By-products of the rice processing obtained by controlled debranning as substrates for the production of probiotic bacteria

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Abstract

Cereal debranning is a milling process in which bran layers are sequentially removed by controlled abrasion. Six rice fractions were produced with increasing debranning degrees: A (0-2.3% w/w), B (2.3-3.8% w/w), C (3.8-5% w/w), D (5-6.3% w/w), E (6.3-7.3% w/w) and F (7.3-100% w/w). Fermentation media were prepared from all fractions, and evaluated for supporting the growth of two probiotic strains, *Lactobacillus plantarum* NCIMB 8826 and *Lactobacillus reuteri* NCIMB 8821. Biomass, substrate consumption, and lactic acid production were accurately modelled using an unstructured mathematical model. A 3.7% (w/w) debranning degree resulted optimal in stimulating the growth of the selected probiotic strains. Results obtained suggest that this fraction might be suitable for producing potential synbiotic formulations.

Industrial relevance

There are many health benefits associated with the consumption of synbiotic foods, combining probiotic bacteria and prebiotic compounds. The development of rice-based synbiotic products might be an approach, offering a healthy alternative for individuals with, for instance, lactose intolerance.

Keywords: microbial bioconversion; microbial growth kinetics; mathematical modelling; probiotics; rice grain processing

1. Introduction

Rice is unique among cereals in that it is primarily consumed in the form of cooked whole grains. Although brown rice is more nutritious than well milled white rice, the outer seed coat is a tough, highly fibrous layer which is disliked by many consumers (Hagiwara, Seki, & Ariga, 2004; Roberts, 1979; Saman, Fuciños, Vázquez, & Pandiella, 2011). Milled rice is commonly produced by removing the hull and bran layers of the rough rice kernel in hulling and milling processes, respectively. Milling of rice grain brings considerable losses of nutrients, whose extent depends on the degree of pearling (Doesthale, Devara, Rao, & Belavady, 1979).

Rice bran is major by-product obtained in the rice processing. Bran constitutes approximately 8-10% (w/w) of the whole rice kernel (Danielski, Zetzi, Hense, & Brunner, 2005; Doesthale et al., 1979; Sohail, Rakha, Butt, Iqbal, & Rashid, 2017). Based on data from the Food and Agriculture Organization (FAO), the global annual production of rice was reported to be about 680 million tons (<http://www.fao.org/worldfoodsituation/csdb/en/>). Therefore, approximately 60 million tons of bran are produced every year. Currently, the rice bran is used mainly in animal feeding. However, this use is not considered to be economically productive, and research is required in the search for value-added products from rice bran.

Rice bran contains three main layers. The outer layer is the pericarp which is fibrous, tough and resilient (Hutchinson & Martin, 1970). This layer is a source of hemicellulose, cellulose and protein. Next to pericarp is the tegmen or seed coat. It contains mainly fatty material. The layer situated beneath the tegmen and enclosing the endosperm is the aleurone layer. This layer can have a thickness from one to seven cells, and it is thicker on the dorsal than along the lateral and ventral surfaces. Aleurone cells are rich in protein (Juliano, 1972).

In several studies, the concentration of a number of interesting components (proteins, starch, fats, vitamins, and minerals), identified in the bran layers and the germ of rice, has been related to the degree of pearling (Ha et al., 2006; Mohapatra & Bal, 2006; Brunschwiler, Heine, Kappeler, Conde-Petit, & Nyström, 2013), which can support the growth of microorganisms. Therefore, most nutrients are concentrated in this fraction compared to the remaining 90% of the kernel consisting mostly of the endosperm (Mohapatra & Bal, 2006). Attempts are being made to increase their pharmaceutical, biotechnological or nutraceutical potential.

Rice bran is also a good source of antioxidants including vitamin E and oryzanol, high-quality oil and protein, cholesterol-lowering waxes and anti-tumour compounds like rice bran saccharide (Schramm, Abadie, Hua, Xu, & Lima, 2007; Pradeep, Jayadeep, Guha, & Singh, 2014; Sharif, Butt, Anjum, & Khan, 2014; Sohail, Rakha, Butt, Iqbal, & Rashid, 2017). Rice bran also contains a high level of dietary fibre. It provides a good proportion of the daily requirements of potential faecal bulk and promotes the transit of faecal and faecal matter through the large bowel (Monro, 2002; Goodyear et al., 2015). Increased rate of transit may protect against a range of large bowel disorders by ensuring constant throughout of moist faecal bulk, reducing dehydration and hardening of the faecal mass, preventing colonic stagnation with a putrefactive production of nitrogenous and other toxins, and replenishing substrates for bacterial production of butyrate or other beneficial products (Schneeman, 1987; Kennedy, Cryan, Dinan, & Clarke, 2014; Marchesi et al., 2016).

Some researchers have attempted to improve the properties of rice bran and enhance its functionality (Lee, Rhee, & Cho, 2014; Saman, Fuciños, Vázquez, & Pandiella, 2011). However, little attention has been given to the relationship between the degree of pearling and the fermentability of milled rice and, in particular, its fractions, which might have an enormous potential for the development of novel functional food products. For instance, there are many health benefits associated to the

consumption of synbiotic foods, combining probiotic bacteria and prebiotic compounds (Asemi, Khorrami-Rad, Alizadeh, Shakeri, & Esmailzadeh, 2014). However, most of these products are dairy-based, which limits the options of individuals with lactose intolerance or allergy to milk. Rice-based synbiotic food products might represent an alternative approach, offering an alternative for individuals with lactose intolerance or allergy to milk, and at the same time providing a solution for the valorisation of by-products from the cereal industry, which may represent up to 50% in weight of the whole grain (Moraes et al., 2014). However, for this purpose, it is required to develop and implement milling techniques such as the controlled debranning of grains (Dexter & Wood, 1996), and a careful classification of the resulting fractions with characteristic nutrient compositions that might support the growth of probiotic bacteria. In this regard, lactic acid bacteria are amongst the most commonly used microorganisms for the production of fermented functional food products due to their health promoting activity and their capacity for stabilising and improving the safety of food products (Speranza, Bevilacqua, Sinigaglia, & Corbo, 2012; Paiva et al., 2016; Viana de Souza & Silva Dias, 2017). However, they are fastidious microorganisms in terms of nutrient requirements (Subhasree & Bhakayaraj, 2013; Vázquez et al., 2016; Mladenoviæ, Djukiæ-Vukoviæ, Kociæ-Tanackov, Pejin, & Mojoviæ, 2016). This work is aimed at evaluating the potential of rice fractions obtained by controlled debranning for supporting the growth of two human-isolated probiotic *Lactobacillus* sp. Cell biomass production and chemical changes were monitored during fermentation. A set of mathematical equations from an unstructured kinetic model were accurately applied to fit the experimental data, and the obtained parameters were compared for all the fractions tested.

2. Materials and methods

2.1. Preparation and composition of rice fractions

The paddy rice RD6 (glutinous rice cultivar from Sisaket, Thailand) was de-husked using a laboratory de-husker (THU35A, Satake, Japan). Samples (200 g, moisture content 12%) were abrasively pearled and the rice layers sequentially removed with an abrasive test mill (Model TM05, Satake, Japan) for different processing times to obtain rice fractions. The weight percentage of rice layers (w/w) removed by pearling was calculated from the weight of rice before and after pearling (Lamberts et al., 2007). Thus, the pearling fractions obtained after 0, 5, 10, 20 and 30 s represent 0-2.3%, 2.3-3%, 3.8-5%, 5-6.3% and 6.3-7.3% of the kernels and were labelled as fractions A, B, C, D, and E, respectively. Fraction F is the grain left after 40 s and represents 7.3-100% of the grains (residual kernels).

2.2. Rice fraction media

The rice fraction media were prepared by adding rice fractions individually in distilled water and made up to concentrations of 3% (w/v). Duran bottles (500 mL) were filled with 400 mL of each rice media and autoclaved at 121°C for 15 min.

2.3. Microbial preparation

Two lactic acid bacteria isolated from healthy human saliva: *Lactobacillus plantarum* NCIMB 8826 (ATCC BAA-793) and healthy human gut: *Lactobacillus reuteri* NCIMB 8821, were obtained from National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland, UK. They were selected to test the rice fractions fermentability due to their probiotic properties (Dong, Rowland, & Yaqoob, 2012; Vesa, Pochart, & Marteau, 2000) and technological capacity to develop fermented cereal beverages (Salmeron, Thomas, & Pandiella, 2014; Salmeron, Thomas, & Pandiella, 2015).

The stock cultures of both lactic acid bacteria maintained on MRS agar slants were inoculated separately into MRS broth. The liquid culture was incubated at 37°C until cell growth was observed, usually after 24 to 48 h. The cell suspension was then propagated a further two times in MRS broth, firstly for 24 h and then for a final 18 h. This process ensured that cells used for each experiment (inoculum) were harvested from the early stationary phase by adjusting the inoculum to an optical density (700 nm) of 0.200 (Kedia, Vazquez, & Pandiella, 2008b; Salmeron, Thomas, & Pandiella, 2015).

2.4. Fermentation procedures

Shake-flask fermentations of different rice fractions were performed in triplicate using 500 mL screw-capped glass bottles with 400 mL of medium. Each rice fraction medium was inoculated with 1% (v/v) inoculum. In all cases, the initial microbial concentration was approx. $7.5 \log \text{CFU mL}^{-1}$. Cultures were maintained in an orbital shaker incubator at 37 °C (G-25 Shaker Incubator, New Brunswick, USA), 150 rpm of agitation and without aeration. Fermentations were carried out for 30 h. Samples of fermented media (4 mL) were withdrawn every 3 h and centrifuged at 5,000 g for 10 min (Minifuge T, Heraeus, Germany). Pellets were discarded and the cell-free post incubated media stored at -20 °C for further chemical analysis.

2.5. Cell enumeration

Viable cells were enumerated using the method of Miles and Misra (Silva, Carvalho, Teixeira, & Gibbs, 2002). Decimal dilutions of fermentation broths were prepared using sterile Ringer's solution. Ten μL was dropped onto MRS agar plates. The plates were incubated at 37 °C for 2–3 days and viable cell counts were calculated and shown as colony forming units per millilitre. Dilutions with a colony count lower than 10 or higher than 130 were discarded.

2.6. Chemical analysis

Total fibre, soluble fibre and insoluble fibre were determined according to the method of (Prosky, Asp, Schweizer, DeVries, & Furda, 1988). The chemical composition of the different rice fractions obtained by debranning is shown in Table 1. Cell-free fermented media were used for total reducing sugar (TRS), free amino nitrogen (FAN) and lactic acid analyses. Samples were analysed in triplicates. The concentration of TRS was quantified by following the method of Miller (1959), using 3,5-dinitrosalicylic acid solution. FAN concentration was determined by the EBC-ninhydrin colorimetric method (Lie, 2013).

The concentration of lactic acid was analysed according to Anderson and Hedlund (1983). Culture samples were filtrated through a 0.45 μm membrane, and filtered solutions analysed directly by HPLC. The HPLC system was equipped with an auto-sampler (model 410 Varian, UK) and UV detector (model 330 Varian, Japan). The column was a Thermo Hypersil (200 $\times$ 4.6 mm, 5 μm ; Hypersil, UK) with 6mM sulphuric acid as mobile phase (flow rate= 0.8 mL min⁻¹) at 60°C and an injection volume of 20 μL .

2.7. Mathematical models

In order to describe and compare the kinetics of the lactic acid bacteria on the rice media, an unstructured mathematical model was used (Charalampopoulos, Vazquez, & Pandiella, 2009; Kedia, Vazquez, & Pandiella, 2008a; Vazquez & Murado, 2008). The variables fitted were the biomass concentration (X : as $\ln(N/N_0)$, where N is the cell population and N_0 the initial cell population as colony forming units per millilitre), lactic acid production (L), total reducing sugars (S) and FAN (F). Definition and units of the model parameters and variables are shown in Table 2.

$$X = \ln\left(\frac{N}{N_0}\right) = \frac{K}{1 + \exp\left[2 + \frac{4 \cdot \mu_m}{K}(\lambda - t)\right]} \quad \text{where } N_m = N_0 \exp(K) \quad (1)$$

$$L = L_0 + \frac{\alpha_L \cdot K}{1 + \exp\left[2 + \frac{4 \cdot \mu_m}{K}(\lambda - t)\right]} - \alpha_L \cdot X_0 + \frac{\beta_L \cdot K^2}{4 \cdot \mu_m} \cdot \ln \left[\frac{\exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right) + \exp\left(\frac{4 \cdot \mu_m \cdot t}{K}\right)}{1 + \exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right)} \right] \quad (2)$$

$$S = S_0 + \frac{X_0}{Y_{X/S}} - \frac{1}{Y_{X/S}} \cdot \frac{K}{1 + \exp\left[2 + \frac{4 \cdot \mu_m}{K}(\lambda - t)\right]} - \frac{m_S \cdot K^2}{4 \cdot \mu_m} \cdot \ln \left[\frac{\exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right) + \exp\left(\frac{4 \cdot \mu_m \cdot t}{K}\right)}{1 + \exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right)} \right] \quad (3)$$

$$F = F_0 + \frac{X_0}{Y_{X/F}} - \frac{1}{Y_{X/F}} \cdot \frac{K}{1 + \exp\left[2 + \frac{4 \cdot \mu_m}{K}(\lambda - t)\right]} - \frac{m_F \cdot K^2}{4 \cdot \mu_m} \cdot \ln \left[\frac{\exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right) + \exp\left(\frac{4 \cdot \mu_m \cdot t}{K}\right)}{1 + \exp\left(2 + \frac{4 \cdot \mu_m \cdot \lambda}{K}\right)} \right] \quad (4)$$

2.8. Numerical and statistical methods

Fitting procedures and parametric estimates from the experimental results were performed by minimisation of the sum of quadratic differences between observed and model-predicted values. The non-linear least-squares (Levenberg-Marquardt) method was performed with Mathematica 7 (Wolfram Research, Inc.). The significance of the estimated parameters (Student's *t*-test, $\alpha = 0.05$) by fitting experimental values to the proposed mathematical models and consistency of these equations (Fisher's *F*-test, $\alpha = 0.05$) were also evaluated.

3. Results

Based on the chemical composition of fractions (Table 1) can be observed that an increase of moisture content was observed with the pearling process. The C and D samples are mainly formed by seed coat including spermoderm and nucellar layer. The best levels of fibre were achieved in A, B, and C fractions, and the higher soluble fibre was presented in B and C fractions. The lower fibre presence was detected in E and F (endosperm and embryo) that are rich in starch. Highest concentrations of protein were obtained in B, C, and D fractions.

To investigate the fermentative property of rice fractions obtained from different degrees of pearling, fermentations were carried out by using two strains of probiotic lactobacilli from human origin. *L. plantarum* NCIMB 8826 and *L. reuteri* NCIMB 8821 growth dynamics were compared under the same fermentation conditions. Chemical changes were also monitored throughout a 30-h fermentation period.

3.1. Relationship between pearling time and degree of pearling

Brown rice was abrasively pearled to obtain rice fractions with various degrees of pearling. Figure 1 shows the degree of pearling as a function of pearling time. An increase in pearling time did not result in a complete and unique linear increase in the degree of pearling. Two linear trends were observed, the first one until 10 s with higher slope and longer than 10 s with a lower and constant slope. The transition at a degree of pearling was probably related to a significant difference in hardness between bran and endosperm. The hardness of the bran decreased from outer to inner layers (Lamberts et al., 2007). Six rice fractions were obtained: Fraction A (0–2.3% w/w), B (2.3–3.8% w/w), C (3.8–5% w/w), D (5–6.3% w/w), E (6.3–7.3% w/w) and F (7.3–100% w/w).

3.2. Fermentability and chemical changes of the rice-fractions media

L. plantarum and *L. reuteri* showed similar growth profiles in all the six rice-fraction media (Figure 2). Cultures started to approach exponential phase after 6.2 h (*L. plantarum*) and 7.9 h (*L. reuteri*) of growth lag phases (Tables 3 and 4), and then cell concentration increased sharply until early stationary phase at 18–21 h. Both strains reached the highest and asymptotic cell density at approximately 24 h in the culture of rice fraction C (3.8–5% w/w degree of pearling), followed by cultures of fraction B (2.3–3.8% w/w), D (5–6.3% w/w), A (0–2.3% w/w), E (6.3–7.3% w/w) and F (7.3–100% w/w), respectively. *L. plantarum* showed higher cell counts than *L. reuteri* ($p < 0.05$) in all the rice fractions (Figure 2, Tables 3 and 4). *L. plantarum* maximum biomass increment ranged from $8.06 \pm 0.37 \ln(N/N_0)$ (Fraction F) to $11.23 \pm 0.44 \ln(N/N_0)$ (Fraction C), whereas *L. reuteri* attained maximum growth increments from $6.82 \pm 0.15 \ln(N/N_0)$ (Fraction F) to $10.62 \pm 0.35 \ln(N/N_0)$ (Fraction C). Similar behaviours were obtained for the values of the specific maximum growth rate in the fractions tested with both lactobacilli ($C > B > D > E > A > F$).

Lactic acid is the main metabolite of lactobacilli fermentations. As expected, lactic acid production was concomitant of cell growth. For both lactobacilli, the production of lactic acid began after 3 h in fractions A, B, C, D and E. Lactic acid accumulation in fraction F was not detectable until 6 h. In all cases, an increase of lactic acid levels was found from 6 to 21 h, after which the concentration of lactic acid remained almost constant (Figure 3). Similarly to the growth profiles, the accumulation of lactic acid was higher in fraction C (which had the highest initial TRS content) ($p < 0.05$), followed by fractions B, D, A, E, and F. *L. reuteri* produced less lactic acid than *L. plantarum* in all cultures. The maximum lactic acid production achieved by *L. plantarum* in the fraction C was 0.74 g L^{-1} , while only 0.2 g L^{-1} was obtained with *L. reuteri* in the same fraction (Figure 3).

As expected, pH dropped through fermentation following a profile inverse to the lactic acid production (data not shown). In all the rice fractions, the initial pH was similar ranging from 6.4 to

6.6. After fermentation, pH decreased to 5.2–5.4 in *L. plantarum* and to 5.7–5.8 in *L. reuteri* cultures. In general, biggest changes were observed after 6 h of fermentation. At this moment, pH started to decrease sharply until 24 h, when it remained nearly constant (data not shown).

Considering TRS initial concentration, the lowest value was found in the medium prepared from fraction F (0.7 g L⁻¹). Intermediate levels of TRS were detected in media from fraction E (1.1 g L⁻¹) and A (1.2 g L⁻¹). Fractions B, C, and D showed a maximum initial TRS concentration of 1.3 g L⁻¹. The TRS was consumed by *L. plantarum* more quickly during 6 to 21 h after which the consumption rate was markedly reduced (Figure 4). *L. plantarum* TRS consumptions were 0.75, 0.83, 0.84, 0.81, 0.65 and 0.51 g L⁻¹ in media from fractions A, B, C, D, E and F, respectively. Similarly to *L. plantarum* fermentations, TRS was consumed by *L. reuteri* gradually. Cultures of fractions A, B, C and D showed the higher consumptions (0.65, 0.73, 0.75 and 0.67 g L⁻¹). Lower consumptions were observed in the fraction E (0.48 g L⁻¹), and especially in the culture from fraction F (0.28 g L⁻¹).

The initial FAN concentration in media from fractions A, B, C, and D was very similar (8.9–9.1 mg L⁻¹). The lowest initial FAN concentration was observed in the medium prepared from fraction F (2.9 mg L⁻¹) followed by the medium from fraction E (7.4 mg/L). As for TRS, *L. plantarum* and *L. reuteri* used FAN reserves of fractions A, B, C, D and E more quickly during 6 to 21 h, after which the rate of FAN consumption became relatively constant (Figure 5). FAN content in the medium from fraction F decreased more gradually through the end of fermentation in both *L. plantarum* and *L. reuteri* cultures. Interestingly, FAN consumption was very similar in both probiotic strains independent of the rice fraction used.

The changes in growth, lactic acid, TRS and FAN were adequately described by the proposed models (1–4). Interestingly, the highest biomass yield per sugar ($Y_{x/s}$) and FAN uptake ($Y_{x/f}$) were

obtained by *L. reuteri* in the culture medium from fraction F (42.22 ± 15.48 [ln (N/N_0)] L/[g of sugar] and 8.98 ± 3.36 [ln (N/N_0)] L/[mg of nitrogen], respectively).

In all cases, the fitting of results was satisfactory graphically and statistically. The mathematical equations were consistent (Fisher's *F*-test), and the parametric estimations were significant (Student's *t*-test). Furthermore, the predicted values from the non-linear adjustments showed a high correlation with the observed experimental values ($r^2 > 0.988$).

4. Discussion

In the present work, different fractions of the outer layer of rice were obtained by debranning technology and studied as a substrate for the production of cellular biomass from probiotic lactic acid bacteria (*L. plantarum* and *L. reuteri*). Among the rice fractions obtained from the pearling treatment, fraction A (0–2.3% w/w pearling) contained mainly the outer layer of the rice kernel (Danielski, Zetzl, Hense, & Brunner, 2005; Sohail, Rakha, Butt, Iqbal, & Rashid, 2017). Fraction B (2.3–3.8% w/w), C (3.8–5% w/w) and D (5–6.3% w/w) should consist mainly of nucellus, aleurone, some part of pericarp and spermoderm, while fraction E (6.3–7.3% w/w) and F (7.3–100% w/w) might contain most of the endosperm, embryo and a few aleurone cells. The degree of pearling affects the amount and quality of carbohydrates as primary fermentation substrates, nitrogen sources, growth factors such as vitamins, minerals, buffering capacity, and the concentration of growth inhibitors. Therefore, rice fraction selection played a key role in the fermentation.

Concerning fermentable carbohydrates (approx. $0.7\text{--}0.8$ g L⁻¹ and lactose-free), microorganisms were initially well supplied. Results in Figure 4 show the initial TRS concentration of all the fractions, which were in agreement with the concentrations of free total sugars in cereal grains previously reported (Hammes et al., 2005; Saman, Fuciños, Vázquez, & Pandiella, 2011; Herrera-

Ponce, Nevarez-Morillon, Ortega-Rývas, Perez-Vega, & Salmeron, 2014). All the fermentation media were able to support the growth of *L. plantarum* and *L. reuteri*. This result agrees with the results reported in (Salovaara & Simonson, 2004), who showed that despite the level of sugars in fully matured grains is relatively low (0.5 to 3%), these free sugars can support the initiation of the fermentation process. Recently, another *L. plantarum* strain (CCMA 0743) was used as probiotic starter to develop nondairy beverage based on cassava a rice materials found cell production around 8.0 log (CFU/mL) and lactic acid concentrations of 1.61 g/L (Freire, Ramos, da Costa Souza, Cardoso, & Schwan, 2017).

Cultures of fractions A, B, C, and D contained almost the same initial concentration of FAN while the lowest FAN content was obtained in the culture of fraction F ($p < 0.05$) followed by the culture of fraction E (Figure 5). The limited FAN content in these fractions could affect the growth of both lactobacilli. The uptakes profiles were very similar in all media for the two lactobacilli ($p > 0.05$) but the real consumption was superior in the fractions A-D ($p < 0.05$). Those trends are also in concordance with the defined for TRS. The changes of the FAN kinetics suggest that *L. plantarum* and *L. reuteri* can use the peptide and amino acids present in the fractions to support growth or the own proteases and peptidases can increase the levels of nutritive peptides and amino acids in the culture media (Rathore, Salmerón, & Pandiella, 2012).

The maximum growths of *L. plantarum* and *L. reuteri* were obtained in the media prepared with fractions B and C. This implies that both fractions contain more nutritive elements (soluble fibre and FAN) than the other fractions. They possibly contain most nucellus, spermoderm and aleurone layers which are also sources of growth factors and essential minerals. Also, both lactobacilli fermentations presented a lower growth in the fraction A culture than in cultures of fraction B, C, and D ($p < 0.05$). Fraction A was composed mainly by crude fibre and few nutrients, which possibly limited the

bacterial growth. Nonetheless, the minimum cell biomass was achieved in fraction F, followed by fraction E medium. This could be because both media contained mainly endosperm and less percentage of total fibre, mainly soluble fibre. As Lamberts et al. (2007) reported, the starch content is higher in the endosperm, whereas protein and mineral contents decreased from the outer bran layers to the endosperm. Therefore, fractions E and F could have a limited quantity of essential nutrients. Similar results were obtained with oat fractions in which the fraction rich in soluble fibre was the most adequate for the growth of probiotic LAB (Kedia, Vazquez, & Pandiella, 2008a). In addition, the viable cells of *L. plantarum* produced in fractions B and C were higher than those reported using glutinous and non-glutinous bran from RD6 and RD17 Thai rice varieties, respectively (Saman, Fuciños, Vázquez, & Pandiella, 2011).

At 24 h, *L. plantarum* showed a maximum cell concentration higher than *L. reuteri* in all the rice fractions. This behaviour can be explained by the specific growth conditions required by *L. reuteri*. Nikawa et al. (2004) found that *L. reuteri* is an obligately heterofermentative resident in the gastrointestinal tracts of humans. This bacterium has nutritional requirements such as several B-complex vitamins, many amino acids, peptides, fatty acid, esters, salts and nucleic acid derivatives (Hébert, Raya, & Savoy de Giori, 2004; Salmeron, Thomas, & Pandiella, 2014).

From the modelling viewpoint, the proposal unstructured equations (1-4) were appropriated for the description of experimental data of biomass and lactic acid productions concomitant with the sugars and protein uptakes. Parameters from these equations are very useful for the prediction of the maximum cell-bacterial growths (K) and the calculation of specific maximum growth rates (μ_m) and lag phases (λ). Equations can also estimate the numerical values of production yields (Y_{XS} and Y_{XF}) and cellular maintenance terms (m_S and m_F). Finally, the values of α_L and β_L (Table 2) from lactic acid formation permit to typify this compound as primary or secondary metabolite (Vazquez &

Murado, 2008). In this sense, the lactic acid production was, mainly in the case of *L. plantarum*, proportional to the growth, behaviour that can be therefore defined of primary metabolite with a very little component of mixed metabolite. However, the formation of lactic acid in *L. reuteri* (Table 3) was clearly of a mixed metabolite ($\alpha_L > 0$ and $\beta_L > 0$). In concordance with the results obtained for viable cells, C and B were the rice fractions in which highest productions of lactic acid were achieved in both lactobacilli. Similar equations have been recently applied to simulate the fermentation of vegetable juices by *L. plantarum* NCDC 414 with remarkable outcomes (Sharma & Mishra, 2014).

On the other hand, by plotting the maximum growth (N_m) vs the degree of pearling (DP), it is possible to observe well-defined optima for each strain (Figure 6). In both cases, the dependence of N_m and DP followed a parabolic profile. Thus, biomass data were fitted to a second-order polynomial function:

$$N_m = b_0 + b_1 DP + b_2 DP^2 \quad (5)$$

By solving the roots of the partial derivatives of the functions obtained (Tables 3 and 4) with respect to the degree of pearling, it was possible to calculate the optimum degree of pearling. As Table 5 shows, the optimum degree of pearling was very similar for both strains (approximately 3.7%). This result, from a methodological point of view, represents a big advantage since it suggests that such a rice fraction contains active compounds that equally stimulate the growth of different probiotic strains. This approach can facilitate the subsequent process of isolation of the active compounds to be used in a prebiotic formulation. In this context, fraction C was the best substrate for the growth of bifidobacteria and lactobacteria from human faecal slurry using *in vitro* faecal fermentations (Saman, Tuohy, Vázquez, Gibson, & Pandiella, 2017).

5. Conclusions

In conclusion, the fermentative capacity of rice fractions obtained by debranning technology shows a strong dependence on the specific probiotic lactic acid bacteria. In fact, in all fractions, *L. plantarum* performed considerably better than *L. reuteri* since greater cell biomass, substrate utilisation (TRS and FAN) and lactic acid production were obtained. An optimum degree of pearling of 3.7% was found, that notably increases the maximum biomass production of probiotic strains. This suggests that fractionation using controlled debranning could be implemented as an easy and inexpensive scale-up process to obtain functional ingredients that might be used for the production of potential synbiotic food products. Further studies should focus on the *in vivo* analysis of the prebiotic potential of these rice fractions. In addition, a set of integrated equations, formulated with kinetic and well-defined parameters, were applied to model accurately the growth and lactic acid production jointly the nutrient consumption.

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Conflict of interest

Premsuda Saman declares that she has no conflict of interest.

Pablo Fuciños declares that he has no conflict of interest.

José A. Vázquez declares that he has no conflict of interest.

Severino S. Pandiella declares that he has no conflict of interest.

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FIGURE CAPTIONS

Figure 1. Effect of pearling time on the degree of pearling.

Figure 2. Biomass production by *Lactobacillus* strains in different culture media from rice debranning fractions. Fraction A (0-2.3%), B (2.3-3.8%), C (3.8-5%), D (5-6.3%), E (6.3-7.3%) and F (7.3-100%). *L. plantarum* (●) and *L. reuteri* (■). Symbols: experimental data. Lines: equation 1. The corresponding confidence intervals of independent experiments are not shown ($\alpha = 0.05$, $n = 3$) because they are below 10% of the experimental mean value.

Figure 3. Lactic acid production by *Lactobacillus* strains in different culture media from rice debranning fractions. Fraction A (0-2.3%), B (2.3-3.8%), C (3.8-5%), D (5-6.3%), E (6.3-7.3%) and F (7.3-100%). *L. plantarum* (●) and *L. reuteri* (■). Symbols: experimental data. Lines: equation 2. The corresponding confidence intervals of independent experiments are not shown ($\alpha = 0.05$, $n = 3$) because they are below 10% of the experimental mean value.

Figure 4. TRS consumption by *Lactobacillus* strains in different culture media from rice debranning fractions. Fraction A (0-2.3%), B (2.3-3.8%), C (3.8-5%), D (5-6.3%), E (6.3-7.3%) and F (7.3-100%). *L. plantarum* (●) and *L. reuteri* (■). Symbols: experimental data. Lines: equation 3. The corresponding confidence intervals of independent experiments are not shown ($\alpha = 0.05$, $n = 3$) because they are below 10% of the experimental mean value.

Figure 5. FAN consumption by *Lactobacillus* strains in different culture media from rice debranning fractions. Fraction A (0-2.3%), B (2.3-3.8%), C (3.8-5%), D (5-6.3%), E (6.3-7.3%) and F (7.3-100%). *L. plantarum* (●) and *L. reuteri* (■). Symbols: experimental data. Lines: equation 4. The

corresponding confidence intervals of independent experiments are not shown ($\alpha = 0.05$, $n = 3$) because they are below 10% of the experimental mean value.

Figure 6. Maximum biomass production by *Lactobacillus* strains in different culture media from rice debranning fractions. Fraction A (0-2.3%), B (2.3-3.8%), C (3.8-5%), D (5-6.3%), E (6.3-7.3%) and F (7.3-100%). *L. plantarum* (●) and *L. reuteri* (■). Symbols: experimental data. Lines: equation 5. The corresponding confidence intervals of independent experiments are not shown ($\alpha = 0.05$, $n = 3$) because they are below 10% of the experimental mean value.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Table 1. Chemical composition of rice fractions (% in g/100 g of dry fractions).

	Chemical Composition				
	Total Fiber (%)	Soluble Fiber (%)	Insoluble Fiber (%)	TRS (g L ⁻¹)	FAN (mg L ⁻¹)
A: 0-2.3% Pearling Fraction	34.5	1.8	32.7	1.2	8.9
B: 2.3-3.8% Pearling Fraction	28.9	13.3	15.6	1.3	9.0
C: 3.8-5% Pearling Fraction	19.2	9.2	10.0	1.3	9.1
D: 5-6.3% Pearling Fraction	10.5	4.0	6.5	1.3	9.1
E: 6.3-7.3% Pearling Fraction	8.2	3.1	5.1	1.1	7.4
F: 7.3-100% Pearling Fraction	4.6	0.9	3.7	0.7	2.9

Table 2. Notation used with units.

Table: Notation used with units.	
N :	Cell population as colony forming units per millilitre, CFU mL ⁻¹
N_0 :	Initial cell population as colony forming units per millilitre, CFU mL ⁻¹
t :	Time, h
N_m :	Maximum cell population, CFU mL ⁻¹
μ_m :	Specific maximum growth rate, [log ₁₀ (N/N ₀)] h ⁻¹
λ :	Growth lag phase, h
K :	Empirical parameter, dimensionless [log ₁₀ (N/N ₀)]
X :	Relative cell population, dimensionless [log ₁₀ (N/N ₀)]
X_0 :	Initial relative cell population, dimensionless [log ₁₀ (N/N ₀)]
L :	Lactic acid concentration, g L ⁻¹
L_0 :	Initial lactic acid concentration, g L ⁻¹
α_L :	Growth-associated constant for lactic acid production, g L ⁻¹ [log ₁₀ (N/N ₀)] ⁻¹
β_L :	Non growth-associated constant for lactic acid production, g L ⁻¹ [log ₁₀ (N/N ₀)] ⁻¹ h ⁻¹
S :	Total reducing sugars concentration, g L ⁻¹
S_0 :	Initial total reducing sugars concentration, g L ⁻¹
Y_{xs} :	Yield coefficient for relative cell formation on sugar, [log ₁₀ (N/N ₀)] [g (sugar)] ⁻¹ L
m_s :	Maintenance coefficient of relative cell on sugar, g (sugar) L ⁻¹ [log ₁₀ (N/N ₀)] ⁻¹ h ⁻¹
F :	Total free amino nitrogen concentration, mg L ⁻¹
F_0 :	Initial total free amino nitrogen concentration, mg L ⁻¹
Y_{xf} :	Yield coefficient for relative cell formation on nitrogen, [log ₁₀ (N/N ₀)] [mg (nitrogen)] ⁻¹ L
m_f :	Maintenance coefficient of relative cell on nitrogen, g (nitrogen) L ⁻¹ [log ₁₀ (N/N ₀)] ⁻¹ h ⁻¹

Table 3. Parametric estimations corresponding to the kinetic models (1–4), applied to the cultures of *L. plantarum* in rice fermentation media obtained by debranning. CI: confidence intervals ($\alpha = 0.05$). F : F-Fisher test ($df_1 =$ model degrees freedom and $df_2 =$ error degrees freedom). $r^2 =$ correlation coefficient between observed and predicted data. NS = Not Significant.

VARIABLES	<i>L. plantarum</i>					
	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E	Fraction F
Growth (X)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
K	9.969 $\pm$ 0.554	10.822 $\pm$ 0.462	11.234 $\pm$ 0.442	10.519 $\pm$ 0.607	9.301 $\pm$ 9.301	8.062 $\pm$ 0.365
μ_m	0.833 $\pm$ 0.180	0.932 $\pm$ 0.158	0.997 $\pm$ 0.163	0.856 $\pm$ 0.185	0.839 $\pm$ 0.839	0.830 $\pm$ 0.183
λ	6.164 $\pm$ 1.426	6.400 $\pm$ 1.090	6.274 $\pm$ 1.020	6.187 $\pm$ 1.464	6.376 $\pm$ 1.339	6.369 $\pm$ 1.198
F ($df_1=3$, $df_2=8$; $\alpha=0.05$)	1093.89	1813.45	2052.39	1051.86	1172.4	1349.55
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	0.998	0.999	0.999	0.997	0.997	0.998
Lactic acid (L)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
L_0	NS	NS	NS	NS	NS	NS
α_L	0.048 $\pm$ 0.010	0.053 $\pm$ 0.008	0.053 $\pm$ 0.009	0.053 $\pm$ 0.008	0.040 $\pm$ 0.010	0.015 $\pm$ 0.004
β_L	0.001 $\pm$ 0.001	0.001 $\pm$ 0.001	0.001 $\pm$ 0.001	0.001 $\pm$ 0.000	0.001 $\pm$ 0.001	<0.001 $\pm$ 0.000
F ($df_1=3$, $df_2=8$; $\alpha=0.05$)	863.31	1336.16	1183.01	1654.39	586.22	489.33
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	0.997	0.997	0.998	0.998	0.995	0.995
Sugars (S)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
S_0	1.252 $\pm$ 0.020	1.323 $\pm$ 0.025	1.363 $\pm$ 0.026	1.305 $\pm$ 0.025	1.132 $\pm$ 0.015	0.656 $\pm$ 0.046
$Y_{X/S}$	12.476 $\pm$ 0.894	12.817 $\pm$ 1.067	14.197 $\pm$ 1.267	12.151 $\pm$ 1.024	14.662 $\pm$ 0.939	20.165 $\pm$ 5.724
m_S	NS	NS	<0.001 $\pm$ 0.000	NS	NS	NS
F ($df_1=3$, $df_2=8$; $\alpha=0.05$)	11141.90	7900.84	7933.28	7711.35	17063.70	553.09
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	>0.999	>0.999	>0.999	>0.999	>0.999	0.995
FAN (F)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
F_0	9.003 $\pm$ 0.165	9.177 $\pm$ 0.157	9.218 $\pm$ 0.178	9.129 $\pm$ 0.173	7.336 $\pm$ 0.076	2.715 $\pm$ 0.129
$Y_{X/F}$	5.250 $\pm$ 1.292	5.540 $\pm$ 1.243	6.162 $\pm$ 1.635	5.142 $\pm$ 1.261	6.464 $\pm$ 0.922	8.976 $\pm$ 3.165
m_F	0.004 $\pm$ 0.003	0.004 $\pm$ 0.003	0.004 $\pm$ 0.003	0.003 $\pm$ 0.003	0.002 $\pm$ 0.001	NS
F ($df_1=3$, $df_2=8$; $\alpha=0.05$)	13309.90	15262.40	12084.70	12414.60	42884.50	1783.33
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999

Table 4. Parametric estimations corresponding to the kinetic models (1-4), applied to the cultures of *L. reuteri* in oat fermentation media obtained by debranning. CI: confidence intervals ($\alpha = 0.05$). *F*: F-Fisher test (df_1 = model degrees freedom and df_2 = error degrees freedom). r^2 = correlation coefficient between observed and predicted data. NS = Not Significant.

VARIABLES	<i>L. reuteri</i>					
	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E	Fraction F
Growth (X)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
K	9.099 $\pm$ 0.311	10.103 $\pm$ 0.407	10.620 $\pm$ 0.346	9.490 $\pm$ 0.412	8.209 $\pm$ 0.228	6.824 $\pm$ 0.153
μ_m	0.784 $\pm$ 0.096	0.850 $\pm$ 0.121	0.917 $\pm$ 0.110	0.821 $\pm$ 0.128	0.786 $\pm$ 0.088	0.784 $\pm$ 0.086
λ	8.378 $\pm$ 0.777	7.936 $\pm$ 0.931	7.944 $\pm$ 0.763	8.425 $\pm$ 0.987	8.550 $\pm$ 0.650	8.840 $\pm$ 0.533
<i>F</i> ($df_1=3$, $df_2=8$; $\alpha=0.05$)	3143.86	2282.2	3359.08	1938.83	4211.52	5502.05
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	0.999	0.999	0.999	0.999	0.999	>0.999
Lactic acid (L)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
L_0	0.031 $\pm$ 0.017	0.037 $\pm$ 0.022	0.035 $\pm$ 0.022	0.034 $\pm$ 0.019	0.028 $\pm$ 0.016	0.019 $\pm$ 0.011
α_L	0.017 $\pm$ 0.006	0.019 $\pm$ 0.007	0.019 $\pm$ 0.006	0.018 $\pm$ 0.006	0.018 $\pm$ 0.006	0.016 $\pm$ 0.004
β_L	NS	NS	NS	NS	NS	NS
<i>F</i> ($df_1=3$, $df_2=8$; $\alpha=0.05$)	227.68	212.42	250.10	230.63	249.23	292.65
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	0.988	0.988	0.989	0.989	0.989	0.991
Sugars (S)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
S_0	1.157 $\pm$ 0.087	1.247 $\pm$ 0.078	1.305 $\pm$ 0.073	1.214 $\pm$ 0.069	0.936 $\pm$ 0.060	0.468 $\pm$ 0.022
$Y_{X/S}$	12.437 $\pm$ 4.698	11.624 $\pm$ 3.287	10.980 $\pm$ 2.575	11.148 $\pm$ 2.894	19.176 $\pm$ 7.910	42.215 $\pm$ 15.476
m_S	NS	NS	<0.001 $\pm$ 0.002	<0.001 $\pm$ 0.002	NS	<0.001 $\pm$ 0.001
<i>F</i> ($df_1=3$, $df_2=8$; $\alpha=0.05$)	523.86	737.69	872.55	867.85	801.24	1358.40
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	0.995	0.996	0.997	0.997	0.997	0.998
FAN (F)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
F_0	8.854 $\pm$ 0.048	9.139 $\pm$ 0.101	9.146 $\pm$ 0.083	9.017 $\pm$ 0.054	7.324 $\pm$ 0.106	2.534 $\pm$ 0.106
$Y_{X/F}$	4.433 $\pm$ 0.332	4.167 $\pm$ 0.548	4.550 $\pm$ 0.498	4.110 $\pm$ 0.309	3.790 $\pm$ 0.551	8.987 $\pm$ 3.364
m_F	0.001 $\pm$ 0.001	NS	NS	NS	NS	NS
<i>F</i> ($df_1=3$, $df_2=8$; $\alpha=0.05$)	134461.00	32840.50	48578.40	107351.00	18065.70	2057.96
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
r^2 (obs-pred)	>0.999	>0.999	>0.999	>0.999	>0.999	0.999

Table 5. Parametric estimations corresponding to equation (5), applied to study the empirical relationship between maximum growth (N_m) and degree of pearling (DP) for both lactobacilli. CI: confidence intervals ($\alpha = 0.05$). F : F-Fisher test (df_1 = model degrees freedom and df_2 = error degrees freedom). r^2 = correlation coefficient between observed and predicted data.

	<i>L. plantarum</i>	<i>L. reuteri</i>
	values $\pm$ CI	values $\pm$ CI
b_0	8.537 $\pm$ 1.808	7.429 $\pm$ 2.307
b_1	1.400 $\pm$ 1.034	1.652 $\pm$ 1.319
b_2	-0.188 $\pm$ 0.128	-0.226 $\pm$ 0.163
N_m	11.146	10.453
Optimum DP	3.728%	3.661%
F ($df_1=3$, $df_2=2$; $\alpha=0.05$)	3551.77	1837.85
p -value	0.0003	0.0005
r^2 (obs-pred)	>0.999	>0.999

Highlights

- **Rice-bran fractions were obtained by debranning technology.**
- **Those rice fractions support probiotics growth.**
- **Unstructured mathematical model accurately predicted the experimental data.**
- **Optimal probiotic growth is achieved in fractions at 3.7% (w/w) debranning degree.**

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