Electronic Supplementary Material (ESI) for Food & Function.
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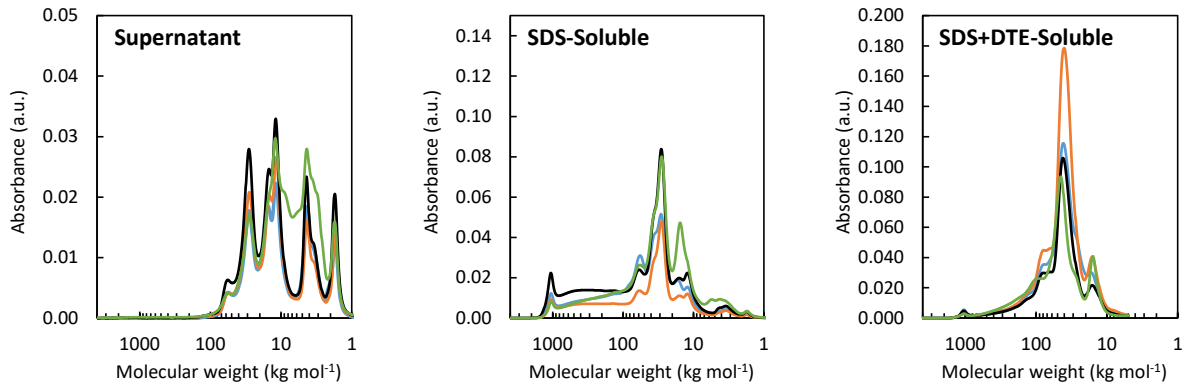
Supplementary material

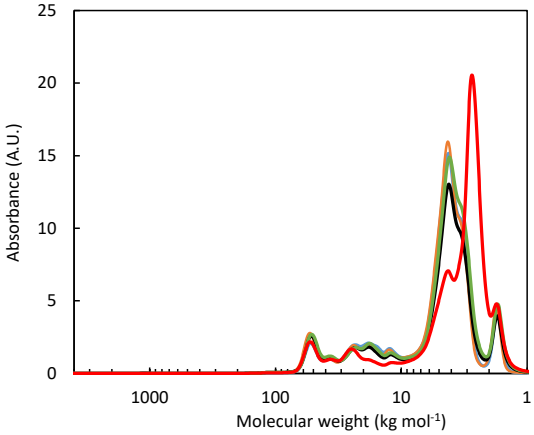
Supplementary material 1. Sequential extraction of proteins from "enzyme-free" control samples, also called blank: protein soluble in the supernatant, pellet proteins soluble in denaturing buffer (SDS) and pellet proteins soluble in reducing buffer (SDS+DTE). The sum of the three gives the yield of extraction of the analysis method. Each parameter is expressed in % of total dough proteins.

Doughs	*Soluble (%)*	*SDS-soluble (%)*	*SDS+DTE soluble (%)*	*Yield of extraction (%)*
Control	12.7 ± 1.0	38.5 ± 5.5	36.8 ± 3.8	88.0 ± 2.1
RuBisCO-enriched	13.8 ± 2.0	39.2 ± 0.5	37.4 ± 0.3	90.3 ± 2.7
Gluten-enriched	10.4 ± 4.2	21.4 ± 0.3	61.5 ± 0.4	93.2 ± 0.3
Pea protein-enriched	10.0 ± 2.4	33.3 ± 11.3	50.4 ± 8.6	93.7 ± 2.8

Supplementary material 2. SE-HPLC profiles of proteins of "enzyme-free" control samples: in supernatant (left), in pellet soluble in SDS (middle) and in pellet insoluble in SDS but soluble in SDS+DTE (right). Black lines stand for control dough, green lines for RuBisCO-enriched dough, blue lines for pea protein-enriched dough and orange lines for gluten-enriched dough. Elution profile of SDS+DTE soluble proteins is represented only from 5 kg mol^{-1} because iodoacetamide molecules used to alkyl thiol function are eluted below.



Supplementary material 3. SE-HPLC profiles of intestinal supernatant: black line stands for control dough, green line for RuBisCO-enriched dough, blue line for pea protein-enriched doughs, orange line for gluten-enriched dough and red line for enzyme signal.



Supplementary material 4

The Expasy tool PeptideMass was used to compared the predicted peptide sizes of RuBisCO and wheat gliadins and glutenins subunits. To do so, the UniProt accession numbers as well as the sequences of lettuce RuBisCO, wheat gliadins and wheat glutenins were retrieved from UniprotKB. The UniProt accession number were used to compute the masses of the peptides generated with Trypsin hydrolysis only and with Trypsin/chymotrypsin hydrolysis on the online Expasy tool PeptideMass. The median of the generated peptides was computed as well as the number of peptide normalised to a molecular weight of 30000 g/mol to account for the differences in molecular weight of each sequence.

UniProt accession number	Protein name	Pepsin digestion			Trypsin/Chimotrypsin		
		Sequence coverage (%)	Normalised number of peptides	Median molecular weight (g/mol)	Sequence coverage (%)	Normalised number of peptides	Median molecular weight (g/mol)
P02863	Alpha/beta-gliadin	10.8	4	607	97.0	13	1591
P04724	Alpha/beta-gliadin A-IV	14.8	6	581	98.2	13	1593
P04722	Alpha/beta-gliadin A-II	15.1	6	662	96.7	13	1522
P04721	Alpha/beta-gliadin A-I	14.5	4	792	97.1	13	1609
P04725	Alpha/beta-gliadin A-V	16.7	6	629	98.0	9	2393
P04723	Alpha/beta-gliadin A-III	15.6	6	612	96.9	10	1587
P04730	Gamma-gliadin (Gliadin B-III)	28.3	10	621	92.6	11	1514
P08079	Gamma-gliadin	18.5	7	601	99.1	10	2850
P06659	Gamma-gliadin B	20.6	8	646	98.2	12	1641
P21292	Gamma-gliadin	24.7	9	643	99.3	10	2201
P08453	Gamma-gliadin	16.2	6	602	99.0	9	1568
P04729	Gamma-gliadin B-I	17.1	6	634	95.0	17	1462
P10388	Glutenin, high molecular weight subunit DX5	13.7	3	557	97.1	12	1603
P10386	Glutenin, low molecular weight subunit 1D1	19.7	6	658	94.0	14	1508
P10385	Glutenin, low molecular weight subunit	22.8	8	664	96.4	15	1343
P10387	Glutenin, high molecular weight subunit DY10	12.6	4	594	96.2	15	1413
P16315	Glutenin, low molecular weight subunit PTDUCD1	24.4	9	647	96.0	16	1519
P08488	Glutenin, high molecular weight subunit 12	12.5	4	640	95.3	14	1730
P08489	Glutenin, high molecular weight subunit PW212	13.7	3	557	97.3	13	1592
P48706	RuBisCO large subunit	37.7	17	630	89.3	23	921
Q40250	RuBisCO small subunit	41.6	13	610	94.4	43	1022

Supplementary material 5. Analysis of the protein aggregation state of the initial doughs as determined by sequential solubility in denaturing buffer (SDS-soluble) and reducing buffer (SDS-DTE). Methods from Ducrocq *et al.*, 2020 were used.

	SDS soluble proteins	SDS+DTE soluble proteins
	% of extractable proteins	
Control dough	79%	21%
RuBisCO-enriched	65%	35%
Gluten-enriched	75%	25%
Pea protein-enriched	76%	24%

Table 1. Proportion of SDS-soluble and SDS+DTE soluble proteins in control and protein-enriched doughs at 37.4% RuBisCO, 35.0% gluten and 38.7% pea proteins. Results are means of duplicates, the variation coefficient of this method is below 5%.

The aggregation state of proteins in the control and protein-enriched doughs was estimated from the amount of SDS-soluble and SDS+DTE soluble proteins. These last two protein extracts represent respectively the small and weakly bound polymers and the large disulphide linked polymers.

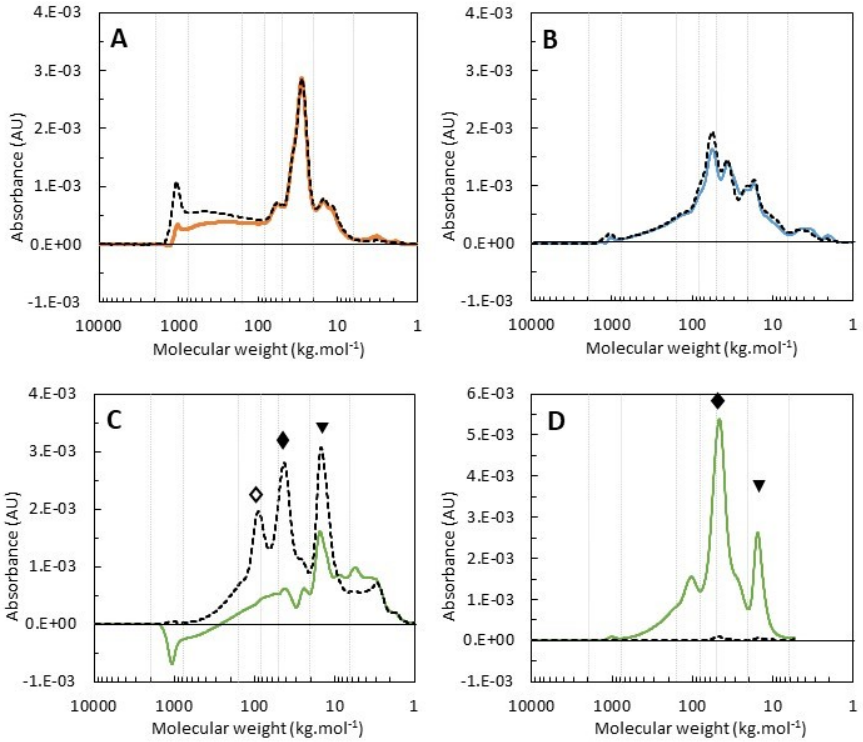


Figure 1 SE-HPLC SDS soluble protein differential profile (solid line) of thermal treated doughs enriched with 14.9% gluten (A), 17.1% pea proteins (B) and 16.3% RuBisCO (C) and SE-HPLC elution profile of SDS soluble protein (dotted line) extracted from gluten (A), pea proteins (B) and RuBisCO (C) concentrates in proportion of respective enriched doughs. D: SE-HPLC SDS+DTE soluble protein differential profile (solid line) of 16.3% thermal treated RuBisCO-enriched dough and SDS+DTE soluble protein profile (dotted line) from RuBisCO concentrates in respective proportion. Symbols represent RuBisCO subunits: small chain (SC; ▼), large chain (LC; ◆) and large

chain dimer (LC dimer; ◊). For the sake of readability, elution profile of figure D is represented only until 5 kg mol^{-1}.

Supplementary material 6. Raw FT-IR spectra of the amide I region of raw (dotted lines) and thermally treated (solid lines) doughs. Black lines stand for control dough, light green lines for RuBisCO- enriched dough at 16.3%, dark green lines for RuBisCo-enriched dough at 37.4%, blue lines for pea protein-enriched and orange lines for gluten-enriched doughs. Each spectrum corresponds to the average of 5 consecutive measurements on the exactly same sample.