Electronic Supplementary Material (ESI) for Food & Function.
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Supplementary data – The particle size of milled wheat fractions affects *in vitro* starch digestibility and quality parameters of wire-cut cookies made thereof

Supplementary Table 1

Supplementary Table S1. Cookie formulations with varying levels of white flour (WF) substituted by A) fine farina (FF) or B) coarse farina (CF), while maintaining a constant starch content on dry matter basis. The amounts of each ingredient are given "as is". Formulations highlighted in shades of orange could not be produced due to issues with dough handling.

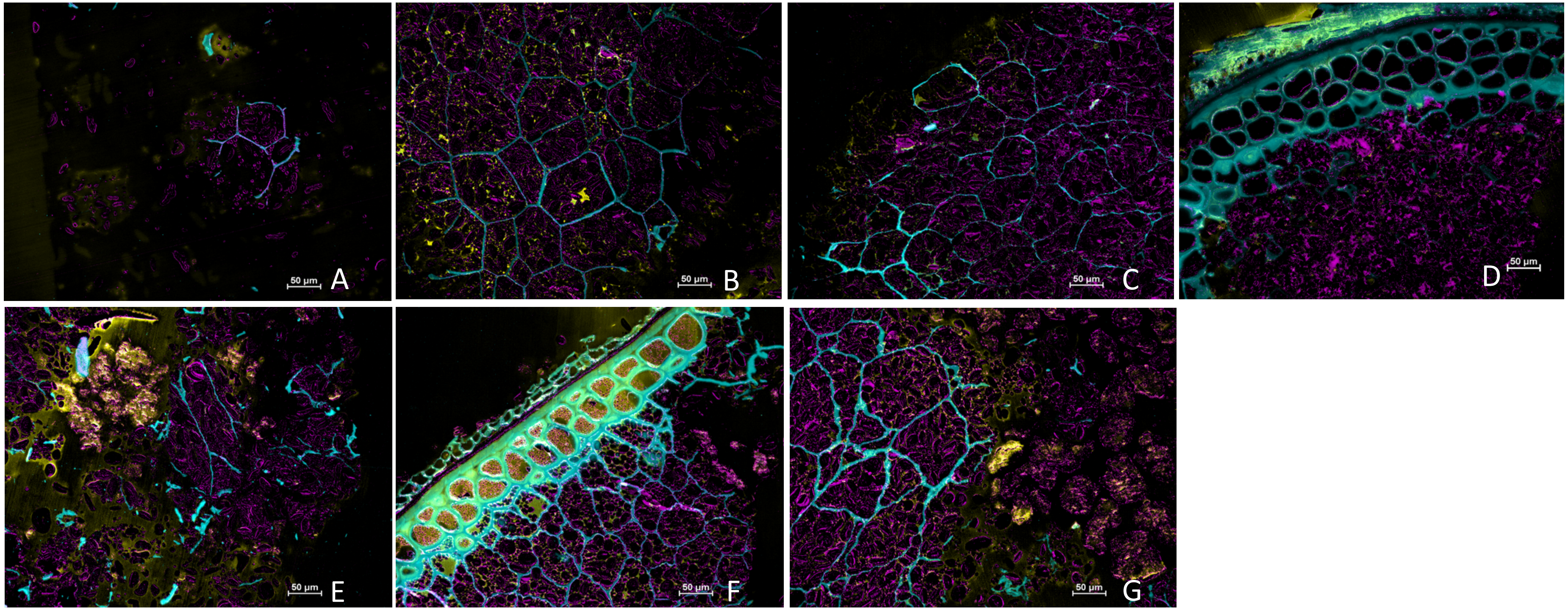
A

Formulations	White flour (WF) (g)	Fine farina (FF) (g)	Bran (BR) (g)	Sucrose (g)	Shortening (g)	Distilled water (mL)	Dextrose solution (11.9 g D-glucose / 100 mL) (mL)	Sodium bicarbonate (g)	Sodium chloride (g)
Control	198.0		22.0	81.7	64.0	21.8	16.5	2.5	2.1
100% FF		213.6		80.7	67.4	19.9	16.6	2.6	2.2
80% FF	42.8	173.0		81.7	68.3	21.0	16.6	2.8	2.4
70% FF	64.3	151.5		81.8	68.3	21.1	16.6	2.7	2.3
65% FF	75.0	140.7		81.8	68.6	21.2	16.6	2.7	2.3
60% FF	85.7	129.8		81.8	68.6	21.3	16.6	2.7	2.3
40% FF	128.6	86.6		81.8	69.8	21.5	16.6	2.3	1.9
20% FF	171.9	43.4		82.0	68.9	22.3	16.6	2.8	2.3

B

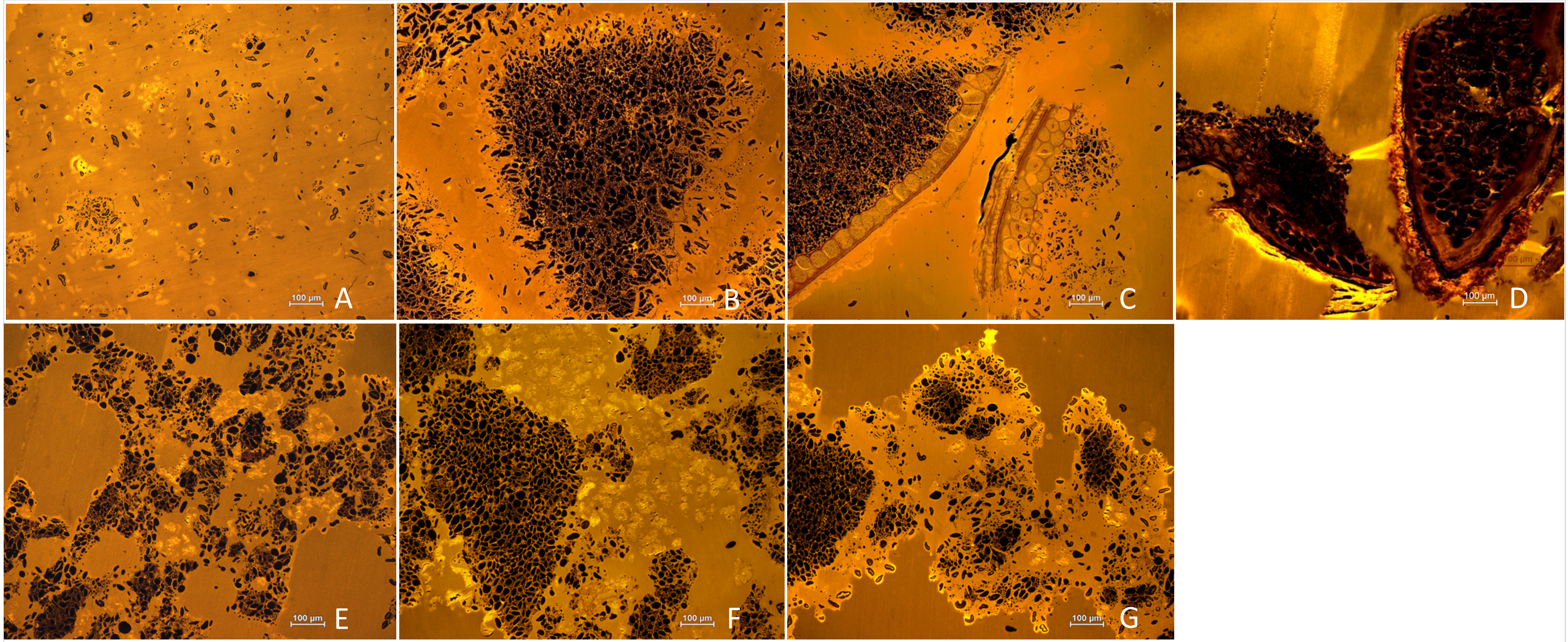
Formulations	White flour (WF) (g)	Coarse farina (CF) (g)	Bran (BR) (g)	Sucrose (g)	Shortening (g)	Distilled water (mL)	Dextrose solution (11.9 g D-glucose / 100 mL) (mL)	Sodium bicarbonate (g)	Sodium chloride (g)
Control	198.0		22.0	81.7	64.0	21.8	16.5	2.5	2.1
100% CF		242.0		81.7	48.1	21.0	12.4	2.6	2.2
80% CF	43.0	194.0		82.0	52.0	22.0	12.4	2.8	2.4
70% CF	63.4	167.1		81.0	53.0	22.1	12.4	2.7	2.3
65% CF	72.9	152.9		79.5	54.0	22.0	12.4	2.7	2.3
60% CF	82.1	139.1		78.4	54.0	21.7	12.4	2.7	2.3
40% CF	127.2	95.8		80.9	60.5	23.9	12.4	2.3	1.9
20% CF	167.7	47.4		80.0	62.5	24.4	12.4	2.8	2.3

Supplementary Figure S1



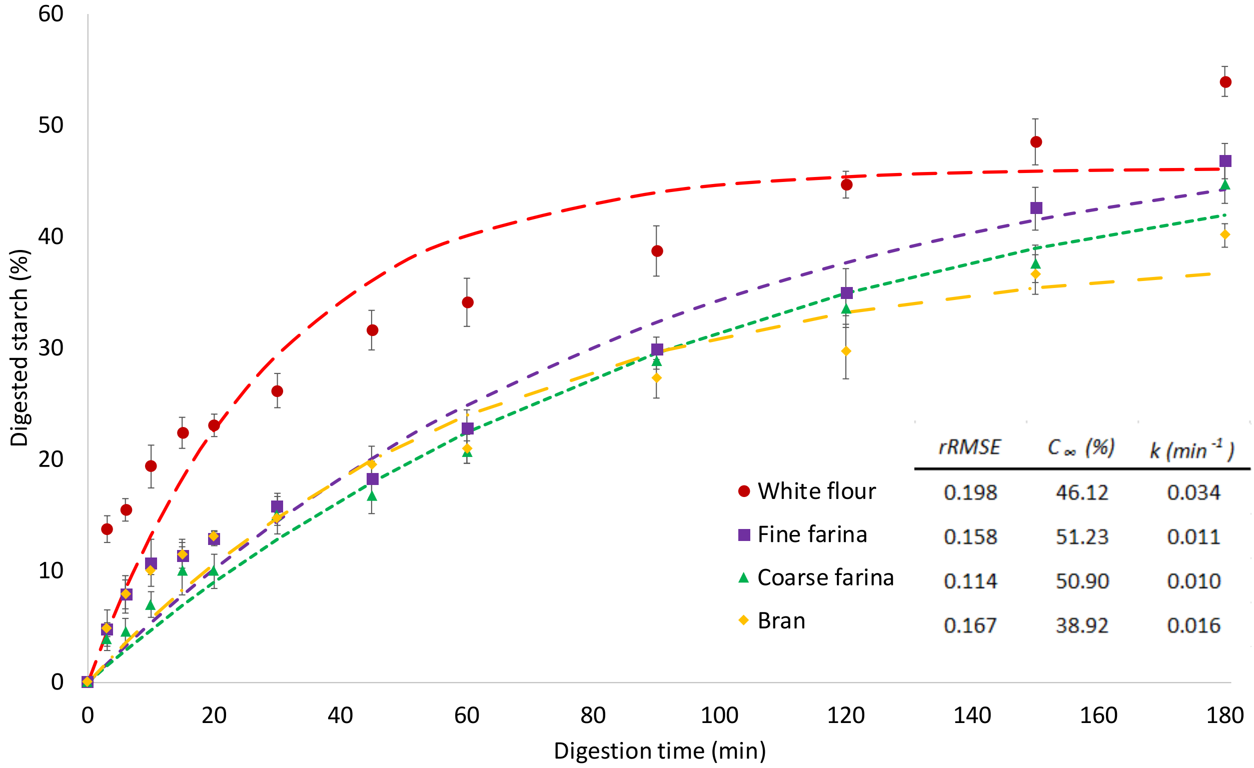
Supplementary Figure S1. Microscopic images of wheat fractions [A: white flour (WF); B: fine farina (FF); C: coarse farina (CF); D: bran BR)] and of wire-cut cookies (E: control; F: 65% FF; G: 65% CF) taken at 20X magnification. Cell wall β-glucans were stained with Calcofluor, starch granules with Lugol's solution and proteins with acid fuchsin. The images shown are composites, with β-glucans, starch and proteins assigned the colours cyan, magenta and yellow, respectively, to improve visibility of the images. Scale bars (50 µm) are represented in each individual image.

Supplementary Figure S2



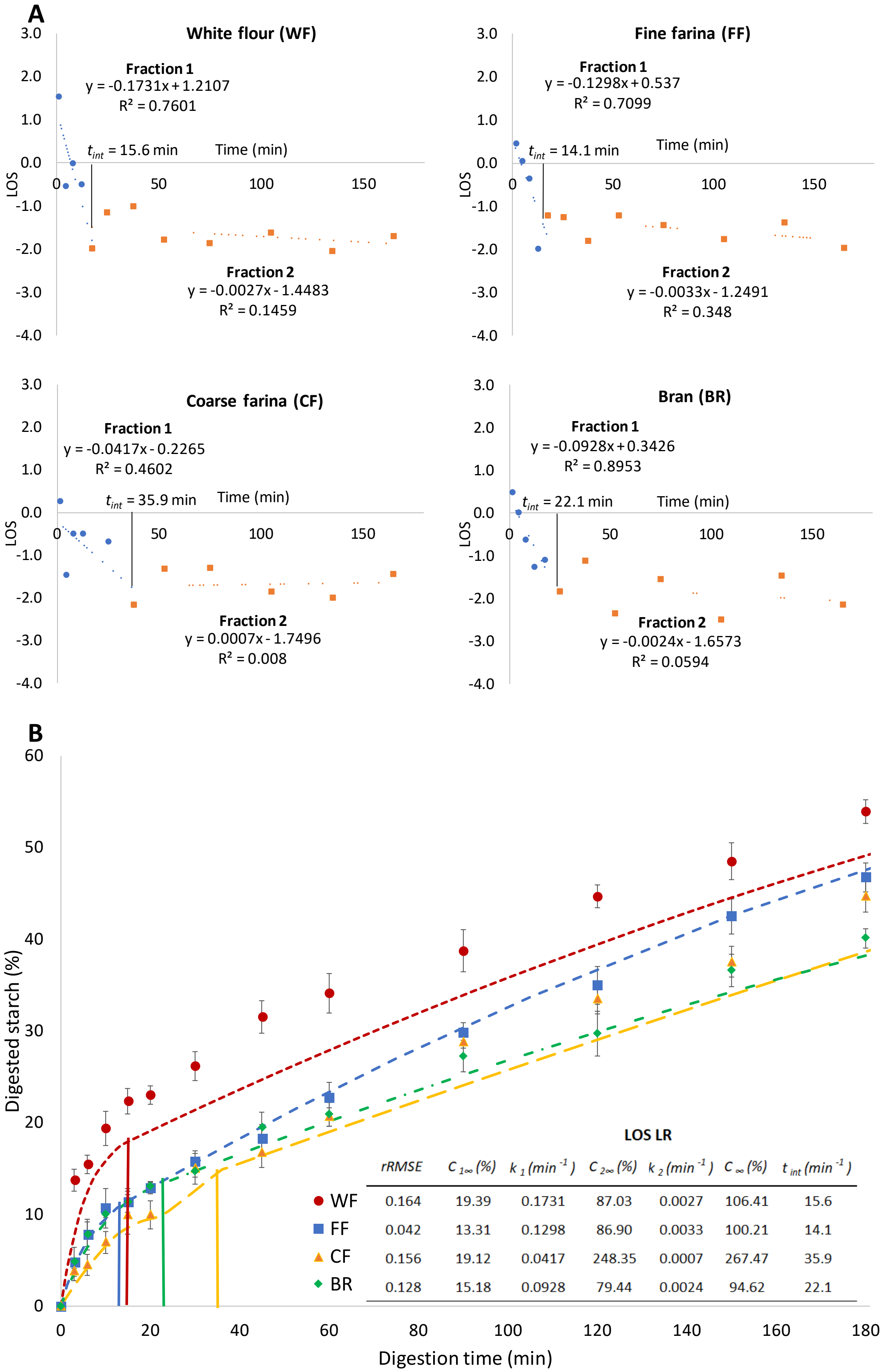
Supplementary Figure S2. Microscopic images of wheat fractions [A: white flour (WF); B: fine farina (FF); C: coarse farina (CF); D: bran BR)] and of wire-cut cookies (E: control; F: 65% FF; G: 65% CF). Proteins were stained with acid fuchsin and visualised with a TRITC filter and 400 ms exposure time. Scale bars (100 µm) are represented in each individual image.

Supplementary Figure S3



Supplementary Figure S3. In vitro *starch digestion of four wheat fractions differing in particle size, i.e. white flour (WF), fine farina (FF), coarse farina (CF) and bran (BR). The extent of digestion is expressed on the y-axis as percentage of digested starch from the measured maltose equivalents in function of digestion time (up to 180 min on the x-axis). For each time point studied, means and standard deviations of three technical repeats are shown. Fits with the single first-order kinetic (SFOK) model are shown for each fraction and the accompanying kinetic parameters are shown in the table within the figure.* C_∞ *(%) indicates the digestible starch and k* (min^{-1}) *the reaction rate constant. The relative root mean square error (rRMSE) is reported as a measure of spread between experimental data and kinetic fit.*

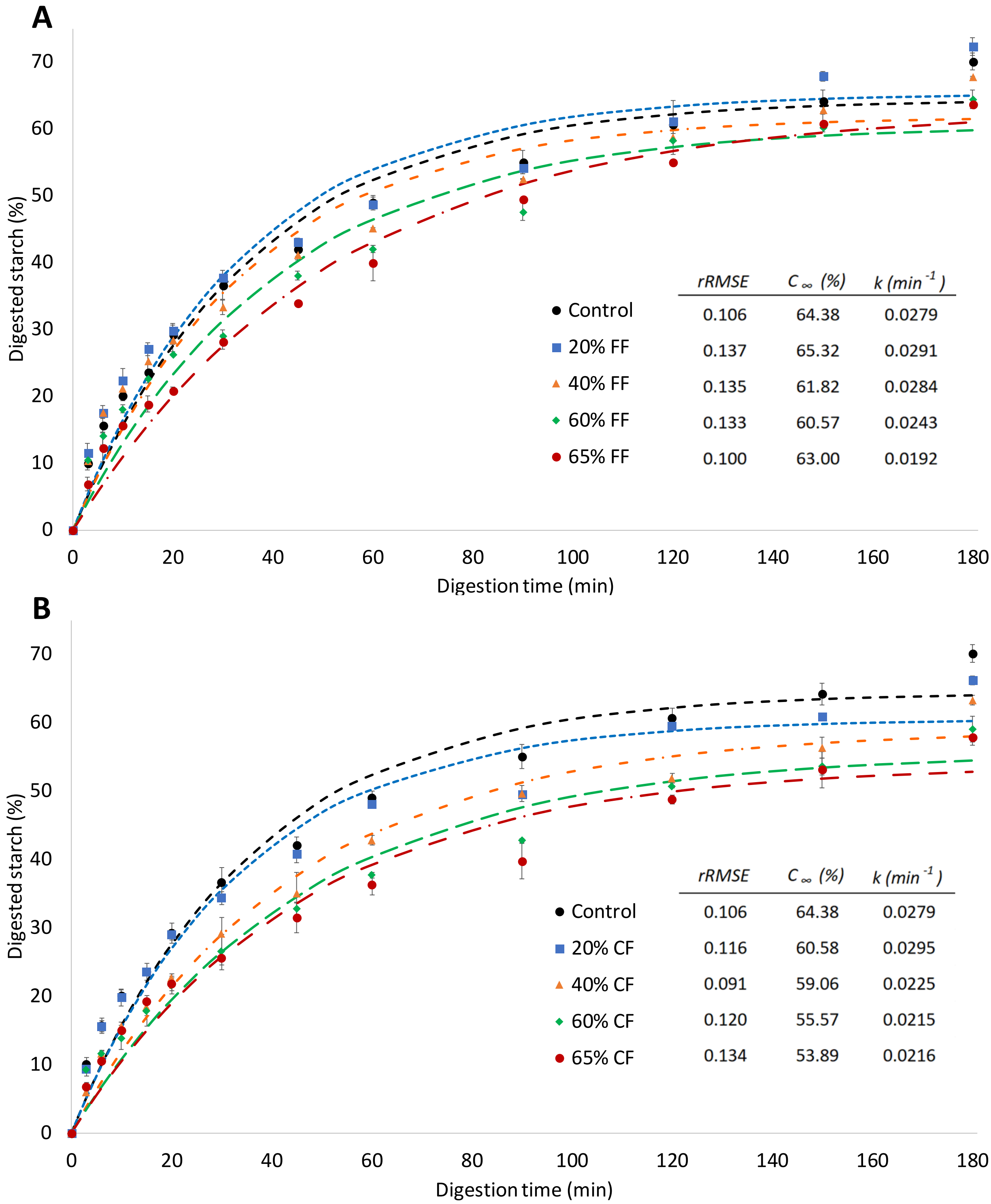
Supplementary Figure S4



	rRMSE	$C_{1\infty}$ (%)	k_1 (min^{-1})	$C_{2\infty}$ (%)	k_2 (min^{-1})	C_∞ (%)	t_{int} (min^{-1})
WF	0.164	19.39	0.1731	87.03	0.0027	106.41	15.6
FF	0.042	13.31	0.1298	86.90	0.0033	100.21	14.1
CF	0.156	19.12	0.0417	248.35	0.0007	267.47	35.9
BR	0.128	15.18	0.0928	79.44	0.0024	94.62	22.1

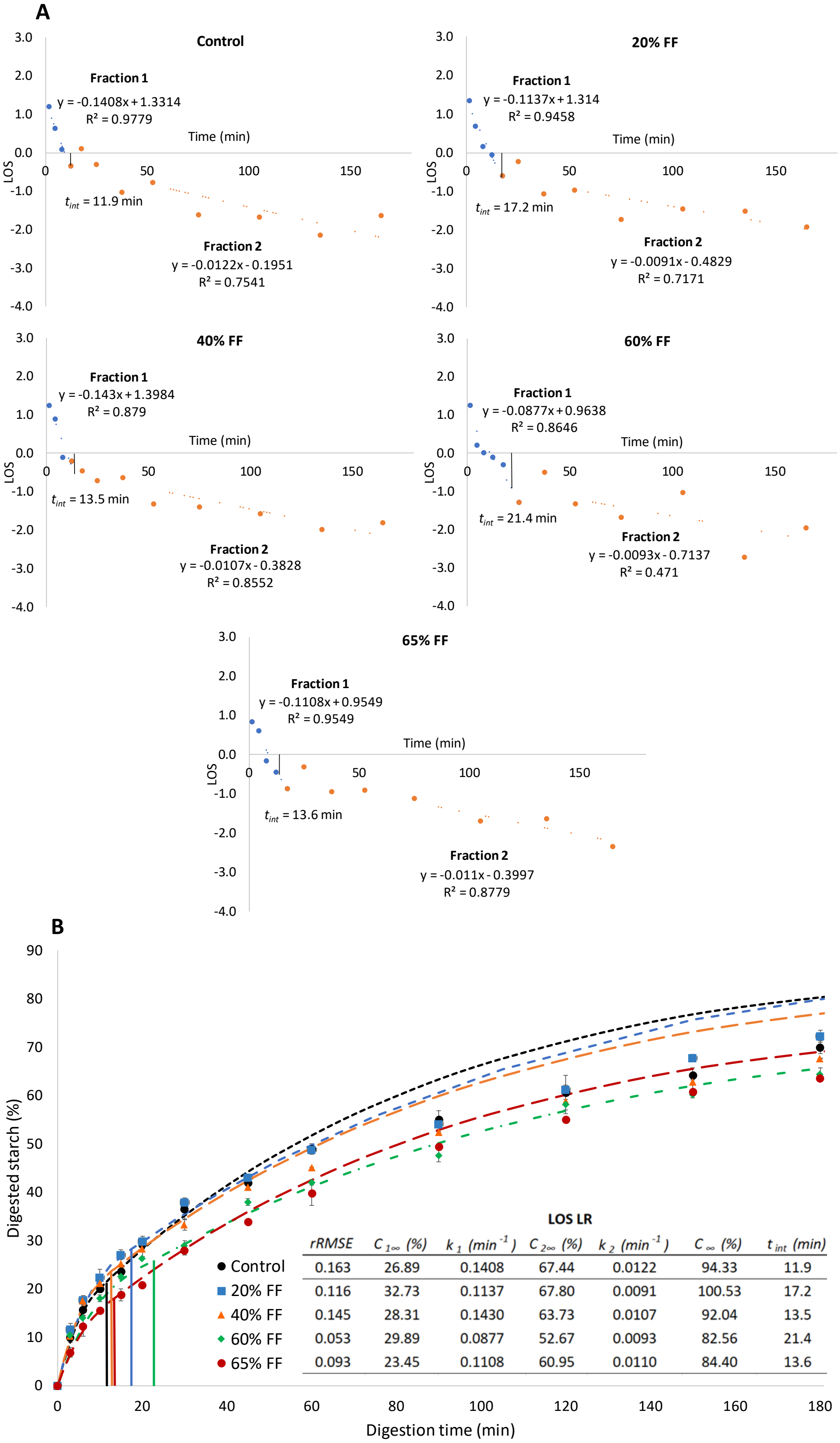
Supplementary Figure S4. Logarithm of slope (LOS) plots created by linear regression (LOS LR) (A) and corresponding kinetic fits (B) of the in vitro *starch digestion of four wheat fractions differing in particle size, i.e. white flour (WF), fine farina (FF), coarse farina (CF) and bran (BR). In (A)* t_{int} *(min) indicates the start of the digestion of the second starch fraction. In (B) the extent of digestion is expressed on the y-axis as percentage of digested starch from the measured maltose equivalents in function of digestion time (up to 180 min on the x-axis). For each time point studied, means and standard deviations of three technical repeats are shown. The inlet table in (B) presents the kinetic parameters of the model.* $C_{1\infty}$ *and* $C_{2\infty}$ *(%) indicate the total digestible starch and* k_1 *or* k_2 *(*min^{-1}*) the reaction rate constants of the hydrolysis of starch fractions 1 or 2, respectively.* C_{∞} *(%) is the sum of* $C_{1\infty}$ *and* $C_{2\infty}$ *and indicates the total digestible starch. The relative root mean square error (rRMSE) is reported as a measure of spread between experimental data and kinetic fit.*

Supplementary Figure S5



Supplementary Figure S5. In vitro *starch digestion of cookies produced with varying proportions of white flour (WF) substituted by A) fine farina (FF) or B) coarse farina (CF), while maintaining a constant starch content on dry matter basis. The extent of digestion is expressed on the y-axis as percentage of digested starch from the measured maltose equivalents in function of digestion time (up to 180 min on the x-axis). For each time point studied, means and standard deviations of three technical repeats are shown. Fits to the single first-order kinetic (SFOK) model are shown for each cookie type. C_{∞} indicates the total digestible starch and k (min^{-1}) the reaction rate constant. The relative root mean square error (rRMSE) is reported as a measure of spread between experimental data and kinetic fit.*

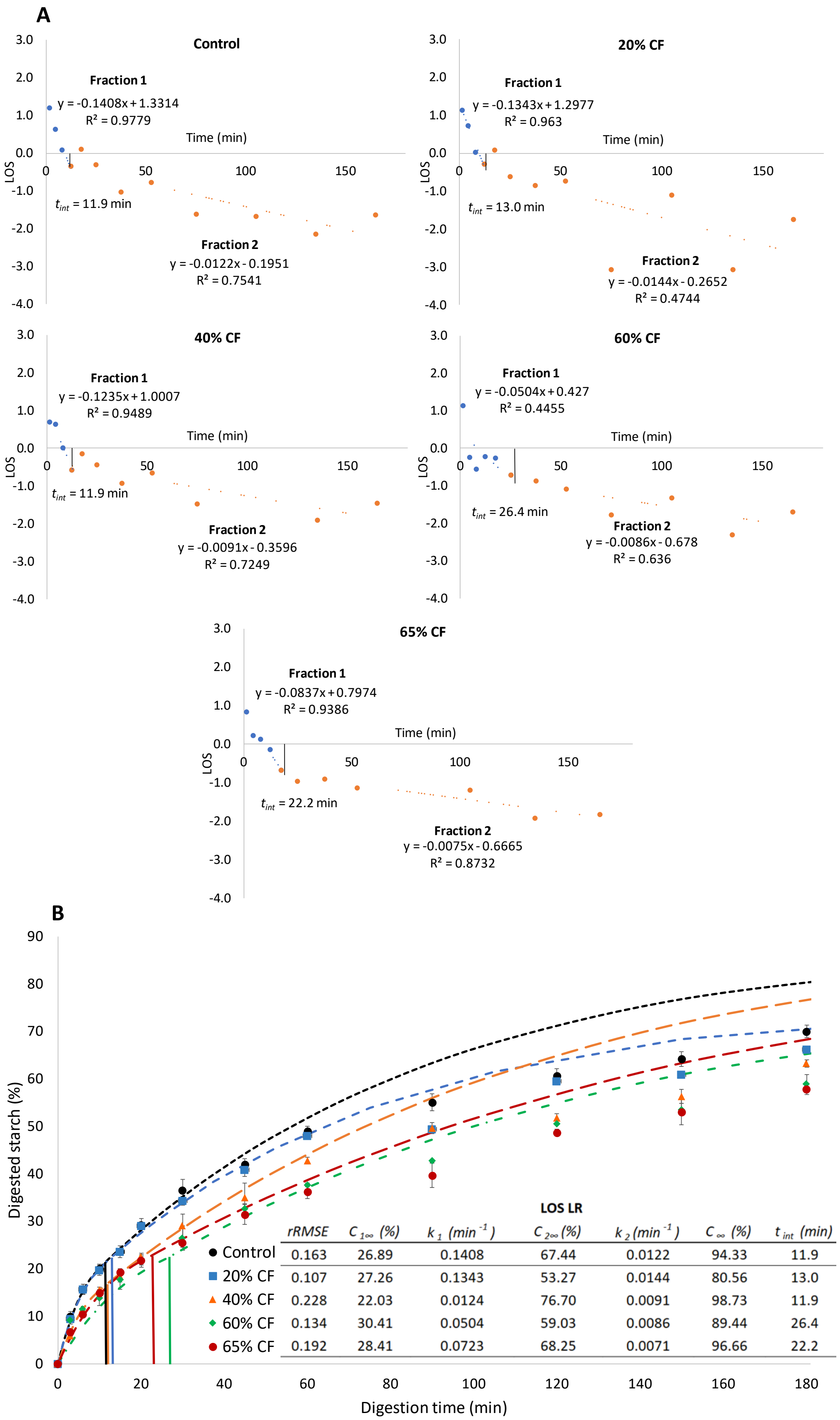
Supplementary Figure S6



	rRMSE	$C_{1\infty}$ (%)	k_1 (min^{-1})	$C_{2\infty}$ (%)	k_2 (min^{-1})	C_∞ (%)	t_{int} (min)
Control	0.163	26.89	0.1408	67.44	0.0122	94.33	11.9
20% FF	0.116	32.73	0.1137	67.80	0.0091	100.53	17.2
40% FF	0.145	28.31	0.1430	63.73	0.0107	92.04	13.5
60% FF	0.053	29.89	0.0877	52.67	0.0093	82.56	21.4
65% FF	0.093	23.45	0.1108	60.95	0.0110	84.40	13.6

Supplementary Figure S6. Logarithm of slope (LOS) plots created by linear regression (LOS LR) (A) and corresponding kinetic fits (B) of the in vitro *starch digestion of cookies produced with varying proportions of white flour (WF) substituted by A) fine farina (FF) or B) coarse farina (CF), while maintaining a constant starch content on dry matter basis. In (A) t_{int} (min) indicates* *the start of the digestion of the second starch fraction. In (B) the extent of digestion is expressed on the y-axis as percentage of digested starch from the measured maltose equivalents in function of digestion time (up to 180 min on the x-axis). For each time point studied, means and standard deviations of two technical repeats are shown. The inlet table in (B) presents the kinetic parameters of the model. $C_{1\infty}$ and $C_{2\infty}$ (%) indicate the total digestible starch and k_1 or k_2 (min^{-1}) the reaction rate constants of the hydrolysis of starch fractions 1 or 2, respectively. C_{∞} (%) is the sum of $C_{1\infty}$ and $C_{2\infty}$ and indicates the total digestible starch. The relative root mean square error (rRMSE) is reported as a measure of spread between experimental data and kinetic fit.*

Supplementary Figure S7



Supplementary Figure S7. Logarithm of slopes (LOS) plots created by linear regression (A) and corresponding kinetic fits (B) of the in vitro *starch digestion of cookies produced with varying proportions of white flour (WF) substituted by A) fine farina (FF) or B) coarse farina (CF), while maintaining a constant starch content on dry matter basis. In (A)* t_{int} *(min) indicates the start of the digestion of the second starch fraction. In (B) the extent of digestion is expressed on the y-axis as percentage of digested starch from the measured maltose equivalents in function of digestion time (up to 180 min on the x-axis). For each time point studied, means and standard deviations of two technical repeats are shown. The inlet table in (B) presents the kinetic parameters of the model.* $C_{1\infty}$ *and* $C_{2\infty}$ *(%) indicate the total digestible starch and* k_1 *or* k_2 *(*min^{-1}*) the reaction rate constants of the hydrolysis of starch fractions 1 or 2, respectively.* C_∞ *(%) is the sum of* $C_{1\infty}$ *and* $C_{2\infty}$ *and indicates the total digestible starch. The relative root mean square error (rRMSE) is reported as a measure of spread between experimental data and kinetic fit.*