



Récents développements de l'effet du levain sur les bénéfices nutritionnels

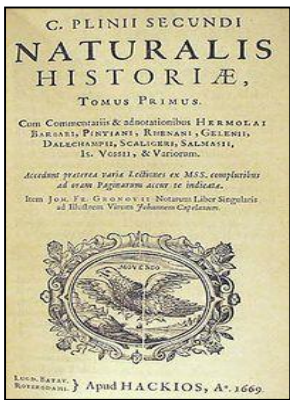
Marco Gobbetti

marco.gobbetti@uniba.it

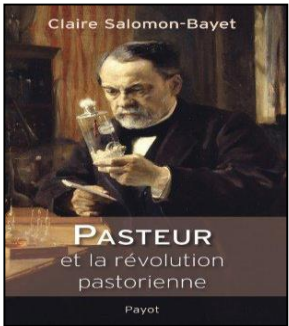
Le Fonds de Dotation EKIP, Paris
28 Novembre 2016



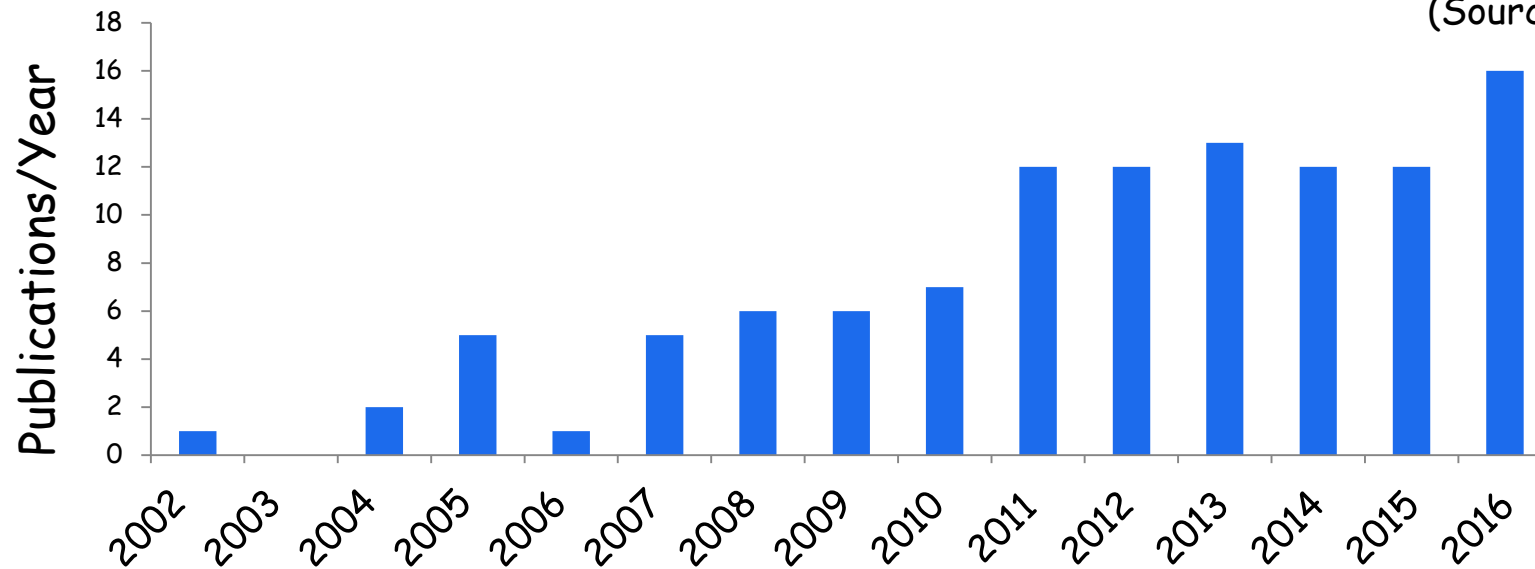
Historical excursus on the «leavening» of baked goods



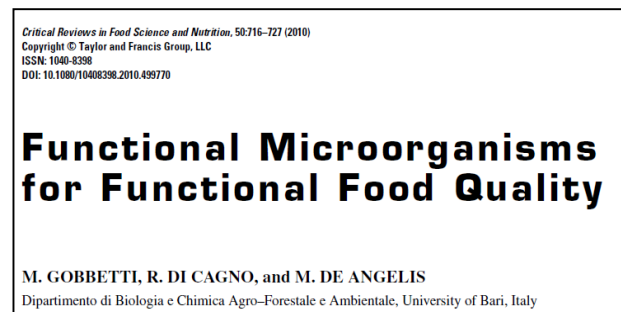
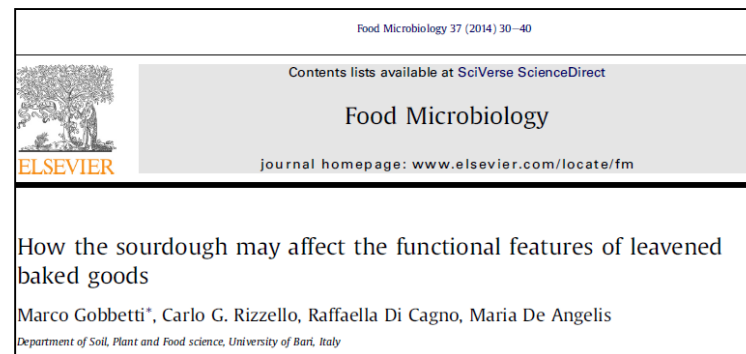
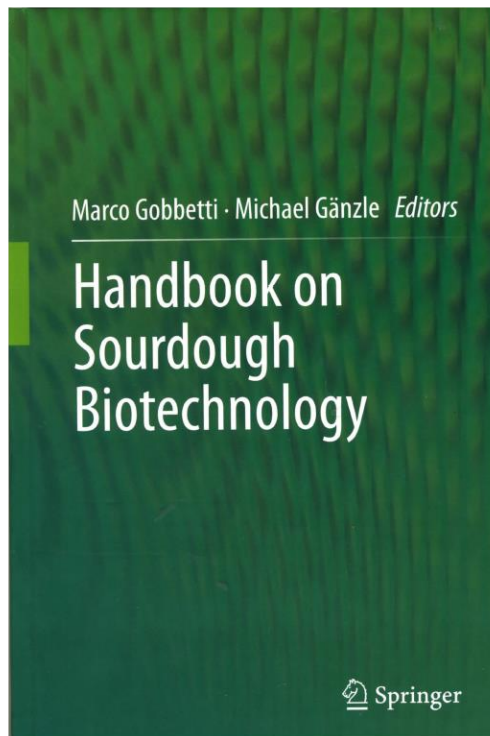
*"Tacuini Sanitatis
among the six elements needed
for the leavening of breads of
the 19th century (1822 - 1895)
"give birth to the bread"
the author of the 19th century
completely fermented..." (XI
century, Codex 4182,
Biblioteca Casanatense Rome)*



(Source: Web of Science)



Nutrition
and
sourdough



Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Italy

For instance ... the Italian market

Fibers

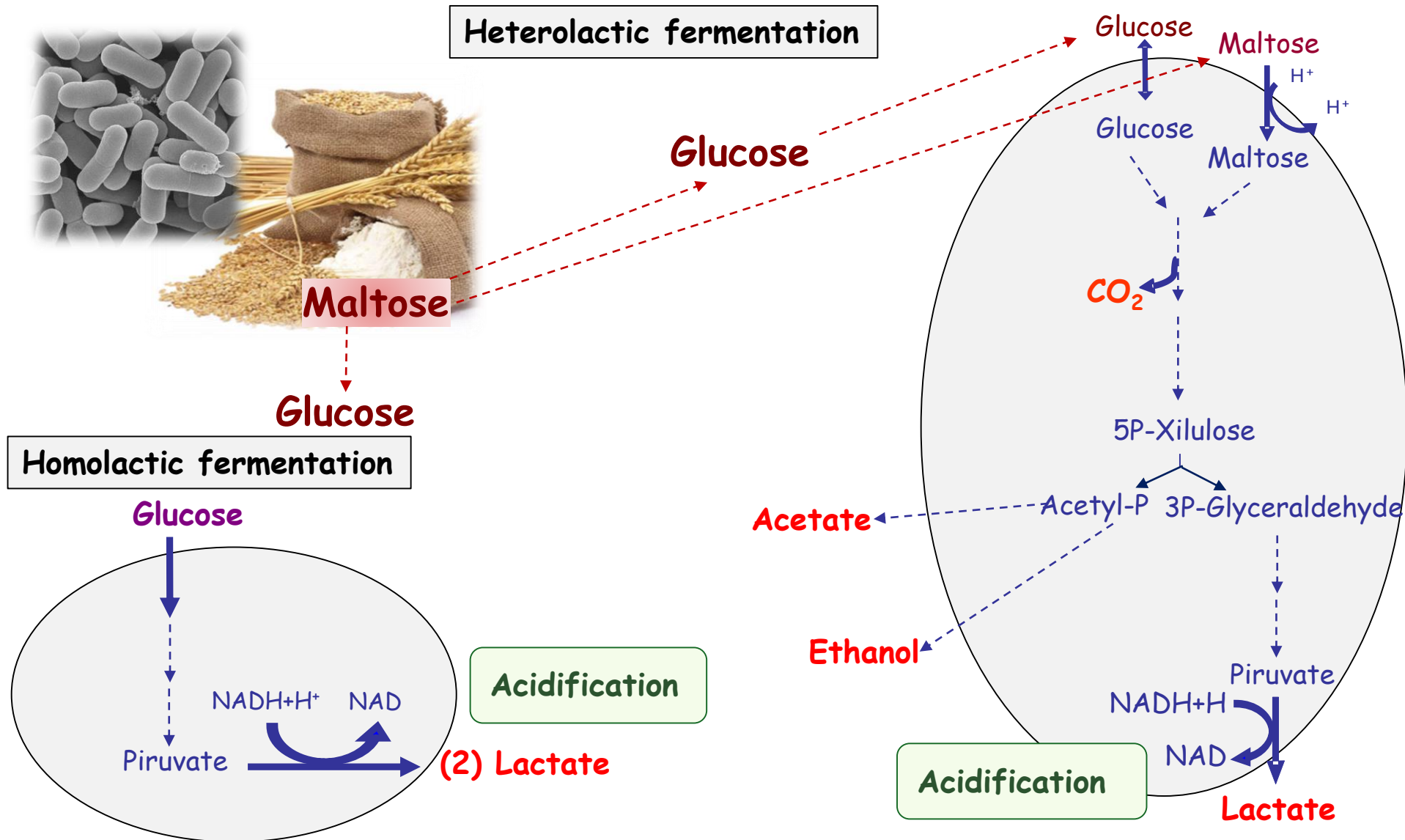
Beta-glucans



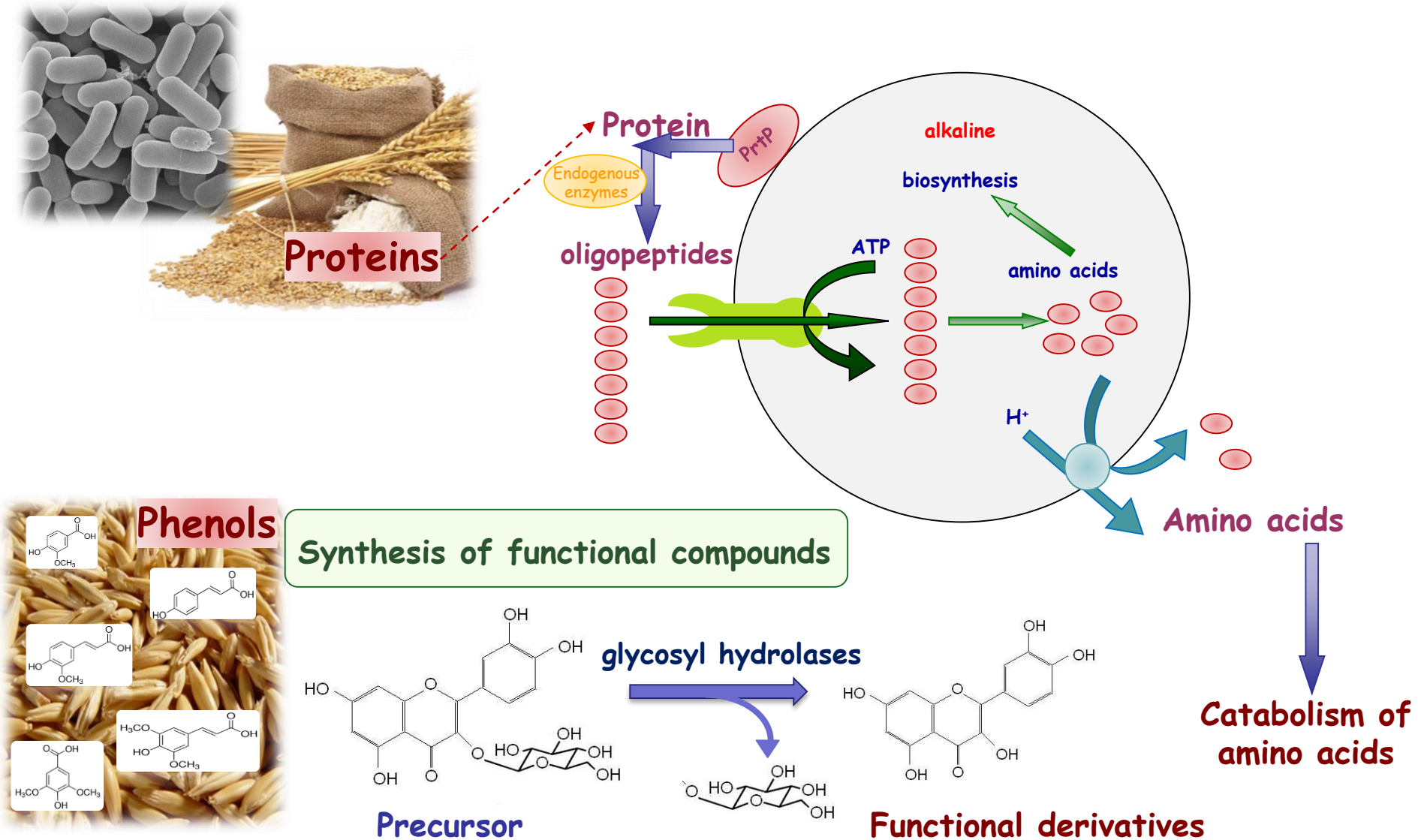
...and minerals



How may sourdough lactic acid bacteria contribute to nutritional value?



How may sourdough lactic acid bacteria contribute to nutritional value?



The sourdough fermentation may affect the nutritional/functional features of leavened baked goods through:

- Pre-treatment of raw materials (e.g., bran and wheat germ)
- Modification/increase of the bio-availability of dietary fibres, minerals and vitamins
- Decrease of glycaemic index/response
- Synthesis of bioactive peptides and amino acid derivatives
- Synthesis/liberation of antioxidant compounds
- Bio-preservation
- Decrease/elimination of the intolerance/sensitivity to gluten

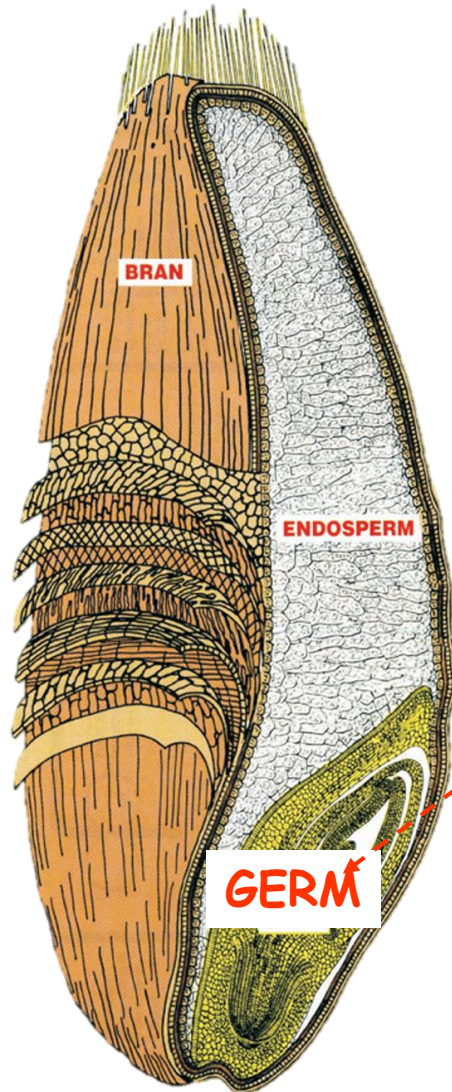


Pre-treatment of bran

- Changes on the quality of dietary fibre and bio-availability of phytochemicals (Broekaert et al. 2011, *Crit Rev Food Sci Nutr* 51:178)
- Improved sensory and structure (e.g., loaf volume and crumb softness) characteristics (Katina et al. 2006, *LWT Food Sci Technol* 16:104; Katina et al. 2007, *Food Microbiol* 24:175)



Pre-treatment of wheat germ



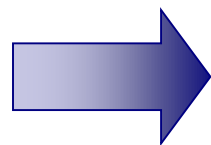
- ✓ α -Tocopherol
- ✓ Vitamins B
- ✓ Dietary fibre
- ✓ Minerals
- ✓ Proteins
- ✓ Phytochemicals (flavonoids, sterols,...)
- ✓ Unsaturated fatty acids

Antinutritional factors

- ✓ Raffinose
- ✓ Phytic acid
- ✓ Wheat germ agglutinin



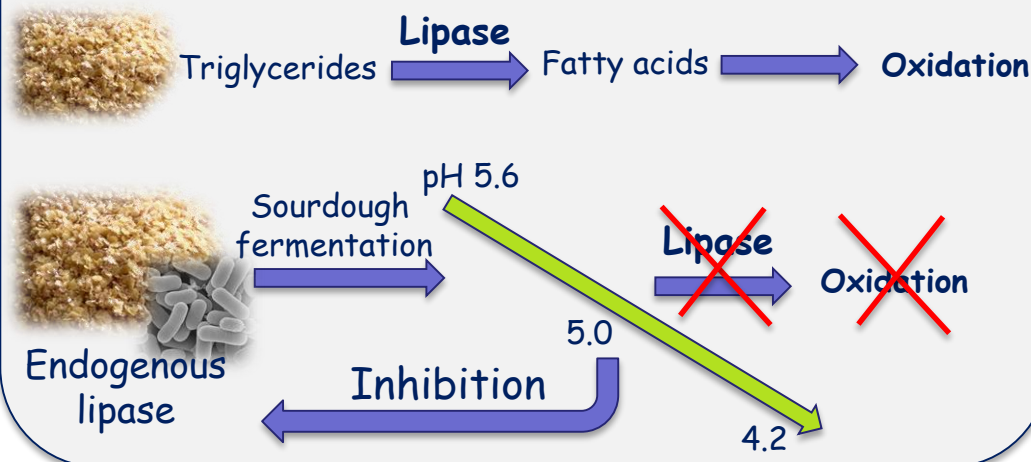
Pre-treatment of wheat germ



Sourdough fermentation

(selected, autochthonous lactic acid bacteria)

Lipase activity

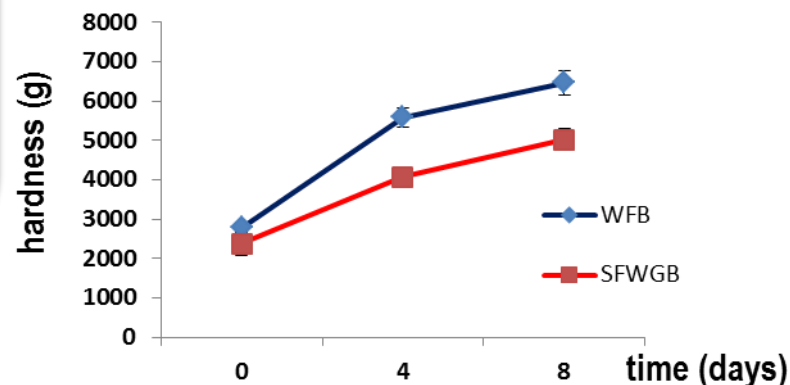


- Lipase activity **ca. 2.6-fold** lower than that found in raw wheat germ
- After 40 days of storage, a very low concentration of hexanal and other markers of lipid oxidation were found in fermented wheat germ (SPME analysis)

SFWG as ingredient for bread making

(4%, replacement of wheat flour)

- Specific volume + **16-18%**
- Improving of textural properties/ firmness delay:



Rizzello et al., 2010 *Eur Food Res Technol* 230:645
Rizzello et al., 2010 *Food Chem* 119:1079
Rizzello et al., 2011 *Food Chem* 127:952

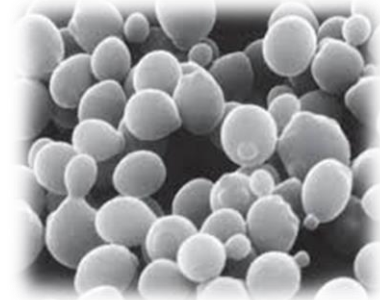
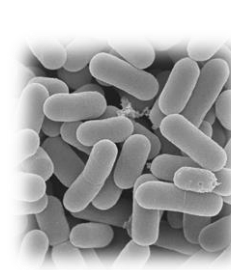
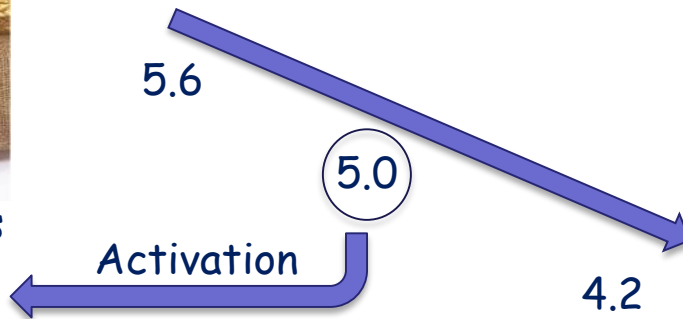


Modification/increase of the bio-availability of dietary fibres, minerals and vitamins

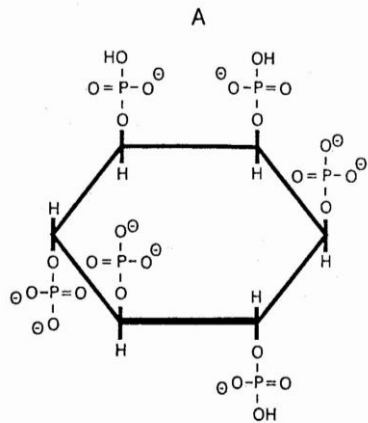


Grain endogenous phytase

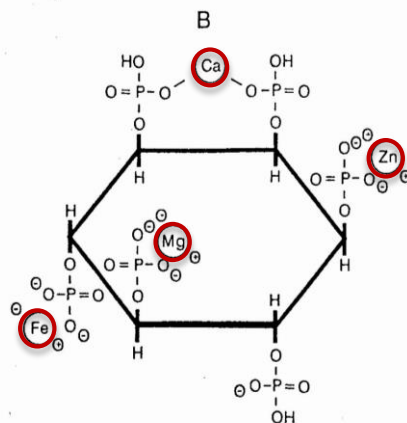
Sourdough fermentation



Microbial phytases
(lactic acid bacteria and yeasts)



Phytic acid



Phytic acid chelate

Phytase

EC 3.1.3.8
EC 3.1.3.26

- Inorganic phytate;
- Myo-inositol phosphate esters;
- Free minerals (e.g., Fe^{3+} , Zn^{2+} , Ca^{2+})
- Proteins, peptides, amino acids

Gobbetti et al., 2014, *Food Microbiol*, 1-11

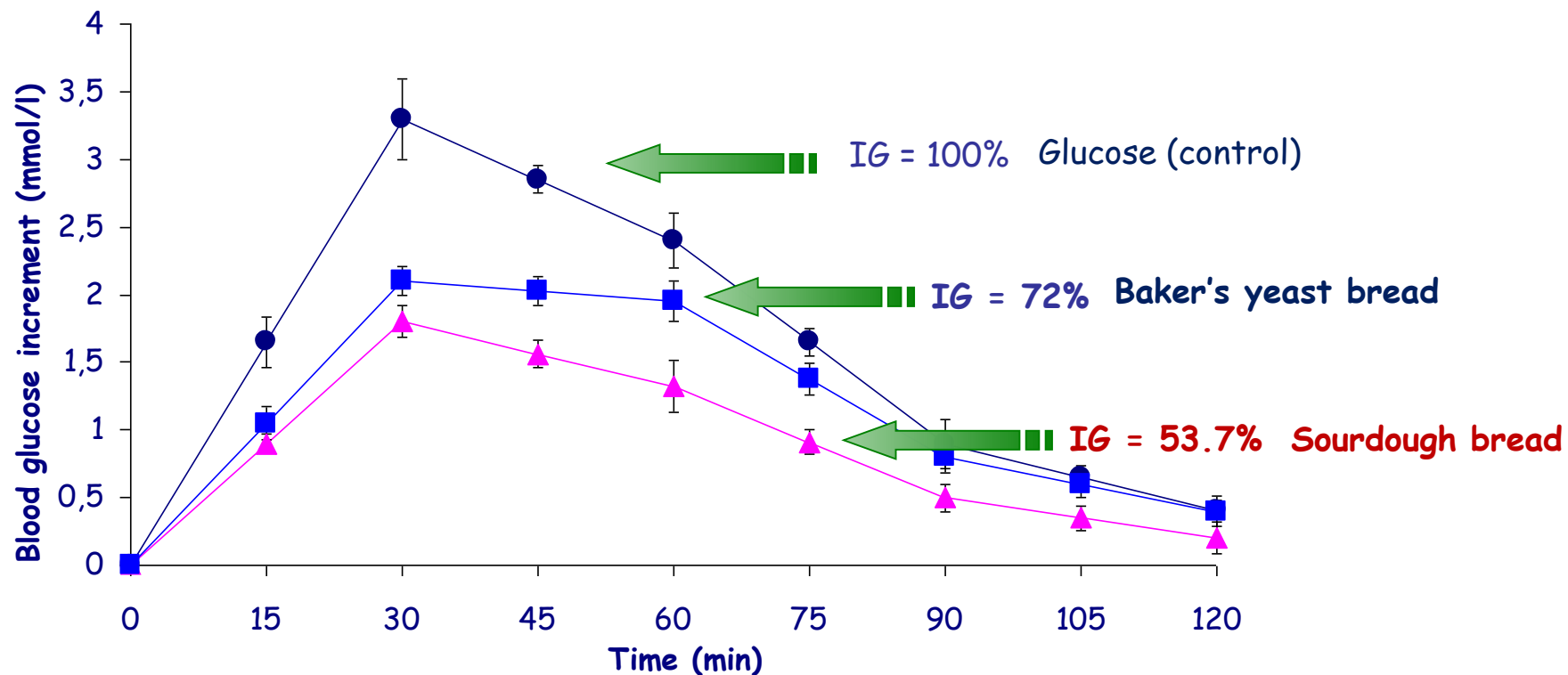


Decrease of glycaemic index/response

- Sourdough fermentation, possibly combined with the addition of soluble fibre, is an effective tool to decrease glycaemic index (GI) and insulin index (II) (Liljeberg et al. 1995; Juntunen et al. 2003; De Angelis et al., 2006; 2009; Fardet et al. 2006; Maioli et al. 2009; Najjar et al. 2009)
- Not all the sourdoughs impact on GI/II (Hardman Fredensborg et al. 2010)



Decrease of glycaemic index/response



De Angelis et al., 2007 *British J Nutr*, 98: 1196-1205



Hypotheses to explain the effect of sourdough fermentation

- Synthesis of organic acids, especially lactic acid (Liljeberg et al. 1995, *J Nutr* 125:1503; Liljeberg et al. 1998, *Eur J Clin Nutr* 52:368)
- Low pH (Burton and Lightowler 2006, *Br J Nutr* 96:877)
- Chemical changes (Östman 2003, PhD Thesis Lund University)
- Liberation of peptides and amino acids, and free phenolic compounds (Nilsson et al. 2007, *Am J Clin Nutr* 85:996; Katina et al. 2007, *Food Microbiol* 24:175)



Bioactive peptides

Bioactive or biogenic peptides are defined as specific protein fragments that have a positive impact on the body function or condition, and may, ultimately, influence the human health

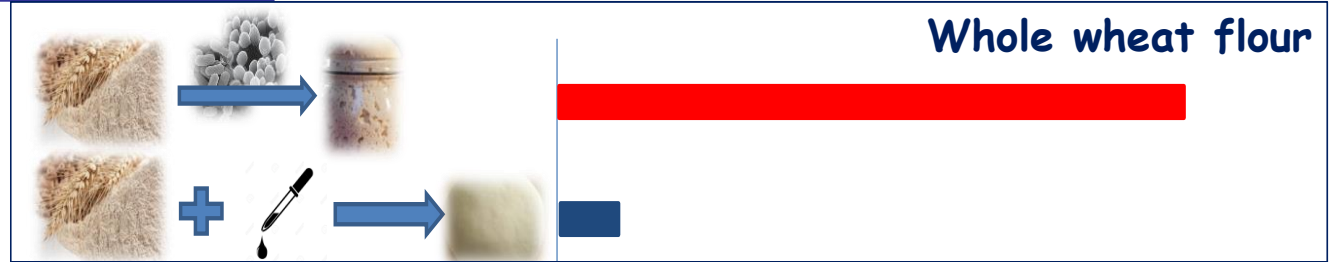
(Kits et al. 2003, *Curr Pharm Des* 9:1309)



Antioxidant activity

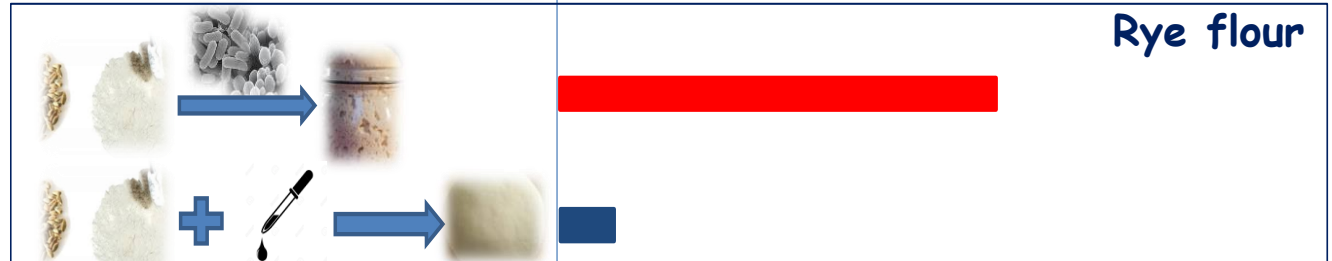
Sourdough fermentation

Chemical acidification



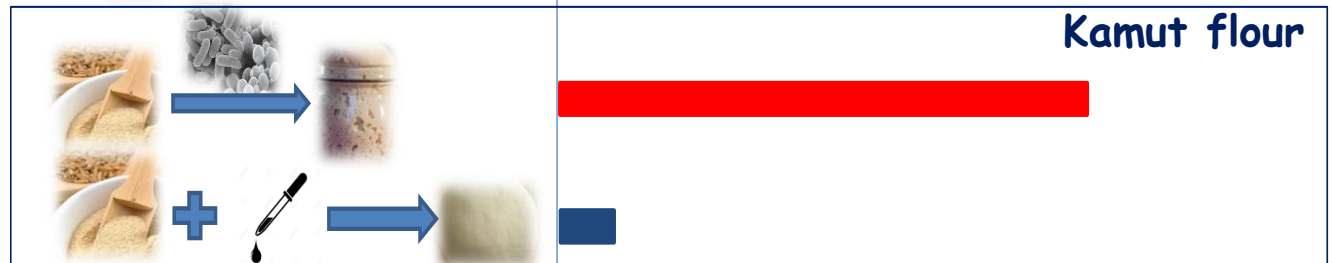
Sourdough fermentation

Chemical acidification



Sourdough fermentation

Chemical acidification



Sourdough fermentation

Chemical acidification



Antioxidant activity (%)

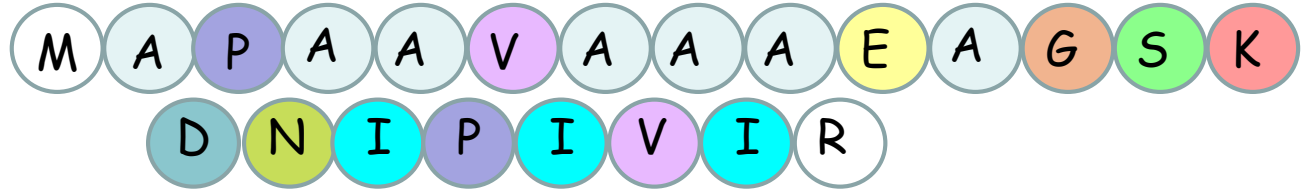
10 20 30 40 50 60



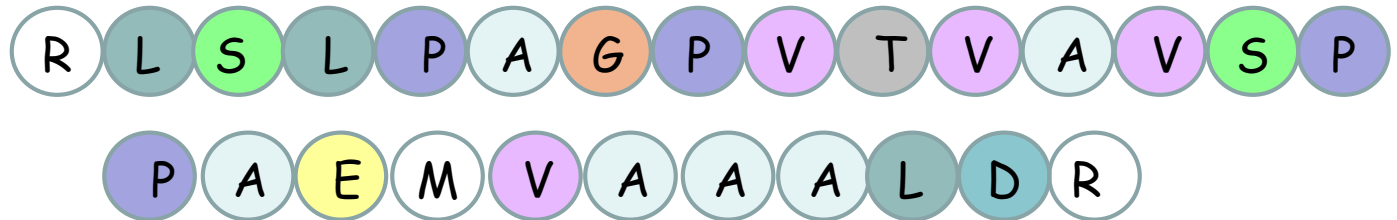
Antioxidant peptides



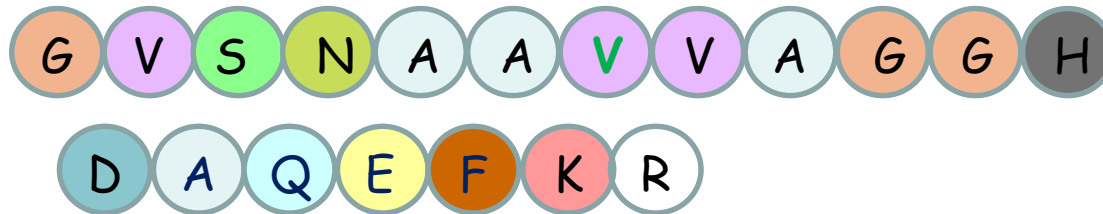
Whole wheat



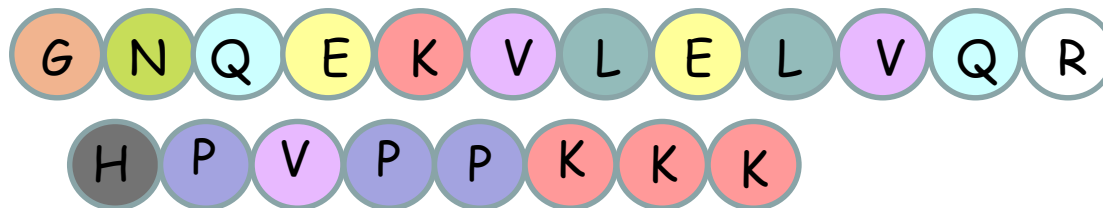
Rye



Kamut



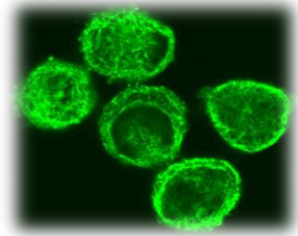
Spelt



Coda et al., 2012, *Appl Environ Microbiol* 78:1087



Antioxidant peptides



Whole wheat flour



M A P A A V A A A E A G S K
D N I P I V I R

Rye flour



R L S L P A G P V T V A V S P
P A E M V A A A L D R

Kamut flour



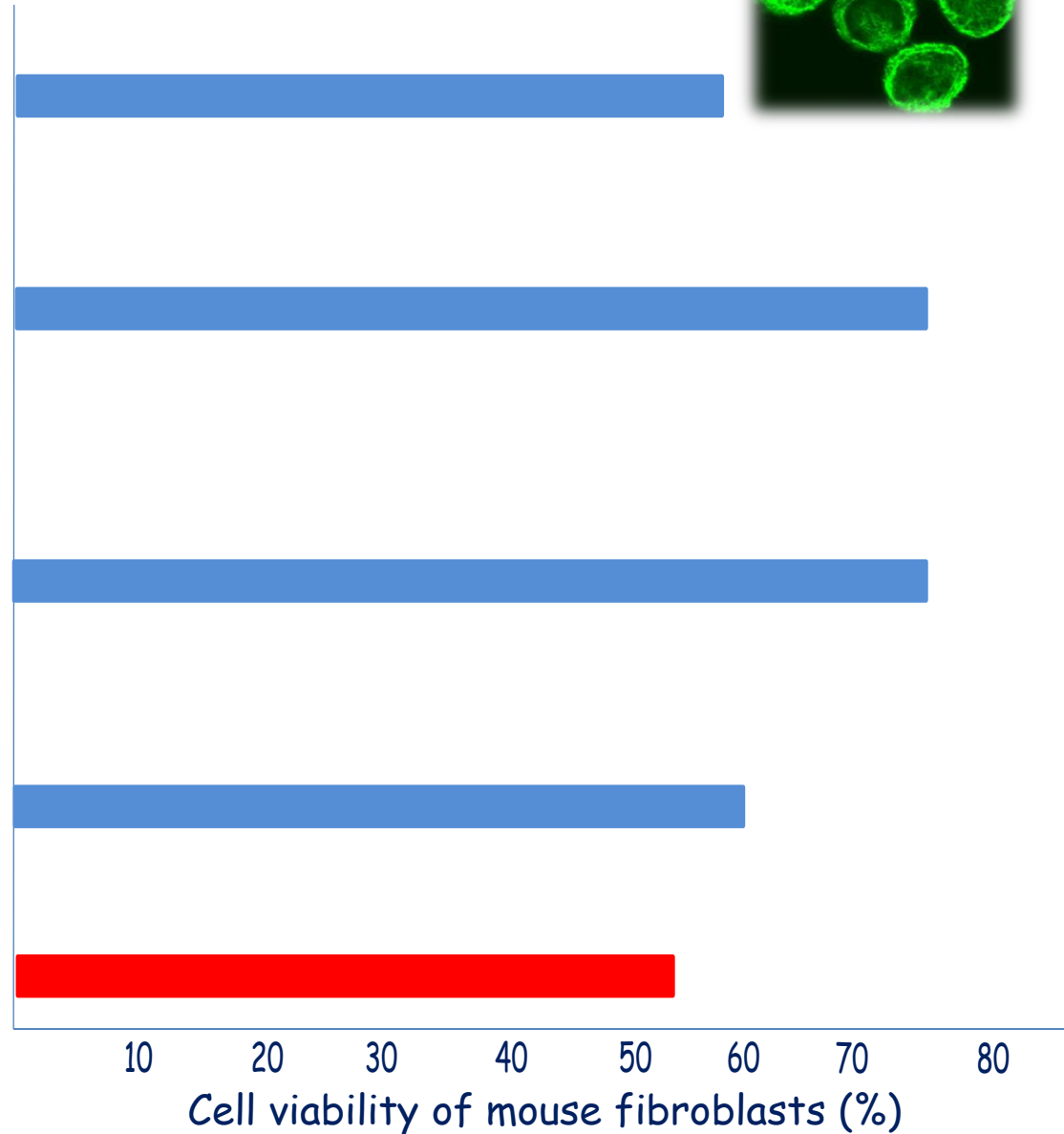
G V S N A A V V A G G H
D A Q E F K R

Spelt flour



G N Q E K V L E L V Q R
H P V P P K K K

CTRL (after oxidative stress)



Coda et al., 2012, *Appl Environ Microbiol* 78, 1087-1096



Lunasin



- initially isolated from soy (small subunit of albumin Gm2S-1); further isolated from barley, wheat, amaranth, rye, *Solanum nigrum*)
- carcinogenesis suppressor (inhibitory effect against the core histone acetylation) (Galvez et al. 2011 *Cancer Res* 61:7473)
- antiinflammatory activity (Dia et al. 2009, *J Agric Food Chem* 57:1260)
- resistant to gastrointestinal digestion: it can reach the blood plasma after oral ingestion (Park et al. 2007, *J Agric Food Chem* 55:10703)



Rizzello et al. 2012, *Nutrition and Cancer* 64:111



Lunasin



Sourdough fermentation



L. curvatus



Chemical acidification



Whole wheat

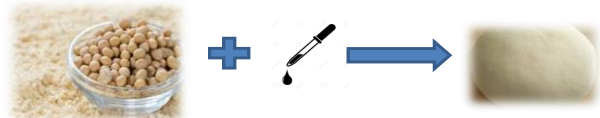
Sourdough fermentation



L. curvatus



Chemical acidification



Soybean

Sourdough fermentation



L. curvatus



Chemical acidification



Barley

Sourdough fermentation



L. curvatus



Chemical acidification



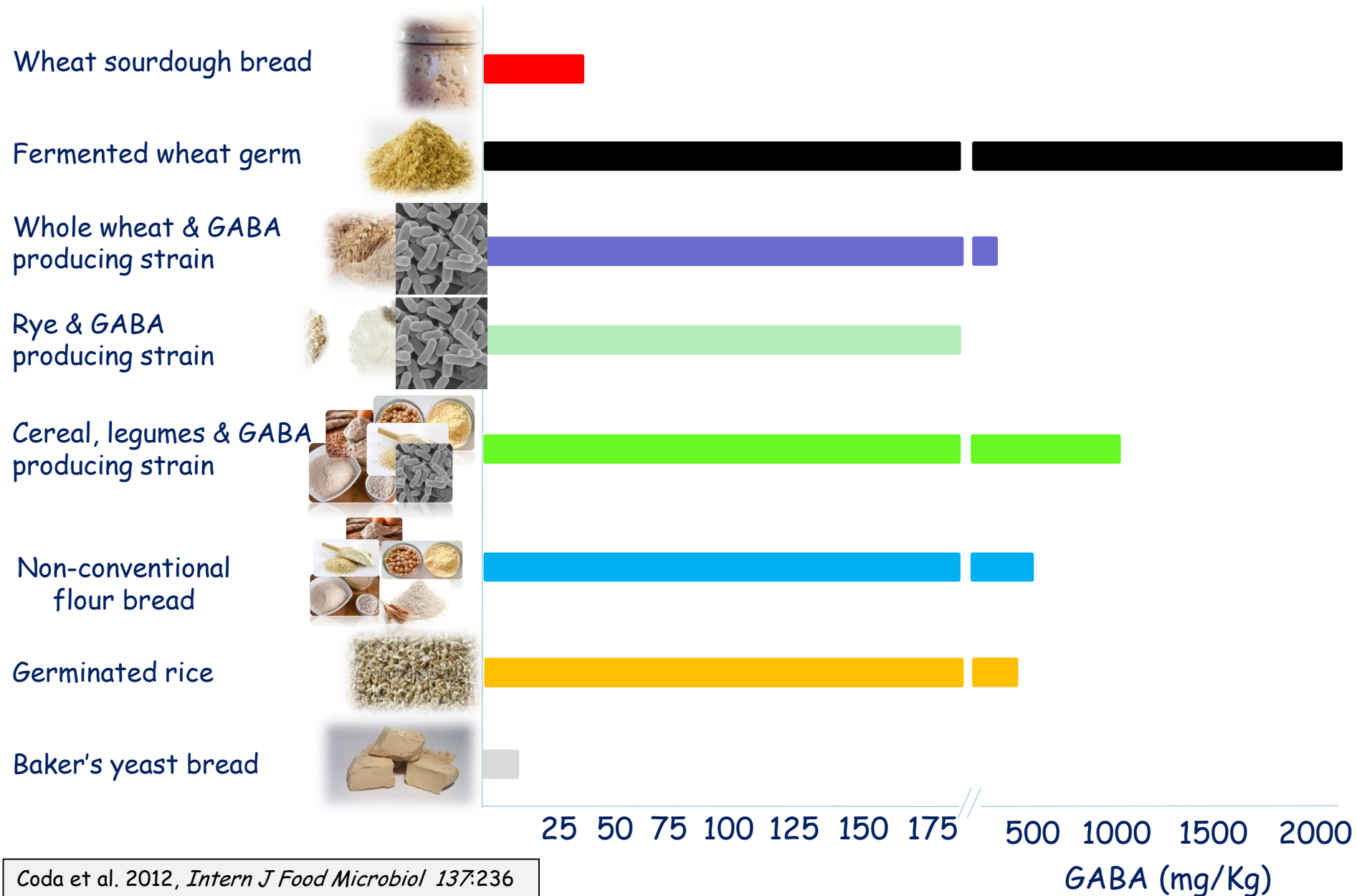
Amaranth

1 2 3 4 5 6
Lunasin (mg/dough)

Rizzello et al. 2012, *Nutrition and Cancer* 64:111



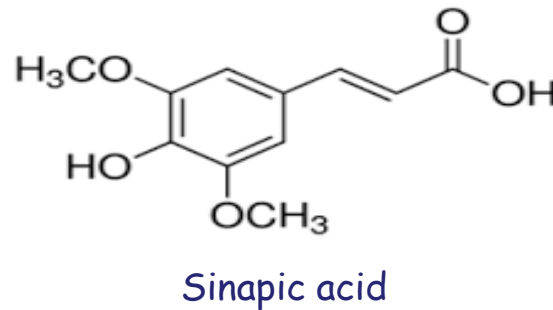
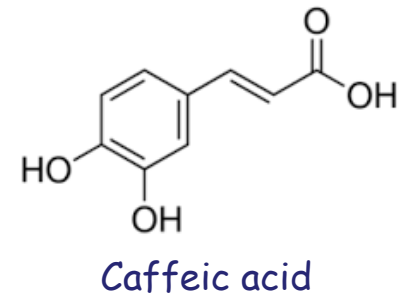
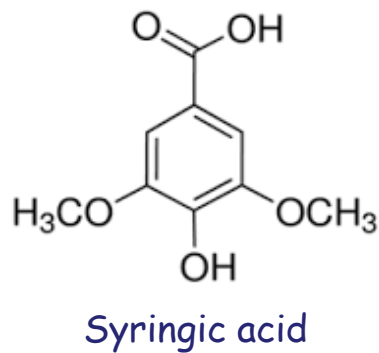
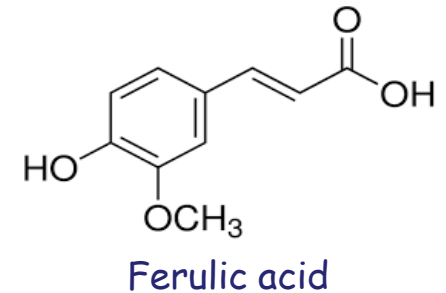
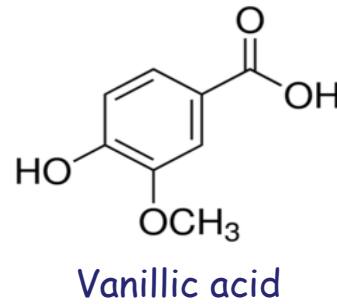
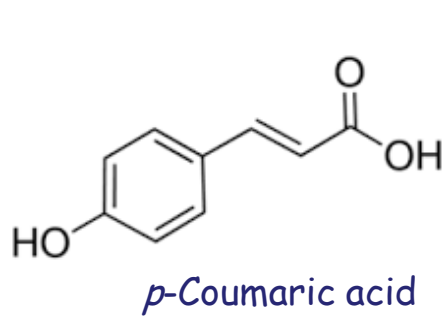
γ -Amino butyric acid (GABA)



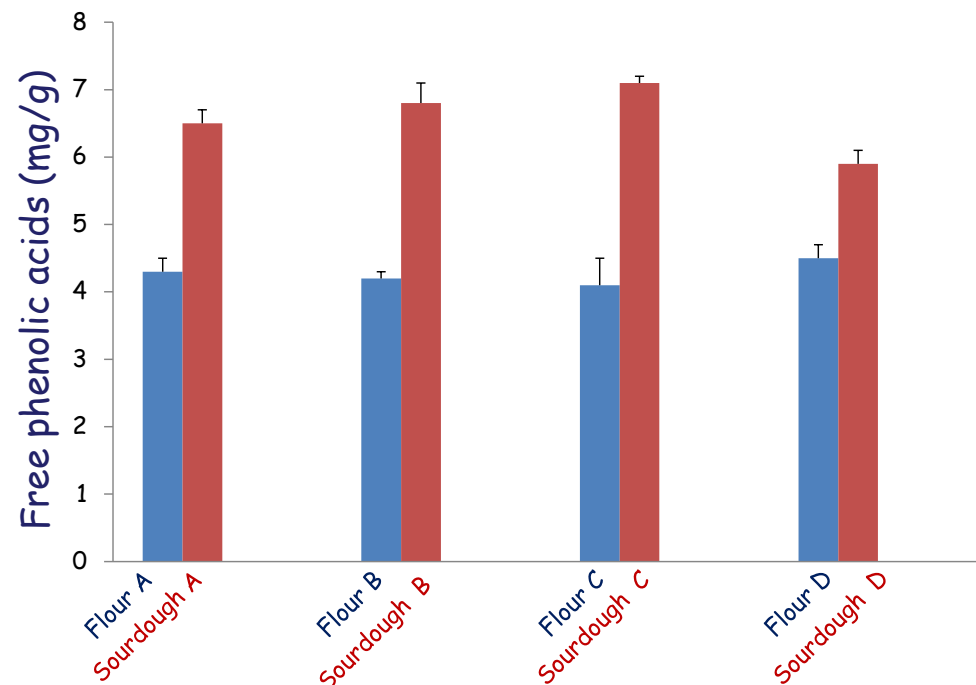
Coda et al. 2012, *Intern J Food Microbiol* 137:236



Synthesis/liberation of antioxidant compounds (phenols)

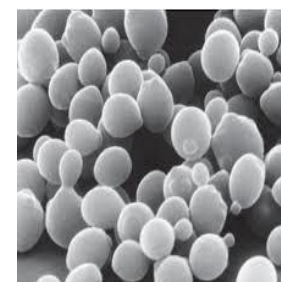
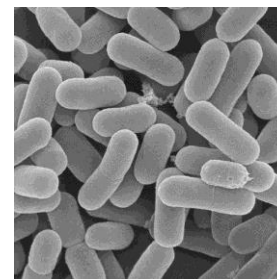


Hydrolysis of phenolic compounds



5.6

Sourdough fermentation



pH

4.2

Microbial activity
(lactic acid bacteria and yeasts)
EC 3.2.1.21 β -Glucosidase
EC 3.1.1.73 Feruloyl-esterase

- Solubilization of phenolic compounds
- Increase of the concentration

Rizzello et al., 2014, *Appl. Environm. Microbiol.* 81: 3192-3204



Bio-preservation



VS



Additives

- ✓ Sorbic acid or potassium sorbate or calcium sorbate;
- ✓ Propionic acid or salts (e.g., sodium-, calcium-, and potassium-propionate);
- ✓ Ethanol;
- ✓ Acetic acid or salts (e.g., sodium-, calcium-, and potassium-acetate);
- ✓ Lactic acid or salts (e.g., sodium-, calcium-, and potassium-lactate)

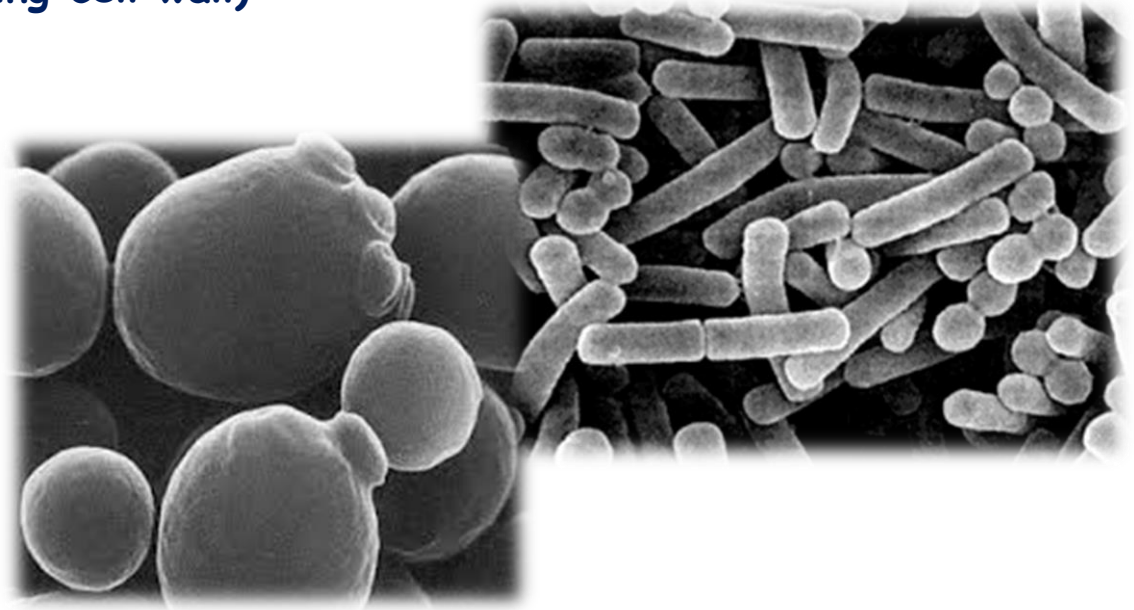


Antifungal activity and baked goods

- ✓ Vegetable matrices (proteins and peptides)



- ✓ Selected lactic acid bacteria (organic acids/antifungal peptides) and yeasts (ethanol + ethyl-acetate/enzymes degrading cell wall)



Fungal spoilage of experimental breads

Controls (baker's yeast, no extracts, no propionate)

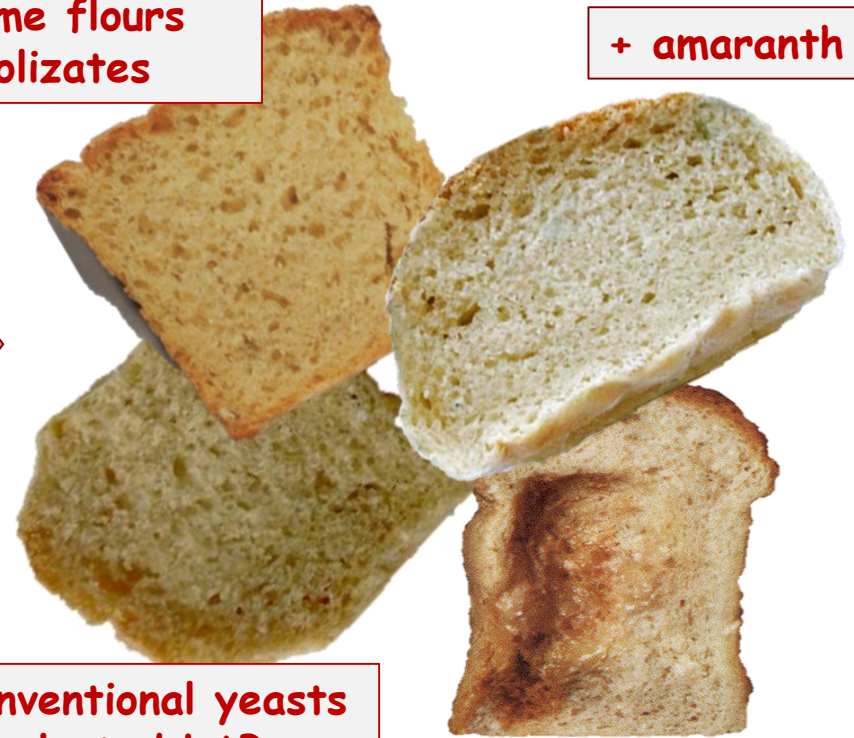


+ legume flours
hydrolyzates

+ amaranth

+ non conventional yeasts
and selected LAB

+ calcium propionate



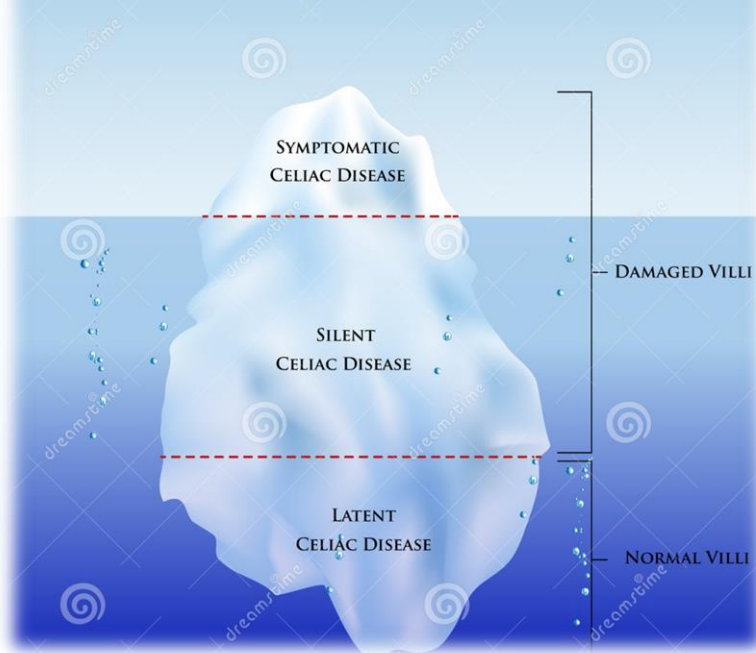
(10² conidia/ml)

Delay of the fungal contamination of two weeks



Novel therapies for celiac disease (Caputo et al., 2010. *Enzyme Res* 103:75)

THE CELIAC ICEBERG



- Wheat
- Barley
- Rye
- Spelt
- Kamut
- Triticale
- Oat*

Non-tolerated

- Oral administration of bacterial endopeptidases (*Flavobacterium meningosepticum*, *Sphingomonas capsulata*, *Myxococcus xanthus*, *Aspergillus niger*)

(Shan et al. 2004. *Biochem J* 383:311; Marti et al. 2005. *J Pharm Exp Therap* 312:19; Gass et al. 2005. *Biotechnol Bioeng* 92:674; Mitea et al. 2008. *Gut* 57:25)

- Transamidation of gliadin

(Gianfrani et al. 2007. *Gastroenterology* 133:780)

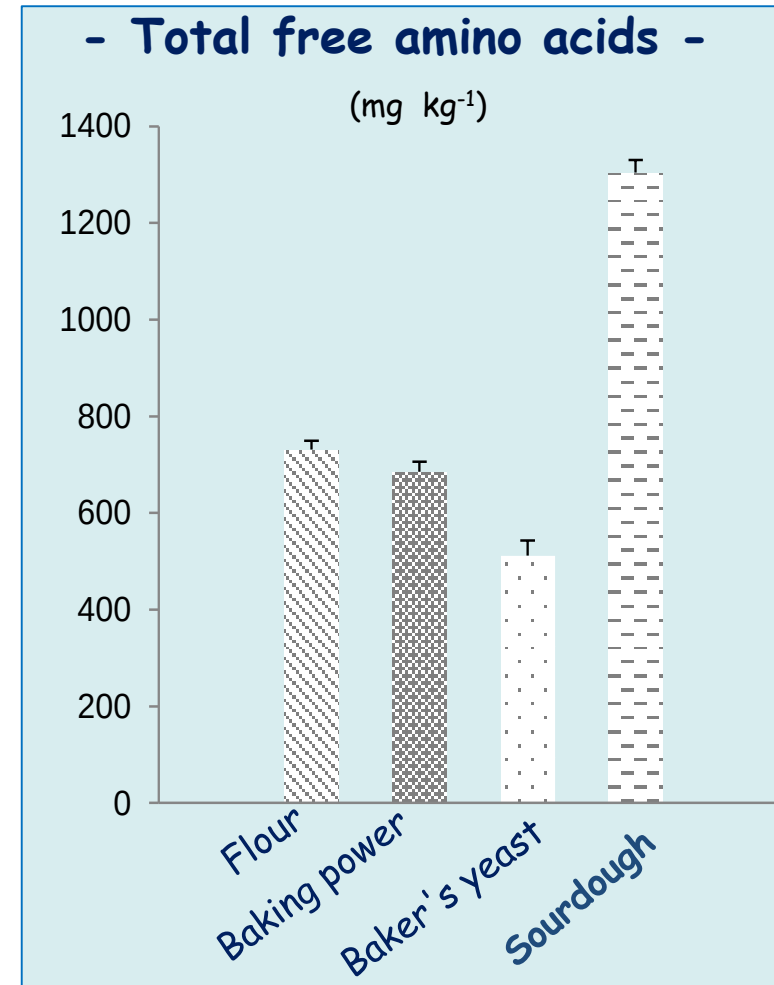
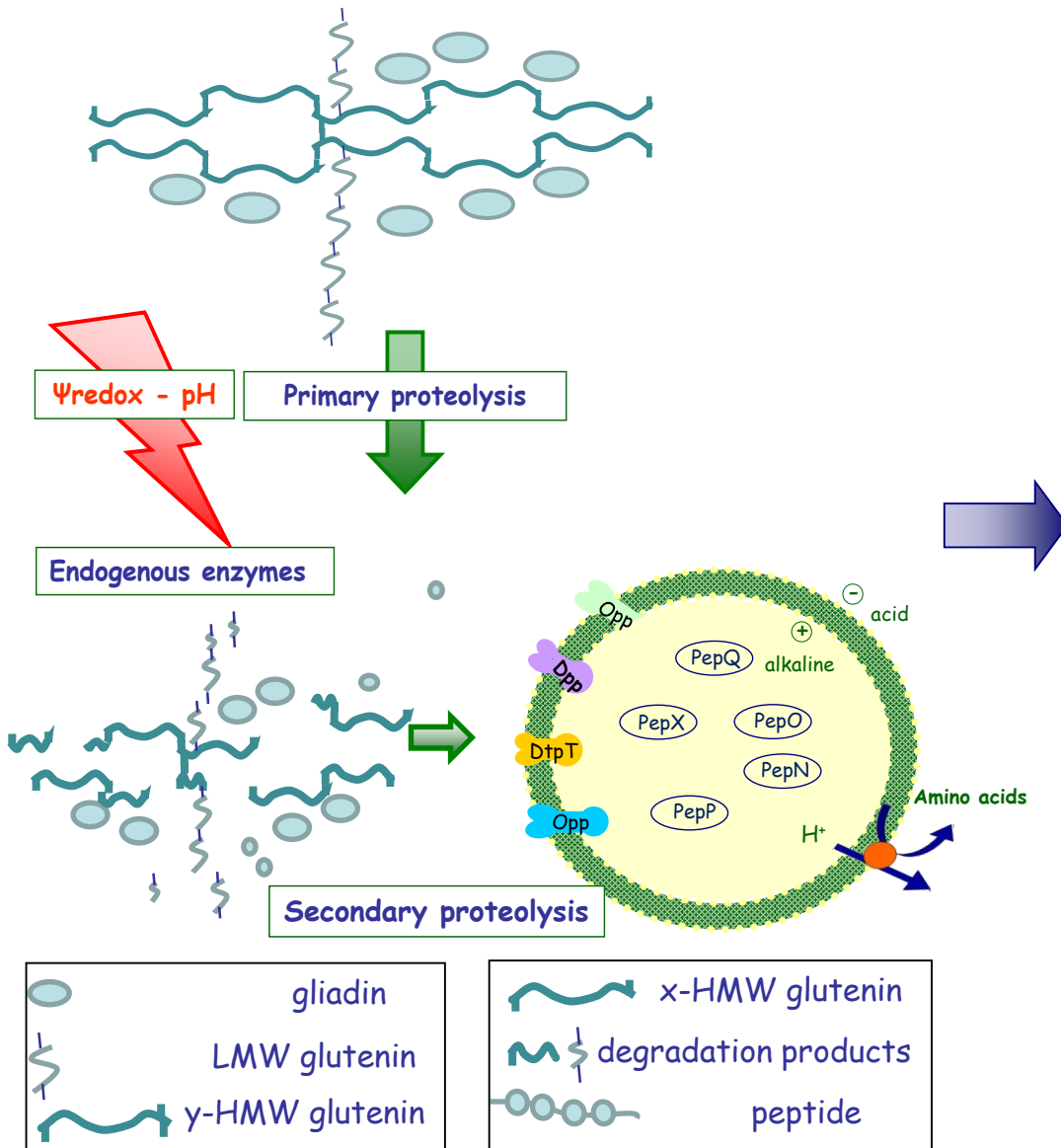
- Transglutaminase inhibitors

Esposito et al. 2007. *Cur Med Chem* 14:2572; Wodzinska et al. 2005. *Med Chem* 5:279; Dolyuchuk 1996. *Plast Reconst Surgery* 98:752; Hoffmann et al. 2009. *J Agric Food Chem* 57:10150).



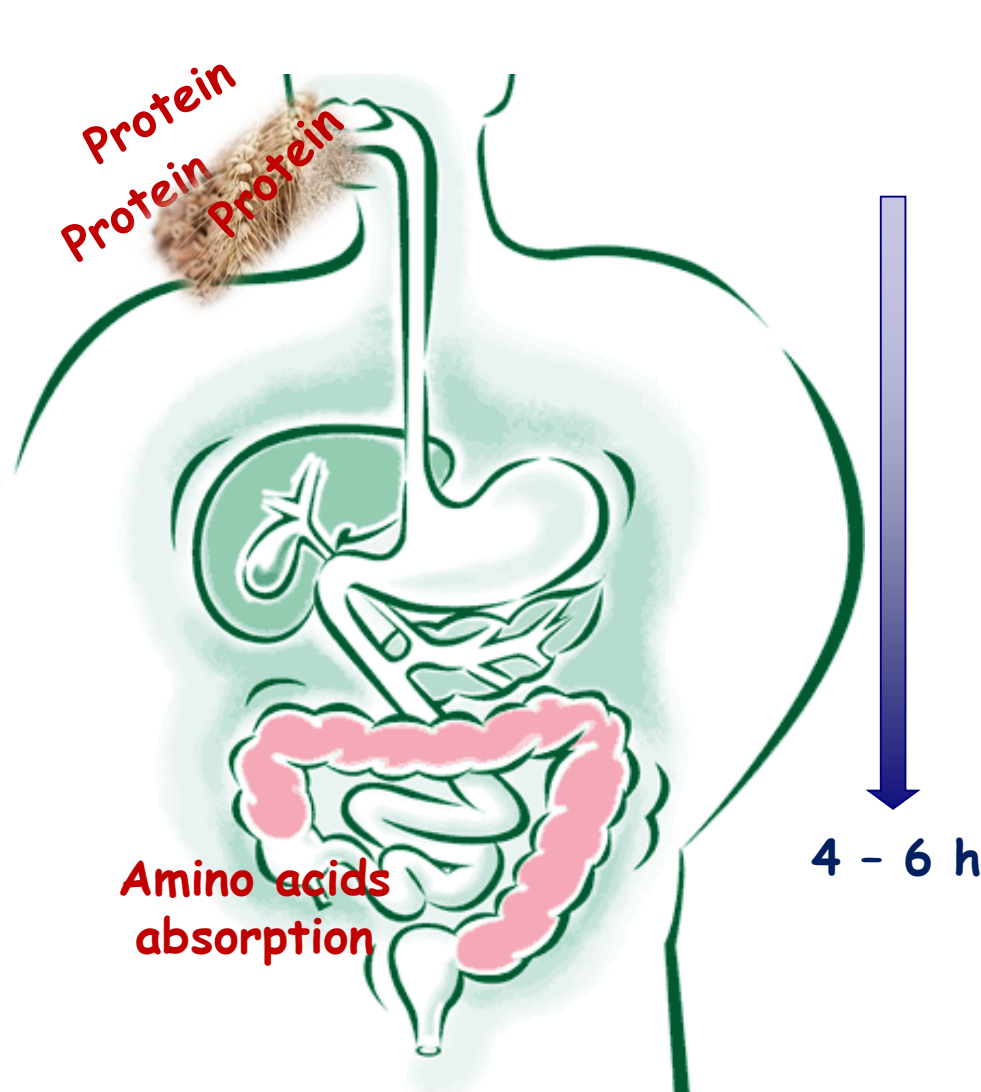
Proteolysis during sourdough fermentation

Ganzle *et al.* 2008, Trends Food Sci Technol 19:513



Gobbetti *et al.*, 1994 J. Food Sci. 59: 881-884

Human digestion



Sourdough fermentation



Altomare *et al.*, 1999. Dis. Colon Rectum, 42: 231-240;
Perri *et al.*, 2010. Dig. Liver Dis., doi:10.1016/j.dld.2010.01.001



Sourdough Bread Made from Wheat and Nontoxic Flours and Started with Selected Lactobacilli Is Tolerated in Celiac Sprue Patients

Raffaella Di Cagno,^{1†} Maria De Angelis,^{2†} Salvatore Auricchio,³ Luigi Greco,³ Charmaine Clarke,⁴ Massimo De Vincenzi,⁵ Claudio Giovannini,⁵ Massimo D'Archivio,⁵ Francesca Landolfo,³ Giampaolo Parrilli,³ Fabio Minervini,¹ Elke Arendt,⁴ and Marco Gobbetti^{1*}

ACKNOWLEDGMENTS

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We thank P. F. Fox for critical revision of the paper and Giuditta Alfonsi and Valeria Ancona for technical support. M.G. thanks his father for the practical suggestions which promoted the idea of this work.

File Messaggio

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Zoom Zoom

Da: ☐ Rising Creek <risingcreekbakery@gmail.com>
 A: ☐ gobbetti@agr.uniba.it
 Cc:
 Oggetto: bread risen with bacteria

Invia: giovedì 09/10/2014 14:26

Hello Dr. Gobbetti, At our bakery we make a fermented bread that utilizes wild bacteria, instead of yeast, to raise the dough and enhance the flavor of the bread.

The bread we make goes beyond sourdough Our bread reduces the gluten or the fructan content - which may explain why many people say eating our bread soothes the stomach. We think you may discover an important new food product to help the gluten-intolerance diet.

We will be glad to supply this bread for your research. Thanks, Jenny

--
 Jenny Bardwell
 Rising Creek Bakery
 PO Box 303
 115 Main St
 Mt. Morris, PA 15349
 724-324-5030



List of articles leading with gluten degradation

- Di Cagno et al., 2002. Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and Environmental Microbiology*, 68:623-633.
- Di Cagno et al., 2004. Sourdough bread made from wheat and nontoxic flours and started with selected lactobacilli is tolerated in celiac sprue patients. *Applied and Environmental Microbiology*, 70:1088-1096.
- Di Cagno et al., 2005. Pasta made from durum wheat semolina fermented with selected lactobacilli as a tool for a potential decrease of the gluten intolerance. *Journal of Agricultural and Food Chemistry*, 53 (11):4393-4402.
- De Angelis et al., 2006a. VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue. *Biochimica et Biophysica Acta*, 1762:80-93.
- De Angelis et al., 2006b. Fermentation by selected sourdough lactic acid bacteria to decrease the intolerance to rye and barley flours. *Journal of Cereal Science*, 43:301-314.
- Gobbetti et al., 2007. Sourdough lactobacilli and celiac disease. *Food Microbiology*, 24(2):187-196.
- Rizzello et al., 2007. Highly efficient gluten degradation by lactobacilli and fungal proteases during food processing: new perspectives for celiac disease. *Applied and Environmental Microbiology*, 73(14):4499-4507.
- Di Cagno et al., 2009. Different fecal microbiotas and volatile organic compounds in treated and untreated children with celiac disease. *Applied and Environmental Microbiology*, 75(12):3963-3971.
- De Angelis et al., 2010. Mechanism of degradation of immunogenic gluten epitopes from *Triticum turgidum* L. var. *durum* by sourdough lactobacilli and fungal proteases. *Applied and Environmental Microbiology*, 76(2):508-518.
- Di Cagno et al., 2010. Gluten-free sourdough wheat baked goods appear safe for young celiac patients: a pilot study. *Journal of Pediatric Gastroenterology & Nutrition*, 51(6):777-783.
- Greco et al., 2011. Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing. *Clinical Gastroenterology and Hepatology*, 9(1):24-29.
- Di Cagno et al., 2011. Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. *BMC Microbiology*, 11,
- Rizzello et al., 2014. Use of fungal proteases and selected sourdough lactic acid bacteria for making wheat bread with an intermediate content of gluten. *Food Microbiology*, 37:59-68.
- Curiel et al., 2014. Manufacture and characterization of pasta made with wheat flour rendered gluten-free using fungal proteases and selected sourdough lactic acid bacteria. *Journal of Cereal Science*, 59:79-87.
- Rizzello et al. 2016. Characterization of the bread made with durum wheat semolina rendered gluten free by sourdough biotechnology in comparison with commercial gluten-free products. *Journal of Food Science*, doi:10.1111/1750-3841.13410.



Pool 1 (ca. log 9/ml)

Lb. brevis 14G
Lb. alimentarius 15M
Lb. hilgardii 51B
Lb. sanfranciscensis 7A

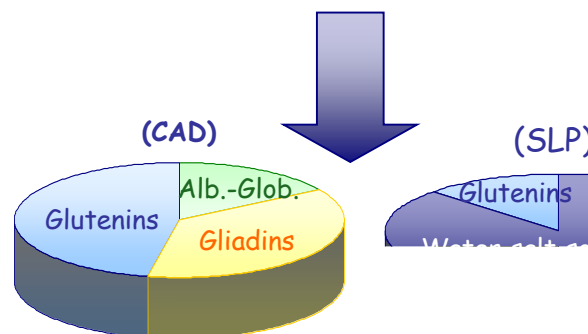
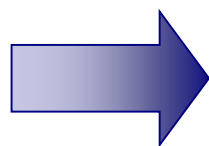
+

Pool 2 (ca. log 8/ml)

Lb. sanfranciscensis E14
Lb. sanfranciscensis E21
Lb. sanfranciscensis 174
Lb. sanfranciscensis 13
Lb. sanfranciscensis A1
Lb. sanfranciscensis 274

+

Fungal proteases (200 ppm)

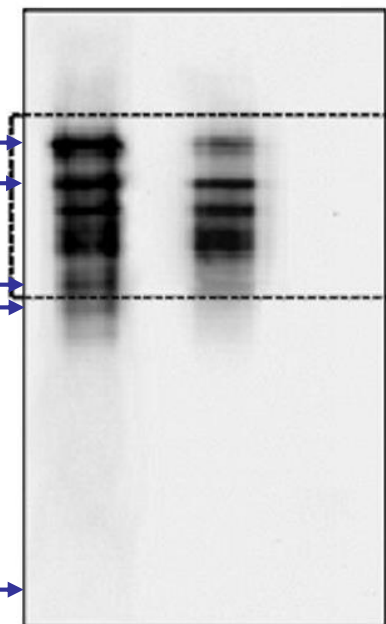


ω -gliadins

α , β , ω -gliadins

hydrolyzed
gliadins

St 1 2



St, European gliadin standard;
1, chemically acidified dough (CAD);
2, fermented dough (20% wheat flour
with pool 1, 2 and proteases (SLP))

R5-ELISA
SLP < 10 ppm

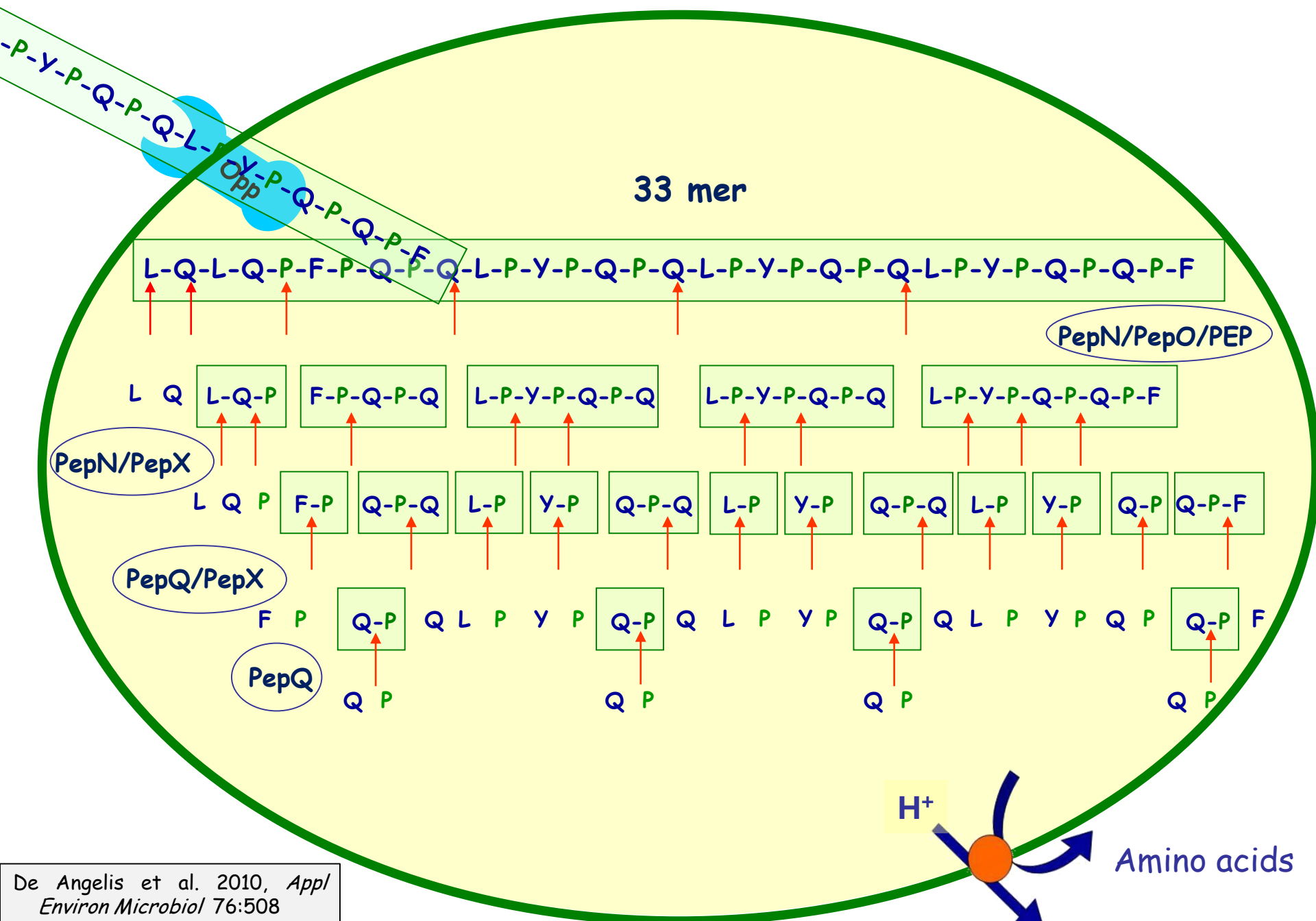
Organic nitrogen concentration of spray-dried flour

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2007, p. 4499-4507
0099-2240/07/\$08.00+0 doi:10.1128/AEM.00260-07
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Vol. 73, No. 14

Highly Efficient Gluten Degradation by Lactobacilli and Fungal Proteases during Food Processing: New Perspectives for Celiac Disease[▽]

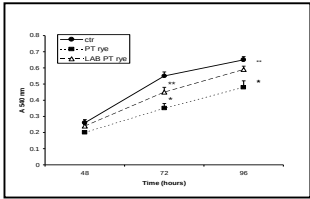
Carlo G. Rizzello,¹ Maria De Angelis,¹ Raffaella Di Cagno,¹ Alessandra Camarca,² Marco Silano,³
Ilario Losito,⁴ Massimo De Vincenzi,⁵ Maria D. De Bari,⁴ Francesco Palmisano,⁴
Francesco Maurano,² Carmen Gianfrani,² and Marco Gobbetti^{1*}



De Angelis et al. 2010, *Appl Environ Microbiol* 76:508

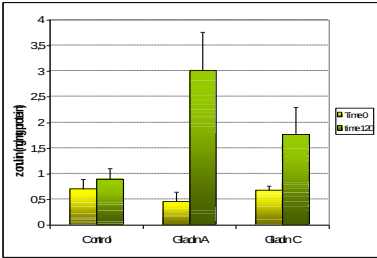


Viability of Caco-2/TC7 cells

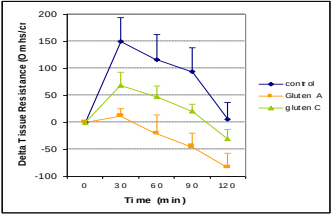


(De Angelis et al. 2006a)

Zonulin Release (IEC-6)

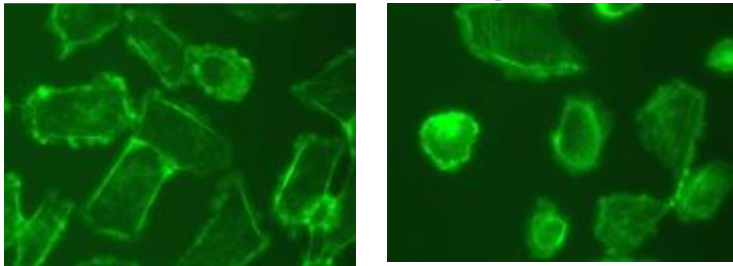


Tissue epithelial electrical resistance

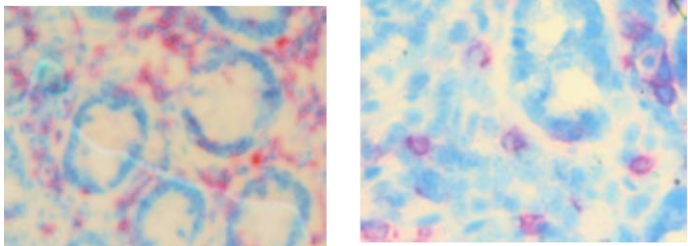
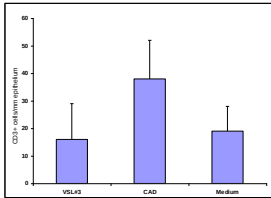


(De Angelis et al. 2006b)

Intracellular F-actin reorganization (IEC-6)

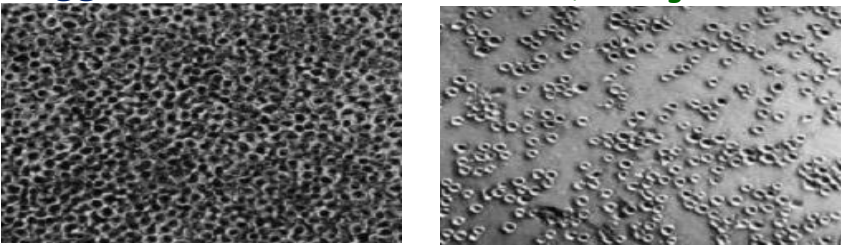


CD3+ intraepithelial lymphocytes infiltration



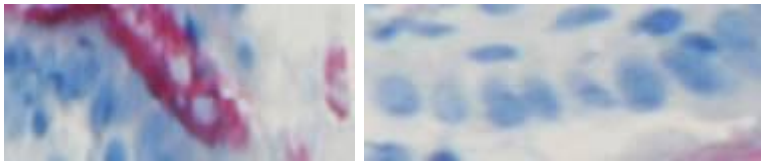
Agglutination test (K562)

(De Angelis et al. 2006b)



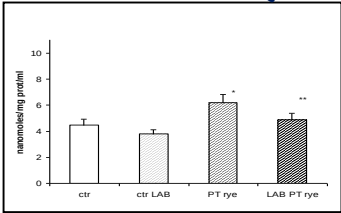
(Di Cagno et al., 2004; De Angelis et al. 2006a, 2006b)

Fas expression (biopsies)

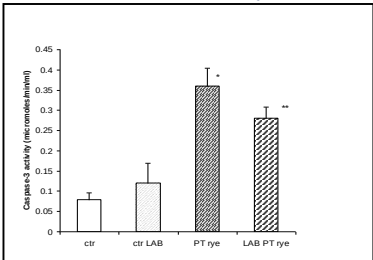


(De Angelis et al. 2006)

Synthesis of NO (Caco-2)



Caspase 3 activity (Caco-2)



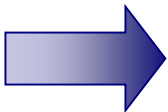
(De Angelis et al. 2006a)



Clinical Challenge 2: Pediatrics and European Laboratory for the Study of Food Induced Diseases University of Naples, Federico II, Italy



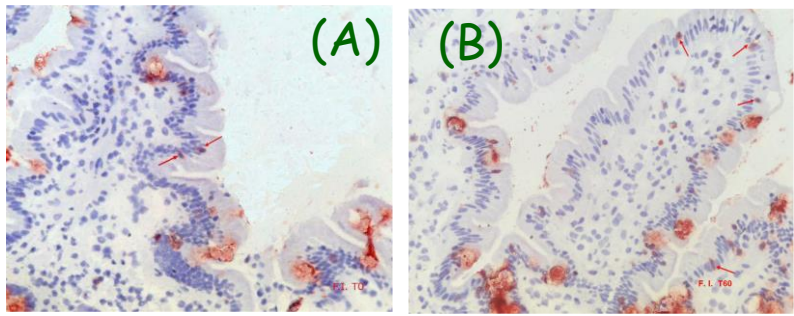
Biscuits (200 g) corresponding to 100 g of processed wheat flour which contained 8 g of hydrolyzed gluten



- Clinical challenge**
- Sixteen CD patients (age median 19, range 12-23 years),
 - Daily consumption of ca. 200 g of biscuits,
 - The challenge lasted 60 days

Greco et al. 2011, *Clin Gastroenterol Hepatol* 78:1087

Density of $\sigma\delta^+$ intraepithelial lymphocytes in jejunal biopsy from patient 4 F.I. at the beginning (panel A) and after 60 day of challenge (panel B)



	TIME	tTG	MARSH GRADE	CD3	$\gamma\delta$	CD25
F.I.	t ₀	1.6	T0	39	5.6	6
	t ₆₀	1.0	T0	38	8.6	5
I.C.	t ₀	1.9	T0	3.7	0.9	11
	t ₆₀	1.1	T0	11	3.8	9
R.R.	t ₀	0.3	T1	53	11.5	3
	t ₆₀	0.3	T1	56	17.8	4
I.I.	t ₀	0.5	T0	31	8.4	21
	t ₆₀	0.3	T0	36	12.8	21
C.C.	t ₀	0.4	T0	32	21	3
	t ₆₀	0.7	T0	47	18	7

tTG, anti-tissue transglutaminase antibodies; T0, normal duodenal mucosa; CD3, T1, architecturally normal duodenal mucosa with increased intraepithelial lymphocytes infiltration; and CD3, $\gamma\delta$ CD25 are markers for intraepithelial lymphocytes in duodenal mucosa



Water, **wheat flour**,
fungal proteases,
selected sourdough
lactic acid bacteria



- Long-time
- Semi-liquid sourdough fermentation

1

Addition of gluten-free ingredients (rice, mais, starch, gums)

(short-time baker's yeast fermentation)

Wheat gluten-free baked goods



2

Spray dryer



Wheat gluten-free flour

(short-time baker's yeast fermentation)

(structuring agents)



Patent number(s) WO2014033765-A1,
US2014065262-A1. Giuliani et al. 2014



**Yes,
you
can**

revolutionises
the gluten-free world

Bontà di Pane
the first bread made
of deglutenised wheat



FROM TRADITION TO INNOVATION

- The first gluten-free wheat bread
- Years of study of the natural rising process have permitted Giuliani to select patented enzymes and lactic cultures that eliminate gluten from wheat flour
- These patented enzymes and lactic cultures combined with the traditional bread-making method featuring a slow rising process permit creation of the first bread with deglutenised wheat flour

UNIQUE BREAD

- With all the properties of traditional bread
- Light and tasty
- All the flavor and fragrance of real bread
- All the nutritional properties of wheat, with complete fibre, vitamin, mineral and amino acid content
- Long-lasting
- Perfect for coeliacs and for anyone who is sensitive to gluten, has difficulty digesting it or stands to benefit from a gluten-free diet

OFFICIALLY REPORTED AND TESTED

- Reported to the Italian Health Ministry
- Guaranteed gluten-free with testing of every lot of deglutenised wheat flour and every lot of finished product
- Clinical tests have demonstrated perfect tolerance among coeliacs

**Yes,
you
can**

revolutionises
the gluten-free world

Bontà di Pane
the first bread made
of deglutenised wheat



PRODUCT REPORTED TO
THE HEALTH MINISTRY.
GUARANTEED GLUTEN-FREE



"The advantages of the bread made with hydrolyzed gluten"

✓ Better flavour



"The advantages of the bread made with hydrolyzed gluten"

✓Extended shelf life



"The advantages of the bread made with hydrolyzed gluten"



Wheat gluten-free bread

✓ Better technology features

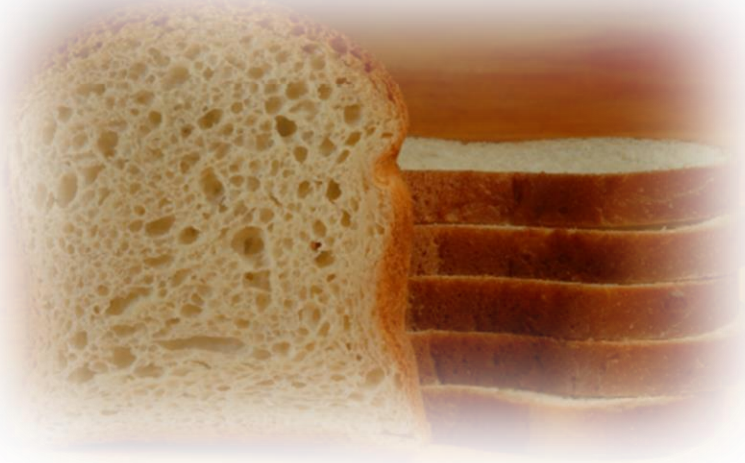


Naturally gluten-free bread



"The advantages of the bread made with hydrolyzed gluten"

✓ Better nutritional profile



INFORMAZIONI NUTRIZIONALI - Valori medi per 100 g NUTRITIONAL INFORMATION - Values calculated on 100 g	
ENERGIA ENERGY	KJ/kcal 1477/353
GRASSI FATS	1,3 g
Di cui acidi grassi saturi Of which saturated	0,3 g
Di cui colesterolo Of which cholesterin	< 0,1 mg
CARBOIDRATI CARBOHYDRATES	77,5 g
Di cui zuccheri Of which sugar	0,3 g
FIBRE ALIMENTARI DIETARY FIBRES	1,8 g
PROTEINE PROTEINS	6,9 g
SALE SALT	0,02 g



Carbohydrates

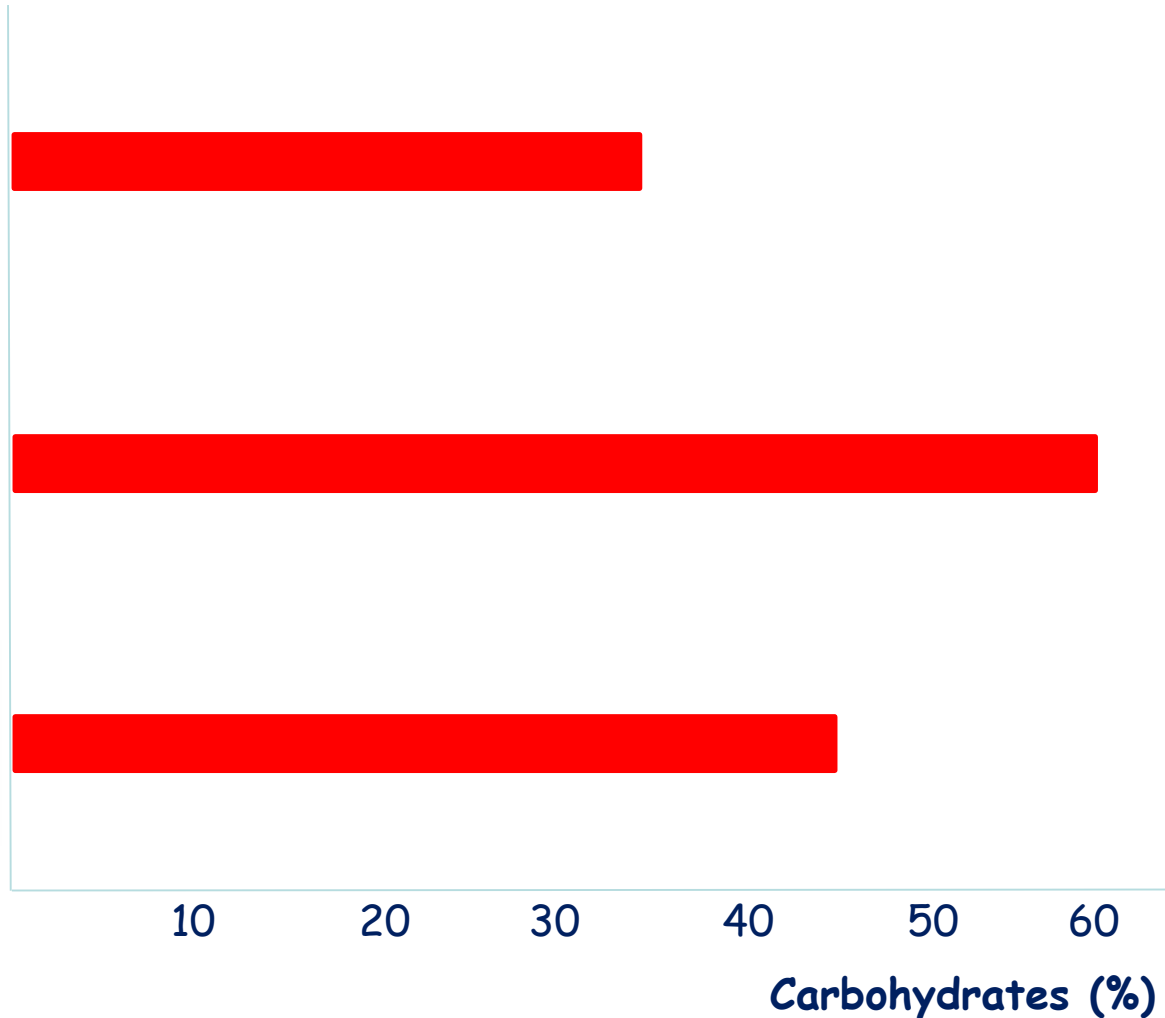
Wheat
gluten-free bread



Naturally
gluten-free bread



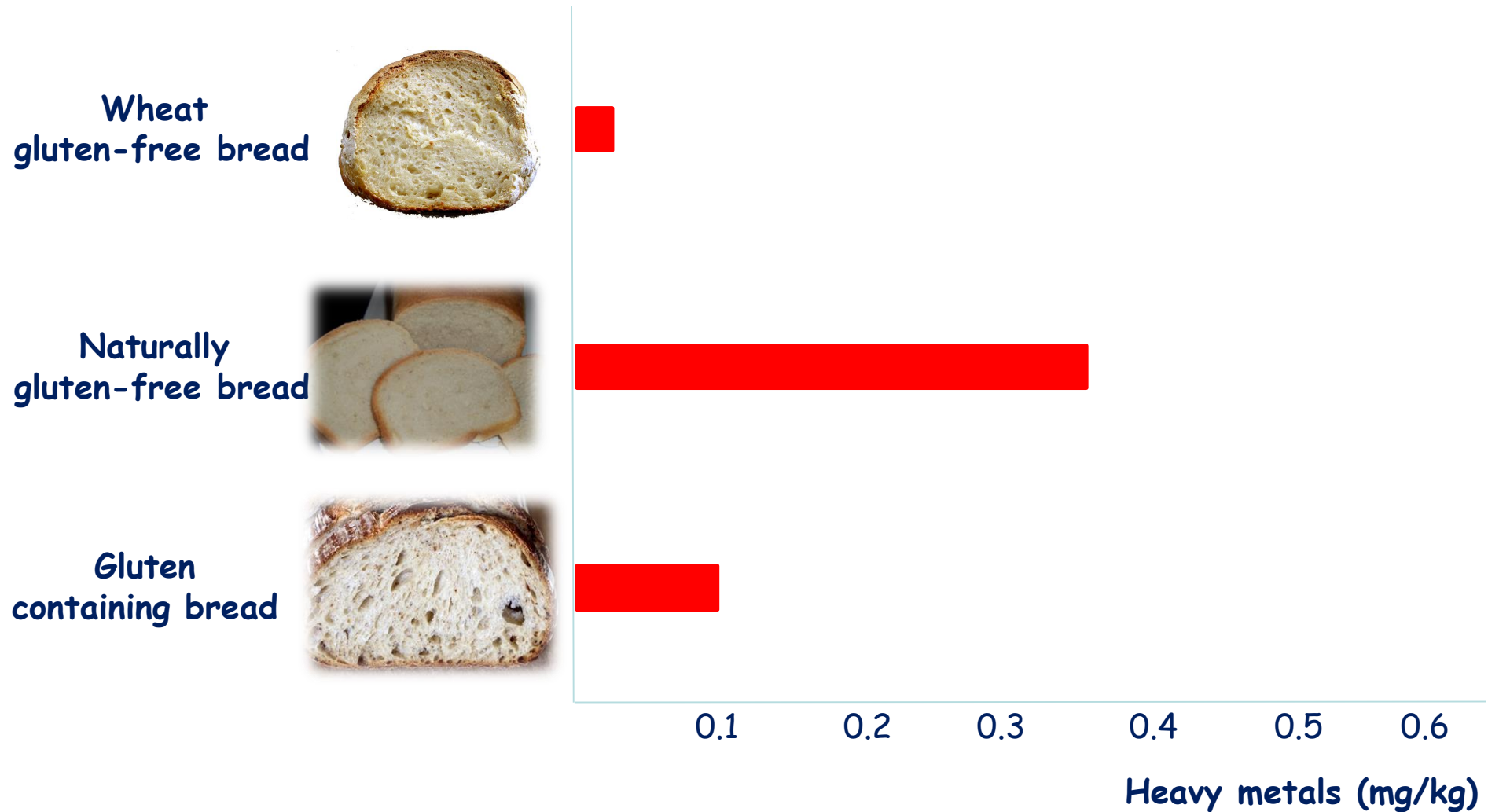
Gluten
containing bread



Rizzello et al., 2016. *J Food Sci*, doi: 10.1111/1750-3841.13410



Heavy metals



Rizzello et al., 2016. *J Food Sci*, doi: 10.1111/1750-3841.13410



Mycotoxins

	Wheat gluten-free bread	Gluten containing bread	Naturally gluten-free bread
Content ($\mu\text{g}/\text{kg}$)			
Aflatoxins	n.d.	n.d.	n.d.
T2	n.d.	n.d.	n.d.
HT2	n.d.	n.d.	n.d.
Ochratoxin	n.d.	0.4 ± 0.2	n.d.
Fumonisin	n.d.	n.d.	19.2 ± 0.8
Zearalenone	n.d.	n.d.	45.0 ± 1.1
Deoxynivalenol	n.d.	46.3 ± 0.8	254.5 ± 1.2

Rizzello et al., 2016. *J Food Sci*, doi: 10.1111/1750-3841.13410



NCGS and IBS

Non-celiac gluten sensitivity (NCGS)

People with celiac disease

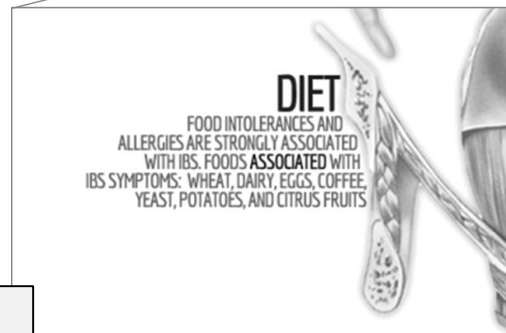
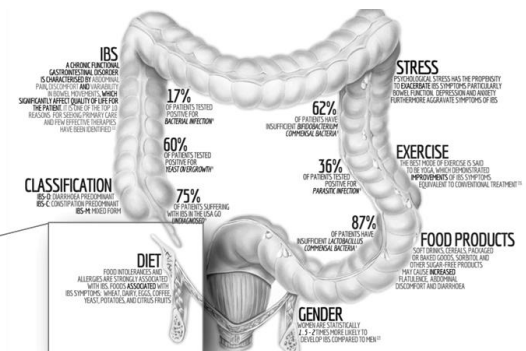
People who will develop celiac disease

People with non-celiac gluten sensitivity



10% of the world's population

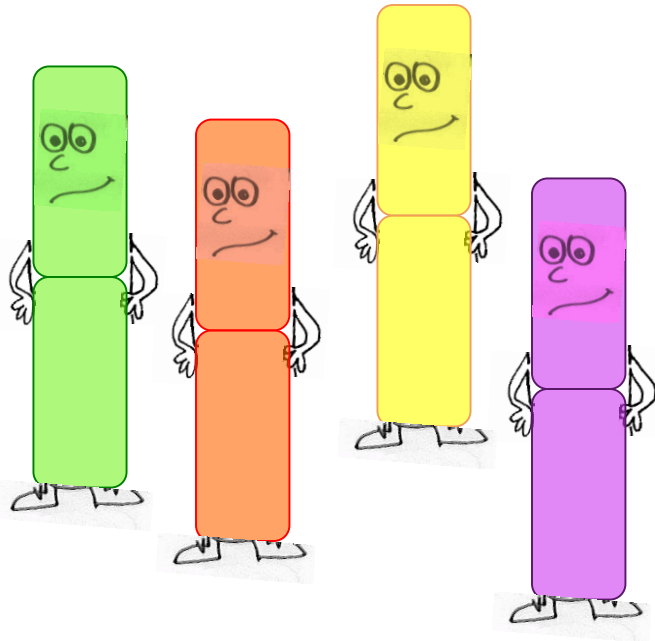
Irritable bowel syndrome (IBS)



Chang, 2014, *World J Gastroenterol.* 20:2499-2514
Catassi et al. 2013, *Nutrients.* 5:3839-3853



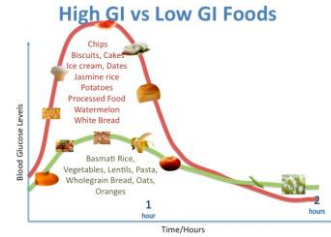
Interim prospects



Taking care of our «friends»



- ✓ Low glycemic index
- ✓ High content of fibres
- ✓ Enriched of high value of by-products (e.g., wheat germ)



- ✓ Naturally preserved
- ✓ Null-, low-, intermediate-gluten content

