

Supplementary Information

Enzymatic hydrolysis using bacterial cultures as a novel method for obtaining antioxidant peptides from brewers' spent grain

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Table S1. List of bioactive peptides obtained from barley proteins as a result of *in silico* analysis.

Biological activity	Peptide sequence	Peptide length	Peptide location	Peptide source	Enzyme name	Molecular weight
Antioxidative peptide	TY	2	385-386	globulin	subtilisin	282.3
	VY		107-108			B hordein
			209-210			
			936-937	D hordein		
	PQIQEQF	7	194-200	B hordein	subtilisin, oligopeptidase F	857.9
			921-927	D hordein	subtilisin oligopeptidase F	
	IR	2	205-206	B hordein	coccolysin, thermolysin	
	LK		932-933	D hordein	coccolysin,	
			271-272	B hordein	coccolysin, thermolysin	259.3
			998-999	D hordein	coccolysin	
			266-267	glutelin	coccolysin, thermolysin	
	558-559					
	697-698	D hordein	thermolysin			
	LPM	3	276-278	gamma 3 hordein	coccolysin, thermolysin	359.5
	LKP		269-271	glutelin	oligopeptidase B	356.5
	LLR		234-236			
EL	2	29-30	gamma 3 hordein	proteinase K, subtilisin , oligopeptidase F	260.3	

	KP		270-271	glutelin	proteinase K	243.3
Glucose uptake stimulating peptide	VL	2	184-185	B hordein	subtilisin	230.3
			257-258	gamma 3 hordein	subtilisin, oligopeptidase F	
			911-912	D hordein	subtilisin	
			6-7		subtilisin, oligopeptidase F	
			278-279	gamma 1 hordein	oligopeptidase F	
			255-256		subtilisin	
			8-9	C hordein	subtilisin, oligopeptidase F	
			228-229	gamma 2 hordein	oligopeptidase F	
	IL	2	147-148	B hordein	subtilisin	244.3
			874-875	D hordein		
			535-536	glutelin		
	LV	2	9-10	B hordein	coccolysin	230.3
			5-6	D hordein		
			15-16			
			736-737			
271-272			gamma 3 hordein			
106-107			glutelin			
IV	185-186	coccolysin, proteinase P1				

	II		208-209	B hordein	coccolysin	244.3
			12-13	D hordein		
			935-936			
			124-125	C hordein	proteinase P1	
			237-238	C hordein		
			495-496	glutelin		
Immunostimulating peptide	YG	2	139-140	glutelin	proteinase P1	238.2
Inhibitor of Prolyl Oligopeptidase	VPL	3	163-165	gamma 3 hordein	subtilisin	327.4
CaMPDE inhibitor (Calmodulin-dependent cyclic nucleotide phosphodiesterase inhibitor)	KF	2	517-518	glutelin	proteinase K	294.3
			267-268		proteinase K, subtilisin, proteinase P1, oligopeptidase F	293.4
			559-560		proteinase K, subtilisin, oligopeptidase F	
			572-573		proteinase K, proteinase P1	
			205-206	B hordein	coccolysin, thermolysin	287.3
			932-933	D hordein	coccolysin,	
Renin inhibitor	IR	2	205-206	B hordein	coccolysin, thermolysin	287.3
			932-933	D hordein	coccolysin,	
			EF		517-518	glutelin

	KF		572-573		proteinase P1	293.4
			267-268		proteinase K, subtilisin, proteinase P1, oligopeptidase F	
			559-560		proteinase K, subtilisin, oligopeptidase F	
			572-573		proteinase K	
Stimulating vasoactive substance release	SE	2	281-282	C hordein	V-8-protease, proteinase P1	234.2
			382-383	glutelin	proteinase P1	
	VPL	3	163-165	gamma 3 hordein	subtilisin	327.4
	EE	2	279-280	C hordein	proteinase P1	276.2
301-302			glutelin			
Coeliac toxic peptide	QQQP	4	60-63	gamma 3 hordein	proteinase K	499.5
			66-69			
			102-105			
			127-130			
			249-252			
Anxiolytic peptide (neuropeptide)	YL	2	162-163	gamma 1 hordein	oligopeptidase F	294.3
			110-111	gamma 2 hordein		

ACE inhibitor (angiotensin converting enzyme inhibitor)	VF	2	190-191	glutelin	subtilisin	264.3	
			483-484				
			568-569				
			156-157	B hordein			
			261-262				
			9-10				
	IF		3	5-6	globulin	subtilisin, oligopeptidase F	287.3
				6-7			
				212-213	B hordein	subtilisin	
	VG		3	306-307	gamma 1 hordein gamma 2 hordein globulin	subtilisin, thermolysin	174.2
				304-305			
				252-253			
		58-59					
	GL	3	105-106	B hordein	subtilisin, oligopeptidase F, proteinase K	188.2	
			27-28	gamma 3 hordein			
	IL	3	535-536	glutelin	subtilisin,	244.3	
			147-148	B hordein			
	LQP	3	263-265	gamma 2 hordein gamma 1 hordein C hordein gamma 3 hordein	coccolysin, thermolysin	356.4	
			81-83		coccolysin		
			131-132		coccolysin, thermolysin		
159-161							
LQQ	3	237-239	gamma 3 hordein		387.4		

			148-150	B hordein			
	FG	2	113-114	gamma 3 hordein	proteinase P1	222.2	
			293-294				
			104-105	B hordein	coccolysin, proteinase P1, thermolysin		
			300-301				
	134-135		C hordein			coccolysin, thermolysin	
	135-136						
	266-267						
	162-162						
	167-168						
	206-207			gamma 1 hordein			
	156-157	gamma 2 hordein	coccolysin, thermolysin				
	50-51	glutelin					
	AH		269-270	B hordein	coccolysin, proteinase P1, thermolysin	226.2	
	GV			226-227	B hordein	proteinase P1	174.2
				307-308		proteinase K, proteinase P1	
				11-12	gamma 3 hordein	proteinase P1	
				238-239	gamma 1 hordein		
				261-262			
			305-306		proteinase P1, proteinase K		

		59-60	globulin	proteinase K	
		22-23		proteinase P1	
		59-60			
		567-568	glutelin	proteinase K, proteinase P1	
		82-83		proteinase K	
	AF	85-86	B hordein	proteinase P1	236.3
		268-269	C hordein		
	MF	176-177	B hordein		296.4
		292-293			
	VP	163-164	gamma 3 hordein	thermolysin	214.3
	GI	301-302	B hordein	proteinase K	188.2
		240-241	glutelin	proteinase P1, proteinase K	
	AI	11-12	B hordein	proteinase K	202.2
		205-206	gamma 3 hordein		
		187-188	glutelin		
	PL	92-93	C hordein	subtilisin, oligopeptidase F	228.3
		174-175			
		213-214			
		247-248			
		270-271			
		293-294			
522-523	D hordein				

			454-455	glutelin	subtilisin	
	TF		6-7	C hordein	subtilisin, oligopeptidase F, proteinase K	266.3
			53-54	gamma 2 hordein	subtilisin,	
			105-106	gamma 1 hordein	subtilisin, proteinase P1, oligopeptidase F, proteinase K	
			176-177	D hordein	subtilisin,	
			75-76		proteinase P1	
			FP		66-67	
	91-92					
	173-174					
	212-213					
	246-247					
	269-270					
	292-293					
	354-355					
	LG		10-11	gamma 3 hordein	thermolysin	188.2
			239-240	glutelin	coccolysin, thermolysin	
			536-537			
			566-567			
			YA		342-343	
	FR		315-316	321.4		

	IG		188-189			188.2	
			81-82	glutelin	proteinase P1, thermolysin		
	SY			42-43	C hordein	proteinase P1	268.3
				171-172	D hordein	proteinase P1, proteinase K	
				194-195		proteinase P1	
				337-338		proteinase P1	
				125-126	gamma 1 hordein	proteinase P1, proteinase K	
				185-186		proteinase P1, proteinase K	
				KY		514-515	
	320-321		C hordein			proteinase K	
	TP				61-62		216.2
	GS			140-141	globulin	subtilisin	162.1
				294-295	gamma 3 hordein		
				495-496	D hordein		
				683-684			
	YP			252-253	D hordein	coccolysin, thermolysin	278.3
				380-381			
	AV			10-11	D hordein	coccolysin, proteinase P1, proteinase K	188.2
				8-9			
				GY		292-293	

			320-321				
			455-456				
	AA		706-707				gamma 3 hordein
	GE		656-657				
	SG		26-27				D hordein
			284-285				
			302-303				
			347-348				
	449-450						
	ST		362-362				glutelin
		474-475					
		53-54					
	YE	100-101	D hordein				
	GHG	3		402-404			
	AQLLAAQLPAMCR- inhibitor z glutenu	13		702-714	oligopeptidase B		
	YH	2		686-687	thermolysin		
	KW			133-134	proteinase K		
	HP			618-619			
	AP			301-302	gamma 1 hordein	coccolysin, thermolysin	
				249-250	gamma 2 hordein	coccolysin,	
183-184		glutelin		coccolysin, thermolysin			
570-571	proteinase K						
GG		136-137	globulin	proteinase P1			
					160.2		
					204.2		
					162.1		
					206.2		
					310.3		
					269.2		
					1417.5		
					318.3		
					332.4		
					352.3		
					186.2		
					132.1		

			311-312	gamma 1 hordein		
	SF		118-119	glutelin	proteinase P1, oligopeptidase F, proteinase K	252.3
			119-120			
			578-579			
			10-11			
		171-172	gamma 3 hordein	proteinase P1, proteinase K		
	YL		162-163	gamma 1 hordein	oligopeptidase F	294.3
	AEL	3	223-225	globulin	proteinase K	331.4
	PQR		177-179		oligopeptidase B	399.4
	VRP		60-62		thermolysin	370.4
	LNP		115-117	gamma 2 hordein	coccolysin	342.4
	LGV		10-12	gamma 3 hordein		287.4
			566-568	glutelin		
	LN	2	146-147	gamma 3 hordein	coccolysin, thermolysin	245.3
			434-435	glutelin		
	KR		186-187	gamma 3 hordein	proteinase P1	302.4
	GM		243-244	glutelin	proteinase K	206.3
			28-29			
	RP		174-175	gamma 3 hordein		271.3
			61-62	globulin		
QP	41-42		gamma 3 hordein	243.3		
	100-101					
HG			157-158	globulin	subtilisin	212.2

	AR		227-228		coccolysin, thermolysin	245.3		
	FQ		63-64			293.3		
	YG		139-140		proteinase P1	238.2		
	EV		133-134]			246.3		
	VY				189-190	glutelin	coccolysin	280.3
	PRY	107-108		subtilisin	434.5			
	AGP	3	98-100		coccolysin, thermolysin	243.3		
	LKP		366-368				thermolysin	356.5
	GQP		269-271		proteinase K	300.3		
	AQL		307-309			330.4		
	AY		24-26		subtilisin	252.3		
	GF	547-548	222.2					
	KF	2	114-115		subtilisin, proteinase P1, oligopeptidase F, proteinase K	293.4		
			229-230				subtilisin, oligopeptidase F, proteinase K	
			267-268					proteinase P1, proteinase K
			AG		559-560	glutelin	coccolysin, thermolysin	146.1
					572-573			
			63-64					
			171-172					

	IE		516-517			260.3	
			90-91				
	IA		167-168			202.2	
			544-545				
	GA		537-538]			146.1	
	TG		321-322			176.2	
			336-337				
	VR		232-233			thermolysin	273.3
	RL		233-234			proteinase K	287.3
	RY		99-100				337.4
	HL		445-446				268.3
	KP		270-271				243.3

Table S2. List of bioactive peptides obtained from wheat proteins as a result of *in silico* analysis.

Biological activity	Peptide sequence	Peptide length	Peptide location	Peptide source	Enzyme name	Molecular weight
Antioxidative peptide	TY	2	180-181	β -Gliadin	subtilisin	282.3
	VY		256-257	α -Gliadin		280.3
			305-306	β -Gliadin		
	LQPGQGQQG	9	758-766	Glutenin	coccolysin, thermolysin	912.0
	IR	2	242-243	γ -Gliadin	proteinase P1	287.3
	LK		832-833	Glutenin	thermolysin	259.3
		43-44				
	LWQ	3	201-203	α -Gliadin	coccolysin, thermolysin	445.5
	LWS		152-154	γ -Gliadin		404.5
	WYY		662-664	Glutenin	proteinase P1	530.6
	SYY		124-126			431.4
	GYY	3	155-157	Glutenin	proteinase P1	401.4
			176-178			
			301-303			
			375-377			
390-392						
437-439						
452-454						
514-516						

			541-543			
			695-697			
			710-712			
			766-768			
			795-797			
			816-818			
	AH	2	169-170	β -gliadin	coccolysin, thermolysin	226.2
	AY		178-179	α -Gliadin,	proteinase K	252.2
	KP		2-3	γ -Gliadin		243.3
	PW		338-339	ω -Gliadin	subtilisin	301.3
Glucose uptake stimulating peptide	VL	2	246-247	α -Gliadin	subtilisin	230.3
				γ -Gliadin	subtilisin, oligopeptidase F	
			175-176	β -Gliadin	subtilisin	
			162-163		subtilisin, oligopeptidase F	
			49-50	ω -Gliadin	oligopeptidase F	
			6-7	Glutenin		
	IL		6-7	α -Gliadin,	oligopeptidase F	244.3
β -Gliadin						

	LV		9-10	γ -Gliadin	subtilisin	230.3
			245-246		Glutenin	
			5-6	proteinase P1		
			15-16			
	79-80		α -Gliadin,			
	272-273			ω -Gliadin	subtilisin	
	12-13		320-321			
	57-58			244.3		
	14-15		244.3			
	320-321			244.3		
II	244.3					
		244.3				
Inhibitor of Prolyl Oligopeptidase-antiamnestic activity	VPL		3	209-211	α -Gliadin,	subtilisin
		β -Gliadin				
		γ -Gliadin			subtilisin, oligopeptidase F	
CaMPDE inhibitor (Calmodulin-dependent cyclic nucleotide phosphodiesterase inhibitor)	KF	2	291-292	ω -Gliadin	proteinase P1,	293.4
	IR		242-243	γ -Gliadin		287.3
Renin inhibitor	IR	2	242-243			proteinase P1

	KF		291-292			293.4
Stimulating vasoactive substance release	SE	2	26-27	Glutenin	proteinase P1	234.2
	VPL	3	209-211	α -Gliadin	subtilisin	327.4
				γ -Gliadin	subtilisin, oligopeptidase F	
39-41	β -Gliadin	subtilisin				
Antiinflammatory peptide	IPP	3	300-302	α -Gliadin, β -Gliadin	coccolysin, thermolysin	325.4
			186-188	ω -Gliadin		
			251-253			
			259-261			
	275-277					
YPP		258-260	γ -Gliadin	thermolysin	311.4	
Alpha-glucosidase inhibitor	YP	2	251-252	α -Gliadin, β -Gliadin	coccolysin, thermolysin	278.3
Pain regulation - analgesic effect	YR	2	280-281	γ -Gliadin	coccolysin, proteinase P1, thermolysin	337.4
Opioid delta receptor ligand	GYG	3	155-157	Glutenin	proteinase P1,	401.4
			176-178			
			301-303			
			375-377			

			390-392				
			437-439				
			452-454				
			514-516				
			541-543				
			695-697				
			710-712				
			766-768				
			795-797				
			816-818				
Celiac toxic peptides	QQPY	4	74-77	α -Gliadin	subtilisin	534.5	
	YPQPQ	5	90-94		γ -Gliadin	thermolysin	631.7
			97-101				
	QQQP	4	15-18	ω -Gliadin	proteinase K	499.5	
			116-119				
	ACE inhibitor (angiotensin converting enzyme inhibitor)	VY	2	298-299	α -Gliadin	subtilisin	280.3
256-257				α -Gliadin, γ -Gliadin			
305-306				β -Gliadin			
PL		252-253		α -Gliadin	228.3		
		443-444		Glutenin			
		547-548					
		701-702					
		330-331		ω -Gliadin			subtilisin, oligopeptidase F
KY	9-10	subtilisin,	309.3				

			110-111	β -Gliadin	proteinase K		
					proteinase P1, proteinase K		
	VP			39-40	α -Gliadin	thermolysin	214.3
				20-21		subtilisin, thermolysin	
				23-24			
				42-43	γ -Gliadin	thermolysin	
				209-210			
	GS		180-181	α -Gliadin	subtilisin	162.1	
	AI		11-12		subtilisin, proteinase K	202.2	
	VR			18-19	β -Gliadin	subtilisin, thermolysin	273.3
				21-22	α -Gliadin		
	IL			6-7	α -Gliadin , β -Gliadin	subtilisin, oligopeptidase F	244.3
	YP			251-252	α -Gliadin	coccolysin, thermolysin	278.3
	AP			307-308			coccolysin, thermolysin, proteinase K
				267-268	α -Gliadin, γ -Gliadin	coccolysin, thermolysin,	
				148-149	ω -Gliadin	proteinase K	
				163-164			
				231-232			
				278-279			
	316-317	β -Gliadin	coccolysin, thermolysin				

	FG		309-310	α -Gliadin,		222.2	
			318-319	β -Gliadin			
	LQ		78-79	α -Gliadin,		259.3	
			702-703	Glutenin			
			75-76	β -Gliadin			
			193-194				
	AH		169-170			226.2	
	FR		110-111	α -Gliadin,		proteinase P1	321.4
			265-266	γ -Gliadin		coccolysin, thermolysin	
	SF		113-114			proteinase P1,	252.3
			243-244	α -Gliadin,		proteinase P1, proteinase K	
			257-258			proteinase P1	
			97-98	β -Gliadin		proteinase P1, proteinase K	
			250-251				
			264-265				
	QP		29-30	α -Gliadin,		proteinase K	243.2
			65-66				
			67-68				
			81-82				
			85-86				
92-93							
99-100							
26-27		β -Gliadin					

			62-63		
			64-65		
			78-79		
			82-83		
			84-85		
			94-95		
			113-114		
			266-267		
			279-280		
			223-224		
			549-550	Glutenin	
			759-760		
			786-787		
			106-107	α -Gliadin, γ -Gliadin	
			108-109	α -Gliadin,	
			259-260		
			272-273		
			21-22	γ -Gliadin	
			36-37		
			51-52		
			61-62		
			232-233		
			332-333	ω -Gliadin	
			340-341		
			356-357		

	AV		10-11	Glutenin	proteinase P1, proteinase K	188.2
	GI		20-21	α -Gliadin,	proteinase K	
			310-311		proteinase P1	
			222-223	γ -Gliadin		
			319-320	β -Gliadin		
			105-106	Glutenin	proteinase K	
	RP		111-112	α -Gliadin,		271.3
	AY		178-179		252.2	
	QG		203-204	γ -Gliadin	subtilisin	203.2
	TF		264-265		proteinase P1	266.3
			40-41			
			17-18	ω -Gliadin	proteinase K	
	TP		120-121			216.2
	AG		274-275	γ -Gliadin	coccolysin, thermolysin	146.1
	IR		242-243		proteinase P1	287.3
	IF		33-34			
	GV		204-205			174.2
	EV		240-241		proteinase P1, proteinase K	246.2
	YV		257-258		proteinase P1	280.3
	HY		19-20		ω -Gliadin	subtilisin, proteinase K
IP	132-133	coccolysin, thermolysin	228.3			

			175-176				
			106-107	Glutenin			
	YK		8-9	ω-Gliadin		309.3	
	FP		307-308			262.3	
	KF		291-292			293.4	
	RF		282-283			proteinase P1	321.4
	ST		92-93			206.2	
	QK		290-291		oligopeptidase B	274.3	
	HK		38-39			283.3	
	NF		22-23		proteinase K	279.3	
	KP		2-3			243.3	
	SG				149-150	Glutenin	proteinase P1
				295-296			
				384-385			
				446-447			
				461-462			
				523-524			
				671-672			
				733-734			
				748-749			
			775-776				
			810-811				
TG		804-805		176.2			
KG		478-479		203.2			

	GR		203-204			231.2	
	AA		829-830			160.2	
	YE		841-842			310.3	
	YH		75-76			318.3	
	GE		821-822			204.2	
	SY		23-24			268.3	
	GY		124-125			proteinase K	238.2
			176-177				
			212-213				
			301-302				
			375-376				
			390-391				
			437-438				
			452-453				
		514-515					
		541-542					
	IAY	556-557	subtilisin	365.4			
		695-696					
	IPP	316-317	coccolysin, thermolysin	325.4			
		177-179					
		300-302					
186-188							
	251-253	ω-Gliadin					
	259-261						
	3						

			275-277				
	LQP		77-79	β -Gliadin		356.4	
	LQQ			80-82	α -Gliadin		387.4
				156-158			
				248-250			
				307-309	Glutenin		
				147-149	β -Gliadin		
			184-186				
	VPP		258-260	γ -Gliadin	thermolysin	311.4	
	TTN			122-124	ω -Gliadin	proteinase P1	334.3
				165-167			
	VSP			66-68	Glutenin	thermolysin	301.3
	AQQLAAQLPAMCR	13		837-849		oligopeptidase B	1417.5