1 **Fermentability of whole oat flour, PeriTec flour and bran by**
2 ***Lactobacillus plantarum***

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ABSTRACT

Whole oat flour obtained by hammer milling was fermented with *L. plantarum* along with white flour and bran in order to compare the suitability of these substrates for the production of a probiotic beverage. The three substrates show a viable cell concentration at the end of fermentation above the minimum required in a probiotic product. The highest cell concentration was observed in white flour (9.16 Log₁₀ CFU/mL) and the lowest in the bran sample (8.17 Log₁₀ CFU/mL).

Keywords: *Lactobacillus plantarum*, probiotic, fermentation, oat flour, unstructured mathematical model.

INTRODUCTION

There has been a recent increase in the use of dietary components that help to maintain, or even improve, the gut micro flora balance. Previous studies have shown that cereals are good substrate for the proliferation of probiotic lactic acid bacteria and could also be used as prebiotics and symbiotic (Charalampopoulos, Pandiella, Wang, & Webb, 2002). Oat grains are packed with nutrients and impart valuable health benefits. They contain biologically active ingredients like dietary and functional fibres that are part of the bran and germ of the grain. Some of these parts of the grain are removed in conventional flour milling to produce white oat flour. Whole grains would have added health benefits by maximising the intake of fibre (Nyman, Siljestroem, Pedersen, Bach Knudsen, Asp, Johansson, & Eggum, 1984).

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43 Due to their perceived health benefits, probiotic bacteria have been incorporated into
44 yoghurts and fermented milks for a number of decades (Saarela, Mogensen, Fonden,
45 Matto, & Mattilda-Sandholm, 2000). Lactobacilli and bifidobacteria are the most
46 commonly used microorganisms and are generally associated with habitats rich in
47 nutrients, such as various food commodities (vegetable, milk, meat). However, some are
48 also habitants of the normal flora of the oral cavity and the gastrointestinal and
49 genitourinary tract of animals and humans (Axelsson, 1998).

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51 The objective of this work is to study the fermentability by probiotic lactic acid bacteria
52 of three different oat samples: whole oat flour produced by hammer milling, PeriTec
53 white flour and bran obtained by debranning technology (Mousia & Pandiella, 2004).
54 Cell growth, metabolic product formation and substrate uptake will be monitored and the
55 results will be fitted to an unstructured mathematical model.

56

57 **MATERIALS AND METHODS**

58

59 **Dry milling of oat to obtain flour and bran**

60 The whole oat flour was obtained by milling the oat in a hammer mill (Falling Number
61 AB, England) fitted with a sieve of 850 μm aperture size, whereas bran and white flour
62 were obtained by combined debranning and dry milling of oat using the Satake STR-100
63 mill. This process consisting of two break grindings, both utilising a pair of 14 flutes per
64 inch corrugated rolls operated under a dull to dull roll disposition, along with four

reduction grindings by a pair of smooth rolls, separated the groats pearled for 20 s into six flour fractions (white flour) and four bran fractions (bran) (Wang, Koutinas, & Campbell, 2007). The overall flour extraction rate reached 51% of the original groat weight. The weight ratio of the bran fractions together with the pearlings, 49%, satisfied one of the four criteria in the definition for oat bran by the AACC (not more than 50% of the starting material; Fulcher & Miller, 1993).

Fermentation monitoring

Microorganism and inocula

L. plantarum (NCIMB 8826) originally isolated from human intestine was used for the fermentation of the oat sample and the strain was stored on slopes of MRS at 4°C.

To obtain sufficient cells for parallel experiments each inoculum was proliferated from the slopes twice in universal bottles containing 20 mL MRS suspension. After 48 h, 0.5 mL of the broth from the first incubation were transferred into freshly sterilized MRS suspension to propagate for another 24 h.

Fermentation procedures

Shake-flask fermentations were performed in duplicate using 500 mL screw-capped glass bottles. In all fermentations, 5% w/v suspensions of the different fractions were prepared and autoclaved at 121°C for 15 min. Bottles were inoculated with a 2% v/v of lactic acid bacteria and incubated at 150 rpm and 37°C for 30 h. Samples were regularly taken for

total cell counting and centrifuged fermented media (10 min, 4500 rpm) were stored at -20°C for later analysis.

Cell enumeration

Viable cells were enumerated using the method of Miles and Misra (Collins, 1984). Decimal dilutions of fermentation broths were prepared using sterile Ringer's solution. 12 µL were dropped onto 3-4 day old MRS agar plates and then incubated at 37°C for 2-3 days. Viable cell counts were calculated as log₁₀ colony forming units per mL. Dilutions with less than 10 or more than 130 colonies were discarded.

Chemical analyses

Total dietary fibre, soluble fibre and insoluble fibre were determined according to the method of Prosky & al. (1992). β-glucan was measured by the McCleary & Codd method (McCleary & Codd, 1991) using an assay kit from Megazyme. The concentration of soluble free amino nitrogen (FAN) during fermentation was assayed by the EBC-ninhydrine colorimetric method (European-Brewery-Convention, 1973). The protein content was calculated by multiplying the total Kjeldahl nitrogen by a factor of 6.25. Total reducing sugar (TRS) was assayed by the dinitrosalicylic acid method (Miller, 1959), and the concentration of lactic acid was obtained using an analytical kit from Megazyme.

Mathematical models

In order to describe and compared the kinetics of the lactic acid bacteria on the oat flour media, an unstructured mathematical model was used (Vázquez & Murado, 2008; Vázquez & Murado, 2008). The variables fitted by this approach were the biomass concentration (X : as $\log_{10}C$, being C the colony forming units per mL), the lactic acid concentration (L), the total the reducing sugars (S) and the free amino nitrogen (N). The definition and units of the model parameters and variables are shown in table 1.

$$X = \frac{X_m}{1 + \exp\left[2 + \frac{4 \cdot v_{mx}}{X_m} \cdot (\lambda_x - t)\right]} \quad (1)$$

$$L = \frac{L_m}{1 + \exp\left[2 + \frac{4 \cdot v_{ml}}{L_m} \cdot (\lambda_l - t)\right]} \quad (2)$$

$$S = S_0 + \frac{X_0}{Y_{x/s}} - \frac{1}{Y_{x/s}} \cdot \frac{X_m}{1 + \left(\frac{X_m}{X_0} - 1\right) \cdot e^{-\frac{4 \cdot v_{mx}}{X_m} \cdot t}} - \frac{m_s \cdot X_m^2}{4 \cdot v_{mx}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mx}}{X_m} \cdot t} - 1\right) + X_m}{X_m} \right] \quad (3)$$

$$N = N_0 + \frac{X_0}{Y_{x/n}} - \frac{1}{Y_{x/n}} \cdot \frac{X_m}{1 + \left(\frac{X_m}{X_0} - 1\right) \cdot e^{-\frac{4 \cdot v_{mx}}{X_m} \cdot t}} - \frac{m_n \cdot X_m^2}{4 \cdot v_{mx}} \cdot \ln \left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mx}}{X_m} \cdot t} - 1\right) + X_m}{X_m} \right] \quad (4)$$

Numerical and statistical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimisation of the sum of quadratic differences between observed and model-predicted values, using the non linear least-squares (quasi-Newton) method provided by the macro ‘Solver’ of the Microsoft Excel XP spreadsheet. Statistica 6.0 software

(StatSoft, Inc. 2001) was used to evaluate the significance of the estimated parameters by fitting the experimental values to the proposed mathematical models, and the consistency of these equations.

RESULTS

The characterisation of the different oat samples is shown in table 2. Figure 1 shows the growth of *L. plantarum* and the chemical changes during fermentation in the three media with time. The numerical values of the kinetic parameters obtained from fitting the experimental data to the unstructured mathematical models, as well as the statistical analysis of the equations and parameters validation are summarised in the table 3. According to these results, the medium prepared from white flour led to the highest maximum biomass production and the maximum growth rate (v_{mx}). Whole flour produced a slightly lower cell concentration, and in bran a maximum cell concentration one Log_{10} CFU/mL below white flour was achieved. The numerical values of the parameter $-\lambda_x$ are not shown in table 3 because they were negatives (not realistic) and therefore not useful for comparative purposes.

The highest concentration ($p < 0.05$) and maximum production rate of lactic acid (v_{ml}) was also obtained in white flour (1.2 g/L and $0.09 \text{ g L}^{-1} \text{ h}^{-1}$ respectively). The evolution of pH decreased accordingly to this organic acid formation. The final TRS concentrations after 30 h decreased to 1.7 g/L, 2.5 g/L and 2.8 g/L for bran, whole flour and white flour, respectively. The sugar consumption was adequately described by the proposed model (3)

(see statistical analysis in table 3). The maximum value of $Y_{x/s}$ was obtained in whole flour followed white flour and bran. In all three fermentation FAN decreases after a small initial rise in the first 4 h of fermentation. For the whole flour broth, FAN increases from 74.86 mg/L to 80.44 mg/L and then decreases to 36.42 mg/L after 30 hours. Similar trends were observed for the white flour and bran media.

DISCUSSION

Due to the complexity of the cereal substrates used, the main compositional changes that were monitored to justify their fermentability were FAN and sugars. Research studies using semi-defined synthetic media have identified these compounds as the most crucial factors for LAB growth (Bethin & Villadsen, 1996; Taillandier, Gilis, Portugal, Laforce, & Strehaiano, 1996; Loubiere, Coccagn-Bousquet, Matos, Goma, & Lindley, 1997). The fractions used had a maximum particle size of 850 μm (the aperture size of the sieve in the hammer mill was 850 μm). The broths prepared from them are homogeneous liquid media with some non-fermentable insoluble bran particles in suspension. It is then possible to compare the different cultures and to fit the numerical data to the mathematical models defined in the materials and methods section.

The growth of LAB in the whole flour was comparable with the results obtained from previous workers in oat and other cereals. *L. plantarum* has also been reported to grow well in wheat and barley without the need for additional nutrients, where along with other LAB it is used for the industrial production of lactic acid (Hofendahl & Hahn-Hagerdal,

1997). Patel & al. (2004) reported a maximum growth of *L. plantarum* in malt, barley and wheat of 9.15 Log₁₀ CFU/mL, 8.46 Log₁₀ CFU/mL and 8.39 Log₁₀ CFU/mL respectively. The maximum cell concentrations in whole and white flours were 8.97 Log₁₀ CFU/mL and 9.16 Log₁₀ CFU/mL respectively, comparable with these results.

LAB are able to assimilate nitrogen in both inorganic and organic forms, although the availability of amino acids is critical for the growth of fastidious bacteria such as lactobacilli (Plessis, Dicks, Vescovo, Torriani, & Dellaglio, 1996; Vescovo, Torriani, Dellaglio, & Bottazi, 1993). The nitrogen uptake in the fermentations was monitored by measuring FAN. Though the FAN concentration decreased over the course of the fermentations, small increments were observed in the stationary phase of growth.

The white flour showed the maximum cell concentration, which is probably due to the fact that does not contain bran and non-fermentable outer layers. The bran fraction obtained by this method will contain most of the outer non-fermentable layers of the grain but also fermentable fractions, which justifies the considerably high value of cell concentrations obtained. All values exceed the level suggested by Sanders & Huis in 't Veld (1999) for a probiotic product formulation (10⁶ CFU/g or CFU/mL).

In summary, the three substrates studied demonstrate their capability to support a probiotic fermentation by human *L. plantarum*. The highest cell populations were obtained for white oat flour. According to our results the bran fraction, usually discarded

by the flour milling industry, could be fermented with a probiotic microorganism. This could lead to the development of novel probiotic beverages.

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FIGURE CAPTIONS

Figure 1. Fermentation of *Lactobacillus plantarum* in the oat samples. Continuous lines represent the mathematical models used to fit experimental data represented by points. ○, White Flour; ●, Bran; ▲, Whole Flour. L: Lactic acid; TRS: Total reducing sugars; N: Nitrogen.

TABLE CAPTIONS

Table 1. Notation used with units.

Table 2. Characterisation of the oat samples used (mean $\pm$ standard deviation for n=3).

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

FIGURE 1

TABLE 1

X :	Biomass as logarithm of colony forming units per millilitre, $\log_{10}$ (CFU/mL)
t :	Time, h
X_m :	Maximum biomass, $\log_{10}$ (CFU/mL)
X_0 :	Initial biomass, $\log_{10}$ (CFU/mL)
μ_{mx} :	Maximum growth rate, [$\log_{10}$ (CFU/mL)]/h
λ_x :	Growth lag phase, h
L :	Lactic acid concentration, g/L
L_m :	Maximum lactic acid, g/L
μ_{ml} :	Maximum lactic acid production rate, $\text{g L}^{-1} \text{h}^{-1}$
λ_l :	Lactic acid production lag phase, h
S :	Total reducing sugars concentration, g/L
S_0 :	Initial total reducing sugars concentration, g/L
$Y_{x/s}$:	Yield coefficient for biomass formation on sugar, $\log_{10}$ (CFU/mL) g^{-1} (sugar) L
m_s :	Maintenance coefficient, $\text{g (sugar) L}^{-1} [\log_{10} (\text{CFU/mL})]^{-1} \text{h}^{-1}$
N :	Free amino nitrogen concentration, mg/L
N_0 :	Initial free amino nitrogen, mg/L
$Y_{x/n}$:	Yield coefficient for biomass formation on nitrogen, $\log_{10}$ (CFU/mL) mg^{-1} (nitrogen) L
m_n :	Maintenance coefficient, $\text{mg (nitrogen) L}^{-1} [\log_{10} (\text{CFU/mL})]^{-1} \text{h}^{-1}$

TABLE 2

Oat Sample	Chemical Composition (%)					
	Moisture	Protein	Total dietary Fiber	Soluble Fiber	Insoluble Fiber	β -Glucan
Whole Oat Flour	11.91 $\pm$ 0.86	15.31 $\pm$ 0.31	12.82 $\pm$ 0.41	5.93 $\pm$ 0.08	6.66 $\pm$ 0.18	4.05 $\pm$ 0.10
White Oat Flour	12.94 $\pm$ 0.75	9.31 $\pm$ 0.17	4.32 $\pm$ 0.12	1.61 $\pm$ 0.13	2.66 $\pm$ 0.08	2.20 $\pm$ 0.14
Oat Bran	11.31 $\pm$ 0.94	12.76 $\pm$ 0.22	17.42 $\pm$ 0.32	7.43 $\pm$ 0.21	9.76 $\pm$ 0.12	5.06 $\pm$ 0.22

TABLE 3

VARIABLES	OAT SAMPLES		
	Whole flour	White flour	Bran
GROWTH (X)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
X_m	8.973 $\pm$ 0.910	9.161 $\pm$ 0.703	8.172 $\pm$ 0.469
X_0	4.800 $\pm$ 0.120	4.785 $\pm$ 0.121	4.966 $\pm$ 0.101
v_{mx}	0.314 $\pm$ 0.173	0.431 $\pm$ 0.198	0.324 $\pm$ 0.132
F (df ₁ =3, df ₂ =7; α =0.05)	793.16	911.60	2063.20
p -value	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9604	0.9653	0.9756
LACTIC ACID (L)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
L_m	0.974 $\pm$ 0.086	1.246 $\pm$ 0.048	0.711 $\pm$ 0.038
v_{ml}	0.051 $\pm$ 0.012	0.088 $\pm$ 0.017	0.040 $\pm$ 0.008
λ_l	4.032 $\pm$ 1.539	4.207 $\pm$ 1.088	2.950 $\pm$ 1.363
F (df ₁ =3, df ₂ =8; α =0.05)	963.68	1777.39	1428.59
p -value	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9965	0.9980	0.9973
SUGARS (S)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
S_0	3.697 $\pm$ 0.079	4.143 $\pm$ 0.092	2.944 $\pm$ 0.183
$Y_{x/s}$	11.789 $\pm$ 1.674	9.248 $\pm$ 4.489	3.393 $\pm$ 1.845
m_s	0.002 $\pm$ 0.000	0.002 $\pm$ 0.000	0.001 (NS)
F (df ₁ =3, df ₂ =7; α =0.05)	15626.30	16140.11	1699.37
p -value	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9895	0.9895	0.9757
NITROGEN (N)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
N_0	83.715 $\pm$ 8.442	65.292 $\pm$ 7.993	40.495 $\pm$ 6.381
$Y_{x/n}$	0.096 $\pm$ 0.079	0.326 (NS)	0.176 $\pm$ 0.172
m_n	0.012 (NS)	0.040 (NS)	0.009 (NS)
F (df ₁ =3, df ₂ =7; α =0.05)	511.99	439.36	221.41
p -value	<0.0001	<0.0001	<0.0001
r (obs-pred)	0.9625	0.9080	0.9251