

Sviluppo di un pane funzionalizzato mediante integrazione con farina di fagiolo fermentata

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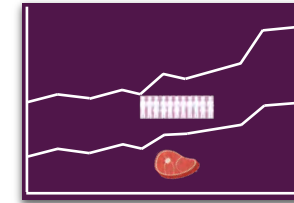
NUTRAGE, OPPORTUNITÀ & INNOVAZIONE
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Future challenges

Introduction

- Feed 9 billion people by 2050: as the world’s population grows so does protein production
- Animal-derived proteins account for almost 40% of humanity’s total protein consumption
- Environmental costs of livestock farming: meat and dairy products impact on greenhouse-gas emissions (although debated)
- Effects of consuming large amount of animal protein on human health



Transition towards more sustainable alternatives

Plant-based proteins



PULSES



PULSES

Introduction

Environment

Sustainability

- Crop rotation
- Enhancement of soil composition

Biodiversity

- Enriched agrobiodiversity
- Enhanced soil biodiversity via interaction with soil microbiome

Mitigation climate changes

- Zero waste
- Reduction green house gases
- Low C and H₂O footprints

Agriculture

- High adaptability to harsh conditions
- Low N needs
- N₂ fixation
- Self-fertilizing
- Livestock fodder



Human

Nutritional and food security

- High **protein**, high fiber
- Low fat
- Bioactive compounds
- Rich in micronutrients (minerals, vitamins)
- Low glycaemic index

Health

- Obesity
- Diabetes mellitus
- CVD prevention
- Cancer
- Gut health
- Malnutrition

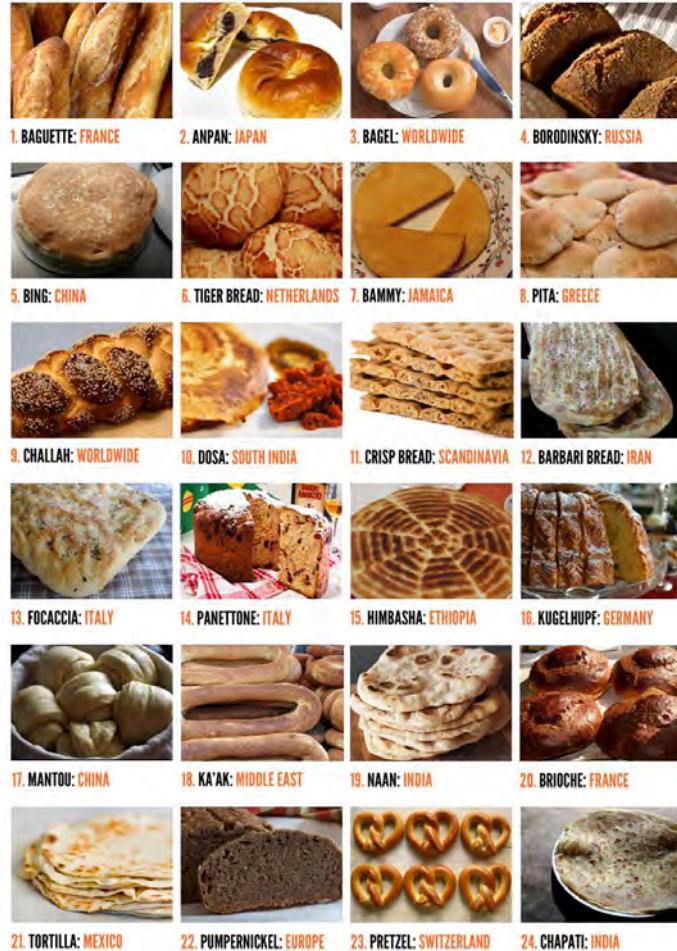
Economy

- Affordable source of proteins
- Valuable low cost feed
- Crop production with low investment



How to increase pulses consumption: bread fortification

Introduction



Bread is one of the most popular food worldwide
(average consumption of 70 kg per year per capita)

Technological and nutritional limits in replacing wheat flour with pulses flour:

- inability to form gluten networks
- reduction of the formation of viscoelastic dough
- air incorporation and gas retention during leavening affected
- antinutritional factors
- unpleasant flavors

Possible solutions:

- technological treatments (fermentation, air classification,...)
-tailored genotypes



Common bean (*Phaseolus vulgaris* L.)

Introduction

The most grown food legume crop worldwide

2018

30.4 million tons of dry seeds
34.5 million hectares cultivated

High energetic value

Excellent source of macro- and micro-nutrients

Nutritional value of seeds

- 22-28% **proteins**
- 50-60% **complex carbohydrates**
- 10% **dietary fiber**
- 2% **lipids**
- **Vitamins** (thiamin, riboflavin, niacin, vitamin B6, folate)
- **Minerals** Fe (40-100 ppm), Zn (20-50 ppm)



Common bean (*Phaseolus vulgaris* L.)

Antinutrient (bioactive) compounds

- Phytic acid _____
 - Phenolic compounds _____
 - Raffinosaccharides _____
 - Lectins (2-5% of total proteins) _____
 - α -Amylase inhibitor _____
 - Trypsin inhibitor _____
- mineral bioavailability
- flatulence
- nausea, vomiting, diarrhoea
- macronutrient (starch, proteins) availability

Fermentation

Introduction



- ✓ improves the functional properties of pulses flour such as water absorption index, water holding capacity, fat absorption and emulsifying properties;
- ✓ reduces the concentration of antinutrients (RFOs, phytic acid, trypsin inhibitor, ...);
- ✓ improves the nutritional value of the flours in terms of total dietary fiber, protein digestibility, mineral, amino acid, fat and antioxidant contents of composite breads;
- ✓ enhances physical properties (specific volume and crumb firmness; yellowness index; reduced crumb lightness (Maillard reaction products))
- ✓ improves appearance, texture, color and overall acceptance
- ✓ provides useful metabolites (free aa, GABA, EPS, antioxidants, etc)

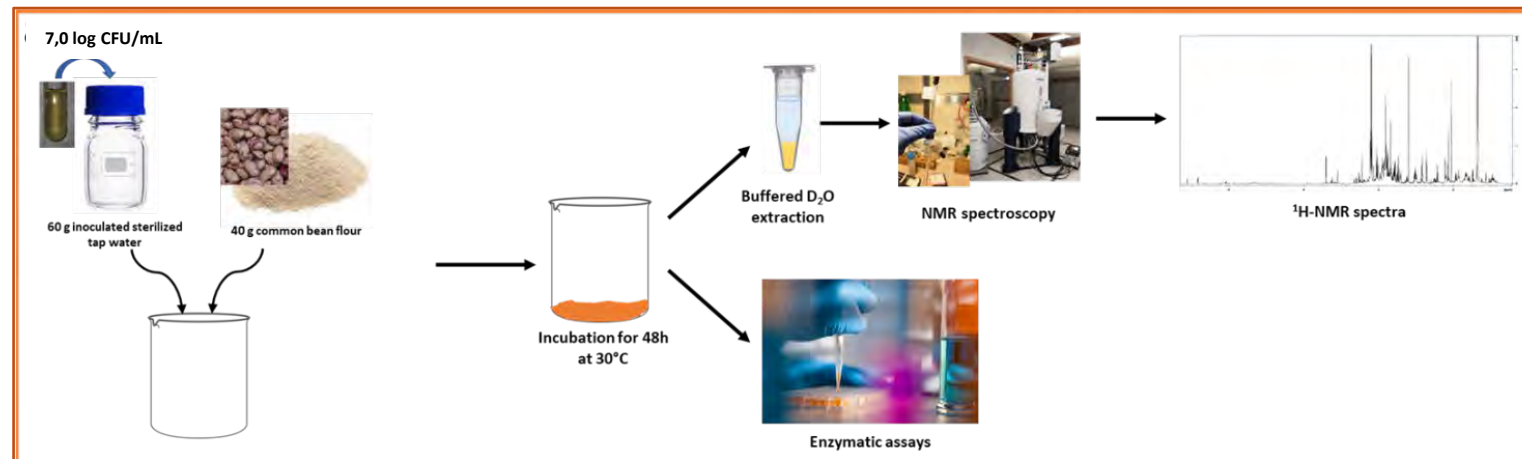
Fermentation

Results

3 LABs , 2 yeasts

- antimicrobial activity (defence against pathogens and moulds)
- different fermentation profile (CO₂)
- nutritional requirements (homo-/hetero-fermentants)
- production of metabolites (EPS)

	Antimicrobial activity	EPS	CO ₂
<i>Lacticaseibacillus rhamnosus</i> LRH01			-
<i>Lentilactobacillus buchneri</i> LBC01	<i>S. aureus</i>	+	+
<i>Leuconostoc lactis</i> LN01			+
<i>Kazachstania humilis</i> 2			+
<i>Saccharomyces cerevisiae</i> 2B			+



Pilot scale fermentation

NMR-based Metabolic & antinutritional profile

Results

FAA	CRTL nf ^a	LRH01	LBC01	LN01	<i>K. humilis</i>	<i>S. cerevisiae</i>
Leucine	0,255	1,47	1,95	1,66	1,8	1,41
Isoleucine	0,131	0,453	0,637	0,56	0,917	0,847
Valine	0,178	0,61	1,28	0,83	1,56	1,51
Alanine	0,7	1,54	2,74	1,86	4,46	4,53
Asparagine	1,56	0,878	1,17	0,917	0,843	0,675
Methionine	3,6	1,81	1,98	2,19	1,79	1,23
Tyrosine	nd ^b	0,23	nd ^b	0,17	0,12	nd ^b
OTHERS						
GABA ^c	1,4	2,34	2,78	2,24	3,25	4,09
RFOs	7,78	0,273	0,307	0,178	nd ^b	nd ^b

^a nf: not fermented bean flour; ^b nd: not detected.

The drying temperature (105°C) does not affect the chemical composition of the fermented flour

Improved nutritional characteristics



Fermented bean-enriched bread preparation

Results



Physical & Technological properties

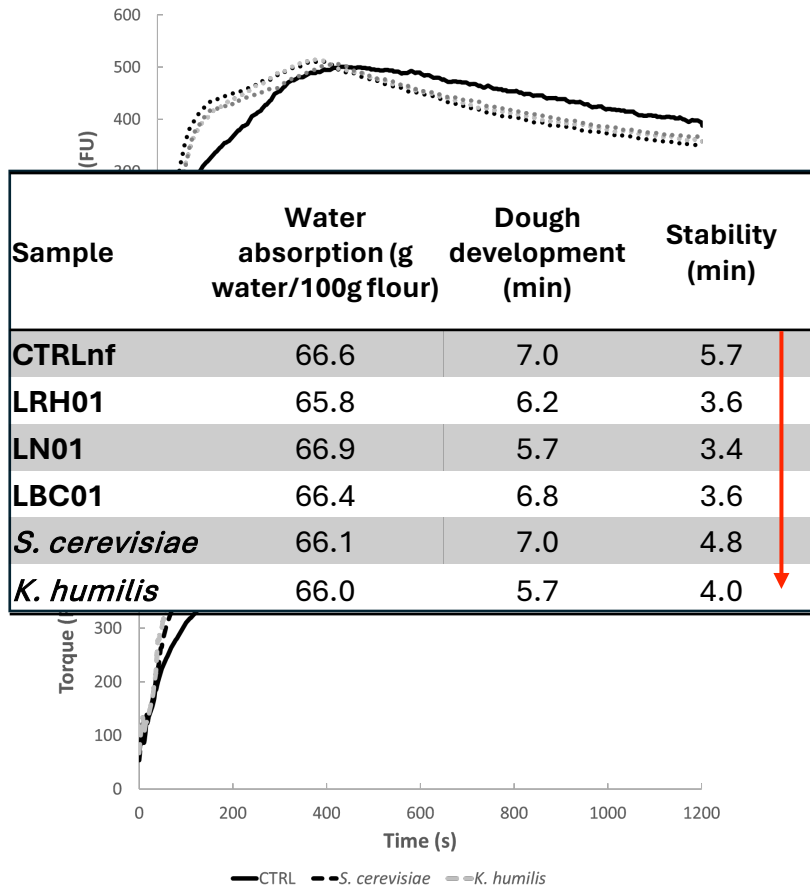
- Water absorption capacity
- Dough development time
- Stability
- Dough behaviour during rising
- Colour, specific volume and consistency of bread



Fermented bean-enriched bread: technological properties

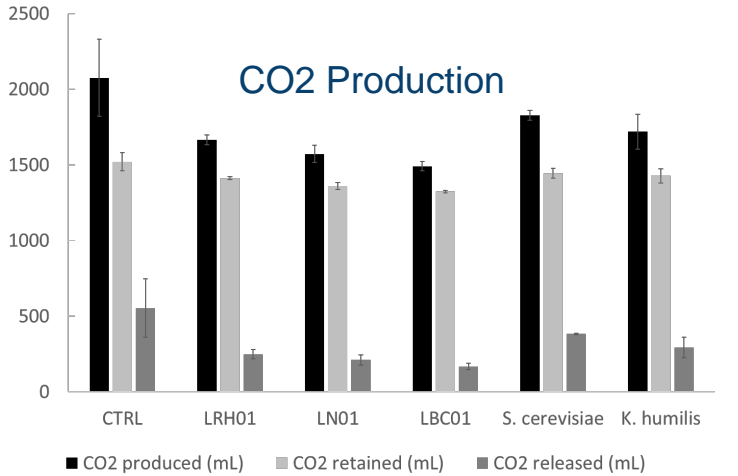
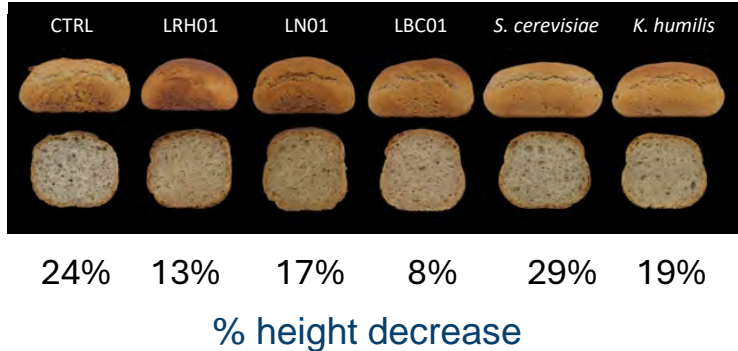
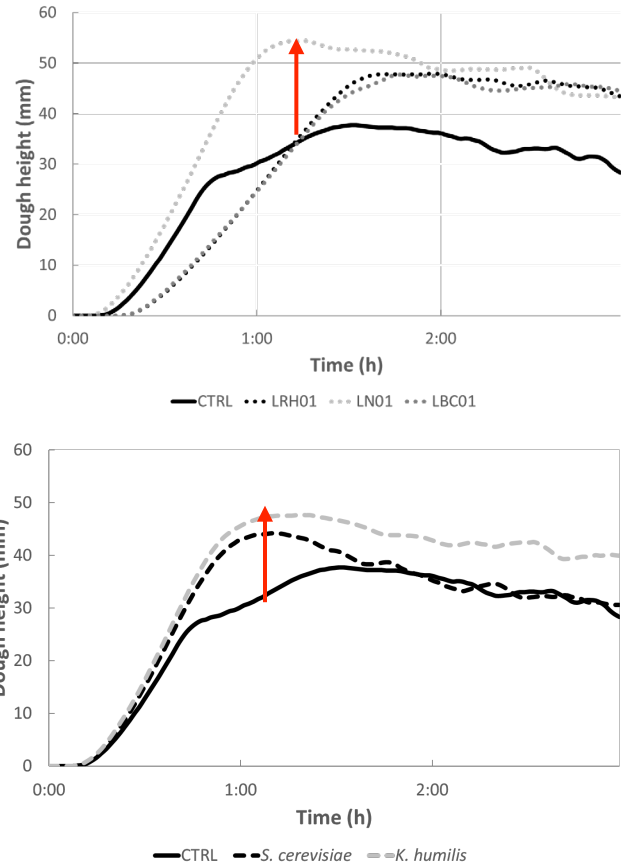
Results

Mixing properties



Leavening

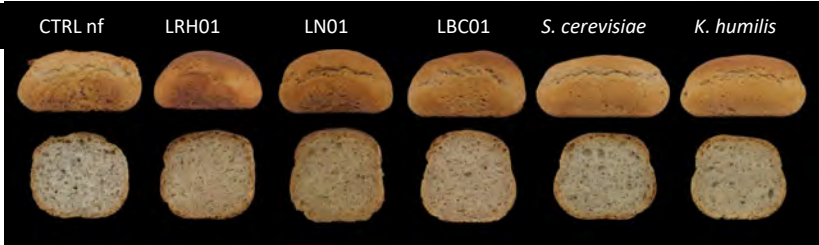
Time to maximum height



Fermented bean-enriched bread: Color, specific volume and consistence

Results

	CTRLnf	LRH01	LN01	LBC01	<i>S. cerevisiae</i>	<i>K. humilis</i>
Crust luminosity	47.82±4.77 ^{ab}	45.78±4.19 ^a	45.09±2.63 ^a	45.37±4.33 ^a	49.81±2.57 ^b	51.11±3.49 ^b
Crust redness	15.36±1.16 ^a	16.88±0.48 ^b	17.30±0.77 ^b	17.25±0.94 ^b	16.58±0.68 ^b	17.33±1.02 ^b
Crust yellowness	25.24±2.20 ^a	24.31±2.71 ^a	24.67±2.36 ^a	24.35±4.09 ^a	27.92±1.52 ^b	29.84±1.92 ^b
Crumb luminosity	58.96±3.02 ^{bc}	57.53±1.90 ^{ab}	55.82±2.31 ^a	59.98±1.70 ^c	58.97±1.20 ^{bc}	59.36±1.53 ^{bc}
Crumb redness	5.44±0.33 ^a	7.93±0.17 ^c	8.52±0.24 ^d	7.70±0.24 ^c	5.61±0.18 ^a	6.26±0.41 ^b
Crumb yellowness	10.92±0.42 ^a	18.73±0.33 ^d	23.62±0.44 ^e	16.91±0.52 ^c	14.86±0.29 ^b	17.40±0.83 ^c
Specific Volume (mL/g)	2.46±0.12 ^a	2.42±0.12 ^a	2.84±0.26 ^b	2.57±0.11 ^a	2.49±0.01 ^a	2.40±0.06 ^a
Firmness after baking (N)	7.43±1.57 ^a	9.02±0.50 ^{ab}	9.29±0.49 ^b	9.70±0.97 ^b	11.44±1.50 ^c	11.52±1.81 ^c
Firmness after 1 day (N)	17.20±2.66 ^{bc}	16.97±2.48 ^b	14.46±0.98 ^{ab}	12.37±2.66 ^a	17.25±2.36 ^{bc}	21.09±3.86 ^c



Different letters in the same row indicate a significant difference among samples (one-way ANOVA; Tukey HSD test; p ≤ 0.05).

- Fermentation changes red (all) and yellow (yeast) colour;
- There are no major changes in specific volume except for LN01;
- Hardness increases after cooking, but fermentation slows it down after 24 hours

Fermented bean-enriched bread: NMR-based Metabolic profile

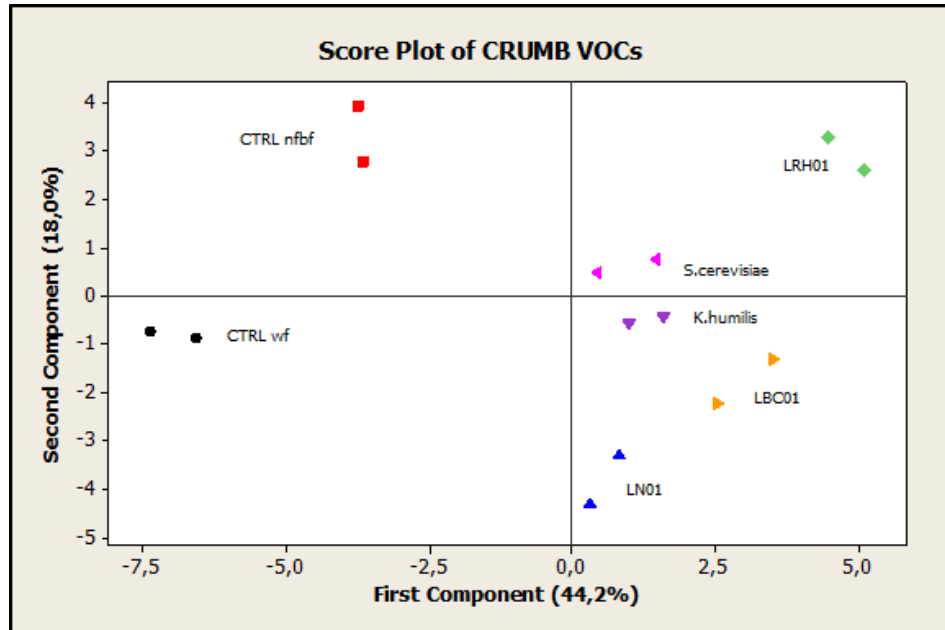
Results

	CTRL wheat		CTRL nf ^a		LRH01		LBC01		LN01		<i>K. humilis</i>		<i>S. cerevisiae</i>	
	crust	crumb	crust	crumb	crust	crumb	crust	crumb	crust	crumb	crust	crumb	crust	crumb
FAA														
Leucine	nd ^b	0,135	0,114	0,177	0,607	0,840	1,180	1,220	1,000	0,960	0,855	0,820	0,693	0,610
Isoleucine	0,053	0,803	0,049	0,074	0,217	0,277	0,440	0,390	0,307	0,357	0,490	0,413	0,413	0,350
Valine	0,094	0,105	0,086	0,116	0,423	0,510	0,823	0,833	0,673	0,753	0,907	0,900	0,840	0,783
Alanine	0,623	0,600	0,640	0,720	1,220	1,460	2,510	2,400	2,100	2,030	3,400	3,190	3,700	3,630
Asparagine	nd	nd	0,374	0,482	0,846	0,965	0,962	0,977	0,651	1,030	nd	1,050	0,948	0,953
Methionine	nd	nd	1,190	1,610	1,140	1,560	1,030	1,190	1,660	1,700	0,810	0,907	0,687	0,750
Tyrosine	nd	nd	nd	nd	0,116	0,105	nd	nd	nd	nd	nd	nd	nd	nd
OTHERS														
GABA ^c	1,03	1,34	nd	1,88	1,51	1,73	2,48	2,59	2,54	2,25	2,92	2,62	3,26	2,55
RFOs	nd	nd	1,1	1,2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

- Crusts and crumbs do not show major fluctuations in the content of the quantified metabolites;
- All the FBF-enriched loaves contain higher levels of FAAs and GABA when compared to both controls;
- RFOs are not detected

Fermented bean-enriched bread: Volatilome profile

Results



compound	class	CTRL wf	CTRL nfb	LRH01	LN01	LBC01	S. cerevisiae	K. humilis
3 methyl 1 butanol	alc	6,07	6,06	5,84	5,89	5,68	5,65	5,76
2 phenylethanol	alc	5,92	5,91	5,53	5,27	5,27	5,64	5,78
2,3 butanedione (diacetyl)	ket	5,16	5,16	5,69	5,08	5,34	5,49	5,39
3 hydroxy 2 butanone (acetoin)	ket	5,17	5,29	6,30	5,21	5,86	5,81	5,66
2 methylpropanoic acid	aci	5,43	5,37	5,53	5,54	5,54	5,28	5,41
3 methylbutanoic acid	aci	5,12	5,04	5,43	5,36	5,41	5,12	5,18
phenylacetaldehyde	ald	4,39	4,35	5,14	5,13	5,24	4,70	4,84
furfural	ald	4,46	4,41	5,43	5,06	4,78	4,18	4,27
2 methylpyrazine	het	4,13	4,57	5,45	4,21	4,70	4,60	4,45
benzaldehyde	ald	4,61	4,40	5,08	5,09	4,82	4,75	4,73
butyric acid	aci	4,41	4,36	4,73	4,81	4,89	4,74	5,23

- Control samples are distinguished from fermented ones
- The second component separates the two controls, and the bread produced with homofermenting LAB from the other samples

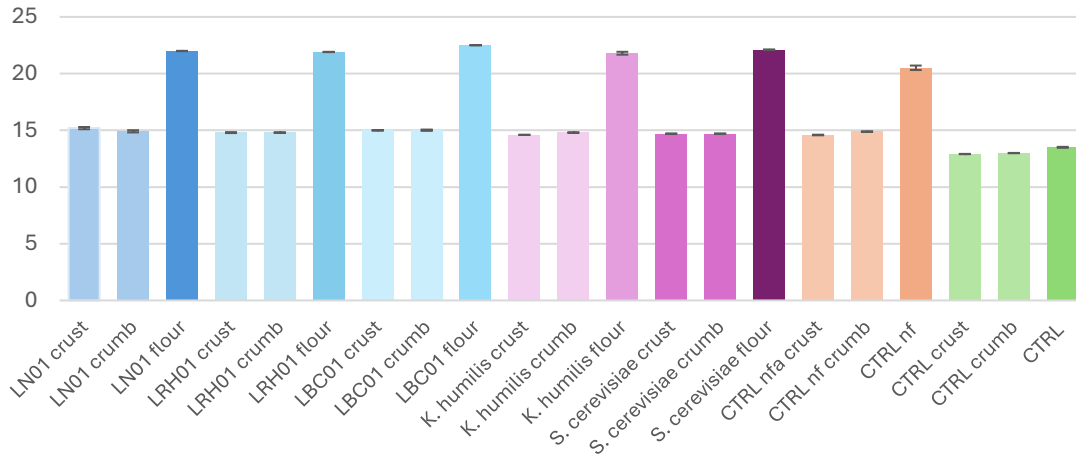
Compounds **significantly different** between the samples and **positively** or **negatively** correlating with bread aroma



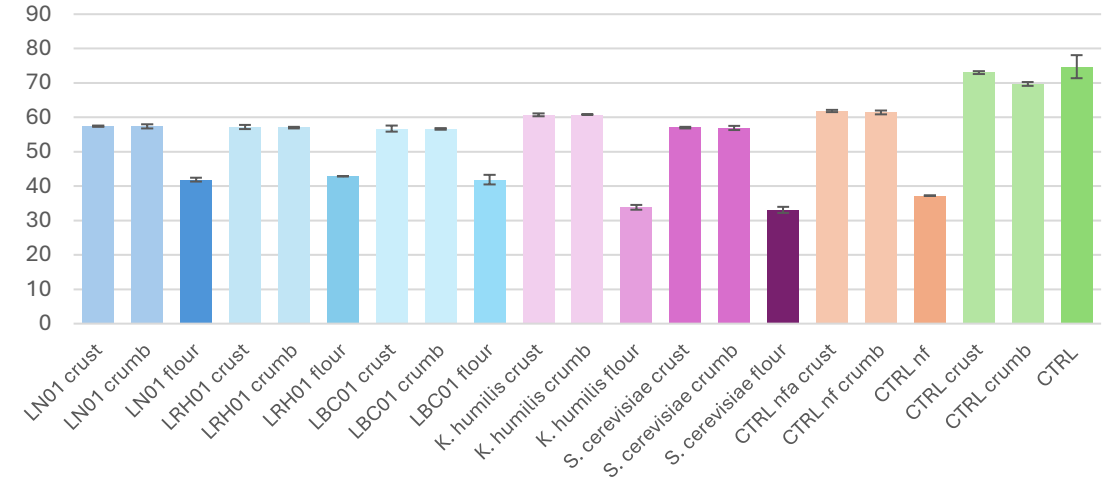
Fermented bean-enriched bread: Nutritional profile

Results

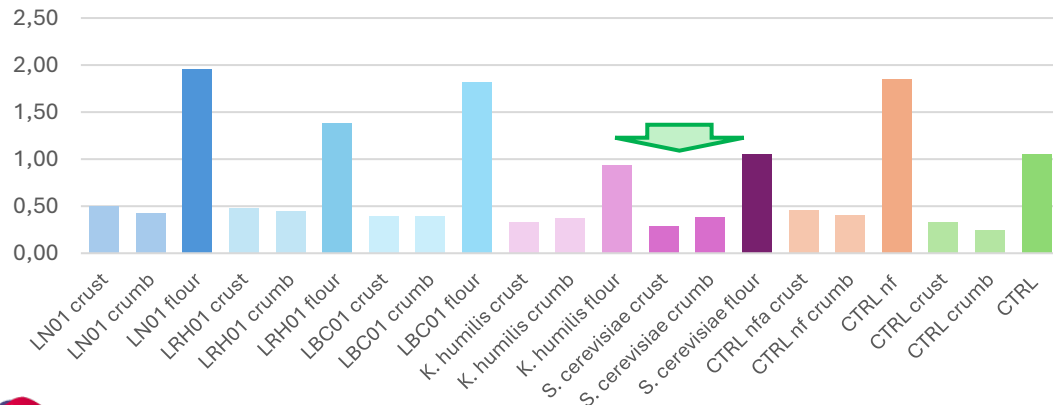
Crude Protein 




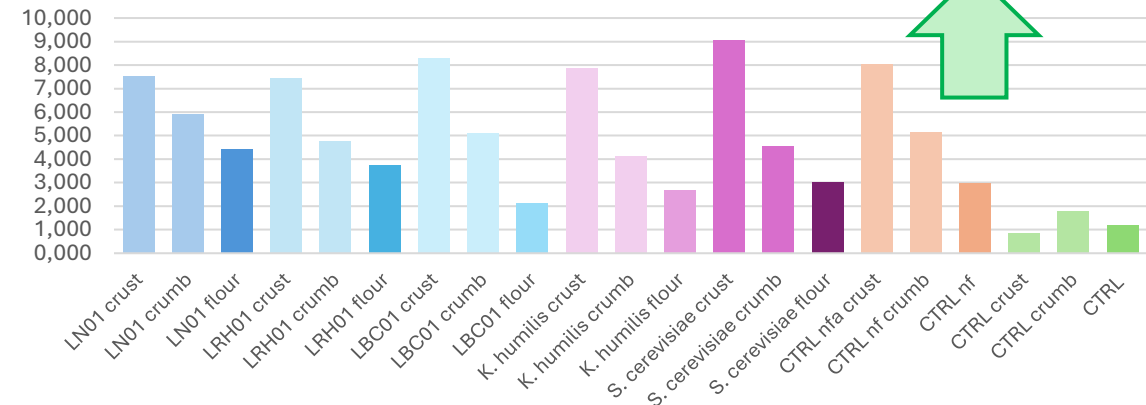
Starch 



Phytic acid g/100g



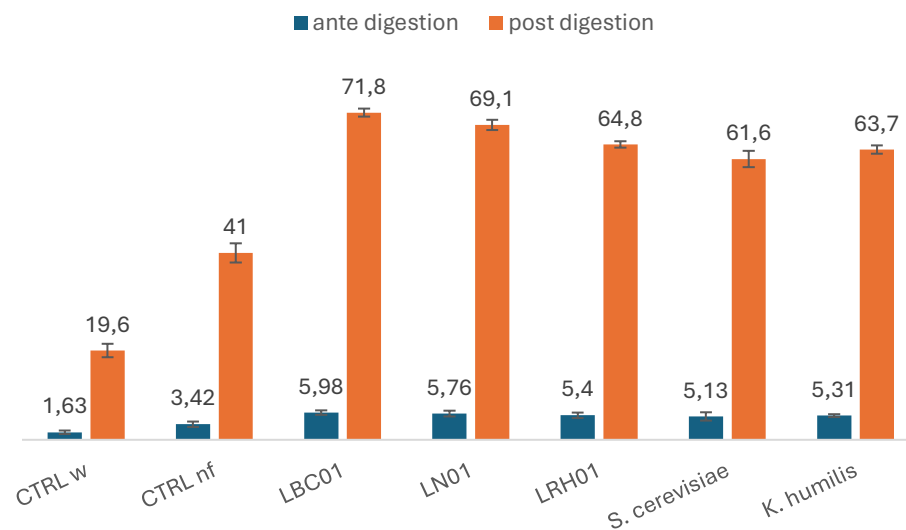
DPPH mean % inib/mg DW 



Fermented bean-enriched bread: protein *in vitro* digestibility

Results

Protein breakdown (%)



Fermentation increases protein digestibility

Fermented bean-enriched bread

CONCLUSIONS

Fermentation based on LABs and yeasts is a **useful process**

- lower some ANFs (e.g. RFOs),
- increase/enrich in useful bioactive compounds (GABA, FAA, and DPPH)
- make nutrients more accessible for digestion

Composite flours based of FBF are technologically more adapted than NFBF to produce bread

We did not observe significant residual amounts of phytic acid and lectins after fermentation

Use the flour of tailored bean genotypes devoid for lectins and phytic acid (*lec-*, *lpa*) that we already proved to be a valid and healthy ingredient for producing biscuits and other bean-based foods



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Small scale fermentation

Metabolic & antinutritional profile

Results

	CRTL nf ^a	VC201	VC213	LP01	VS513	LRH01	LBC01	LN01	CE65	WS01	<i>K. humilis</i>	<i>S. cerevisiae</i>
 Leu, Ile, Val Asn GABA  RFOs Phytic Acid												
FAAs												
Leucine	0,26	0,69	0,78							1,03	0,96	0,56
Isoleucine	0,13	0,24	0,27							0,48	0,51	0,39
Valine	0,18	0,42	0,46							0,89	1,04	0,61
Alanine	0,70	1,06	1,10							1,98	3,17	2,43
Asparagine	1,56	1,05	1,02							0,97	0,69	1,15
Methionine	3,60	2,61	2,48							2,39	3,50	3,25
Tyrosine	nd ^b	0,18	0,24							0,21	nd	nd
OTHERS												
GABA	1,40	2,54	2,26	2,27	2,17	3,04	4,59	2,61	2,31	3,83	4,83	4,80
RFOs	7,78	0,91	0,32	0,33	0,84	0,46	0,21	1,04	1,90	1,22	nd	0,13
Phytic Acid g/100g	1,472	1,521	1,491	1,783	1,573	1,407	1,528	0,21	1,557	1,567	0,838	na ^c
Lectin activity (HAU/g)	12800	6400	3200	12800	3200	3200	6400	6400	6400	6400	na ^c	na ^c

^a nf: not fermented bean flour; ^b nd: not detected; ^c na: not analysed.

