Novel synbiotic African cereal-based product: nutritional, physicochemical, and microbiological characterization

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Introduction

The development of fermented functional probiotic cereal-based products has been gaining interest, motivated by the high prevalence of lactose[1] or gluten[2] intolerances, and also by established trends such as vegetarianism[3]. Lactic acid bacteria have been used as starter cultures on controlled fermentation, contributing successfully to product quality, safety and functionality, with exopolysaccharides[4] (EPS) having an important technological role, bringing the advantage of being a suitable natural alternative to chemical food additives, such as hydrocolloids.

Objectives

- To select a bacterial consortium (including the probiotic *Lactiplantibacillus plantarum* 299v strain and one of four indigenous *Weissella confusa/cibaria* strains, previously isolated from traditionally fermented African cereal-based products) to be used as a starter culture for the development of a novel synbiotic cereal-based product;
- To characterize the nutritional, physicochemical and microbiological profiles of the resulting fermented whole grain cereal-based product.

Methods

Grains' **collection** from local producers (Kenya and Burkina Faso)

Dry milling
Two-stage process

< 500 µm

Sterilization
(120 ºC, 15 min)

Suspension in a sucrose-based solution

Strains' **inoculation** (1%)
1 indigenous and/or 1 probiotic strain

Fermentation
(30 ºC, 200 rpm, 8 h, orbital shaker)
 # total of 12 runs

Each run: 1 type of cereal + 2 types of bacterial cultures

Red sorghum
Sorghum bicolour (L.) *Moench*

Finger millet
Eleusine coracana (L.) *Gaertn*

Pearl millet
Pennisetum glaucum (L.)

Plain 1 of 4 EPS-producer indigenous strains:
Weissella confusa 2LABPT05
Weissella confusa/cibaria 32LABPT05
Weissella confusa/cibaria C2
Weissella confusa/cibaria C5

Combined 1 commercial probiotic strain
Lactiplantibacillus plantarum 299v
+ 1 of the 4 previous indigenous strains

Selection of the best bacterial consortium and cereal matrix

Fermentation run at the same conditions
Output: fermented cereal-based yoghurt-like beverage (YLB)

CHARACTERIZATION

Microbiological
- Microbial growth:
  - fermentation
  - storage

(fresh YLB, at 4ºC; freeze-dried YLB, at room temperature)

Physicochemical
- Acidification
- Organic acids
- Apparent viscosity
- Dextran

Nutritional
- Macronutrient composition
- Protein digestibility
- Amino acids
- Minerals

Results

Selection of the best bacterial consortium and cereal matrix

Red sorghum *vs.* Finger millet (FM) *vs.* Pearl millet (PM)

- Sorghum was not fermented by the strains, except for the strain C2 (both cultures) ($p \leq 0.05$);
- Generally, *Lactiplantibacillus* and *Weissella* strains grew better in Finger millet.

Plain *vs.* combined cultures

- The probiotic strain did not influence impactfully the performance of the indigenous strain;
- *L. plantarum* grew better when combined with the strains 32LABPT05 and 2LABPT05;
- 32LABPT05 and 2LABPT05 strains' fermentation produced a more viscous cereal matrix.

L. plantarum 299v + *W. confusa* 2LABPT05
Finger millet

Microbiological, physicochemical and nutritional characterization

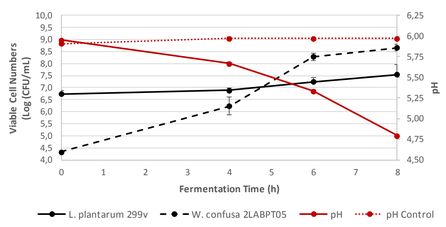

Figure 1 Bacterial growth (black lines) and acidification (red lines) in the finger millet slurry, during the fermentation process. Error bars represent the standard deviation of independent replicate slurries.

Table 1 Physicochemical characterization of unfermented and fermented Finger millet yoghurt-like beverage (YLB), by *W. confusa* 2LABPT05 co-cultured with *L. plantarum* 299v, over 8 h, at 30 ºC and 200 rpm, in an orbital incubator.

	Unfermented slurry	Fermented YLB (F-YLB)
Lactic Acid (g/kg YLB)	< LOD[1]	2.69 ± 0.09
Acetic Acid (g/kg YLB)	< LOD[1]	0.70 ± 0.08
Sucrose (g/kg YLB)	64 ± 8 [a]	38 ± 6 [a]
Glucose (g/kg YLB)	10 ± 2	7.0 ± 0.8 [a]
Fructose (g/kg YLB)	4.9 ± 0.8 [a]	21 ± 3 [b]
Protein digestibility (%)	25 ± 2 [a]	66 ± 2 [b]
Fe (mg/kg YLB)	4.74 ± 0.09 [a]	4.6 ± 0.3 [a]
Mg (mg/kg YLB)	134 ± 1 [a]	126 ± 1 [b]
Mn (mg/kg YLB)	18.3 ± 0.7 [a]	17.1 ± 0.1 [a]
K (mg/kg YLB)	303 ± 2 [a]	312 ± 8 [a]
Na (mg/kg YLB)	3.4 ± 0.1 [a]	32 ± 2 [b]
P (mg/kg YLB)	244 ± 3 [a]	238 ± 2 [b]
Ca (mg/kg YLB)	365 ± 9 [a]	319 ± 2 [b]
Zn (mg/kg YLB)	1.13 ± 0.04 [a]	0.95 ± 0.01 [b]
Apparent viscosity (mPa.s), 20 ºC	11.9 ± 0.4 [a]	35 ± 2 [b]
Apparent viscosity (mPa.s), 8 ºC	13.2 ± 0.4 [a]	102 ± 35
Average Dextran (%), dry weight	0.3 ± 0.09 [a]	16.1 ± 0.9

Different letters within each row are significantly different ($p \leq 0.05$), using the Paired sample T-test or Wilcoxon test. [1]LOD: Limit of detection (Acetic and lactic acids: 0.05 g/L).

Strains Stability Storage

Refrigerated, at 4 ºC, for 7 days: both strains above 10^8 CFU/mL. Storage conditions were not critical for their survival;

Freeze-dried, at room temperature, for 12 weeks: sorbitol (1%) protected both strains. *Lactiplantibacillus*' viable counts reduced 5% from the ninth week on ($p \leq 0.05$).

Nutritional Label

Nutrients	Per 100g of fermented *smoothie*
Energy (kcal/kJ)	57/238
Fat (g)	0.1
saturated (g)	< 0.01
Carbohydrates (g)	15.2
sugars (g)	6.2
Fibre (g)	4.0
Protein (g)	0.7
Salt (g)	< 0.1

✓ High Fibre ✓ Fat-free

EPS (dextran) characterization

Composed by α–(1 → 6) glycosidic linkages (97%) in the main chain and few α–(1 → 3) branched linkages (3%).

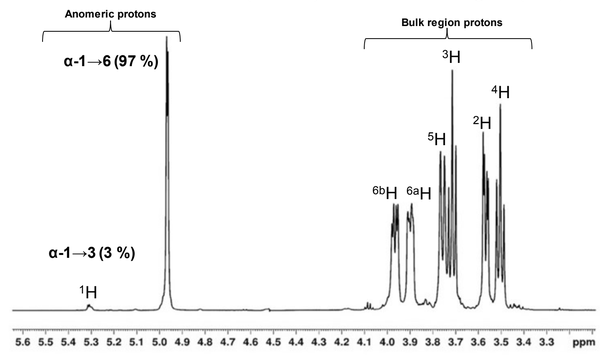

Figure 2 The 1D 1H nuclear magnetic resonance spectrum of EPS produced by W. confusa 2LABPT05) recorded at 600 MHz in D20 at 50 ºC. The peaks are referenced to internal acetone (1H = 2.225 ppm).

Conclusions

- The bacterial consortium allowed the development of a **novel functional finger millet-based product**, characterized by its content of **probiotic** microorganisms within the minimum required threshold (10^7 CFU/g), and an interesting slimy and viscous texture, improved by the production of microbial EPS, which acted as a natural texture improver;
- The resulting product was proven to be an **added value product**: innovative, and with valuable nutritional profile (high fibre, interesting amino acids content and protein digestibility);
- A freeze-dried version of the yoghurt-like beverage can be easily transported due to **longer shelf life**, targeting **international markets**, such as Europe and Africa, and different population groups from children to the elderly and lactose or gluten intolerants.

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