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USE OF *Weissella cibaria* BAL3C-5 B2 TO PRODUCE FUNCTIONAL BREADS ENRICHED IN VITAMIN B_2 AND FORTIFIED WITH DEXTRAN

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INTRODUCTION & AIM

Fermentation can improve the sensory, nutritional, and safety qualities of food. Furthermore, producing functional foods that contain bioactive compounds generated *in situ* by fermentative microorganisms is more sustainable than adding chemically or biotechnologically synthesized compounds.

Using optimized protocols for quantifying riboflavin (RF) and dextran, as well as gastrointestinal (GIDM) and colonic fermentation (CFDM) dynamic models, we have analyzed the functional properties of experimental breads ($BREAD_{LAB}$) made from doughs inoculated with *Weissella cibaria* BAL3C-5 B2, a RF-overproducing spontaneous mutant of the dextran-producing lactic acid bacteria (LAB) *W. cibaria* BAL3C-5, which was isolated from rye sourdough.

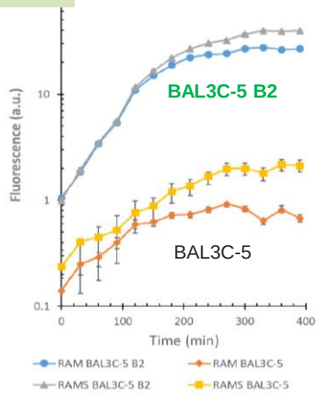
Doughs made with binary (50:50) flour mixtures (wheat/chickpea, wheat/quinoa, wheat/teff, rice/quinoa and buckwheat/quinoa) were fermented with or without (control) *W. cibaria* BAL3C-5 B2. Dextran (~2% w/w) was only detected in the LAB-fermented doughs, which had a vitamin B_2 content that was 2- to 3-fold higher than that of the control doughs. GIDM revealed that the bioaccessible vitamin B_2 content in wheat/chickpea B_{LAB} was ~3.7-fold higher than in control breads ($BREAD_C$). Using CFDM, buckwheat/quinoa $BREAD_{LAB}$ and $BREAD_C$ were shown to significantly induce SCFA production by the gut microbiota, although $BREAD_{LAB}$ resulted in a greater increase (*Lactobacillus* and *Bifidobacterium*) or no decrease (*Akkermansia*) of the abundance of beneficial bacteria.

RESULTS & DISCUSSION

SELECTION OF THE LAB STRAIN

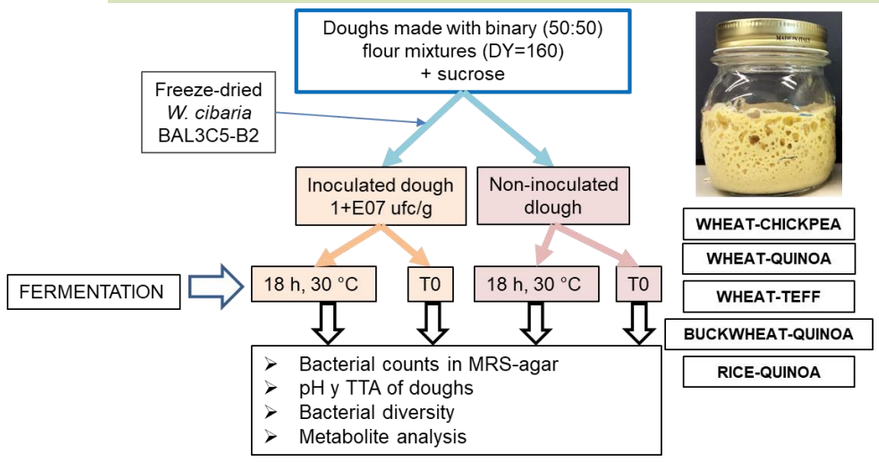
Weissella cibaria BAL3C-5 B2

- LAB isolated from rye sourdough
- Riboflavin-overproducing spontaneous mutant selected after roseoflavin exposure
- Dextran producing strain
- Genome sequenced (2.4 Mb)
- Lacks virulence factors and plasmids involved in antibiotic resistance



Hernández-Alcántara et al. *Front. Nutr.* (2022)

PREPARATION OF EXPERIMENTAL BINARY MINI-DOUGHS



WHEAT-CHICKPEA
WHEAT-QUINOA
WHEAT-TEFF
BUCKWHEAT-QUINOA
RICE-QUINOA

FERMENTATIVE PROCESS IN THE DOUGHS

	pH	TTA	$CFU_{18\,h}/CFU_{0\,h}$
	BAL3C-5 B2	BAL3C-5 B2	BAL3C-5 B2
MATRIX	18 h 30 °C	18 h 30 °C	18 h 30 °C
WHEAT-CHICKPEA	4.8 ↓	15 ↑	1.0E+02 ↑
BUCKWHEAT-QUINOA	4.5 ↓	19.2 ↑	1.9E+02 ↑

Binary (50:50) low-gluten or gluten-free flour doughs (wheat/chickpea, wheat/quinoa, wheat/teff, rice/quinoa, and buckwheat/quinoa) supplemented with 6% sucrose were inoculated with 1×10^7 c.f.u./g of BAL3C-5 B2, while non-inoculated doughs served as controls. All doughs were incubated at 30 °C for 18 h, and samples were collected before (T0) and after incubation (T18 h) to assess bacterial counts, pH, total titratable acidity, bacterial diversity, and metabolite content. After incubation, only the inoculated doughs showed a decrease in pH and an increase in TTA, confirming fermentation. BAL3C-5 B2 fully colonized all fermented doughs and increased by approximately 2 logs in the wheat/chickpea and buckwheat/quinoa mixtures.

IN SITU PRODUCTION OF METABOLITES IN DOUGHS

FREE RIBOFLAVIN
- Extraction in water
- Detection and quantification by fluorescence

TOTAL VITAMIN B_2
Jakobsen, J. *Food Chemistry* 106 (2008)
Yepez, A., et al. *Food Microbiology* 77 (2019)
- Enzymatic method
- Detection and quantification by fluorescence

DEXTRAN
Katina, K., et al. *Food Microbiology* 26 (2009)
- Dextranase based method
- Detection and quantification of isomaltose by oxime derivatization and GC-MS

	FREE-RIBOFLAVIN (mg/100g)			TOTAL VITAMIN B_2 (mg/100g)			DEXTRAN (g/100g)		
	NON-INOCULATED		BAL3C-5 B2	NON-INOCULATED		BAL3C-5 B2	NON-INOCULATED		BAL3C-5 B2
MATRIX	0 h	18 h 30 °C	18 h 30 °C	0 h	18 h 30 °C	18 h 30 °C	0 h	18 h 30 °C	18 h 30 °C
WHEAT-CHICKPEA	**0.05 ± 0.01**	**0.04 ± 0.02**	**0.69 ± 0.04**	**0.33 ± 0.08**	**0.29 ± 0.03**	**0.82 ± 0.03**	0.00	0.00	3.74 ± 0.13
BUCKWHEAT-QUINOA	0.04 ± 0.01	0.05 ± 0.01	0,44 ± 0.01	0.24 ± 0.02	0.24 ± 0,04	0.66 ± 0.01	**0.00**	**0.02 ± 0.01**	**3.85 ± 0.27**

WHEAT-CHICKPEA

TOTAL VITAMIN B_2 IN THE INOCULATED DOUGH
0.82 ± 0.03 (mg/100g)
(52 % of PRI)

BUCKWHEAT-QUINOA

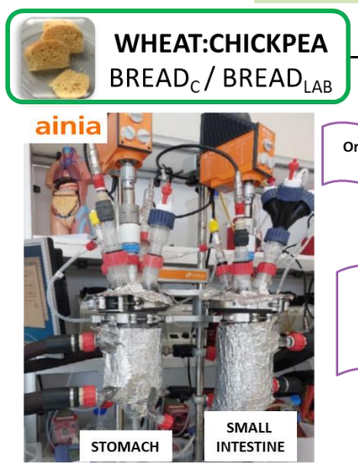
TOTAL DEXTRAN IN THE INOCULATED DOUGH
3.85 ± 0.27 (g/100g)

We optimized protocols to quantify riboflavin and dextran in doughs inoculated with BAL3C-5 B2 and in the resulting breads. All doughs showed increased riboflavin, with the highest rise in the wheat/chickpea mixture, which was selected for vitamin B_2 accessibility analysis in GIDM. Dextran production was observed in the inoculated fermented doughs, and the buckwheat–quinoa gluten-free dough was chosen to evaluate $BREAD_{LAB}$ effects on the human colonic microbiota.

ANALYSIS OF THE FUNCTIONAL PROPERTIES OF THE BREADS

VITAMIN B_2 BIOACCESSIBILITY ANALYSIS

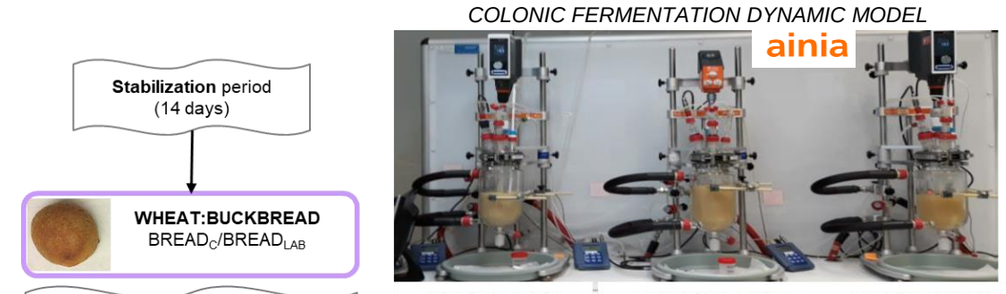
GASTROINTESTINAL DIGESTION DYNAMIC MODEL



	CONTROL BREAD (NON-INOCULATED)	BREAD WITH BAL3C-5 B2	VITAMIN B_2 INCREASE (# FOLD)
	TOTAL VITAMINA B_2 (mg/ 50 g)		
BEFORE GASTRIC DIGESTION	0,135 ± 0,02	0,465 ± 0,04	3,4
SOLUBLE FRACTION OF THE GASTROINTESTINAL DIGESTA	0,078 ± 0,005	0,287 ± 0,007	3,7
%VIT. B_2 BIOACCESIBILITY	57,8 %	61,7 %	1,1

IN VITRO EVALUATION OF THE EFFECT OF $BREAD_{LAB}$ ON THE HUMAN COLONIC MICROBIOTA COMPARED WITH $BREAD_C$

COLONIC FERMENTATION DYNAMIC MODEL



Stabilization period (14 days)

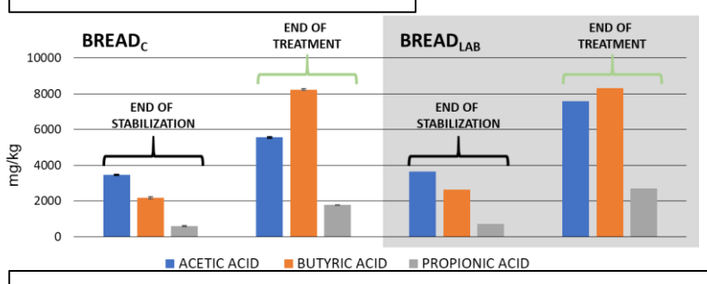
WHEAT:BUCKBREAD
$BREAD_C$/$BREAD_{LAB}$

Treatment period: the system is fed daily with control bread and $BREAD_{BAL}$ (14 days)

Sampling of each section of the colon
- ❑ End of stabilization
- ❑ 7 days of treatment
- ❑ 14 days of treatment

- Determination of **SCFAs**
- Analysis of the **biodiversity** of the human colonic microbiota:
  - ➢ Viable microbiota
  - ➢ Total microbiota (16S metagenomics)

PRODUCTION OF SCFAS



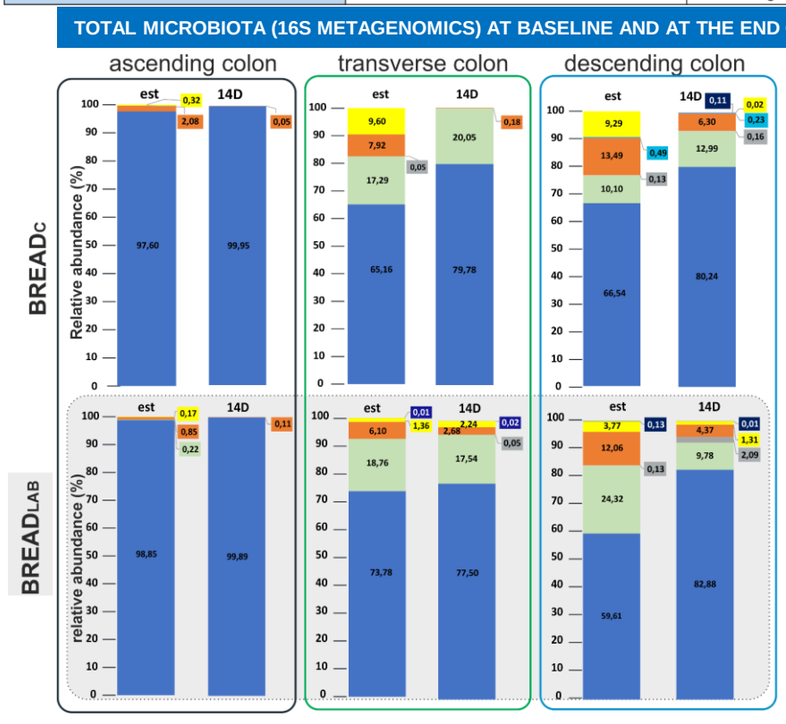
	TOTAL SCFAs (mg/kg)	FOLD INCREASE
TREATMENT WITH $BREAD_C$		
End of stabilization	6205 ± 86	2.5
End of treatment	15557 ± 66	
TREATMENT WITH $BREAD_{LAB}$		
End of stabilization	6962 ± 63	2.7
End of treatment	18591 ± 67	

COMPOSITION OF THE HUMAN COLONIC MICROBIOTA

VIABLE MICROBIOTA AFTER 14 DAYS OF TREATMENT
(SUMMARY OF THE THREE COLON SECTIONS)

	$BREAD_C$ TREATMENT	$BREAD_{LAB}$ TREATMENT
TOTAL ANAEROBS	stable =	stable =
ENTEROBACTERIA	~0.5-log decrease ↓	~0.4 to 2.1-log decrease ↓↓
LACTOBACILLI	~1.5 to 2-log increase ↑	~1.6 to 3-log increase ↑↑
BIFIDOBACTERIA	stable =	~1-log increase in the transverse and descending sections ↑

TOTAL MICROBIOTA (16S METAGENOMICS) AT BASELINE AND AT THE END OF TREATMENT



In both treatments, *Bacillota* increased in the two distal colonic sections. *Bacteroidota* remained stable with $BREAD_C$ but rose in the descending colon with $BREAD_{LAB}$. *Pseudomonadota* decreased under both treatments. *Akkermansia muciniphila* (*Verrucomicrbiota*) dropped sharply with $BREAD_C$, but with $BREAD_{LAB}$ it increased in the transverse colon and, after a slight decline, remained stable in the descending colon.

CONCLUSION

- Buckwheat/quinoa $BREAD_{LAB}$ has a higher prebiotic effect than $BREAD_C$ and increases the number of beneficial bacteria (Lactobacilli and Bifidobacteria), while reducing the number of Enterobacteria.
- Buckwheat/quinoa $BREAD_{LAB}$ and $BREAD_C$ positively modulate the metabolic activity of adult human colonic microbiota. Feeding the CFDM with $BREAD_{LAB}$ for 14 days increases total SCFA production by 2.7-fold.
- Buckwheat/quinoa $BREAD_{LAB}$ modifies the composition of the total colonic microbiota, mainly in the transverse and descending segments.

REFERENCES

Hernández-Alcántara, A. M., Chiva, R., Mohedano, M. L., Russo, P., Ruiz-Masó, J. Á., del Solar, G., Spano, G., Tamame, M., & López, P. (2022). *Weissella cibaria* riboflavin-overproducing and dextran-producing strains useful for the development of functional bread. Frontiers in Nutrition, 9, 978831–978831. https://doi.org/10.3389/fnut.2022.978831