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Short Communication

Can broad bean (*Vicia faba*) and white lupin (*Lupinus albus*) flours serve as carbon sources to support probiotic growth? ☆

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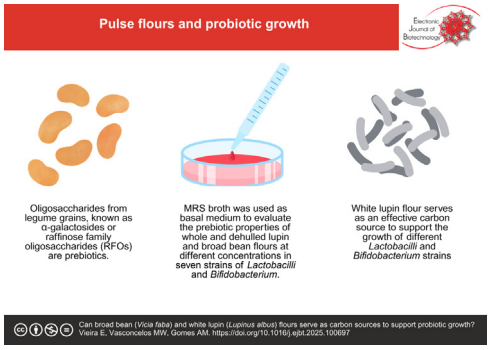
GRAPHICAL ABSTRACT

Can broad bean (*Vicia faba*) and white lupin (*Lupinus albus*) flours serve as carbon sources to support probiotic growth?



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ABSTRACT

Background: There is growing interest in identifying substrates that support the growth of probiotics in foods. Pulses are an excellent source of nutrients and bioactive compounds, including non-digestible oligosaccharides from the α-galactoside group, which are probiotic growth factors. This study aimed to evaluate the potential of white lupin and broad bean flours to support the growth of seven probiotic strains of *Lactobacilli* and *Bifidobacterium*.
Results: Different Man-Rogosa-Sharpe broth media were prepared using whole or dehulled flour as carbon sources at different concentrations (20, 30, 40, and 60 g/L) and inoculated with 2% (w/v) of each probiotic strain. Viable cell numbers and medium acidification were monitored throughout fermentation and compared to negative (MRS without a carbon source) and positive (MRS with 20 g/L glucose) controls. White lupin at 60 g/L concentration proved to be a suitable carbon source for both *Lactobacillus acidophilus* Ki and *Lactobacillus casei* ssp. *paracas*ei L26, while concentrations of 40 g/L and 60 g/L supported *Bifidobacterium animalis* Bb12 growth.
Conclusions: Flour concentration had a greater impact on probiotic growth than composition (hull vs. dehulled). These results suggested that white lupin is a promising ingredient for the development of functional foods.

☆ Audio abstract available in Supplementary material.

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1. Introduction

Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts [1]. Extensive research has confirmed the role of probiotics in treating chronic diseases, modulating host immunity, protecting against infectious and non-infectious diseases, and producing beneficial substances, such as organic acids and short-chain fatty acids [2,3].

Consequently, the consumption of probiotic-enriched food has increased. The most common probiotics used in foods and dietary supplements are bacteria from the genera *Lactobacillus* and *Bifidobacterium* and the yeast species *Saccharomyces boulardii* [1]. To confer health benefits, probiotics must remain viable and at sufficient concentrations after food processing and throughout the product's shelf life. Additionally, they must survive the acidic pH of the gastric environment, reach the small intestine, and colonize the host's gut [4]. Owing to the challenges of maintaining probiotic viability in certain food matrices [3], there is growing interest in investigating substrates that support these microorganisms.

Pulses have emerged as promising candidates because of their health, nutritional, and environmental benefits. They are accessible, versatile, and functional ingredients, particularly in flour form, allowing their incorporation into various food matrices [5]. Pulses are rich in non-digestible oligosaccharides, specifically raffinose family oligosaccharides (RFOs), which include raffinose, stachyose, and verbascose [6]. These RFOs serve as excellent growth factors for probiotics [7].

Lupins (*Lupinus albus*) and broad beans (*Vicia faba*), two pulses traditionally found in the Mediterranean region, are rich in RFOs [7]. Recently, the use of lupin has increased due to its nutritional density and potential health benefits [8]. Additionally, broad beans have shown agronomical advantages, being one of the most efficient nitrogen-fixing legumes with high crop yields [9,10]. The global lupin market is projected to register a Compound Annual Growth Rate (CAGR) of 5.1% [11]. In contrast, the broad bean market is projected to register a 4.0% CAGR [12] during the forecast period of 2021–2026.

Few studies have demonstrated the capacity of lupin and broad beans to support the growth of probiotic bacteria [13,14]. Most of these studies have used extracted fractions rather than whole grains, which are the most common form of consumption. Given the rising interest in these pulses, it is essential to identify their applications. Therefore, this study aimed to comparatively evaluate the effectiveness of whole and dehulled white lupin and broad bean flours at different concentrations as carbon sources to support the growth of seven probiotic strains of *Lactobacillus* and Bifidobacterium. These findings support the development of functional foods.

2. Materials and methods

2.1. Broad bean and lupin flours

Broad beans (*Vicia faba*) and white lupin (*Lupinus albus*) were obtained from a local producer in Mirandela, Portugal. Whole and dehulled grains were milled into fine powder using a Thermomix® TM31 commercial food processor (Vorwerk, Germany).

2.2. Microorganisms

Seven commercial probiotic strains were used in this study: *Lactobacillus acidophilus* Ki and LAFTI® L10, *Lactobacillus casei* LAFTI® L26 (currently Lacticaseibacillus casei), *Lactobacillus rhamnosus* R11 (currently Lacticaseibacillus rhamnosus), *Bifidobacterium animalis* Bb12 and *Bifidobacterium animalis* Bo, and *Bifidobacterium breve* National Collection of Industrial and Marine Bacteria 702258. *L. acidophilus* Ki and *B. animalis* Bo, previously isolated from fermented milk, were obtained from CSK (Netherlands). *B. animalis* Bb12 was obtained from Christian Hansen (Denmark). *L. acidophilus* LAFTI® L10 and *L. casei* LAFTI® L26 were procured from DSM Food Specialties (Australia), and *L. rhamnosus* R11 was obtained from LALLEMAND Bio-Ingredients (Canada). All strains were provided as ultra-frozen concentrates.

The strains were reactivated by pre-inoculation in de Man-Rogosa-Sharpe broth (MRS; Biokar Diagnostics, France) and incubated overnight at 37°C. At least two subsequent culturing steps were performed under the same growth conditions after the initial inoculation of 100–200 µL concentrate in 15 mL MRS broth. The cell biomass was harvested by centrifuging (Sorvall LYNX 4000, Thermo Scientific) at a final volume of 15 ml of MRS broth containing each strain at 5000 rpm for 20 min at 4°C. The biomass was washed once with an equal volume of 8.5 g L^{-1} NaCl solution. After centrifugation, the supernatant was discarded and the pelleted biomass was resuspended in 8.5 g L^{-1} NaCl solution. For *Bifidobacterium animalis* Bb12 and Bo, and *B. breve,* the MRS broth was supplemented with filter-sterilized 0.5 g L^{-1} L-cysteine·HCl (Fluka, Switzerland) to lower the redox potential. These cultures were incubated in a plastic anaerobic jar with a GasPak™EZ sachet (Becton, Dickinson and Company, USA) to maintain the anaerobic conditions.

2.3. Media

MRS broth was used as the basal medium to evaluate the probiotic growth-promoting properties of lupin and broad bean flour. The medium was prepared by substituting the conventional carbon source (glucose) with various other ingredients. The fermentation medium contained 10 g L^{-1} peptone (Sigma-Aldrich, USA), 10 g L^{-1} meat extract (Merck, Germany), 5 g L^{-1} yeast extract (Biokar Diagnostics), 2 g L^{-1} dipotassium phosphate (Merck), 1.08 g L^{-1} Tween 80 (Merck), 5 g L^{-1} sodium acetate (Merck), 2 g L^{-1} ammonium citrate tribasic (Sigma-Aldrich), 0.2 g L^{-1} magnesium sulfate (Merck), and 0.05 g L^{-1} manganese sulfate (Sigma-Aldrich). As a positive control, 20 g L^{-1} glucose (Sigma-Aldrich) was added to the medium. MRS without a carbon source was combined with different concentrations (20, 30, 40, and 60 g L^{-1}) of whole or dehulled lupin and broad bean flour. For *B. animalis* Bb12 and Bo, *B. breve,* and *L. acidophilus* Ki, all media were supplemented with filter-sterilized 0.5 g L^{-1} L-cysteine·HCl and incubated at 37°C under anaerobic conditions.

2.4. Evaluation of pulse flours as a carbon source to support probiotic bacterial growth

The fermentability potential of lupin and broad bean flours was evaluated by screening for flour incorporation, stirring, and steril-

ization methods (data not shown) to select those that would not affect the structure and activity of the flours. The effect of pulse hulls on the acidification of probiotic strains and microbial growth was determined. A test was conducted to compare whole-grain flour with dehulled-grain flour at three concentrations (20, 30, and 40 g L^{-1}), focusing on one *Bifidobacterium* and one *Lactobacillus* strain (*B. animalis* Bb12 and *L. acidophilus* L10, respectively). Subsequently, the concentration range was expanded to 20, 40, and 60 g L^{-1} for lupin and broad bean whole flours. These were tested in MRS medium with seven probiotic strains (*L. acidophilus* Ki, *L. acidophilus* L10, *L. rhamnosus* R11, *L. casei* L26, *B. animalis* Bo, *B. breve*, and *B. animalis* Bb12) to select the optimal pulse type/concentration combination based on microbial growth and acidification.

2.4.1. Incorporation of lupin and broad bean flours and sterilization method

Flour was added to the basal medium without a carbon source at the intended concentration and homogenized using an Ultraturrax (ICA Works, USA) for 3 min at 10.000 rpm to ensure optimum homogenization. The resulting medium was sterilized by autoclaving at 110°C for 10 min.

2.4.2. Determination of the dehulling effect on bacterial growth by viable cell determination

MRS broth was prepared with glucose (20 g L^{-1}) and without any carbon source as the positive and negative controls, respectively. Three concentrations of lupin and broad bean flour (20, 30, and 40 g L^{-1}) were added to the MRS broth without a carbon source. Each sterilized medium was transferred to 50-mL Schott® flasks (*L. acidophilus* L10) or 50-mL flat-bottom glass bottles with narrow necks (*B. animalis* Bb12) and inoculated at 2% (v/v) with the respective strain; less technologically demanding strains were selected for this stage. The assay was performed in duplicate. Inoculated glass flasks were incubated at 37°C with orbital shaking at 150 rpm. Growth was monitored by enumerating viable cell numbers (CFU/mL) at 0, 4, 6, 8, and 24 h (in duplicate). At each sampling time, decimal dilutions were prepared using peptone-saline water (8.5 g L^{-1} sodium chloride; 1 g L^{-1} peptone) up to 10^{-6} and plated on MRS agar using the Miles and Misra [15] method.

2.4.3. Evaluation of probiotic growth-promoting potential by viable cell numbers determination and medium acidification

Seven probiotic strains were selected to confirm the growth-promoting potential of the whole-grain pulse flours: *L. acidophilus* L10, *L. acidophilus* Ki, *L. casei* L26, *L. rhamnosus* R11, *B. animalis* Bb12, *B. animalis* Bo, and *B. breve*. This array of strains was used because it has been demonstrated that the use of a specific carbon source varies among different genera, species, and even strain levels. Growth curves were monitored by enumeration of viable cells and medium acidification was determined by measuring the pH.

MRS basal broth was prepared with 20 g L^{-1} glucose (positive control) or without a carbon source (negative control). Three concentrations (20, 40, and 60 g L^{-1}) of whole-grain pulse flour were added to the MRS basal broth without a carbon source. For aerobic strains (*L. acidophilus* L10, *L. casei* L26, and *L. rhamnosus* R11), 100 mL Schott flasks were used for inoculation. For anaerobic strains (*B. animalis* Bb12 and Bo, *B. breve,* and *L. acidophilus* Ki), flat-bottom glass bottles with narrow necks were used to minimize oxygen contact. Each probiotic strain was inoculated at 2% (w/v) into each experimental medium, and the assay was performed in duplicate for each strain.

Glass bottles (for anaerobic microorganisms) and Schott flasks (for aerobic microorganisms) were incubated as discussed in Section 2.4.2. The decimal dilutions and plating conditions have been described in the previous section.

In addition, acid production was monitored by measuring pH during incubation to confirm substrate utilization as a carbon source during fermentation. The pH meter was calibrated using reference buffer solutions at pH levels of 4.0 and 7.0. The samples were blended before the pH measurement.

2.5. Statistical analysis

Statistical analyses were performed using the SPSS software (version 17.0; SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used to assess data normality. For normally distributed data, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. Non-normally distributed data were analyzed using Kruskal-Wallis and Dunn's post-hoc tests. Differences between the means were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Determination of the dehulling effect on bacterial growth

The growth of *L. acidophilus* L10 and *B. animalis* Bb12 was monitored in media containing three different concentrations (20, 30, and 40 g L^{-1}) of pulse flour over 24 h of fermentation at 37°C by enumerating viable cell numbers (Fig. 1a–d). Glucose (positive control) promoted the best growth of both strains (Fig. 1), compared to lupin and broad bean flours, regardless of the presence or absence of hulls. No significant differences were observed between groups ($p > 0.05$). However, for B. animalis Bb12 incubated in MRS basal medium containing 40 g L^{-1} of lupin flour, with or without hull, viable cell numbers at 24 h of fermentation were 1 log cycle higher than those in the MRS positive control. The absence of hulls did not significantly affect the growth behavior of *B. animalis* Bb12 compared to its whole-grain counterparts. This can be attributed to the presence of growth-promoting factors (primarily fiber and α-galactosides) within the cotyledons [6].

Generally, seed dehulling reduces the anti-nutritional composition and alters the nutrient profile [16]. Moreover, hull removal is associated with lower antioxidant activity [17], a biological property that should ideally be preserved. Given that no significant differences were observed between whole-grain flour, dehulled lupin flour, and broad bean flour, whole-grain flour was selected for subsequent experiments due to its simpler preparation process and reduced nutrient loss.

3.2. Evaluation of the probiotic growth-promoting potential of flours

To assess the potential of pulses to promote probiotic growth and acidification, three concentrations of lupin and broad bean flours were added to MRS basal medium without a carbon source and tested against seven *Lactobacillus* and *Bifidobacterium* strains. The growth curves are illustrated in Fig. 2a–g and the acidification capacity after 36-h of fermentation is shown in Table 1. Generally, lupin flour supported strain growth more effectively than broad bean flour at similar concentrations, sometimes producing results comparable to or better than those of glucose.

The utilization of legume flours as growth promoters by different *Lactobacillus* and *Lacticaseibacillus* strains demonstrated species- and strain-level specificity, consistent with previous findings [18,19]. *L. acidophilus* Ki (Fig. 2a) and *L. rhamnosus* R11 (Fig. 2c) strains effectively metabolized legume flours in basal MRS media. At 60 g L^{-1} lupin flour, *L. acidophilus* Ki growth was significantly better ($p < 0.05$) than that of the positive control after 12 h of fer-

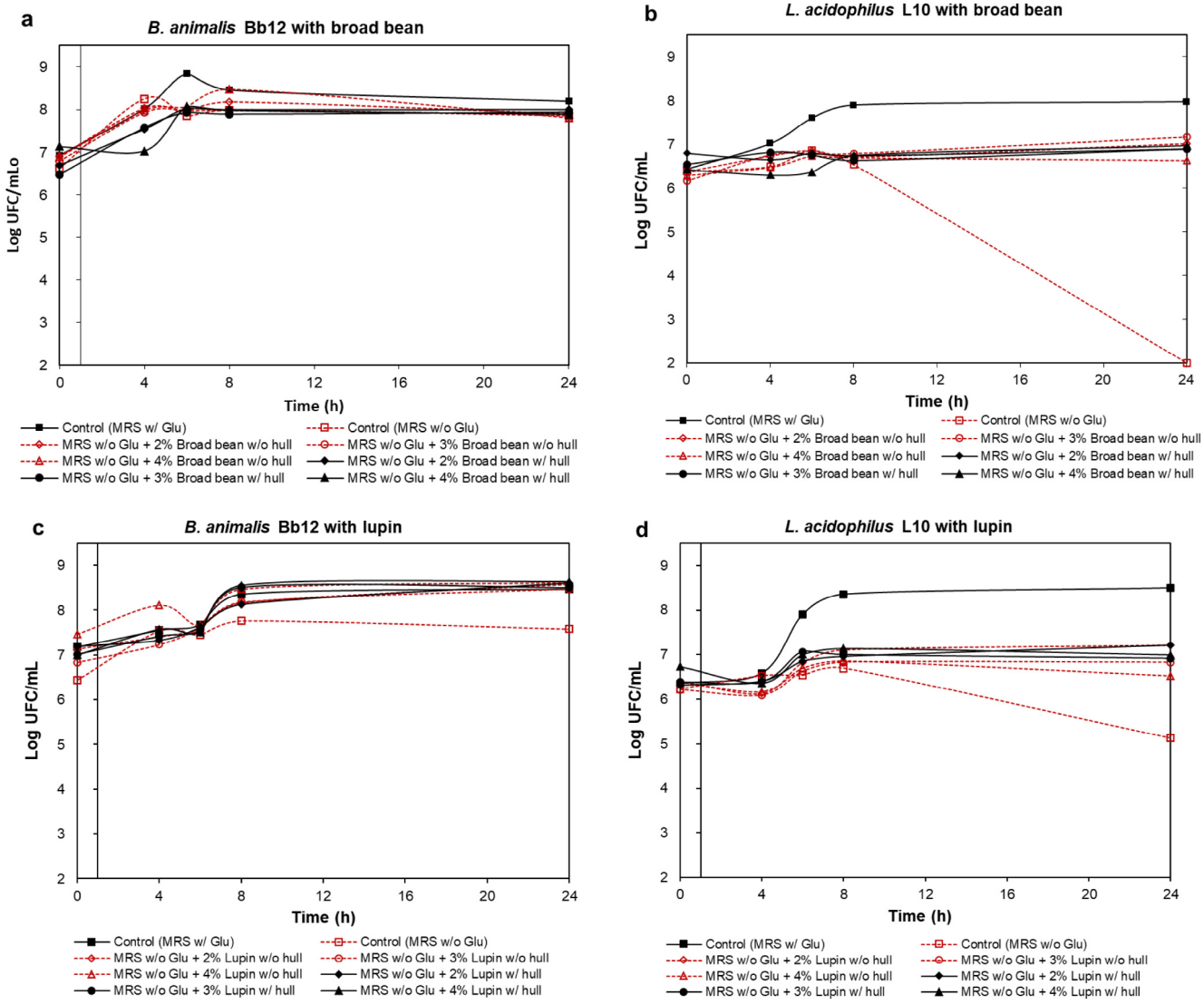

Fig. 1. Growth curves of *B. animalis* Bb12 (a and c) and *L. acidophilus* L10 (b and d) in MRS with or without glucose, and MRS with broad bean (a and b) or lupin (c and d) flour, with or without hull, at different concentrations: with 20 g L^{-1} flour, with 30 g L^{-1} flour, with 40 g L^{-1} flour.

mentation (almost 1 log cycle higher). *L. rhamnosus* R11 achieved growth comparable to that of glucose by 36 h (9.3 log CFU/mL), albeit with a prolonged 12 h adaptation period, compared to the 4 h initiation of the logarithmic growth phase reported for glucose.

L. acidophilus L10 showed no growth impact from either flour, regardless of concentration. The initial viable cell numbers remained relatively constant or decreased over time compared to those reported in glucose medium (Fig. 2b), supporting the strain-specific traits observed for novel prebiotic sources. For *L. casei* L26 (Fig. 2d), the viable cell numbers at 12 h (stationary phase) were significantly higher ($p < 0.05$) in lupin flour (all concentrations) and glucose than in broad bean flour.

All *Bifidobacterium* strains demonstrated a good capacity to use legume flour as a carbon source, albeit to varying degrees. Higher concentrations of both flours (40 g L^{-1} and 60 g L^{-1}) enabled *B. breve* to increase by almost 3.0 log cycles, reaching viable cell numbers similar to those of the positive control after 24 h of fermentation (Fig. 2f). *B. animalis* strains (Fig. 2e and Fig. 2g) showed an increase of about 1.5 log cycles with 40 g/L and 60 g/L of lupin flour.

For B. animalis Bb12, growth curves with lupin flour at 40 g L^{-1} and 60 g L^{-1} were similar to glucose (positive control), with significantly higher ($p < 0.05$) viable cell numbers (8.7 and 8.8 log CFU/mL, respectively) after 36 h of fermentation compared to the other concentrations of both flours (Fig. 2g). *B. animalis* Bo showed higher ($p < 0.05$) viable cell numbers with 60 g L^{-1} of lupin flour ($p < 0.05$) than the positive control.

Metabolic activity, reflected by acidification (pH decrease) during fermentation, was generally aligned with the growth curves of *Lactobacillus, Lacticaseibacillus*, and *Bifidobacterium* strains (Table 1). This indicates the effective metabolism of legume flours, although at varying rates and to varying extents. As expected, glucose (positive control) resulted in the highest acidification rate across all species and strains, whereas acidification was insignificant without a carbon source. MRS basal media with 40 g L^{-1} and 60 g L^{-1} lupin flour and 60 g L^{-1} broad bean flour produced results similar to those reported for the positive control.

White lupin flour is a suitable carbon source for both *Lactobacillus* and *Bifidobacterium* strains, consistent with previous studies [13,20]. Other legume flours have also demonstrated this property

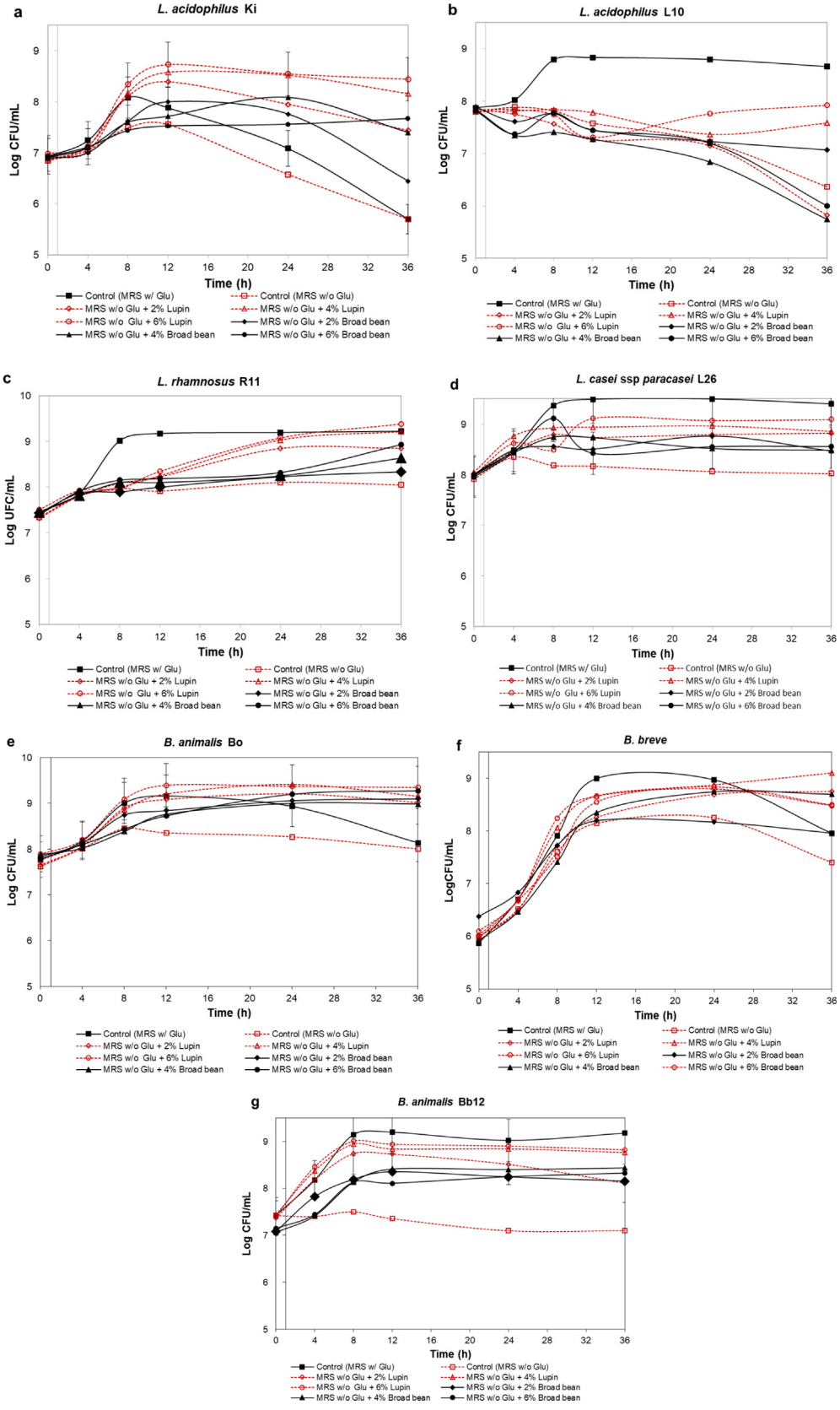

Fig. 2. Growth curves of *L. acidophilus* Ki (a), *L. acidophilus* L10 (b), *L. rhamnosus* R11 (c), *L. casei* ssp *paracasei* L26 (d), *B. animalis* Bo (e), *B. breve* (f), and *B. animalis* Bb12 (g) in MRS with or without glucose, and MRS with broad bean or lupin flour, in different concentrations: with 20 g L^{-1} flour, with 40 g L^{-1} flour, with 60 g L^{-1} flour.

Table 1
Variation of pH for the seven probiotic strains tested in the different MRS culture media with or without glucose or supplemented with either 20, 40 or 60 g L^{-1} of lupin or broad bean flours.

Strain	Incubation time (h)	Glucose 20 g L^{-1}	Without glucose	Lupin Flour 20 g L^{-1}	Lupin Flour 40 g L^{-1}	Lupin Flour 60 g L^{-1}	Broad Bean 20 g L^{-1}	Broad Bean 40 g L^{-1}	Broad Bean 60 g L^{-1}
***L. casei* ssp *paracasei* L26**	0	6.54 ± 0.01^{b}	7.23 ± 0.00^{a}	6.90 ± 0.00^{c}	6.73 ± 0.01^{b}	6.58 ± 0.00^{b}	6.79 ± 0.12^{c}	6.61 ± 0.00^{c}	6.54 ± 0.01^{b}
	4	5.68 ± 0.04^{b}	6.8 ± 0.02^{a}	6.39 ± 0.01^{c}	6.25 ± 0.00^{b}	6.14 ± 0.00^{b}	6.17 ± 0.01^{c}	6.11 ± 0.07^{c}	6.11 ± 0.01^{b}
	8	4.67 ± 0.00^{b}	6.63 ± 0.00^{a}	6.15 ± 0.02^{c}	5.95 ± 0.00^{b}	5.84 ± 0.00^{b}	6.02 ± 0.00^{c}	6 ± 0.00^{c}	5.96 ± 0.00^{b}
	12	4.21 ± 0.00^{b}	6.66 ± 0.02^{a}	5.95 ± 0.02^{c}	5.67 ± 0.00^{b}	5.5 ± 0.02^{b}	5.91 ± 0.00^{c}	5.9 ± 0.02^{c}	5.81 ± 0.00^{b}
	24	3.68 ± 0.00^{b}	6.72 ± 0.01^{a}	5.82 ± 0.02^{c}	5.41 ± 0.00^{b}	5.21 ± 0.00^{b}	5.84 ± 0.01^{c}	5.91 ± 0.17^{c}	5.63 ± 0.02^{b}
	36	3.81 ± 0.00^{b}	6.79 ± 0.02^{a}	5.84 ± 0.04^{c}	5.43 ± 0.01^{b}	5.25 ± 0.02^{b}	5.81 ± 0.01^{c}	5.86 ± 0.18^{c}	5.56 ± 0.08^{b}
	ΔpH	3.02	0.44	1.06	1.30	1.33	0.98	0.75	0.98
***L. acidophilus* Ki**	0	6.49 ± 0.00^{b}	6.78 ± 0.00^{a}	6.55 ± 0.00^{ab}	6.37 ± 0.00^{b}	6.3 ± 0.01^{b}	6.65 ± 0.00^{a}	6.58 ± 0.00^{ab}	6.52 ± 0.00^{ab}
	4	6.23 ± 0.00^{b}	6.66 ± 0.00^{a}	6.36 ± 0.00^{ab}	6.19 ± 0.00^{b}	6.12 ± 0.01^{b}	6.42 ± 0.03^{a}	6.36 ± 0.00^{ab}	6.29 ± 0.00^{ab}
	8	4.81 ± 0.00^{b}	6.26 ± 0.00^{a}	5.54 ± 0.04^{ab}	5.28 ± 0.00^{b}	5.18 ± 0.01^{b}	5.78 ± 0.00^{a}	5.56 ± 0.00^{ab}	5.45 ± 0.00^{ab}
	12	4.32 ± 0.00^{b}	6.26 ± 0.00^{a}	5.15 ± 0.04^{ab}	4.76 ± 0.00^{b}	4.54 ± 0.00^{b}	5.47 ± 0.03^{a}	5.47 ± 0.00^{ab}	5.33 ± 0.00^{ab}
	24	4.01 ± 0.04^{b}	6.21 ± 0.00^{a}	5.12 ± 0.03^{ab}	4.78 ± 0.00^{b}	4.59 ± 0.00^{b}	5.31 ± 0.01^{a}	4.97 ± 0.00^{ab}	5.2 ± 0.00^{ab}
	36	3.96 ± 0.04^{b}	6.24 ± 0.00^{a}	5.14 ± 0.03^{ab}	4.81 ± 0.01^{b}	4.64 ± 0.01^{b}	5.3 ± 0.02^{a}	5.02 ± 0.04^{ab}	4.84 ± 0.01^{ab}
	ΔpH	2.53	0.54	1.41	1.56	1.66	1.35	1.53	1.68
***L. acidophilus* L10**	0	6.72 ± 0.00^{b}	7 ± 0.12^{a}	6.82 ± 0.00^{a}	6.62 ± 0.01^{b}	6.47 ± 0.00^{b}	6.93 ± 0.01^{a}	6.83 ± 0.00^{a}	6.73 ± 0.04^{c}
	4	6.21 ± 0.02^{b}	6.77 ± 0.00^{a}	6.54 ± 0.00^{a}	6.39 ± 0.00^{b}	6.24 ± 0.00^{b}	6.62 ± 0.00^{a}	6.52 ± 0.00^{a}	6.48 ± 0.00^{c}
	8	4.76 ± 0.01^{b}	6.97 ± 0.00^{a}	6.79 ± 0.02^{a}	6.64 ± 0.02^{b}	6.52 ± 0.01^{b}	6.81 ± 0.00^{a}	6.85 ± 0.02^{a}	6.84 ± 0.02^{c}
	12	4.34 ± 0.16^{b}	7.35 ± 0.07^{a}	7.05 ± 0.02^{a}	6.86 ± 0.02^{b}	6.75 ± 0.00^{b}	7 ± 0.00^{a}	6.95 ± 0.00^{a}	6.8 ± 0.02^{c}
	24	3.77 ± 0.00^{b}	7.19 ± 0.01^{a}	6.94 ± 0.00^{a}	6.8 ± 0.00^{b}	6.65 ± 0.00^{b}	7.01 ± 0.01^{a}	6.84 ± 0.02^{a}	6.81 ± 0.00^{c}
	36	3.64 ± 0.01^{b}	7.17 ± 0.02^{a}	6.93 ± 0.02^{a}	6.8 ± 0.00^{b}	6.64 ± 0.01^{b}	6.97 ± 0.02^{a}	6.81 ± 0.01^{a}	6.79 ± 0.00^{c}
	ΔpH	3.08	−0.17	−0.11	−0.18	−0.17	−0.04	0.02	−0.06
***L. rhamnosus* R11**	0	6.81 ± 0.00^{b}	7.19 ± 0.00^{a}	6.9 ± 0.02^{b}	6.71 ± 0.00^{b}	6.52 ± 0.00^{b}	7.03 ± 0.00^{a}	6.91 ± 0.01^{c}	6.82 ± 0.01^{b}
	4	6.13 ± 0.00^{b}	6.72 ± 0.00^{a}	6.46 ± 0.00^{b}	6.33 ± 0.00^{b}	6.18 ± 0.00^{b}	6.59 ± 0.01^{a}	6.48 ± 0.00^{c}	6.42 ± 0.00^{b}
	8	4.66 ± 0.00^{b}	6.71 ± 0.00^{a}	6.49 ± 0.02^{b}	6.35 ± 0.00^{b}	6.19 ± 0.00^{b}	6.65 ± 0.00^{a}	6.54 ± 0.00^{c}	6.49 ± 0.03^{b}
	12	4.21 ± 0.00^{b}	6.75 ± 0.02^{a}	6.58 ± 0.01^{b}	6.38 ± 0.00^{b}	6.19 ± 0.00^{b}	6.66 ± 0.01^{a}	6.58 ± 0.00^{c}	6.5 ± 0.02^{b}
	24	3.55 ± 0.00^{b}	6.87 ± 0.00^{a}	6.26 ± 0.03^{b}	5.83 ± 0.03^{b}	5.62 ± 0.00^{b}	6.64 ± 0.02^{a}	6.43 ± 0.01^{c}	6.36 ± 0.00^{b}
	36	3.57 ± 0.00^{b}	6.95 ± 0.00^{a}	6.08 ± 0.00^{b}	5.58 ± 0.03^{b}	5.33 ± 0.02^{b}	6.59 ± 0.00^{a}	6.45 ± 0.00^{c}	6.31 ± 0.01^{b}
	ΔpH	3.24	0.24	0.82	1.13	1.19	0.44	0.46	0.51
***B. animalis* Bb12**	0	6.82 ± 0.02^{b}	7.12 ± 0.00^{a}	6.83 ± 0.01^{b}	6.62 ± 0.00^{b}	6.47 ± 0.00^{b}	6.99 ± 0.00^{ab}	6.86 ± 0.00^{b}	6.77 ± 0.00^{b}
	4	6.54 ± 0.05^{b}	7.03 ± 0.00^{a}	6.22 ± 0.00^{b}	5.89 ± 0.07^{b}	5.76 ± 0.00^{b}	6.5 ± 0.00^{ab}	6.46 ± 0.00^{b}	6.38 ± 0.00^{b}
	8	5.53 ± 0.00^{b}	7.01 ± 0.00^{a}	5.33 ± 0.00^{b}	4.87 ± 0.00^{b}	4.66 ± 0.00^{b}	5.77 ± 0.02^{ab}	5.35 ± 0.04^{b}	5.13 ± 0.02^{b}
	12	5.01 ± 0.06^{b}	7.05 ± 0.00^{a}	5.35 ± 0.01^{b}	4.87 ± 0.00^{b}	4.66 ± 0.01^{b}	5.66 ± 0.02^{ab}	5.17 ± 0.02^{b}	4.93 ± 0.01^{b}
	24	4.45 ± 0.00^{b}	7.05 ± 0.00^{a}	5.33 ± 0.00^{b}	4.87 ± 0.00^{b}	4.67 ± 0.01^{b}	5.49 ± 0.04^{ab}	5 ± 0.01^{b}	4.76 ± 0.01^{b}
	36	4.32 ± 0.03^{b}	6.98 ± 0.00^{a}	5.31 ± 0.00^{b}	5.02 ± 0.01^{b}	4.86 ± 0.00^{b}	5.42 ± 0.02^{ab}	4.95 ± 0.01^{b}	4.68 ± 0.01^{b}
	ΔpH	3.50	0.14	1.52	1.60	1.61	1.57	1.73	2.09
***B. animalis* Bo**	0	6.6 ± 0.01^{b}	6.9 ± 0.00^{a}	6.6 ± 0.01^{a}	6.5 ± 0.00^{b}	6.3 ± 0.00^{b}	6.8 ± 0.00^{a}	6.8 ± 0.04^{a}	6.6 ± 0.00^{ab}
	4	5.8 ± 0.02^{b}	6.4 ± 0.00^{a}	6.2 ± 0.00^{a}	6.0 ± 0.00^{b}	5.9 ± 0.00^{b}	6.3 ± 0.00^{a}	6.2 ± 0.00^{a}	6.2 ± 0.00^{ab}
	8	4.3 ± 0.00^{b}	6.1 ± 0.04^{a}	5.7 ± 0.06^{a}	5.5 ± 0.04^{b}	5.4 ± 0.00^{b}	6.1 ± 0.04^{a}	6.0 ± 0.00^{a}	6.0 ± 0.00^{ab}
	12	3.9 ± 0.01^{b}	6.1 ± 0.01^{a}	5.5 ± 0.07^{a}	5.3 ± 0.05^{b}	5.1 ± 0.00^{b}	6.1 ± 0.08^{a}	5.9 ± 0.00^{a}	5.9 ± 0.02^{ab}
	24	3.6 ± 0.02^{b}	6.2 ± 0.11^{a}	5.5 ± 0.06^{a}	5.3 ± 0.06^{b}	5.1 ± 0.00^{b}	5.6 ± 0.02^{a}	5.4 ± 0.03^{a}	5.4 ± 0.02^{ab}
	36	3.6 ± 0.00^{b}	6.1 ± 0.11^{a}	5.6 ± 0.07^{a}	5.3 ± 0.06^{b}	5.1 ± 0.00^{b}	5.7 ± 0.13^{a}	5.3 ± 0.00^{a}	5.2 ± 0.00^{ab}
	ΔpH	3.00	0.80	1.00	1.20	1.20	1.10	1.50	1.40
B. breve	0	6.36 ± 0.00^{a}	6.67 ± 0.00^{a}	6.44 ± 0.00^{a}	6.29 ± 0.01^{a}	6.2 ± 0.00^{a}	6.56 ± 0.00^{a}	6.5 ± 0.01^{a}	6.45 ± 0.00^{a}
	4	6.35 ± 0.01^{a}	6.66 ± 0.00^{a}	6.44 ± 0.00^{a}	6.28 ± 0.00^{a}	6.19 ± 0.00^{a}	6.55 ± 0.00^{a}	6.49 ± 0.01^{a}	6.43 ± 0.00^{a}
	8	6.12 ± 0.02^{a}	6.5 ± 0.02^{a}	6.27 ± 0.06^{a}	6.01 ± 0.04^{a}	5.79 ± 0.00^{a}	6.44 ± 0.03^{a}	6.39 ± 0.01^{a}	6.28 ± 0.00^{a}
	12	5.02 ± 0.02^{a}	6.14 ± 0.03^{a}	5.75 ± 0.04^{a}	5.4 ± 0.06^{a}	5.08 ± 0.02^{a}	6.06 ± 0.02^{a}	5.88 ± 0.02^{a}	5.63 ± 0.00^{a}
	24	4.4 ± 0.00^{a}	6.13 ± 0.02^{a}	5.39 ± 0.11^{a}	5.07 ± 0.01^{a}	4.94 ± 0.02^{a}	5.71 ± 0.00^{a}	5.51 ± 0.01^{a}	5.38 ± 0.03^{a}
	36	4.35 ± 0.02^{a}	5.68 ± 0.08^{a}	5.24 ± 0.06^{a}	5.01 ± 0.03^{a}	4.89 ± 0.02^{a}	5.46 ± 0.06^{a}	5.4 ± 0.00^{a}	5.31 ± 0.03^{a}
	ΔpH	2.01	0.99	1.20	1.28	1.31	1.10	1.10	1.14

a: negative control; b: positive control. Values with the same superscript letters show no significant difference ($p \geq 0.05$) in relation to the media at the same strain.

[6,21]. Gullón et al. [13] found that both broad bean and white lupin stimulated the growth of probiotic bacteria such as *Bifidobacterium* spp., *Lactobacillus – Enterococcus* group, among others. However, in the current study, the broad bean flour did not significantly support the growth of probiotics.

The differences in probiotic growth support between white lupin and broad bean may be attributed to variations in α-galactoside content and composition. White lupins contain twice the content of α-galactoside as broad beans [22]. Stachyose is the main α-galactoside in white lupin seeds, whereas verbascose is typically undetectable. In contrast, broad beans have the lowest stachyose content among pulses, with verbascose as the primary oligosaccharide [22,23]. Research has shown that probiotic bacteria exhibit limited fermentation of verbascose [24], whereas lactic acid bacteria have been demonstrated to utilize stachyose and raffinose [20]. Therefore, these variations in oligosaccharide composition between white lupin and broad beans could explain the differences observed in the present study.

Various studies conducted *in vitro* [25], in food matrices [26], *in vivo* [27], and in humans [28] have demonstrated the ability of

lupin to promote probiotic bacterial growth, typically using fiber component extracts. Our study achieved similar results using whole lupin flour without extraction, suggesting that the prebiotic benefits of lupin may be attained with minimal processing.

4. Conclusions

This study demonstrated that white lupin flour, particularly at concentrations of 40 g L^{-1} and 60 g L^{-1} (w/v), serves as an effective carbon source to support the growth of different *Lactobacillus* and *Bifidobacterium* strains, especially *L. acidophilus* Ki, *L. rhamnosus* R11, and *B. animalis* (Bo and Bb12). However, the results indicated specificity at both the species and strain levels. This finding suggests that lupin flour has potential as an ingredient for the production of functional food products.

CRediT authorship contribution statement

Evla Vieira: Writing – original draft, Formal analysis. **Marta W. Vasconcelos:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Ana Maria Gomes:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

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Data availability

Data will be made available on request.

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