



Making Wheat Technically Gluten Free: New Research in Sourdough Methods

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“A mixture of flour (wheat, rye, rice, etc...) and water, fermented by lactic acid bacteria and yeasts, which are responsible for its capacity to leaven a dough, while contemporarily and unavoidably acidifying it”

(Gobbetti, 1998. *Trends Food Sci. Technol.*)

WEB OF SCIENCE™

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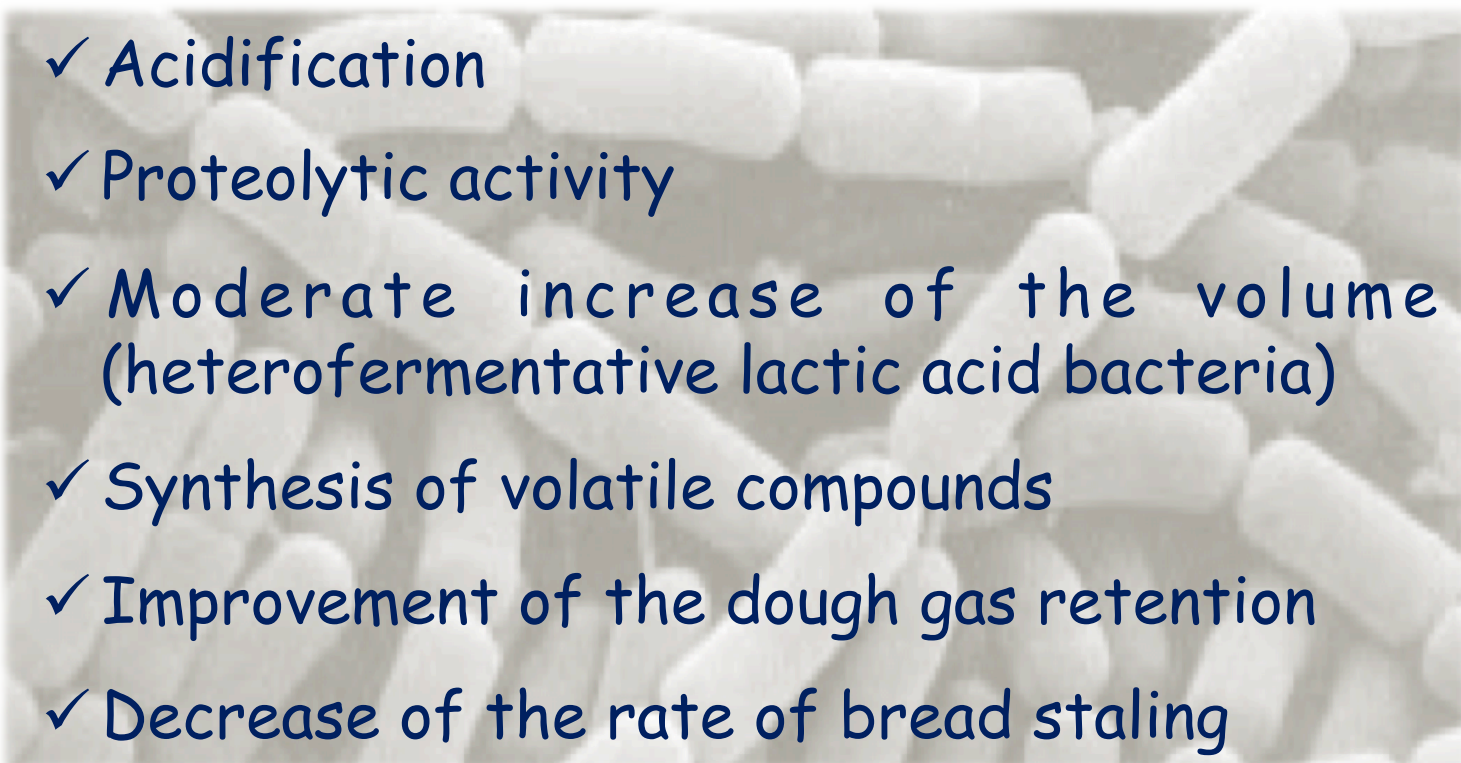
TIMESPAN

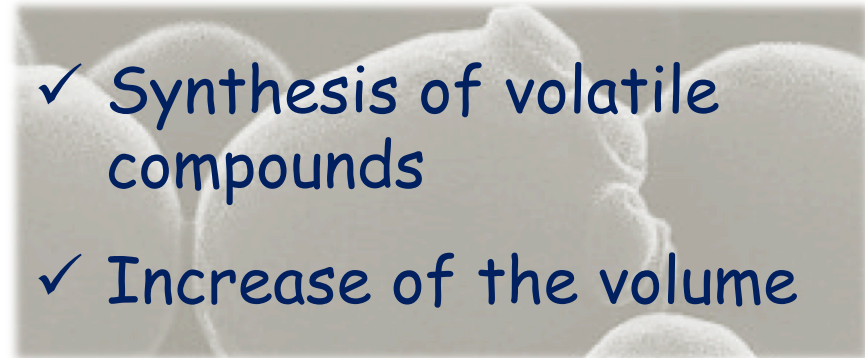
All years

From 2000 to 2014



How sourdough lactic acid bacteria and yeasts contribute to the organoleptic features of baked goods

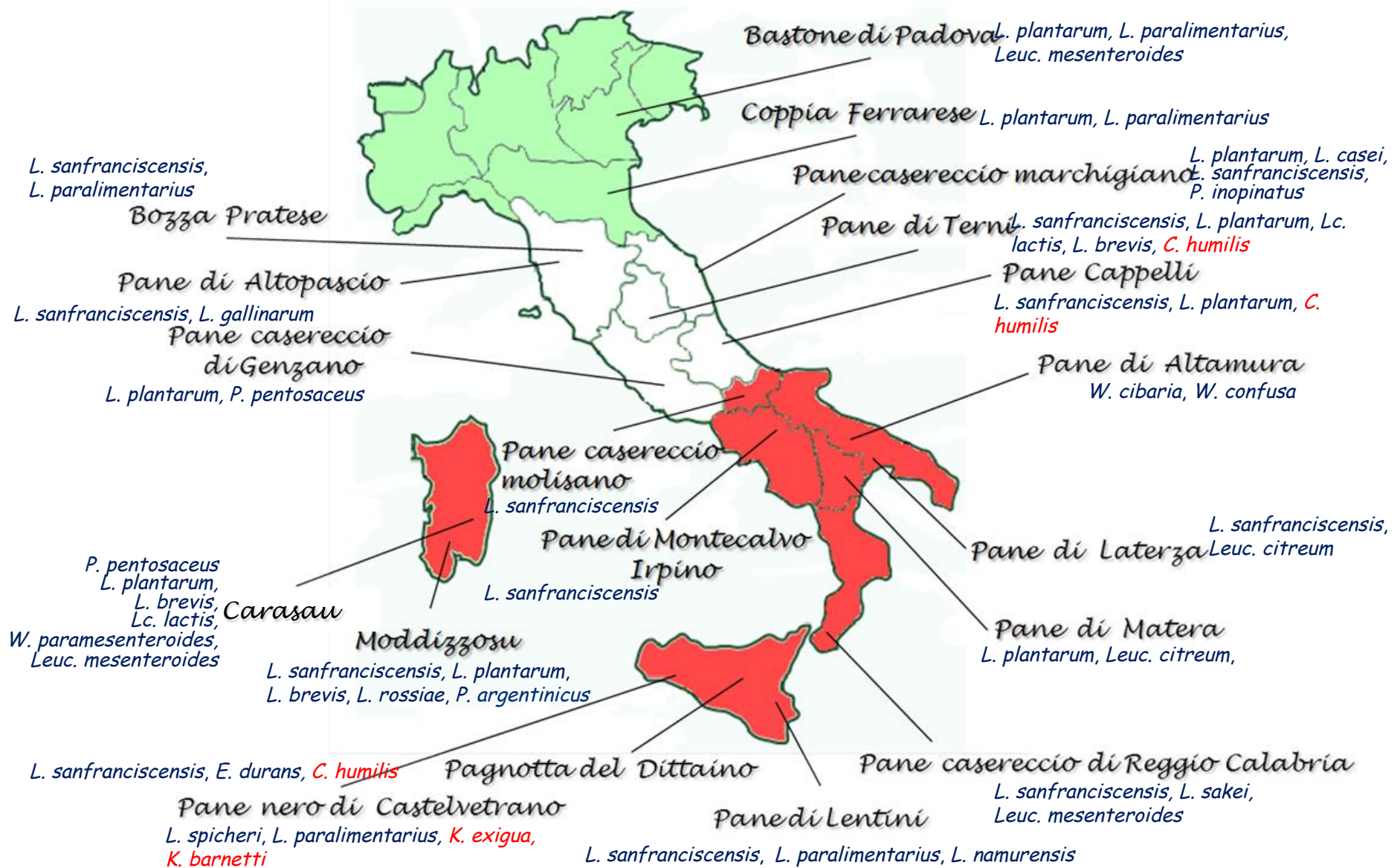
- 
- ✓ Acidification
 - ✓ Proteolytic activity
 - ✓ Moderate increase of the volume (heterofermentative lactic acid bacteria)
 - ✓ Synthesis of volatile compounds
 - ✓ Improvement of the dough gas retention
 - ✓ Decrease of the rate of bread staling

- 
- ✓ Synthesis of volatile compounds
 - ✓ Increase of the volume



Biodiversity of the Italian sourdoughs

Minervini *et al.*, 2012.
Applied Environ Microb, 78:1251-1264



The first sourdough "library" (Saint-Vith, Belgium) in the world



Future therapeutic approaches for celiac disease treatment

(Caputo et al., 2010. *Enzyme Research* doi10.4061/2010/174354; Gujral et al., 2012. *World J Gastroenterol.* 18: 6036-6059)

Therapeutic agent

- Glutenenases and endoprotease
- Prolyl endopeptidase
- **Sourdough lactobacilli**
- Lyophilised bacteria (VSL#3)

- Hexapeptide derived from zonula occludens toxin of *Vibrio cholera*
- Synthetic polymer poly
- Anti-gliadin IgY

- Dihydroisoxazoles
- Cinnamoyltriazole
- Aryl β -aminoethyl ketones

- Three deamidated peptides derived from Wheat α -, ω -gliadin, β -hordein
- Human hookworm inoculation

- HLA-DQ2 blocker
- Interleukin blocker
- R-spondin-1

Mechanism

- Hydrolysis of toxic gliadin

- Prevention of gliadin absorbtion Larazotide

- tTG2 inhibitor

- Peptide vaccination

- Modulate immune response

- Restore intestinal architecture



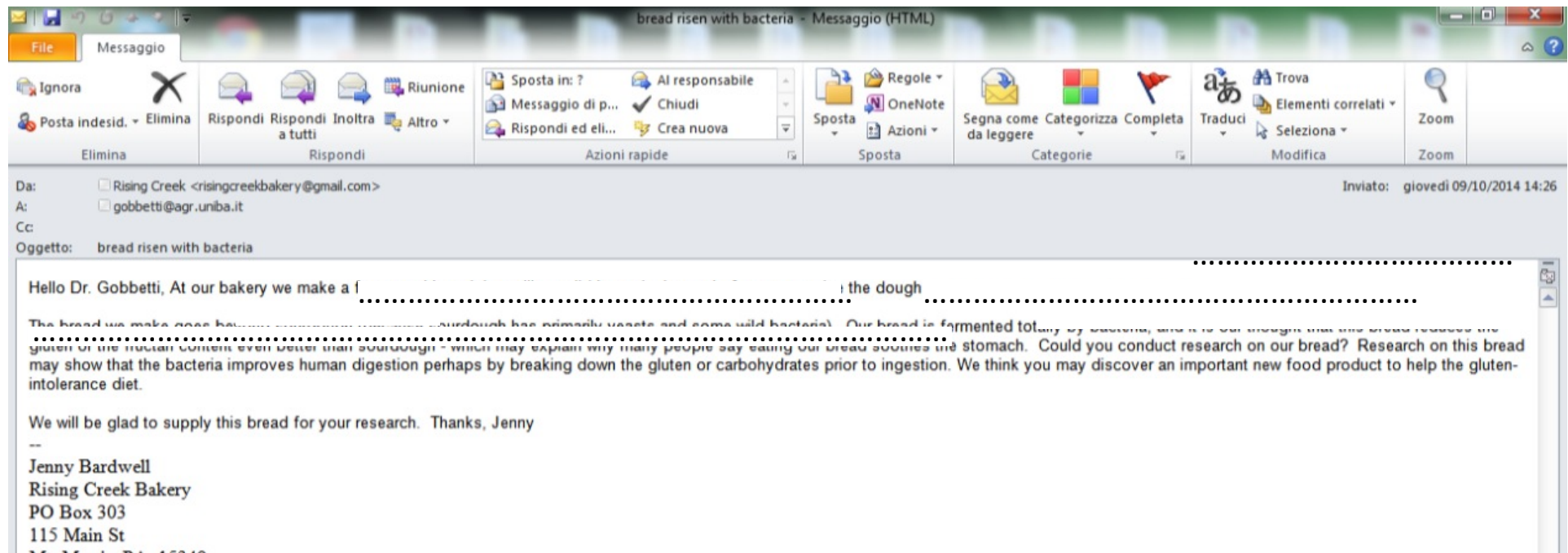
Sourdough Bread Made from Wheat and Nontoxic Flours and Started with Selected Lactobacilli Is Tolerated in Celiac Sprue Patients

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*}

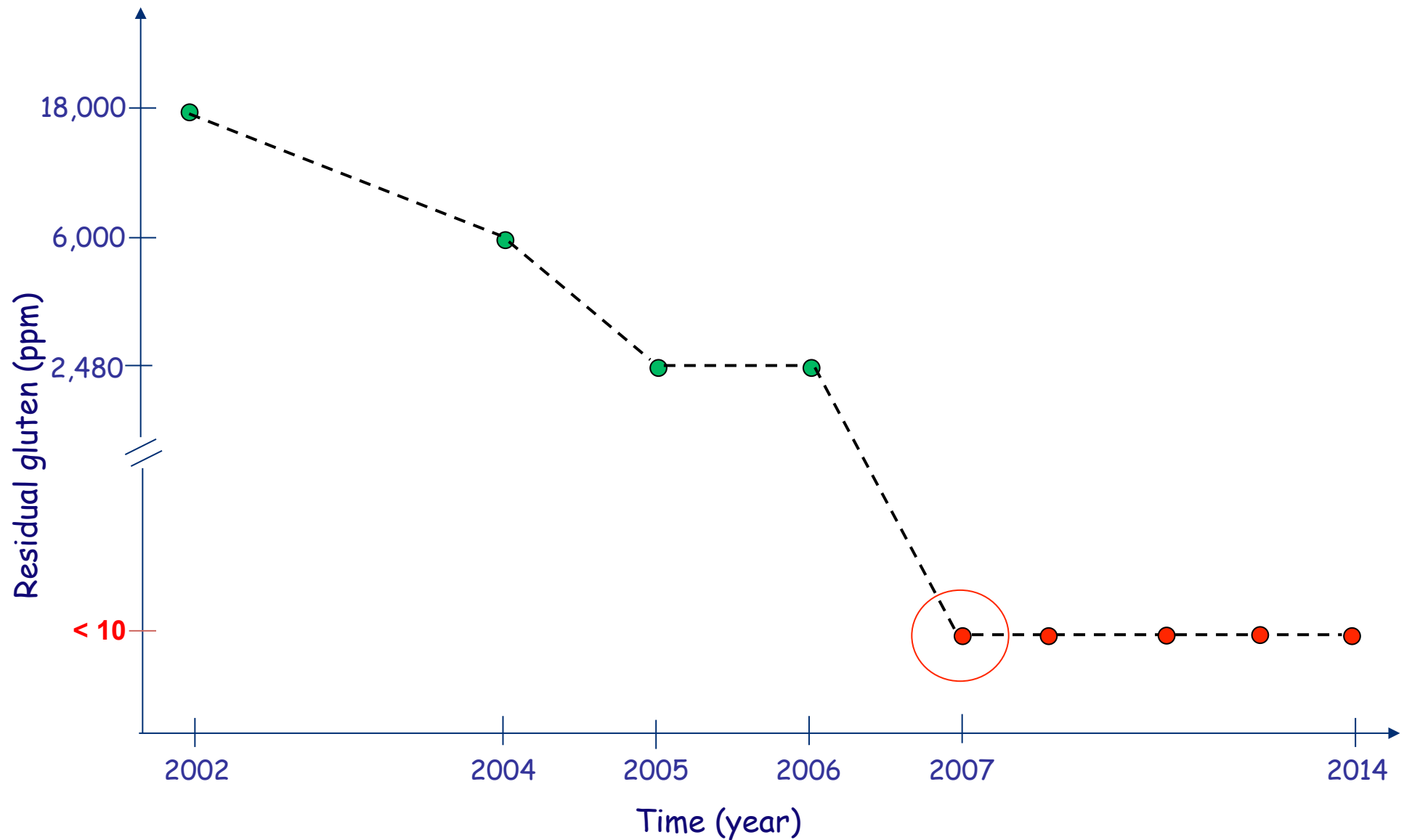
ACKNOWLEDGMENTS

This work was supported by the Italian Ministry of University and Scientific and Technological Research (Murst), Development of Research Networks no. 488/92, cluster C06 + 07, project 6-2.2.

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.



The "struggle" of our research team against the gluten



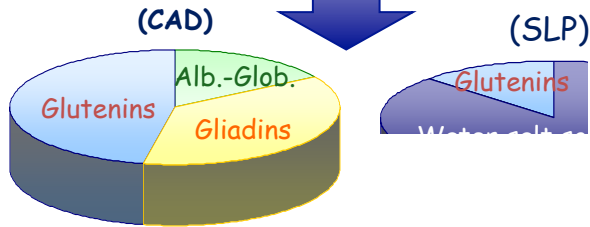
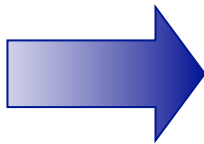
Pool 1 (ca. log 9/ml)
Lb. brevis 14G
Lb. alimentarius 15M
Lb. hilgardii 51B
Lb. sanfranciscensis 7A

+

Pool 2 (ca. log 8/ml)
Lb. sanfranciscensis E14
Lb. sanfranciscensis E21
Lb. sanfranciscensis 174
Lb. sanfranciscensis 13
Lb. sanfranciscensis A1
Lb. sanfranciscensis 274

+

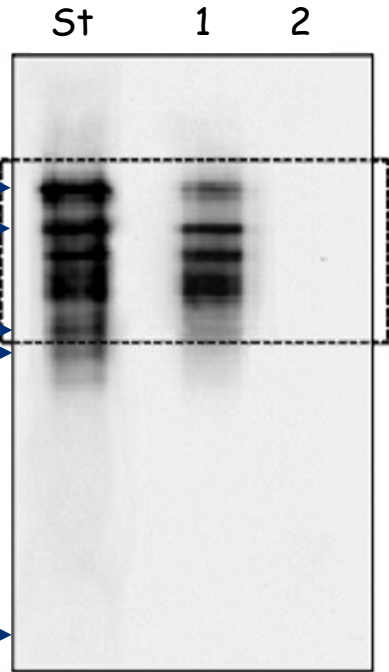
Fungal proteases (200 ppm)



ω -gliadins

α, β, ω -gliadins

hydrolyzed gliadins



St, 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
 0099-2240/07/0075-4499-09 doi:10.1128/AEM.02266-07
 Copyright © 2007, American Society for Microbiology. All Rights Reserved. Vol. 73, No. 14

Highly Efficient Gluten Degradation by Lactobacilli and Fungal Proteases during Food Processing: New Perspectives for Celiac Disease¹

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

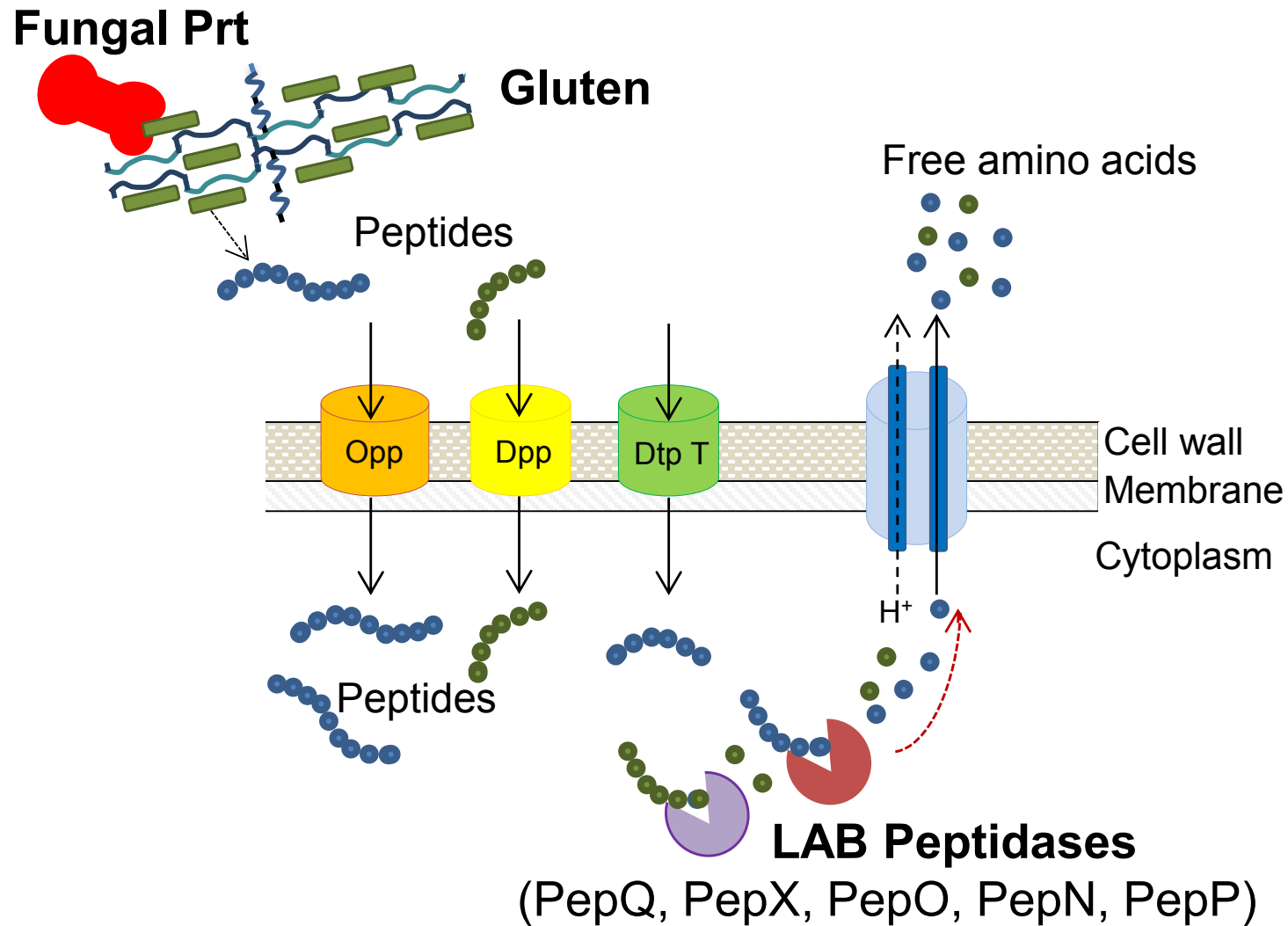
¹Department of Plant Protection and Applied Microbiology, University of Bari, 70126 Bari, Italy²; ³Istituto di Scienze dell'Alimentazione—CNR, 83100 Avellino, Italy⁴; ⁵Division of Food Science, Human Nutrition and Health, Istituto Superiore di Sanità, I-00161 Rome, Italy⁶; and ⁶Dipartimento di Clinica, Centro Interdipartimentale di Ricerca SMART, University of Bari, 70126 Bari, Italy⁶

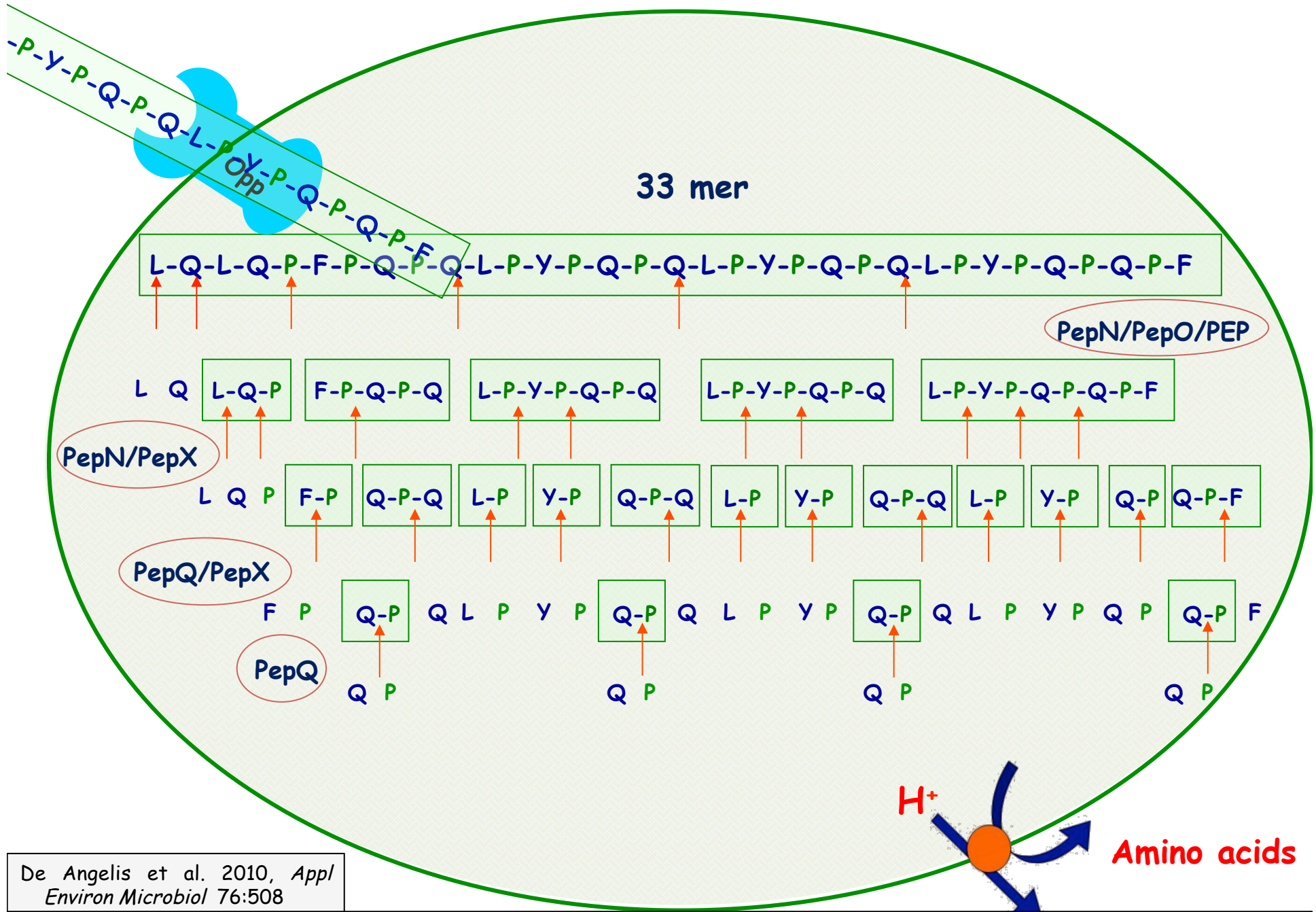
Received 1 February 2007/Accepted 15 May 2007

Presently, the only effective treatment for celiac disease is a life-long gluten-free diet. In this work, we used a new mixture of selected sourdough lactobacilli and fungal proteases to eliminate the toxicity of wheat flour during long-time fermentation. Immunological (R5 antibody-based sandwich and competitive enzyme-linked immunosorbent assay [ELISA]) and R5 antibody-based Western blot, two-dimensional electrophoresis, and mass spectrometry/capillary liquid chromatography-electrospray ionization/strong-cationic-exchange liquid chromatography/capillary liquid chromatography-electrospray ionization/quadrupole-time-of-flight mass spectrometry analyses were used to determine the gliadin concentration. Assays based on the production of peripheral blood mononuclear cells (PBMCs) and gluten intestinal production by PBMCs and intestinal T-cell lines (iTCILs) from 12 celiac disease patients were used to determine the protein toxicity of the peptides again digested from fermented wheat dough (sourdough). As determined by R5-based sandwich and competitive ELISAs, the residual concentration of gliadin in sourdough was 12 ppm. Albumin, globulin, and gliadins were completely hydrolyzed, while ca. 20% of glutenins persisted. Low-molecular-weight epitopes were not detectable by SCX-1FCapLC-ESI-Q-ToF.



Mechanisms of gluten proteolysis





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



Other cereals

Durum wheat cultivars

Ciccio



Colosseo



Duilio



Simeto



Svevo



**Gluten
< 10 ppm**

Other species

Barley



Rye



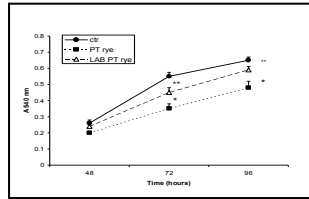
Oat



**Gluten
> 10 ppm**

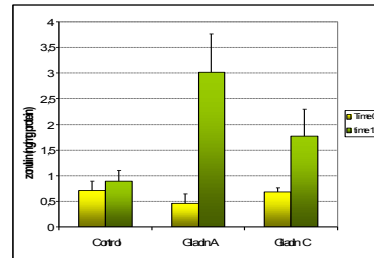


Viability of Caco-2/TC7 cells

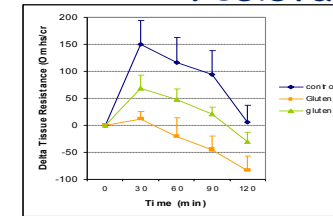


(De Angelis et al. 2006a)

Zonulin Release (IEC-6)

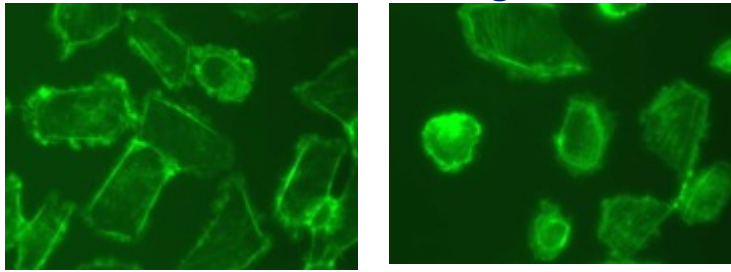


Tissue epithelial electrical resistance

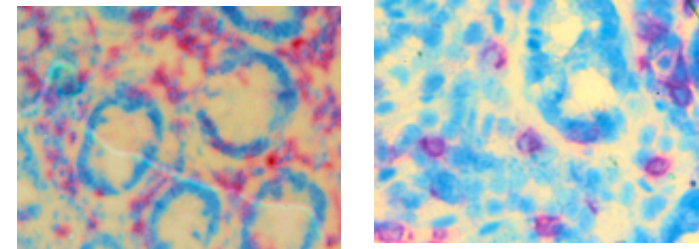
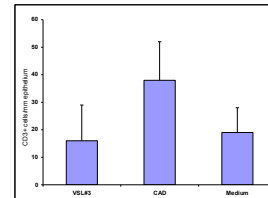


(De Angelis et al. 2006b)

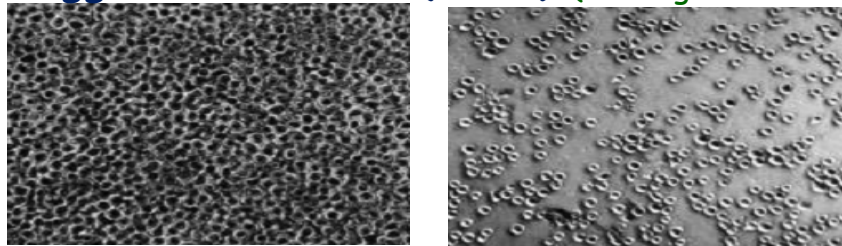
Intracellular F-actin reorganization (IEC-6)



CD3+ intraepithelial lymphocytes infiltration

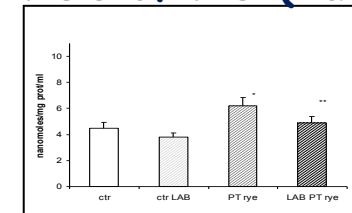


Agglutination test (K562) (De Angelis et al. 2006b)

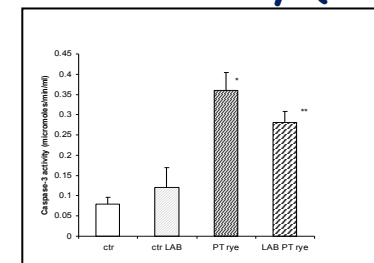


(Di Cagno et al., 2004; De Angelis et al. 2006a, 2006b)

Synthesis of NO (Caco-2)



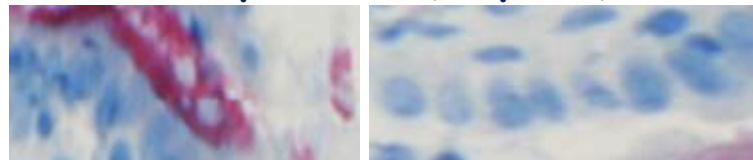
Caspase 3 activity (Caco-2)



(De Angelis et al. 2006)

(De Angelis et al. 2006a)

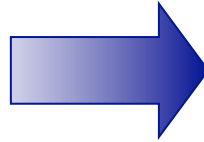
Fas expression (biopsies)



Clinical Challenge 1: Department of Pediatric, Sapienza - University of Roma



Sweet baked goods (200 g) corresponding to 100 g of processed wheat flour which contained 10 g of hydrolyzed gluten



Clinical challenge

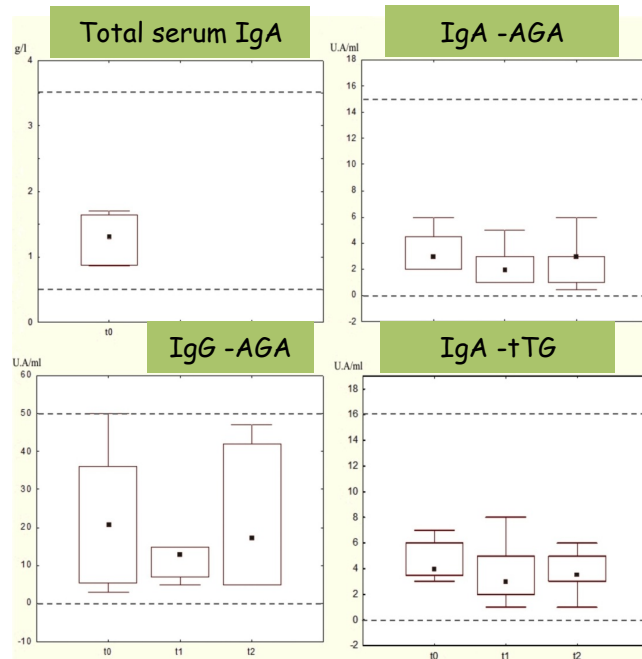
- Eight CD patients (age median 13, range 8-17 years),
- Daily consumption of ca. 200 g of Sweet baked goods,
- The challenge lasted 60 days

ORIGINAL ARTICLE: HEPATOLOGY AND NUTRITION

Gluten-free Sourdough Wheat Baked Goods Appear Safe for Young Celiac Patients: A Pilot Study

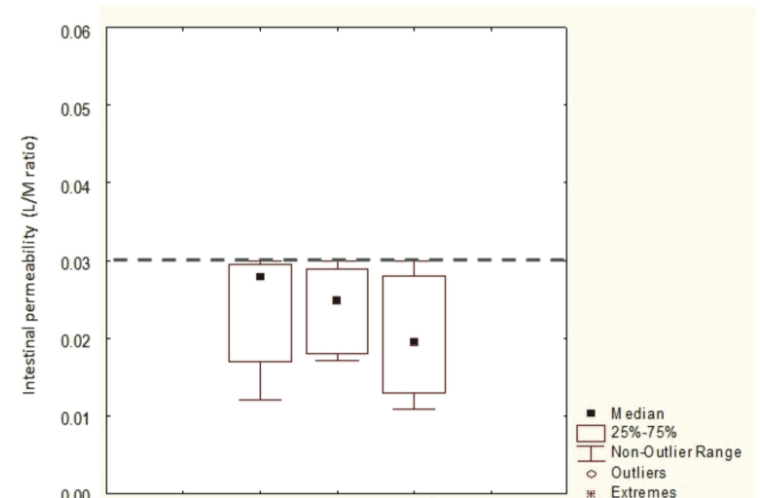
**Raffaella Di Cagno, †Maria Barbato, †Chiara Di Camillo, *Carlo Giuseppe Rizzello, *Maria De Angelis, †Giammaria Giuliani, §Massimo De Vincenzi.*

(A)



Serology analyses (A) and intestinal permeability (B) at 0 (t0), 30 (t1), and 60 (t2) days of the clinical challenge

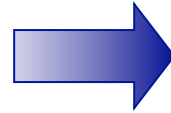
(B)



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

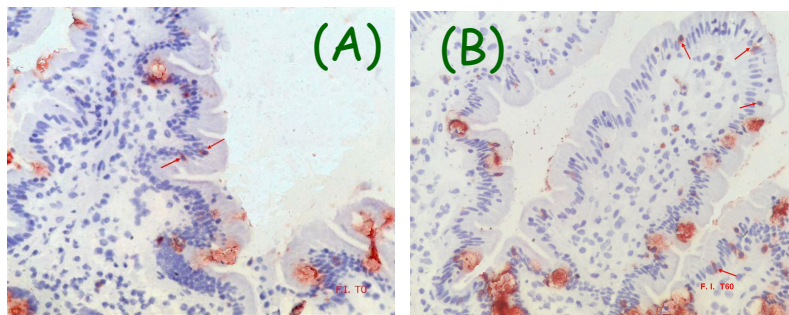


Clinical challenge

- 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)

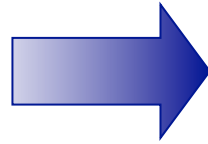


	TIME	tTG	MARSH GRADE	CD3	$\gamma\delta$	CD25
F.I.	t ₀	1.6	T0	39	5.6	6
	t ₆₀	1.0	T0	38	8.6	5
I.C.	t ₀	1.9	T0	3.7	0.9	11
	t ₆₀	1.1	T0	11	3.8	9
R.R.	t ₀	0.3	T1	53	11.5	3
	t ₆₀	0.3	T1	56	17.8	4
I.I.	t ₀	0.5	T0	31	8.4	21
	t ₆₀	0.3	T0	36	12.8	21
C.C.	t ₀	0.4	T0	32	21	3
	t ₆₀	0.7	T0	47	18	7

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

Clinical Challenge 3: Pediatrics and European Laboratory for the Study of Food Induced Diseases University of Naples, Federico II, Italy

Hydrolized wheat flour



CLINICAL CHALLENGE IN PROGRESS
10 CD patients (range 12-23 years)



- Serology analyses
- Morphometric analysis
- Immunohistochemistry

Time: $t_0 - t_1 - t_2 - t_3 - t_4 - t_5 - t_6$ MONTHS

CLINICAL CHALLENGE IN PROGRESS
30 CD patients (range 12-23 years)



Gene Expression Profile of
Peripheral Blood Monocytes



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

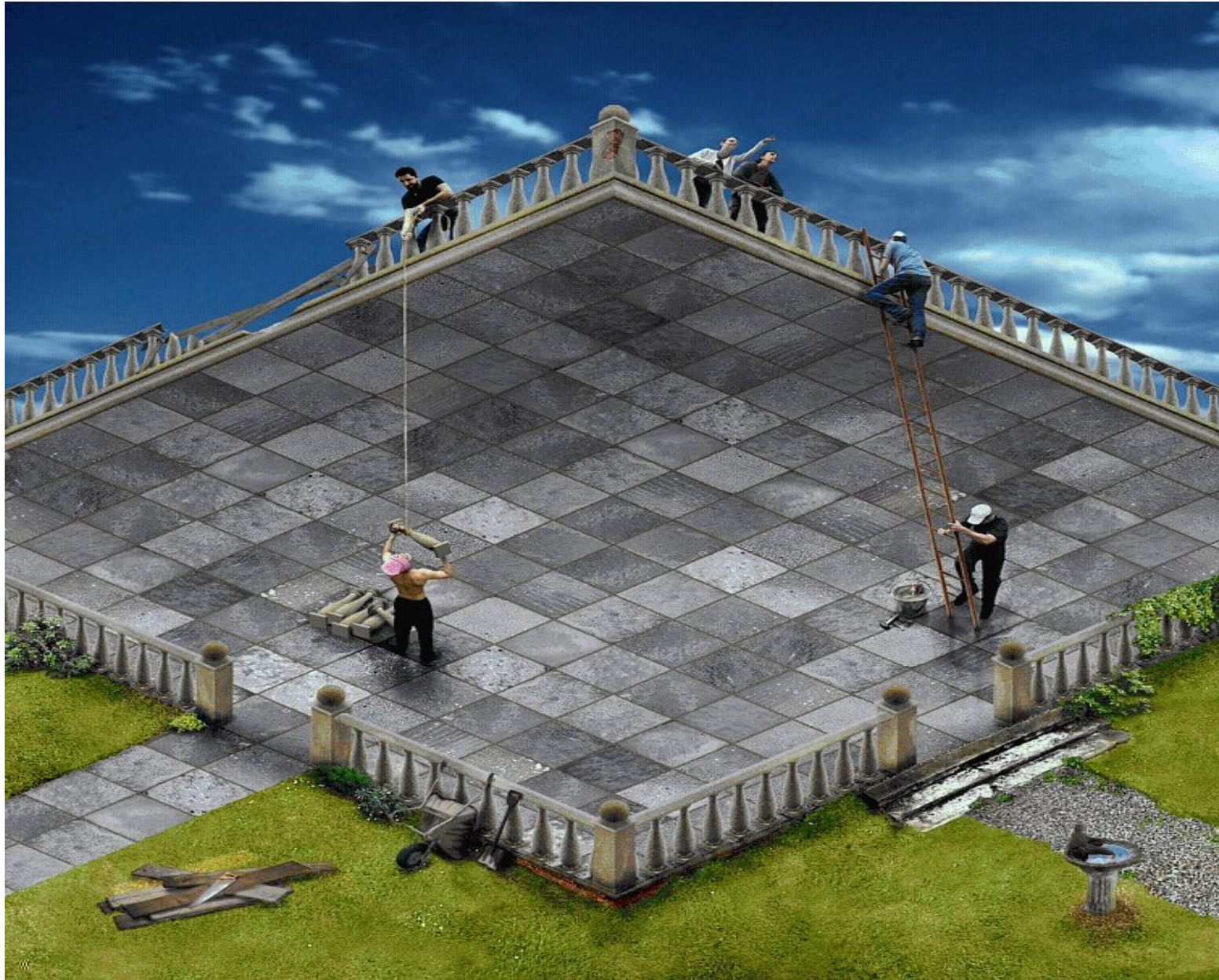
(short-time baker's yeast fermentation)



(structuring agents)

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





"Illusion" Escher, 1974



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

Giusto
SENZA GLUTINE

Sapori Tradizionali

Tutta la bontà della farina di frumento senza glutine

NOVITÀ
ESCLUSIVA

Bontà di Pane



DALLA TRADIZIONE ALL'INNOVAZIONE

Un pane unico, rivoluzionario! Per la prima volta un pane garantito senza glutine ottenuto dalla farina di frumento in modo da garantire tutte le qualità del pane tradizionale: buono, soffice, dall'inconfondibile profumo di pane e dalla tipica crosta dorata, con le caratteristiche nutrizionali e organolettiche proprie del frumento.

Grazie ad anni di studio sulla lievitazione naturale (lievito madre), all'utilizzo del metodo di produzione del pane tradizionale, alla combinazione di una selezione ottimale dei fermenti lattici e ad un prolungato tempo di "riposo", è stato possibile eliminare il glutine dalla farina.

Giusto Sapori Tradizionali "Bontà di Pane" con farina di frumento senza glutine: il piacere ritrovato del buon pane per tutti.

PANE A LIEVITAZIONE NATURALE SENZA GLUTINE

Ingredienti: lievito naturale di frumento senza glutine 50% (acqua, semola di grano duro, correttore di acidità: carbonato acido di sodio; lattobacilli), amido di frumento deglutinato, farina di miglio, olio di oliva 4,6%, lievito, proteine vegetali, addensante: amido modificato di mais; glucosio, emulsionanti: mono- e digliceridi degli acidi grassi; albume d'uovo, addensanti: gomma di xanthan; umidificanti: glicerolo; sale, enzimi: α -amilasi. Prodotto in uno stabilimento che tratta proteine del latte, soia.

- Prodotto dietetico destinato a soggetti intolleranti al glutine
- Prodotto realizzato con ingredienti naturalmente privi o specificamente lavorati per ridurre il contenuto di glutine
- Conservare in luogo fresco e asciutto
- Prodotto confezionato in atmosfera protettiva

VALORI NUTRIZIONALI MEDI PER 100 g:

Energia: 929 kJ/223 kcal; Grassi: 7,1 g, di cui saturi 2,1 g; Carboidrati: 33,1 g, di cui zuccheri 2,4 g; Fibre 1,3 g; Proteine: 6,0 g; Sale: 1,4 g.

SENZA LATTOSIO

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400 g e

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Lotto / Da consumatori preferiti. Fonte: entro fine



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

The advantages

Wheat gluten-free bread



Versus

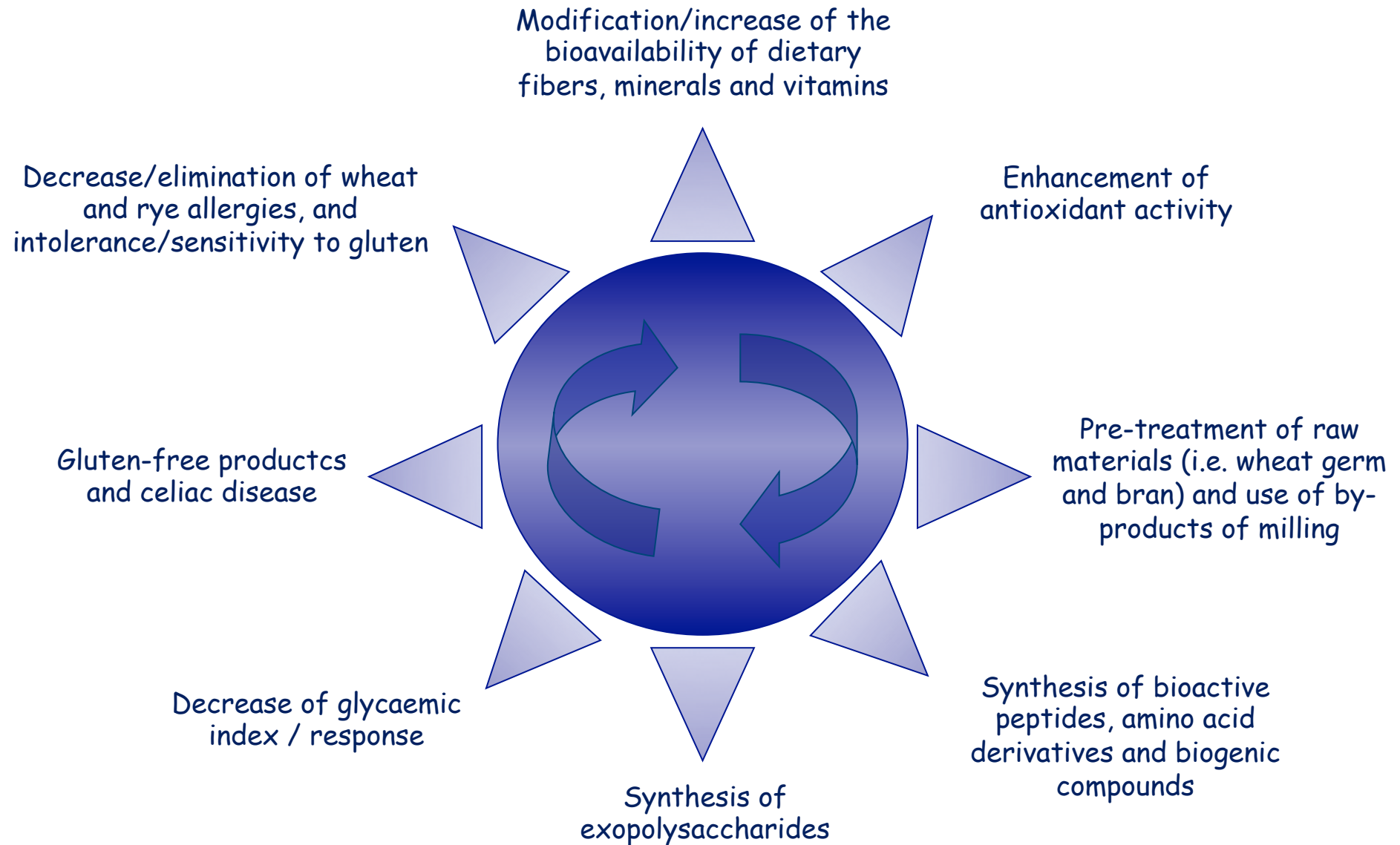
Naturally gluten-free bread



- Better sensory properties
- Better rheology properties and extended shelf life
- Higher mineral, vitamin, amino acid and fiber bioavailability
- Lower costs
- Improved social life



Sourdough lactic acid bacteria and functional features

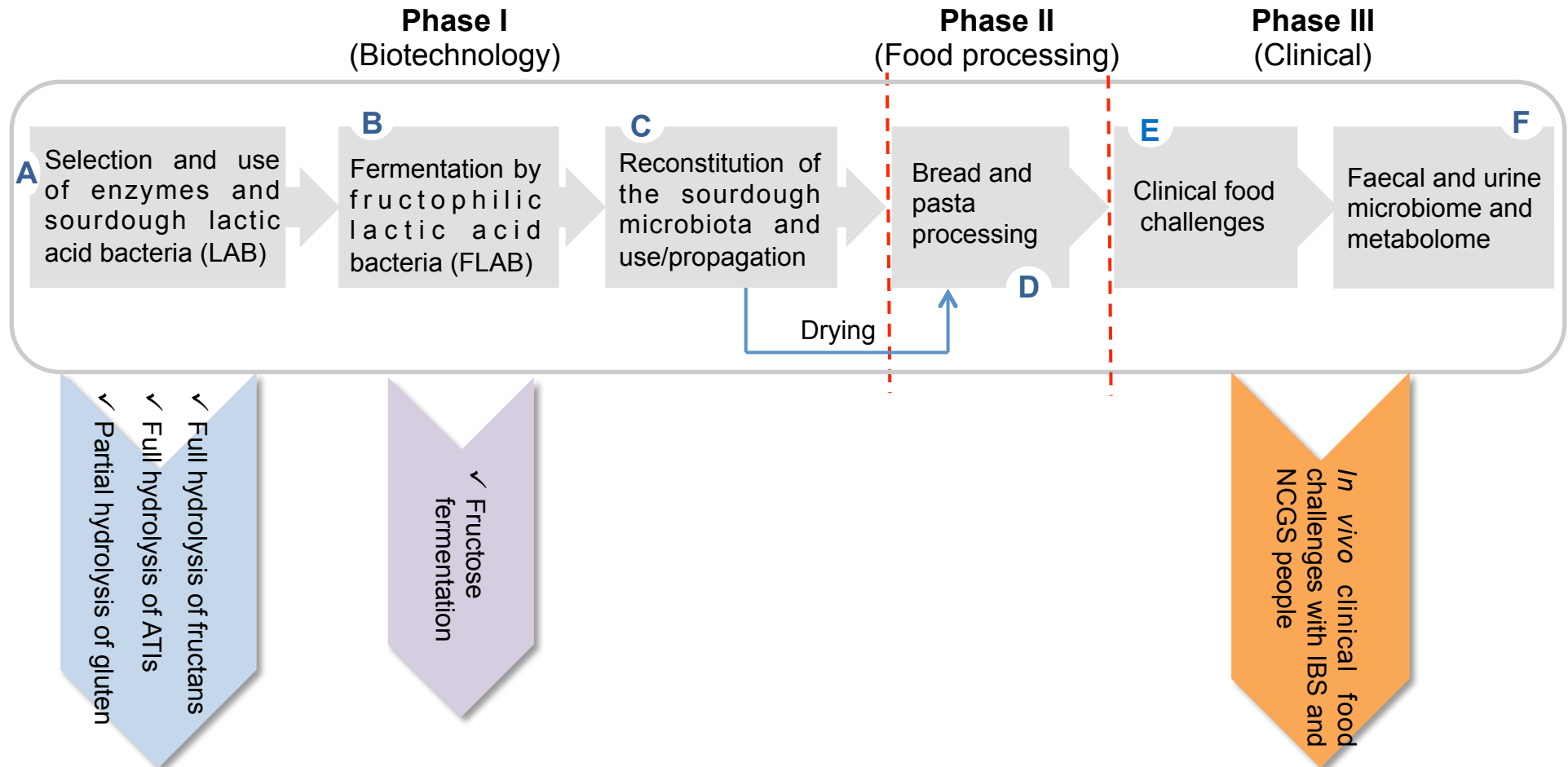


(Katina et al., *Trends Food Sci Technol.* 2005; Katina et al., *Food Microbiol.* 2007; Gobbetti et al. *Food Microbiol.* 2014)

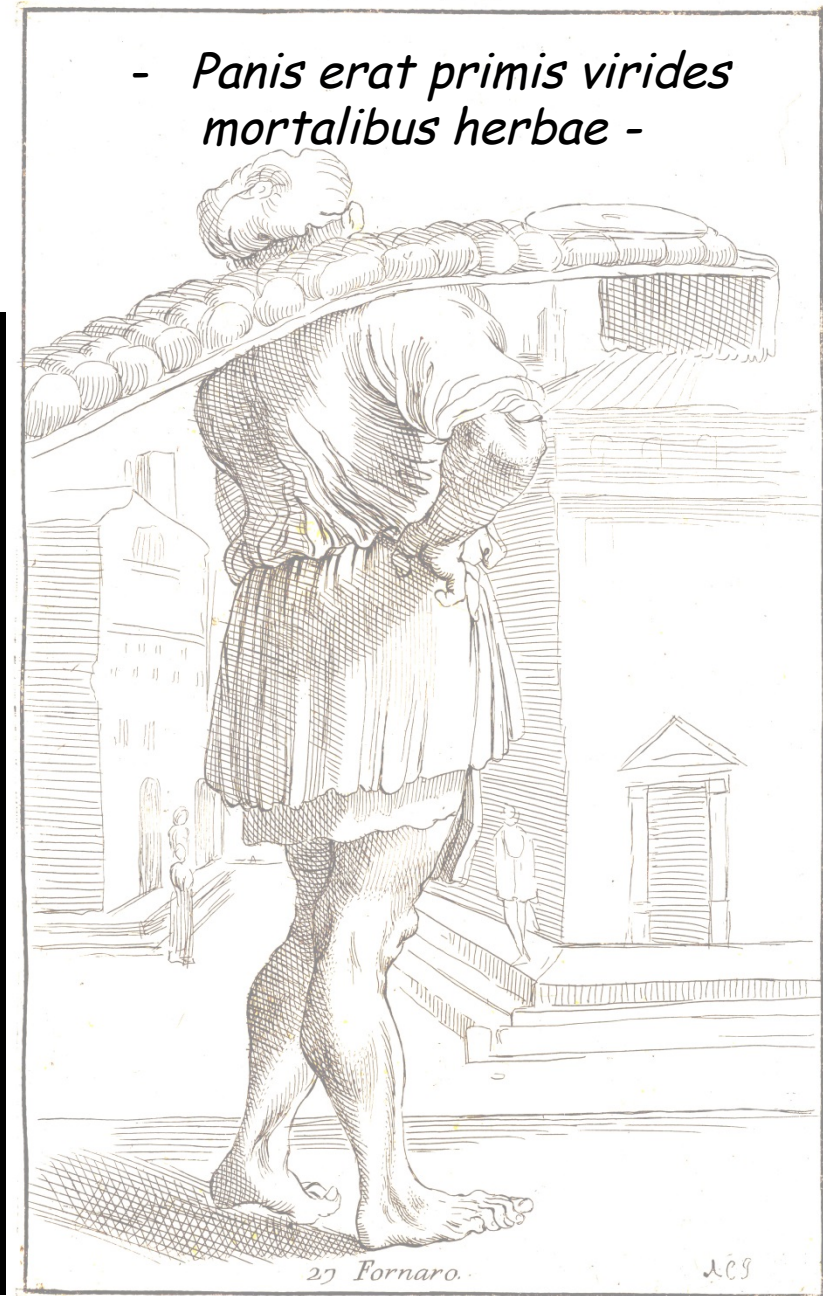
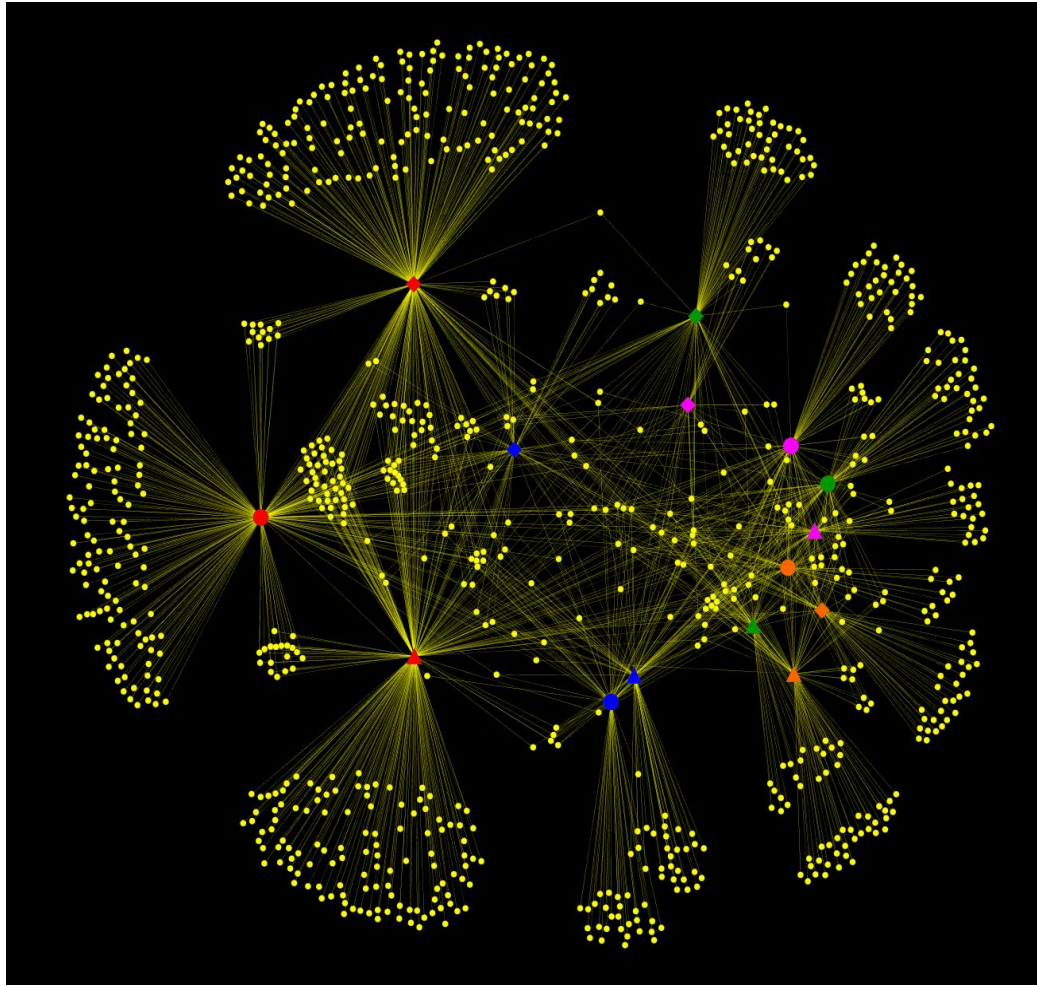


Sourdough biotechnology with lactic acid bacteria and enzymes to produce safe wheat foods for people suffering from irritable bowel syndrome (IBS) and non-celiac gluten sensitivity (NCGS)

WHEATFORALL



- High throughput sequencing of
sourdough microbiota -



(Annibale Carracci, 1646)



