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Le scienze agrarie nella bioeconomia

16/17
FEBBRAIO 2023
DISTAL BOLOGNA

Processi fermentativi nelle strategie "zero-waste":

dagli alimenti funzionali alle bioplastiche

Carlo Giuseppe Rizzello

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Origin and fate of the agri-food side-streams

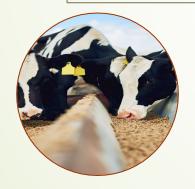
Discarded during supply chain, at any stage:

- Production
- Processing
- Distribution
- Retail
- Consumption

By-products, surplus, defective/unsold/expired products...

edible/not edible side-streams

compost - biogas/bioethanol - feed

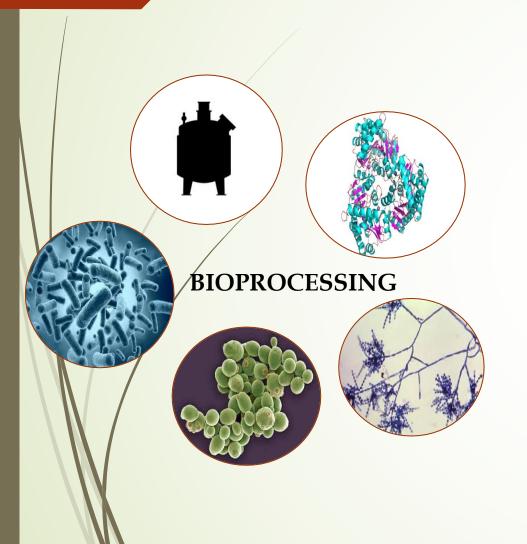


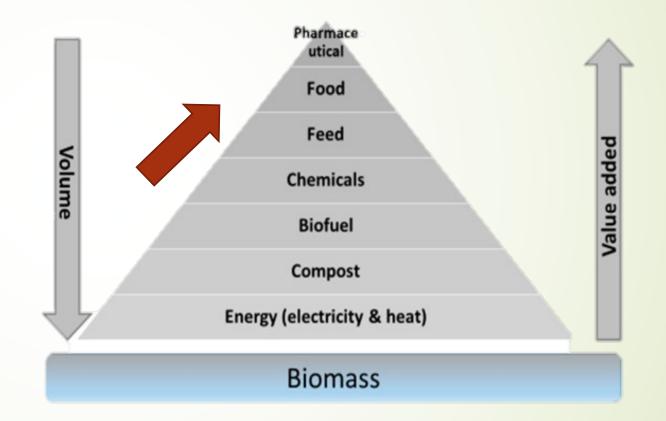
















Recycling and valorisation through bioprocessing

a) use as substrate for microbial growth

microbial biomasses

probiotics

starters (fermented food)

biocontrol/biopreservation

metabolites (biorefinery)

natural or recombinant microrganisms

Bioplastics

Biofungicides

Functional compounds

GABA Bioactive peptides Equol

Compounds for pharmaceutical and cosmetic uses

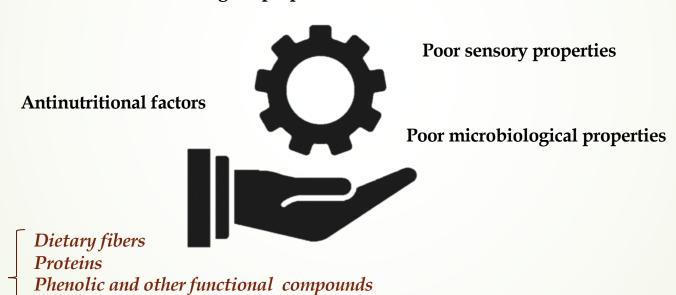




Minerals

b) food ingredients

Poor technological properties





Upcycling side streams into food ingredients

Development of the "strategy"

- Control plan for the side-streams supply chain
- pre-process conditions (thermal treatments, wet milling)
- selection of starter microrganisms and enzymes
- set-up of the biotechnological protocol
- optimization of the process parameters
- stabilization conditions



Agri-food side-streams

fruits and vegetables

post-harvest losses, unsold, peels

cereal industry

surplus wasted bread milling by-products brewer's spent grain

dairy sector

whey ricotta cheese exhausted

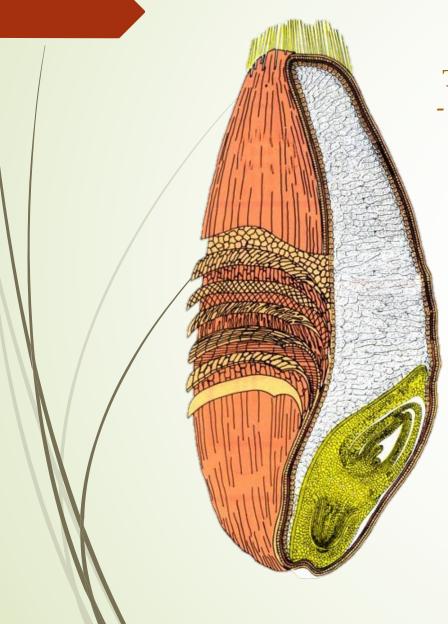
whey

oenological sector

grape pomace



Carlo Giuseppe Rizzello Biotecnologie innovative e strategie zero-waste nelle filiere agroalimentari



Technological issues

- Consumers' acceptability

Source of:

- Fibers
- Minerals
- Proteins
- Phenols

Antinutritional factors

- Phytic acid



Effects of sourdough fermentation on dietary fibre

- Bread with 10 % of fermented bran had the best sensory properties (Eiman et al., 2008)
- Most suitable technology for the manufacture of wholemeal rye (Katina et al., 2005)
- Controls the endogenous xylanase activity and the subsequent solubilisation of arabinoxylans (Katina *et al.*, 2012)

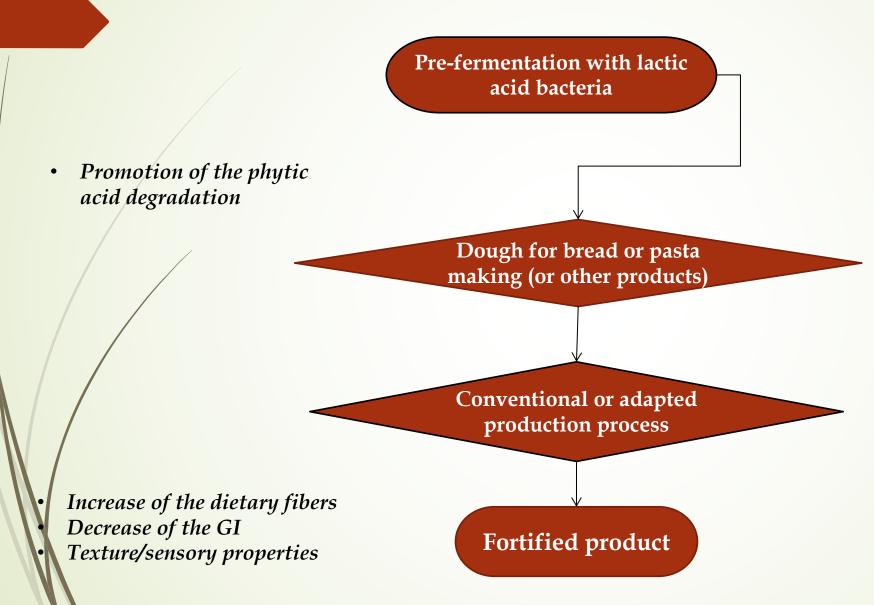




- Without sourdough wholemeal rye or wheat-rye flour mixes are very difficult to process (Katina and Poutanen 2012)
- Improved the loaf <u>volume</u> and crumb softness of high-fibre wheat breads (Katina et al., 2012; Salmenkallio-Marttila et al., 2001; Katina et al., 2006)
- Improves flavour, <u>texture</u> and shelf life of whole grain rye breads (Katina and Poutanen 2012)

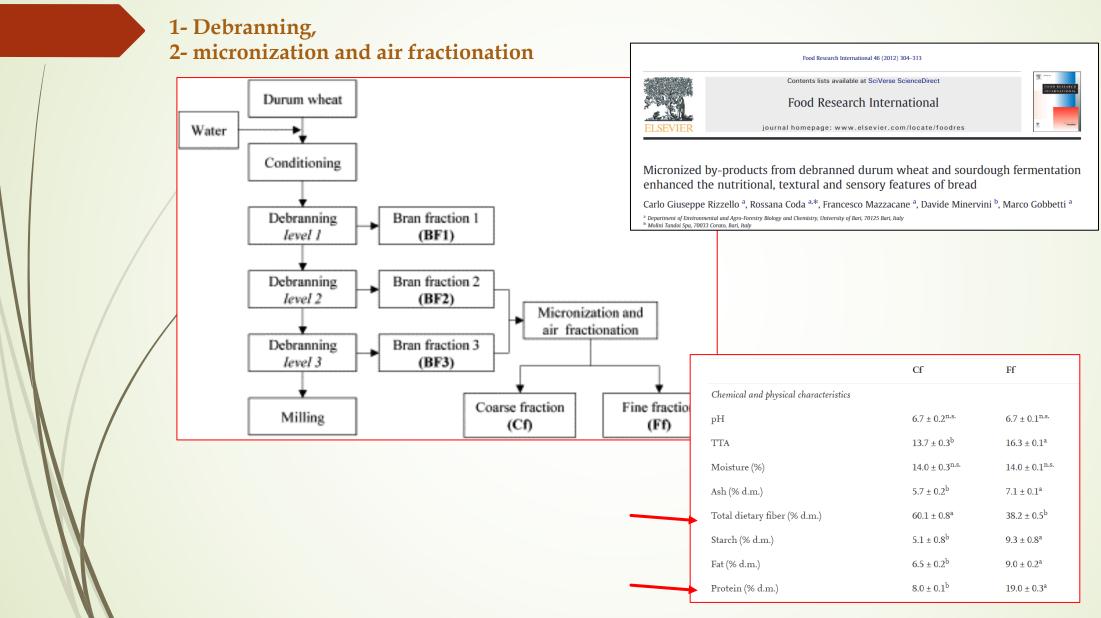


How to use «non-wheat» ingredients





Technological options

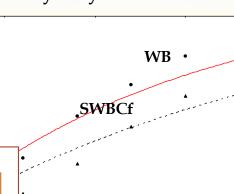




Sourdough applications: pre-treatment of bran and micronized bran

5- Micronization and effect of the particle size





100

Time (min)

• -High concentration of functional compounds (phenols and dietary fibre),

Fermented bran as bread ingredient:

- -Decrease of HI
- Improving of the textural properties,
- Improving of sensory characteristics.
- Increased protein bioavailability (use of xylanases) and digestibility

'drolysis (%)

Innovative Food Science and Emerging Technologies 25 (2014) 19–27

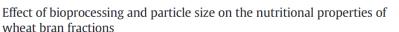
Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

urnal homanaga: www.alsaviar.com/locata/ifeat



journal homepage: www.elsevier.com/locate/ifset





Rossana Coda ^{a,*}, Carlo Giuseppe Rizzello ^b, José Antonio Curiel ^b, Kaisa Poutanen ^{a,c}, Kati Katina ^a

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Food Research International 46 (2012) 304-313



Contents lists available at SciVerse ScienceDirect







Micronized by-products from debranned durum wheat and sourdough fermentation enhanced the nutritional, textural and sensory features of bread

Carlo Giuseppe Rizzello ^a, Rossana Coda ^{a,*}, Francesco Mazzacane ^a, Davide Minervini ^b, Marco Gobbetti ^a

Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari, 70125 Bari, Italy
 Molini Tandoi Spa, 70033 Corato, Bari, Italy

Panel test

150

200

| | WB | SWB | WBCf | SWBCf |
|-------------|-----|-----|------|-------|
| Elasticity | 7.5 | 7.8 | 6.3 | 7.8 |
| Crumb color | 3.8 | 4.5 | 7.3 | 7.6 |
| Crust color | 4.3 | 6.3 | 6.5 | 7.8 |
| Acid flavor | 1.8 | 4.8 | 3.0 | 5.0 |
| Acid taste | 1.5 | 5.3 | 2.8 | 5.8 |
| Sweetness | 4.7 | 4.7 | 5.7 | 5.0 |
| Dryness | 4.3 | 4.0 | 5.0 | 3.8 |
| Taste | 6.3 | 7.0 | 7.3 | 7.8 |
| Salty | 5.3 | 5.7 | 5.7 | 6.7 |

Department of Environmental Biology, Sapienza University of Rome, Italy



Technological options

3- Enzymes



pubs.acs.org/JAFC

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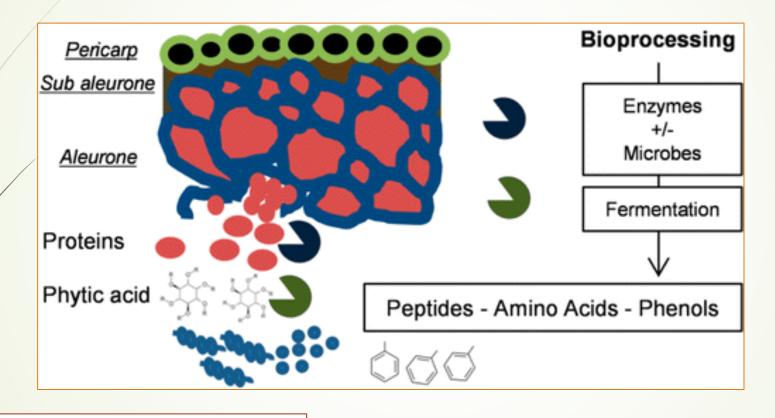
Impact of Enzymatic and Microbial Bioprocessing on Protein Modification and Nutritional Properties of Wheat Bran

Elisa Arte, Carlo G. Rizzello, Michela Verni, Emilia Nordlund, Kati Katina, and Rossana Coda*

[†]Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, FI-00014 Helsinki, Finland

[‡]Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, University of Bari, Via G. Amendola 165/a, Bari 70126, Italy

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Available online at www.sciencedirect.com

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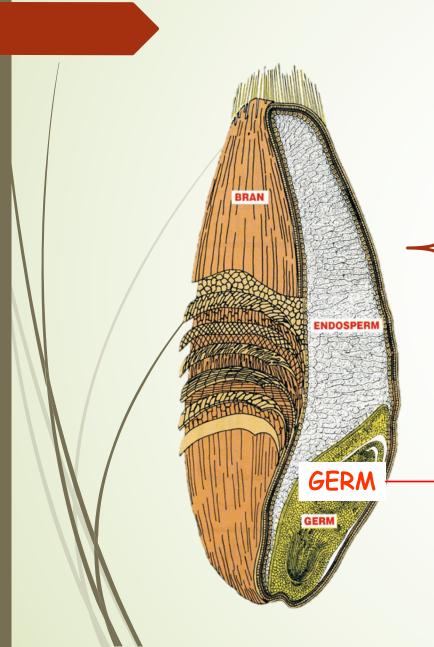


Bran bioprocessing for enhanced functional properties Rossana Coda¹, Kati Katina¹ and Carlo G Rizzello²





Sourdough applications: pre-treatment of wheat germ



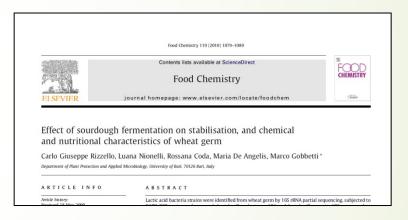
- ✓ a-Tocopherol
- ✓ Vitamins B
- ✓ Dietary fibre
- Minerals
- ✓ Proteins
- ✓ Phytochemicals (flavonoids, sterols,...)
- ✓ Unsatured fatty acids

Antinutritional factors

- Raffinose
- ✓ Phytic acid
- ✓ Wheat germ agglutinin

- Wheat germ-

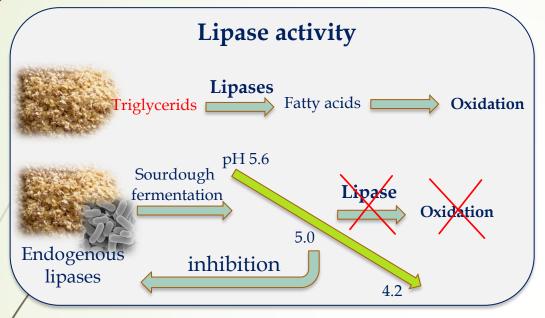
- Technological issues
 - Storage issues
- Consumers' acceptability



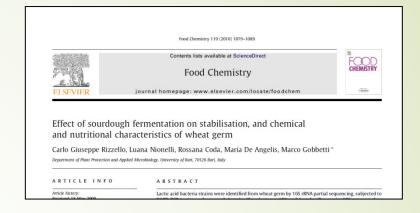




Pre-fermentation of wheat germ with selected lactic acid bacteria



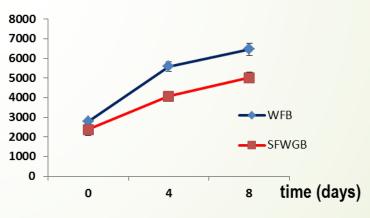
- ✓ Decrease of the lipase activity (2.6 times lower than control)
- ✓ 40 days monitoring of hexanal and volatile markers of lipidic oxidation



SFWG as bread ingredient

(4% on flour weight)

- ✓ Specific volume + 16-18%
- ✓ Increase of the structural features; firmness delay

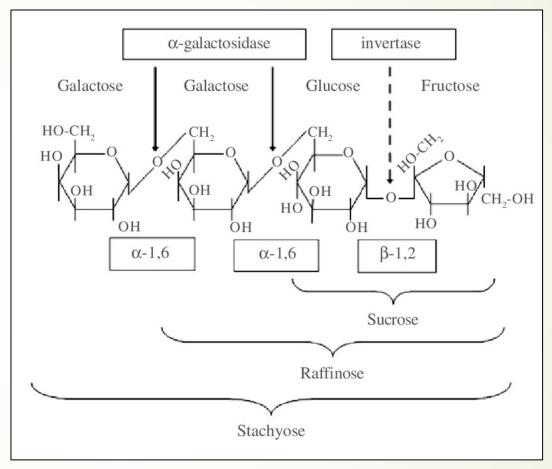




Raffinose (and RFO) degradation

- Most mammals, including man, lack pancreatic alpha-galactosidase (alpha-Gal), which is necessary for the hydrolysis of these sugars.
- RFO can be fermented by gasproducing microorganisms present in the cecum and large intestine, which in turn can induce flatulence and other gastrointestinal disorders in sensitive individuals
- the use of microorganisms expressing alpha-Gal is a promising solution to the elimination of NDO before they reach the large intestine





Leblanc et al., 2004



Pre-fermentation of milling by-products: applications







patent n. 102016000015871 16.2.2016

- Low glycemic index bread (GI *in vivo* 36.9%)
 - "source of fibers" (6%, w/w)



ORIGINAL RESEARCH ARTICLE

Front. Microbiol., 19 March 2019 | https://doi.org/10.3389/fmicb.2019.00561



Maize Milling By-Products: From Food Wastes to Functional Ingredients Through Lactic Acid Bacteria Fermentation

👱 Erica Pontonio¹, 🔔 Cinzia Dingeo¹, 🚊 Marco Gobbetti² and 🚊 Carlo Giuseppe Rizzello¹

¹Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy ²Faculty of Science and Technology, Free University of Bozen-Bolzano, Bolzano, Italy

patent n. WO 2021/260543



International Journal of Food Microbiology 313 (2020) 108384

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro



Brans from hull-less barley, emmer and pigmented wheat varieties: From byproducts to bread nutritional improvers using selected lactic acid bacteria and xylanase



Erica Pontonio^a, Cinzia Dingeo^a, Raffaella Di Cagno^{b,*}, Massimo Blandino^c, Marco Gobbetti^b, Carlo Giuseppe Rizzello^a

- a Department of Soil, Plant and Food Science, University of Bart Aldo Moro, 70126 Bart, Italy
- ^b Faculty of Science and Technology, Free University of Bozen-Bolzano, 39100 Bolzano, Italy
- ^c Department of Agricultural, Forest and Food Sciences, University of Turin, 10095 Grugliasco, Italy



Emmer - barley - pigmented wheat



Chemical composition (g/100 g)

Proximate composition of the defatted wheat germ (DWG).

Table 1

Moisture

Fat (d.m.)

Salt (d.m.)

Ash (d.m.)

Protein (d.m.)a

Carbohydrates (d.m.)

Total dietary fibers (d.m.)

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt



m to produce type-II and III sourdoughs:

Giuseppe Perri ^a, Marcello Greco Miani ^b, Gianfranco Amendolagine ^b, Erica Pontonio ^a, Carl



Defatted durum wheat germ to produce type-II and III sourdoughs: Characterization and use as bread ingredient

Giuseppe Perri ^a, Marcello Greco Miani ^b, Gianfranco Amendolagine ^b, Erica Pontonio ^a, Carlo Giuseppe Rizzello ^{c,*}



Cell density of lactic acid bacteria (log10 cfu/g), pH, concentration of lactic and acetic acids, fermentation quotient (FQ), total titratable acidity (TTA), phytic acid, raffinose and total free amino acids (TFAA) content, of the fermented defatted wheat germ (fDWG) before (0 h) and after (24 h) fermentation at 30 °C with L. plantarum T6B10 and F. sanfranciscensis A2S5. Data refer to wet samples (DY 200).

ELSEVIER

DWG

 7.00 ± 0.28

 0.51 ± 0.20

 25.20 ± 0.77

 28.19 ± 1.30

 35.44 ± 3.13

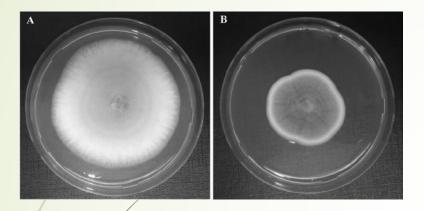
 $\begin{array}{c} 0.02 \pm 0.00 \\ 5.05 \pm 0.55 \end{array}$

| | fDWG | | |
|------------------------------------|-----------------------|-------------------------|--|
| | 0 h | 24 h | |
| Lactic acid bacteria (log10 cfu/g) | 7.43 ± 0.48^{b} | $9.76 \pm 0.20^{\rm a}$ | |
| pH | 6.22 ± 0.15^a | 3.74 ± 0.31^{b} | |
| TTA (ml NaOH) | 2.70 ± 0.11^{b} | 44.14 ± 2.25^a | |
| Lactic acid (mmol/kg) | 0.27 ± 0.02^{b} | 167.7 ± 9.57^{a} | |
| Acetic acid (mmol/kg) | 1.04 ± 0.09^{b} | 15.01 ± 1.15^{a} | |
| FQ | 0.25 ± 0.02^{b} | 11.17 ± 2.50^{a} | |
| TFAA (mg/kg) | 1307.61 ± 118^{b} | 4268.5 ± 301^a | |
| Phytic acid (g/100g) | 1.43 ± 0.24^{a} | 0.77 ± 0.15^{b} | |
| Raffinose (g/100g) | 0.66 ± 0.18^a | 0.06 ± 0.02^{b} | |

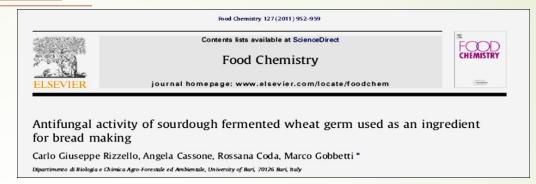




Antifungal organic acids and peptides purified from sourdough fermented wheat germ



| Organic acids | Activity |
|-----------------------------|----------|
| Organic acid mixtureb | +++ |
| Oxalic acid (1.57 mM) | - |
| Lactic acid (24.3 mM) | ± |
| Formic acid (24.7 mM) | ++ |
| Acetic acid (10.8 mM) | ± |
| Citric acid (3.2 mM) | - |
| Citric acid (18.2 mM) | - |
| Phenyl lactic acid (0.4 mM) | + |
| Valeric acid (0.98 mM) | ± |

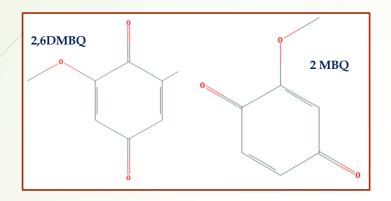


| Sequence | Source Protein | | |
|--------------------|----------------|--|--|
| Sequence | NCBI accession | | |
| VLHEPLF | FH4_ORYSJ, | | |
| | Q8H8K7 | | |
| YNNPIIYVTENGIAEGNN | BGL29_ORYSJ;; | | |
| KSLPITEAL | A3C053 | | |
| ALKAAPSPA | HOX2_ORYSI, | | |
| | Q84U86 | | |
| AILIIVMLFGR | HKT6_ORYSJ, | | |
| | Q6H501 | | |
| AAAAVFLSLLAVGHCAAA | EXPB4_ORYSJ;; | | |
| DFNATDADADFAGNGVD | Q94LR4 | | |
| FNSSDAAVYWGPWTKAR | · | | |



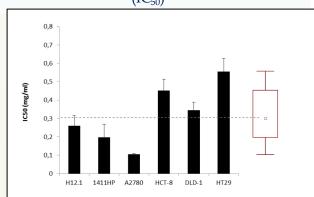


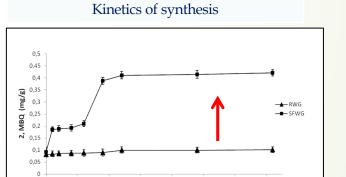
Synthesis of functional compounds during sourdough fermentation of wheat germ





Antiproliferative activity on tumoral human cells (IC_{50})







Biotransformation of brewer's spent grain: increased functionality for novel food applications





70% low-value animal feed (~€35/ton)

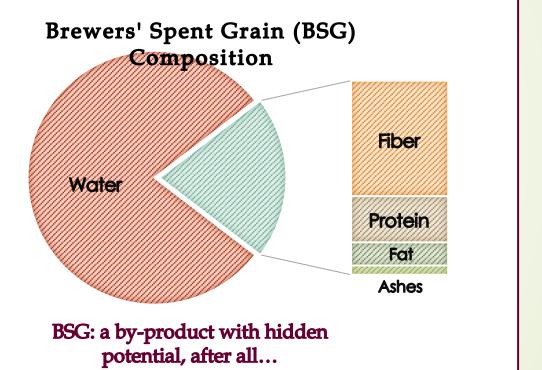


10% biogas

20% landfill



Bianco et al., 2020. Appl. Microbiol. Biotechnol. 1-18.

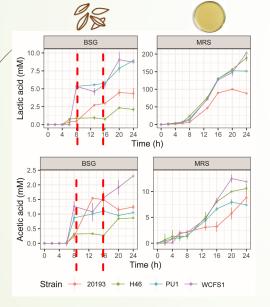




Phenotype switching and gene expression

unibz

- All strains showed **organic acid** production divided into two phases in BSG (diauxic growth).
- Expression of genes related to selected substrates that strains consumed more intensely under BSG conditions during the two phases



microbial biotechnology



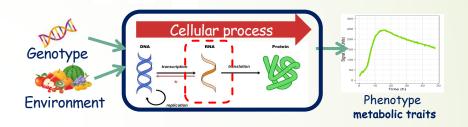
Research Article 🖸 Open Access 💿 🛊 😑 💲

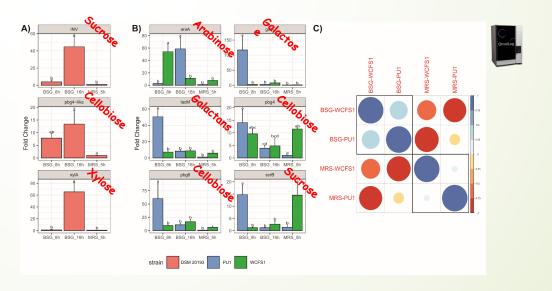




How water-soluble saccharides drive the metabolism of lactic acid bacteria during fermentation of brewers' spent grain

Marta Acin-Albiac, Pasquale Filannino, Rossana Coda, Carlo Giuseppe Rizzello, Marco Gobbetti, Raffaella Di Cagno X



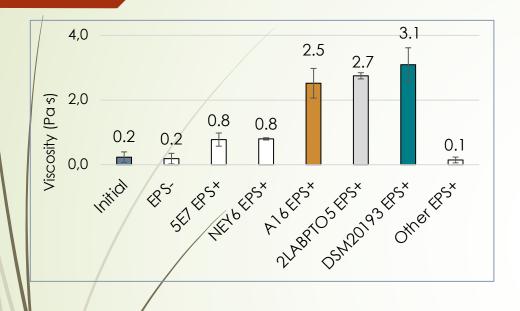




BSG bioprocessing: dextran synthesis by Lactic Acid Bacteria



Viscosity increase



Untreated spent



Fermented spent

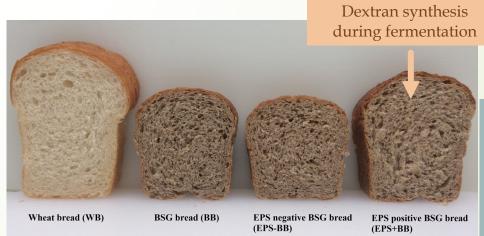


- BSG amount: ca. 33-37% of dough weigh
- Dextran content ca. 1.6% w/w→ 0.59% of dextran in bread (effective as hydrocolloid)

RESEARCH

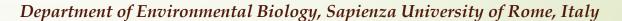
Brewers' spent grain as substrate for dextran biosynthesis by *Leuconostoc* pseudomesenteroides DSM20193 and *Weissella* confusa A16

Prabin Koirala¹, Ndegwa Henry Maina¹, Hanna Nihtilä¹, Kati Katina¹ and Rossana Coda^{1,2*}



BSG bread containing dextran vs native BSG

- Volume + 13%
- Hardness 40%
- Staling rate 33%
- taste/mouthfeel perception

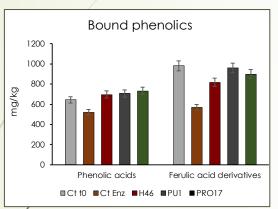


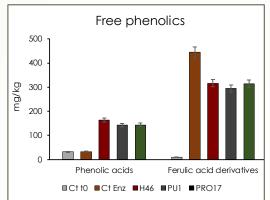


BSG bioprocessing: increase of the antioxidant activity

use of xylanase + lactic acid bacteria fermentation

Phenolic compounds





Xylanase liberated 25% of the phenolic compounds bound to lignocellulosic material and, therefore, not available to exert their function. Whereas LAB metabolized phenolic acids and polymeric forms of proanthocyanidins into more active forms.

Bioactive peptides

LAB proteolytic system enabled the **release of small bioactive peptides** sequences encrypted in barley and maize native proteins, showing common features of antioxidant peptides.





Bioprocessed BSG for functional pasta and extruded snacks







Article

Bioprocessed Brewers' Spent Grain Improves Nutritional and Antioxidant Properties of Pasta

Rosa Schettino ¹, Michela Verni ¹¹⁰, Marta Acin-Albiac ²⁰, Olimpia Vincentini ³⁰, Annika Krona ⁴, Antti Knaapila ⁵, Raffaella Di Cagno ², Marco Gobbetti ², Carlo Giuseppe Rizzello ^{6,*}⁰ and Rossana Coda ^{5,7}⁰



- ■Blend ingredients; total water 26%
- Extrusion at 30/80/90/110/95 °C, 50 rpm
- ■Microwave 12 g at 750 W for 45 s to expand



Technological properties

- ✓ Degraded arabinoxylan structure
- ✓ More homogeneous protein network

Functional properties

- ✓ Rich in phenolic compounds and bioactive peptides
- Protective effects of digested pasta towards induced oxidative stress in Caco-2 cells cultures



Wasted Bread

- **■**European project WASTEBAKE
- Biotechnological functionalization of bakery waste (Call: EUROTRANSBIO)
- Valle Fiorita Catering Srl (Italy) Koivulan Leipomo Oy (Finland) Senson (Finland) Iceberg LLC (Russia)
 University of Bari (Italy) University of
 Helsinki (Finland) ITMO University
 (Russia)







Enrichment in EPS



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International Journal of Food Microbiology

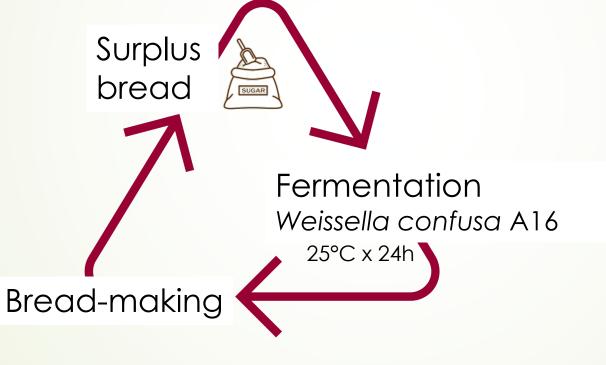
journal homepage: www.elsevier.com/locate/ijfoodmicro



Waste bread recycling as a baking ingredient by tailored lactic acid fermentation



Mikko Immonen^{a,*}, Ndegwa H. Maina^a, Yaqin Wang^a, Rossana Coda^{a,b}, Kati Katina^a









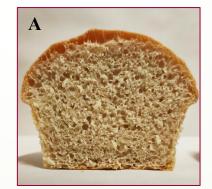
Wasted Bread

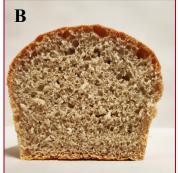
GABA enrichment

- o GABA content 136 mg/kg
- Higher Free Amino Acids
- Higher *In vitro* Protein Digetibility
- Lower predicted Glycaemic Index
- Good technological properties



Image and cross section of common wheat flour bread (A) and bread produced with surplus bread slurry containing 30% of wheat bran and fermented with *L. plantarum* H64 (B)





Alternative options for cereal by-products valorisation:

Substrate for the cultivation of starters



food ingredients

Repurposing discarded dough: Cultivating microbial starters wit wasted bread for industrial bake

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600000

Scientists Want to Feed Old Bread to Microbes - Here's the

Fighting Food Waste: Scientists Repurpose Waste

Plan

Lab Manager

Bread to Feed Microbes

FRONTIERS

scitechdaily.com | 8d



Patent n. 102019000017408, 27.09.2019.

WBM

Wasted Bead Medium

Old bread is being given a new lease of life as scientists create a 'secret sauce' that allows it to be turned into yoghurt, wine and even new bread - all in a bid

waste

Packaging

Taking a bite out of food waste:

Scientists repurpose waste bread

nutrition insight

() FEBRUARY 28, 2020

Credit: CC0 Public Domain

to feed microbes

discarded bread into a platform for yeast to grow in ve to the unused loaves being sent to landfill sites it could then be used in commercial bakeries d by bakeries to recycle their own unused produce

Bread 'trash' is microbial treasure

Saturday, 29 February, 2020

Tonnes of bread end up in landf

Tonnes of bread end up in landfill every year, but researchers have now found a way to repurpose this discarded bread and dough. Research published in *Frontiers in Microbiology* has revealed that old bread can be used as a medium for cultivating microbial

medium for cultivating microbial fermentation starters, which could have applications in food industries like bakeries, dairy and winemaking.

Department of Environmental Biology, Sapienza University of Rome, Italy





Next-generation soil-improvers

a FORK TO FARM approach?

- Supplement for OC and TN
- LAB as PGPM
- Acidification effect
- Antimicrobial activity

Chemical and physicochemical properties of CTR, WBA and FWBA soils

| Samples | pH _{H2O} | pH _{KCI} | EC μ§ m ⁻¹ | 0.C. % | TN % | P _{ava} mg kg ⁻¹ |
|----------|-------------------|-------------------|--------------------------|-----------|---------|---|
| то | 8.2 a | 7.2 | 200 b | 16.0 b | 1.6 bc | 45.5 ab |
| CTR | 8.2 a | 7.3 | 319 b | 15.2 b | 1.5 c | 46.9 a |
| WBA | 7.7 b | 7.3 | 805 a | 20.3 a | 2.1 a | 37.3 bc |
| bWBA | 7.7 b | 7.2 | 764 a | 20.8 a | 1.9 ab | 36.1 c |
| HSD.test | *** | ns | *** | *** | ** | ** |

Mean biometric features of plants at the end of the trial

| Samples | Average n. of leaves/plant | Treated/CTP leaves ratio Average head escarole fresh weight (g) | | Treated/CTP yield ratio |
|----------|----------------------------|---|--------|----------------------------|
| СТР | 13 b | - | 18.2 b | - |
| WBP | 22 a | 1.7 | 64.5 a | 1.97 |
| bWBP | 19 ab | 1.4 | 55.9 a | 1.70 |
| HSD.test | * | ns | *** | ns |





Reuse of Wasted Bread as Soil Amendment: Bioprocessing, Effects on Alkaline Soil and Escarole (Cichorium endivia) Production

Claudio Cacace 10, Carlo Giuseppe Rizzello 20, Gennaro Brunetti 1, Michela Verni 1,*0 and Claudio Cocozza 10

8 weeks



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Potential of native and bioprocessed brewers' spent grains as organic soil amendments

Francesco De Mastro¹, Gennaro Brunetti¹ and Michela Verni^{1*}





Bioplastic production (microbial synthesys of PHBV)

Why Hfx mediterranei?

- PHA-producer
- Halophilic
 - No sterilization
 - Green Extraction
- Starch metabolism
 - Salts supplementation
 - Purity
 - Composition
 - Technological properties



TYPE Original Research
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*CORRESPONDENCE Carlo Giuseppe Rizzello carlogiuseppe.rizzello@uniroma1.it Exploitation of wasted bread as substrate for polyhydroxyalkanoates production through the use of *Haloferax mediterranei* and seawater

Marco Montemurro¹, Gaia Salvatori², Sara Alfano², Andrea Martinelli², Michela Verni¹, Erica Pontonio¹, Marianna Villano^{2,3} and Carlo Giuseppe Rizzello^{4*}







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