0.3199	0.5385	0.2181	0.5385	0.2181	0.2179
$LSD_{0,05}$	T	2.02	n.s.	1.26	n.s.	90.32	1.51
	N	2.86	n.s.	1.78	n.s.	127.73	2.14
	Y	2.47	0.29	1.54	2.08	110.62	1.85
	T × N	4.05	n.s.	2.51	n.s.	180.64	3.02
	T × Y	3.51	0.41	2.18	2.95	156.44	2.62
	N × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Explanations: T – tillage (df_1 = 1, df_2 = 6), N – nitrogen dossage (df_1 = 3, df_2 = 6), Y - year (df_1 = 2, df_2 = 6), T × N – tillage × nitrogen dossage (df_1 = 3, df_2 = 6), T × Y – tillage × year (df_1 = 2, df_2 = 6), N × Y – nitrogen dossage × year (df_1 = 6, df_2 = 6): where df_1 – degrees of variable freedom, df_2 – degress of error freedom; *CV%* - coefficient of variation, Estimantion F of variance analysis, significant difference at (*α = 0.05, ** α = 0.01, *** α = 0.001), p-value of F-variance ratio, LSD – least significant difference, n.s.- not significant.

Table S5. Basic data for the human labour consumptions (hours ha^{-1}) of cultivation technology of spring Triticale (preparation of the Table 4: Energy inputs and their structure in spring Triticale production)

Treatments	Soil tillage x nitrogen dosage							
	TRD0	TRD40	TRD80	TRD120	RED0	RED40	RED80	RED120
Harrowing	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ploughing	2.5	2.5	2.5	2.5	0.0	0.0	0.0	0.0
Cultivation	0.0	0.0	0.0	0.0	0.7	0.7	0.7	0.7
Cultivation	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Mineral fertilization NPK	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Harrowing	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Sowing	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Harrowing	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fertilization N	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Spraying – pathogenes and weeds 3x	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Grain harvest	1.0	1.0	1.2	1.5	1.0	1.0	1.2	1.5
Grain transport	0.5	0.5	1.0	1.0	0.5	0.5	1.0	1.0
Total	9.5	9.5	10.2	10.5	7.7	7.7	8.4	8.7

Table S6. Basic data for energy inputs (MJ ha^{-1}) of the treatments in cultivation technology of spring Triticale (preparation of the table 4: Energy inputs and their structure in spring Triticale production and for the Table 5: Structure of the energy inputs in the cultivation of spring-Triticale)

Treatments	Energy inputs (MJ ha^{-1})	Soil tillage x nitrogen dosage							
		TRD0	TRD40	TRD80	TRD120	RED0	RED40	RED80	RED120
Harrowing	1	118.30	118.30	118.30	118.30	118.30	118.30	118.30	118.30
	2	50.63	50.63	50.63	50.63	50.63	50.63	50.63	50.63
Ploughing	1	591.50	591.50	591.50	591.50	0.00	0.00	0.00	0.00
	2	54.40	54.40	54.40	54.40	0.00	0.00	0.00	0.00
Cultivation	1	0.00	0.00	0.00	0.00	165.62	165.62	165.62	165.62
	2	0.00	0.00	0.00	0.00	58.68	58.68	58.68	58.68
Cultivation	1	165.62	165.62	165.62	165.62	165.62	165.62	165.62	165.62
	2	58.68	58.68	58.68	58.68	58.68	58.68	58.68	58.68
Mineral fertilization NPK	1	118.30	118.30	118.30	118.30	118.30	118.30	118.30	118.30
	2	47.04	47.04	47.04	47.04	47.04	47.04	47.04	47.04
Harrowing	1	118.30	118.30	118.30	118.30	118.30	118.30	118.30	118.30
	2	50.63	50.63	50.63	50.63	50.63	50.63	50.63	50.63
Sowing	1	165.62	165.62	165.62	165.62	165.62	165.62	165.62	165.62
	2	241.84	241.84	241.84	241.84	241.84	241.84	241.84	241.84
Harrowing	1	118.30	118.30	118.30	118.30	118.30	118.30	118.30	118.30
	2	50.63	50.63	50.63	50.63	50.63	50.63	50.63	50.63
Fertilization N	1	141.96	141.96	141.96	141.96	141.96	141.96	141.96	141.96
	2	47.04	47.04	47.04	47.04	47.04	47.04	47.04	47.04
Spraying – pathogenes and weeds 3x	1	283.92	283.92	283.92	283.92	283.92	283.92	283.92	283.92
	2	81.13	81.13	81.13	81.13	81.13	81.13	81.13	81.13
Grain harvest	1	624.00	624.00	748.80	936.00	624.00	624.00	748.80	936.00
	2	997.24	997.24	997.24	997.24	997.24	997.24	997.24	997.24
Grain transport	1	118.30	118.30	236.60	236.60	118.30	118.30	236.60	236.60
	2	63.84	63.84	63.84	63.84	63.84	63.84	63.84	63.84
Total	1	2564.12	2564.12	2807.22	2994.42	2138.24	2138.24	2381.34	2568.54
	2	1743.10	1743.10	1743.10	1743.10	1747.38	1747.38	1747.38	1747.38

1- Direct energy carries, 2 - Investments

Table S7. Basic data for energy inputs (MJ ha^{-1}) of the raw materials and materials in cultivation technology of spring Triticale (preparation of the Table 6: Structure of the energy inputs in the raw materials and materials in production of spring-Triticale)

Treatments	Type	Soil tillage x nitrogen fertilization							
		TRD0	TRD40	TRD80	TRD120	RED0	RED40	RED80	RED120
Fertilizer	N	0.0	3080.0	6160.0	9420.0	0.0	3080.0	6160.0	9420.0
	P	1260.0	1260.0	1260.0	1260.0	1260.0	1260.0	1260.0	1260.0
	K	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Seeds		500	500.0	500.0	500.0	500.0	500.0	500.0	500.0
Plant protection products		448.2	448.2	448.2	448.2	448.2	448.2	448.2	448.2
Total		3208.2	6288.2	9368.2	12448.2	3208.2	6288.2	9368.2	12448.2

Table S8. Basic data for the preparation of the Table 7: Energy intensity of production of spring Triticale and bioethanol

Tillage systems	Nitrogen dossage	Year	Energy value of grain yield	Energy expdenditure on producing yield	Energy intensity	Energy value of bioethanol	Energy value of inputs on grain fermentation	EROI
			MJ ha^{-1}	MJ ha^{-1}		MJ ha^{-1}	MJ ha^{-1}	
TRD	0	2012	76377.6	8275.4	9.229	40665.4	25395.9	1.206
		2013	70135.2	8275.4	8.475	37341.8	23320.3	1.180
		2014	61506.0	8275.4	7.432	32698.3	20420.4	1.137
	40	2012	95839.2	11355.4	8.440	51027.2	31867.0	1.179
		2013	101714.1	11355.4	8.957	54317.7	33921.9	1.198
		2014	88311.6	11355.4	7.777	46873.3	29576.0	1.152
	80	2012	107773.2	14734.5	7.314	57123.1	35673.9	1.132
		2013	107956.8	14734.5	7.327	57737.5	36057.6	1.135
		2014	103917.6	14734.5	7.053	55328.4	34553.1	1.121
	120	2012	109054.8	18025.7	6.050	57717.2	36045.0	1.066
		2013	111445.2	18025.7	6.183	60047.9	37500.5	1.080
		2014	108140.4	18025.7	5.999	57749.3	36065.0	1.067
RED	0	2012	63158.4	7709.8	8.192	33677.5	21032.0	1.164
		2013	66830.4	7709.8	8.668	35635.5	22254.7	1.181
		2014	58017.6	7709.8	7.525	31260.4	19522.5	1.140
	40	2012	76194.0	10789.8	7.062	40567.6	25334.9	1.117
		2013	85741.2	10789.8	7.946	45787.7	28594.8	1.157
		2014	64076.4	10789.8	5.939	34422.8	21497.4	1.060
	80	2012	81151.2	14168.9	5.727	43077.4	26902.3	1.044
		2013	100429.2	14168.9	7.088	53070.2	33142.9	1.117
		2014	81518.4	14168.9	5.753	43858.0	27389.7	1.050

		2012	87760.8	17460.1	5.026	46656.0	29137.1	0.997
	120	2013	105937.2	17460.1	6.067	56234.5	35119.0	1.065
		2014	82803.6	17460.1	4.742	44615.5	27864.8	0.980

Explanations: TRD – traditional tillage system, RED – reduced tillage system

Table S9. Results of statistical calculations for the studied energy features of spring triticale grains and bioethanol - for the preparation of the Table S7.

Variable		Energy valu of grain yiel	Energy expenditure on producing yield	Energy intensity	Energy value of bioethanol	Energy value of inputs on grain fermentation	EROI
		MJ ha^{-1}	MJ ha^{-1}		MJ ha^{-1}	MJ ha^{-1}	
CV%	T	9.00	2.21	6.18	8.93	8.93	2.18
	N	15.55	28.47	13.85	15.53	15.53	4.28
	Y	5.96	0.00	6.14	5.86	5.86	1.87
	T × N	18.22	28.56	15.45	18.18	18.18	4.89
	T × Y	11.23	2.21	9.19	11.04	11.04	3.03
	N × Y	16.86	28.47	15.48	16.83	16.83	4.75
Estimation F	T	107.00***	16.99^{9***}	60.22***	121.92***	121.92***	67.25***
	N	106.58***	943.13^{9***}	100.89***	123.01***	123.01***	86.26***
	Y	23.45***	0.00	29.72***	26.27***	26.27***	24.67***
	T × N	4.06	0.30	4.65*	4.87*	4.87*	4.16
	T × Y	6.38*	0.00	6.88*	5.98*	5.98*	6.55*
	N × Y	1.49	0.00	2.67	1.95	1.95	1.83

p-value	T	0.0000	0.0000	0.0002	0.0000	0.0000	0.0002
	N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Y	0.0015	0.8941	0.0008	0.0011	0.0011	0.0013
	T × N	0.0682	0.8049	0.0524	0.0476	0.0476	0.0650
	T × Y	0.0327	0.9015	0.0280	0.0373	0.0373	0.0310
	N × Y	0.3199	0.5020	0.1285	0.2181	0.2181	0.2408
$LSD_{0,05}$	T	3717.0	0.01	0.28	1842.5	1150.7	0.01
	N	5256.7	0.02	0.39	2605.7	1627.3	0.02
	Y	4552.4	n.s.	0.34	2256.6	1409.3	0.02
	T × N	n.s.	n.s.	n.s.	3685.1	2301.4	n.s.
	T × Y	6438.1	n.s.	0.48	2191.37	1993.1	0.03
	N × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Explanations: T – tillage ($df_1 = 1$, $df_2 = 6$), N – nitrogen dossage ($df_1 = 3$, $df_2 = 6$), Y - year ($df_1 = 2$, $df_2 = 6$), T × N – tillage × nitrogen dossage ($df_1 = 3$, $df_2 = 6$), T × Y – tillage × year ($df_1 = 2$, $df_2 = 6$), N × Y – nitrogen dossage × year ($df_1 = 6$, $df_2 = 6$): where df_1 – degrees of variable freedom, df_2 – degress of error freedom; *CV%* - coefficient of variation, Estimantion F of variance analysis, significant difference at (*$\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$), p-value of F-variance ratio, LSD – least significant difference, n.s.- not significant.

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