ENZYMATIC DIGESTION AND *IN VITRO* FERMENTATION OF OAT FRACTIONS BY HUMAN *LACTOBACILLUS* STRAINS.

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Running Title: *In vitro* fermentation of oat fractions by lactobacilli.

Abstract

Oats have received considerable interest for their high content of soluble and insoluble fibre and for their high fermentability with probiotic lactic acid bacteria. However, these fibres are not uniformly distributed within the oat kernel. Oat fractions were obtained by debranning technology and the pearlings generated were hydrolysed *in vitro* using gastric and pancreatic enzymes of human origin. The indigestible part was separated using dialysis and the soluble and insoluble fibre was obtained by precipitation with ethanol. The suspensions were later fermented by lactic acid bacteria of human origin to evaluate the prebiotic potential of the oat fractions and flours *in vitro*. Of the three probiotic strains tested, *Lactobacillus plantarum* showed in all media a higher maximum growth. The 1-3% pearling oat sample has higher fermentation ability and the indigestible components of this fraction showed the highest growth of lactobacilli.

Keyword: Oat, Prebiotic, Probiotic, Fibre, Debranning, Lactobacillus.

Introduction

In recent years, much research has focused on characterising the physiological effects resulting from human consumption of a wide variety of dietary fibre sources. Dietary fibre comprises a group of low-calorie carbohydrates whose chemical structure prevents them from being digested by humans due to the lack of the digestive enzymes needed for its hydrolysis. There are two basic types of dietary fibre: soluble and insoluble fibre. Soluble fibre dissolves in water and can form viscous media that slow down the rate of digestion in the gut. Other fibres are insoluble in water and do not affect the rate of digestion. Fibre that escapes colonic degradation, bacterial cells arising from fermentation, and water associated with these components all serve to increase faecal bulk, which could have an impact on the reduction of conditions such as colon cancer and irritable bowel syndrome.

Cereals are one of the most suitable components for the production of a foods contain a probiotic microorganism (in most cases lactic acid bacteria or bifidobacteria) and a prebiotic substrate (non-digestible oligosaccharides that feed the gut flora), that is, synbiotic product. The synbiotic concept has recently been proposed to characterise health-enhancing foods and supplements used as functional food ingredients [1]. Cereals contain all of the essential nutrients for fermentation, fibre, carbohydrates, proteins, vitamins, lipids and minerals. These different components are found in specific fractions of the grain and are not distributed uniformly. The bran fraction, which is responsible for protecting the cereal seed, contains high levels of fibre, potassium, sodium, magnesium and calcium [2]. The aleurone layer includes niacin, phytic acid and phosphorus, and the

endosperm mostly contains starch, which is the largest component of the kernel (82 % dry basis). The embryo, responsible for the development of roots and shoot during germination, has the majority of the grain lipids, fats and sugars [3].

Oats, unlike other cereals have received considerable interest as delivery vehicles for probiotics due to their high content of soluble and insoluble fibres resulting in positive effects on blood cholesterol levels [4,5]. It is possible to isolate the fibre rich fraction from cereals by conventional cereal processing like milling and/or debranning technology [6].

Prebiotics promote the increment in numbers and/or activity of beneficial microorganisms in the human large intestine, predominantly bifidobacteria and lactic acid bacteria [7]. Most of the development work on new prebiotic ingredients has focused on non-digestible oligosaccharides, substrates that accomplish two main requisites to be classified as prebiotics: they are capable of resisting hydrolysis and absorption in the stomach or small intestine, and they can stimulate selectively the growth of bacterial groups in the human colon associated with a healthy intestinal tract [8]. Fructose oligomers are the most studied oligosaccharides and their effect on the growth of colon beneficial bacteria has been demonstrated [1,7,9-11]. Nevertheless, other oligosaccharides, such as xylo-oligosaccharides, have also been referred as emerging prebiotics that may present the same or more desirable properties than the established prebiotics, although their use and production are not widespread [12,13].

Too often, ingredients are added to diets on the assumption that because they are “fibre”, or “soluble”, they will also be fermentable and therefore have a positive influence on gut health. However, this is not necessarily the case. Given the increasing interest in the use of fermentable components for human and animal diets, it is important to develop a method to evaluate the potential fermentability in the gut, particularly in response to an appropriate microbial population. Ideally, such evaluations should be preformed *in vivo*, but given the high costs associated with the conduct of human and animal trials, a number of groups have developed *in vitro* methods to predict the physiological effects of dietary fibre consumption [14]. In the method developed for this study, oat fractions were first digested using human digestive enzymes. The digestible sugars and amino acids obtained, which would be absorbed before reaching colon, were later separated by dialysis.

The aim of this work is to study the fermentation of soluble, insoluble and non-digestible fractions of oat fractions separated by debranning, whole oat flour and bran by human *Lactobacillus* strains to test its *in vitro* prebiotic protential. As criteria for comparison, the assessment is based on the kinetic parameters of the cultures, obtained by numerical adjustment of the results to the logistic equation.

Materials and Method

Preparation of the oat fractions and flours

The whole oat flour was obtained by milling the oat grains in a hammer mill (Falling Number AB, England) fitted with a sieve of 850 µm aperture size. The oat bran sample

was obtained by combined debranning and dry milling of oats using the Satake STR-100 mill and the method developed by Wang et al. [6]. Debranning, also known as pearling, is the process of sequentially removing the grain layers by the combined action of friction and abrasion [6]. Debranning of winter oat grains (naked expression) was carried out using the Satake Abrasive Test Mill Model TM05C. Pearlings obtained between 5-20 s and 20-35 s represent 1-3% and 3-4.5% debranning of the oat kernels respectively [15].

In vitro digestion of Oat

To digest the oat fractions *in vitro* each sample (5 g) was mixed in a flask with 100 mL of 20 mM sodium phosphate buffer (pH 6.9) containing 10 mM NaCl. The solution was stirred slowly and then boiled, and the temperature of the mixture was adjusted to 37^{o}C. 250 μL of human salivary α-amylase solution (5 mg/mL in 3.6 mM $CaCl_2$) was added. The mixture was stirred for 30 minutes at 37^{o}C, and the pH of the mixture was adjusted to 2.0 with 6M HCl. 750 μL of pepsin solution (0.5 mg/mL in 0.9% NaCl) were added, and the mixture was stirred for 1 hour at 37^{o}C. After neutralization (pH 6.9) with 3M NaOH, 1.5 mL of pancreatin solution was added (0.5 mg/mL in 20 mM sodium phosphate buffer containing 10 mM NaCl at pH 6.9). After stirring for 3 hours at 37^{o}C the mixture contains both non hydrolysed cereal and the products of the enzymatic hydrolysis. A dialysis membrane of molecular cut-off of 1000 Daltons was used to separate the digested and the undigested fraction in a sodium phosphate buffer of pH 6.9. The buffer was changed twice every 2 hours and then left overnight in order to attain equilibrium and separate any possible micro molecules left in the dialysis bag. The

content from the dialysis bags was removed and used as substrate for fermentation after sterilisation (121℃ for 15 minutes).

Fermentation monitoring

Microorganisms and inocula

Lactobacillus reuteri (NCIMB 11951), *Lactobacillus plantarum* (NCIMB 8826) and *Lactobacillus acidophilus* (NCIMB 8821) originally isolated from human intestine were used for the fermentation of the oat fractions. All the lactobacilli strains were stored on slopes of MRS at 4℃.

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.

Media Preparation

Soluble and insoluble fibers of oat fractions were obtained by hydrolyzing the samples with α-amylase and amyloglucosidase as developed by Prosky et al. [16]. The supernatant was precipitated with 4 volumes of ethanol for 1 hour to separate the soluble fibre, whereas the residue was collected as insoluble fibre. 50 mL of media were prepared using distilled water containing 2% peptone, 2% yeast extract and the soluble or insoluble fibre separated before. The media were sterilised at 121°C for 15 minutes.

To obtain the non-digestible component, a 5% suspension of the samples were prepared and digested with α-amylase, pepsin and pancreatic enzymes as explained in the previous section. The micro molecules obtained after digestion were separated by dialysis. The solution removed from the bag contains the non-digestible components of the oat samples which were sterilised at 121°C for 15 minutes.

Fermentation procedures

Shake-flask fermentations were performed in duplicate using 500 mL screw-capped glass bottles. In all cases 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 the centrifuged fermented media (10 min, 5000×*g*) were stored at -20°C for later analysis. All fermentations were carried out in duplicate.

Cell enumeration

Viable cells were enumerated using the method of Miles and Misra [17]. 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 $\log_{10}$ colony forming units per mL. Dilutions with less than 10 or more than 130 colonies were discarded.

Analytical methods

The protein content in the fractions was determined by multiplying the total Kjeldahl nitrogen by a factor of 6.25. Total dietary fibre, soluble fibre and insoluble fibre were determined according to method of Prosky et al. [16]. β-glucan was determined according to method of McCleary and Codd [18] using an assay kit from Megazyme.

Kinetic model

In order to describe and compare the culture kinetics of lactic acid bacteria on the media, a logistic model was used [19,20].

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

X: Biomass as logarithm of colony forming units per millilitre ($\log_{10}$ CFU/mL).

X_m: Maximum biomass ($\log_{10}$ CFU/mL).

v_m: Maximum growth rate (($\log_{10}$ CFU/mL) h^{-1}).

λ: Lag phase growth (h).

Numerical methods

Fitting procedures and parametric estimations were calculated 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 program (StatSoft, Inc. 2001) was used to evaluate the significance of the parametric estimates (Student's t test, α=0.05) and the consistency of the models (Fisher's F test, α=0.05).

Results

Chemical composition of the oat fractions and flours

The chemical composition of the oat samples was determined using the methods earlier described (see Table 1). The analysis shows a high total dietary fibre in the 1-3% pearling fraction which is probably due to the presence of aleurone cells. The 3-4.5% pearling fraction contains more starch and the dietary fibre content is much lower. These two fractions were selected in this study due to their high fermentability with probiotic lactic acid bacteria [15].

Growth of *Lactobacillus* strains in soluble fibre of oat fractions and flours

Figure 1 shows the growth of *L. plantarum*, *L. reuteri* and *L. acidophilus* in soluble fibre media obtained from the 1-3% pearling fraction, 3-4.5% pearling fraction, whole oat flour and bran. The fit of the model to the data are satisfactory and gives an adequate representation of the cell growth. Parametric estimations to the logistic model are summarised in table 2.

A lag phase was not observed in any of the cultures, which grow exponentially after two hours of inoculation. The maximum cell concentration was reached after approximately 12 hours in all cases. *L. plantarum* maximum biomass concentration (X_m) was 7.3 $\log_{10}$ CFU/mL in whole oat flour, 8.3 in 1-3% pearling fraction, 6.3 in 3-4.5% pearling fraction and 7.9 $\log_{10}$ CFU/mL in bran. Similar growth was observed with *L. reuteri* and *L. acidophilus*. The maximum growth rate (v_{mx}) shows the same tendency that the maximum

biomass concentration. Amongst all fractions, the maximum growth was obtained in the 1-3% pearling fraction for all strains (8.3, 7.8 and 7.6 $\log_{10}$ CFU/mL in *L. plantarum, L. reuteri* and *L. acidophilus* respectively).

Growth of *Lactobacillus* strains in insoluble fibre of oat fractions and flours

In Figure 2 and table 3 the results obtained for insoluble fibre of oat fractions and flours are shown. Comparatively, growth of all strains was much lower in these media. Approximately after two hours of inoculation, exponential growth was observed for *L. plantarum* in the 1-3% pearling fraction and bran. A lag phase of approximately 6 hours was noted in the whole oat flour media and there was no significant growth in the 3-4.5% pearling fraction. A similar behaviour was observed for *L. reuteri*. *L. acidophilus* did not show significant growth or decreased by approximately 1 $\log_{10}$ CFU/mL in the 3-4.5% pearling fraction and whole oat flour. The growth of all strains was limited, especially for *L. acidophilus* where it was not possible to use the kinetic model described in equation (1).

Growth of *Lactobacillus* strains on indigestible components of oat fractions and flours

Figure 3 shows the actual growth and the predicted growth by the logistic model for *L. plantarum*, *L. reuteri* and *L. acidophilus* cells in the different media. The numerical values of the kinetic parameters obtained from these fits as well as their corresponding statistical analysis are summarised in table 4. According to these results, the medium prepared from 1-3% pearlings led to the highest maximum cell population (X_m) and the

maximum growth rate (v_m). *L. plantarum* was the strain that experienced the highest cell growth in all media. However, lactobacilli growth in the 3-4.5% pearling fraction was very low and the cell concentration only increased from 5.2 to 6.2 and 6.5 $\log_{10}$ CFU/mL for *L. reuteri* and *L. plantarum* respectively. The growth of *L. acidophilus* in all media was very poor. In order to test if nutrients required for growth have been removed by dialysis, one of the fermentation broths (whole oat flour media with *L. acidophilus*) was supplemented with 5 g/L of fructo oligosaccharides (a well established carbon source for lactobacilli). This addition did not significantly affect the cell growth, which was very similar to the one observed without the supplement.

Discussion

Fermentation can have both positive and negative effects in the gut, which to a large extent depends on whether fermentation is of carbohydrates or proteinaceous substances. Fermentation of carbohydrates leads to the production of short chain fatty acids resulting in ammonia consumption as N source for microbial growth [21]. However, fermentation of proteins produces branched-chain fatty acids [22], releases ammonia and often other potentially toxic compounds such as amines and short-chain phenols [22-24]. It has also been observed that some potential pathogens are protein-fermenters, and are more likely to grow in conditions that favour protein fermentations [25]. It is therefore preferable to stimulate carbohydrate fermentations and minimize that of proteins along the entire gut.

Fermentation mostly occurs in the large bowel, though some studies suggest that fermentative activities can also take place in the small intestine [26]. The human ileum

has been reported to contain bacterial populations of 10^5–10^6 colonies/g [27]. Small intestinal bacteria could ostensibly affect the digestive processes, but relatively little data exists about the effects of starch and fibre on the small and large intestinal reactions.

Growth of lactobacilli in soluble and insoluble fibres of oat pearlings, whole flour and bran

Soluble fibre is made up of sticky substances like gums and gels and dissolves in water. Studies have shown that foods rich in soluble fibre can lower the blood cholesterol of individuals in a low fat and low cholesterol diet. Soluble fibre increases the passage of bile acids through the digestive system reducing cholesterol levels in blood. Oat fractions with high concentrations of soluble fibre showed high growth of all three *Lactobacillus* strains used in this study [15]. The smaller growth observed in the 3-4.5% pearling fraction could be justified by the fact that this fraction contains only 2.83% of soluble fibre. The 1-3% pearling fraction contains 14.56% of soluble fibre and led to the highest growths for all strains. Similar maximum growths were obtained when *L. plantarum* B28 and *L. casei* spp *paracasei* B29 were fermented with oats [28] and heat-treated oat mash [29]. Other authors have used dietary fibre obtained from oat and barley to increase the β-glucan level at the end of LAB fermentation [30]. Specific oligosaccharides obtained from oat bran have also been fermented with LAB [31], and mixtures of oats and fat-free milk have also been used for the development of novel probiotic formulations [32].

Insoluble fibre is a coarse material that does not dissolve in water. It helps preventing constipation as it swells and softens the stool and stimulates the intestinal muscles. It also

283 prevents intestinal disorders as it reduces pressure in the intestine by increasing the
284 movement of food. Increasing the amount and speed of mass through the intestinal tract
285 also reduces the time for the accumulation of harmful substances, which may also help
286 preventing colonic cancer. Insoluble fibres are poorly fermented by lactobacilli, which
287 justifies the fact that in our study none of the fractions showed significant growth of the
288 *Lactobacillus* strains.
289
290 **Growth of lactobacilli in indigestible oat pearlings, whole flour and bran**
291 In this work, indigestible oat fractions were used as sole carbon source for fermentation
292 by three lactobacillus strains. *L. plantarum* and *L. reuteri* grew well in the 1-3% pearling
293 fraction, whole flour and bran. No significant growth has been observed in the
294 indigestible medium obtained from the 3-4.5% pearling fraction. The reason for this
295 could be attributed to the fact that this sample mostly contains starch, which is digested
296 by the gastric and pancreatic enzymes and removed by dialysis. Previous researchers
297 have investigated the microbial growth in cereal substrates, but the growth on indigestible
298 cereal fractions has not been studied [4,5,28-34]. *L. acidophilus* hardly grows in either of
299 these fractions. This trend has also been observed in the previous two fractions and has
300 been previously reported [33,34], which indicates the growth limitations of this strain in
301 cereal media.
302
303 Of the three broths where growth was significant, the indigestible medium from the 1-3%
304 pearling fraction gave the maximum biomass populations, followed by bran and whole
305 flour, which could be related to the fibre content. The dietary fibre content in the 1-3%

306 pearling fraction, bran and whole flour is 32.3, 17.4 and 12.8% respectively. Whole flour
307 contains less dietary fibre and more starch easily hydrolysed by the digestive enzymes.
308 The hydrolysis products would be removed by dialysis, which would leave less nutrients
309 in the fermentation broth for lactobacilli to grow. The 1-3% pearling fraction and bran
310 contain less digestible components and more fibre, and produce media with more
311 nutrients for the strains to grow.
312
313 **References**

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398

TABLES CAPTIONS

Table 1 Chemical composition of oat fractions and flour.

Table 2 Parametric estimations to the logistic model applied to the Lactobacilli cultures on soluble fibre of oat fractions. CI: confidence intervals with α=0.05. r: correlation coefficient between observed and predicted data.

Table 3. Parametric estimations to the logistic model applied to the Lactobacilli cultures on insoluble fibre of oat fractions. CI: confidence intervals with α=0.05. r: correlation coefficient between observed and predicted data. NS: non significant.

Table 4. Parametric estimations to the logistic model applied to the Lactobacilli cultures on oat indigestible fractions. CI: confidence intervals with α=0.05. r: correlation coefficient between observed and predicted data.

FIGURE CAPTIONS

Figure 1. Cell concentration during growth of *Lactobacillus* strains (●: *L. plantarum*, ■: *L. reuteri*, ▲: *L. acidophilus*) in soluble fibre of oat fractions (A: 1-3% pearling, B: 3-4.5% pearling, C: whole oat flour, D: oat bran) at 37ºC. The error bars are the confidence intervals (α=0.05; n=2).

Figure 2. Cell concentration during growth of *Lactobacillus* strains (●: *L. plantarum*, ■: *L. reuteri*, ▲: *L. acidophilus*) in insoluble fibre of oat fractions (A: 1-3% pearling, B: 3-4.5% pearling, C: whole oat flour, D: oat bran) at 37ºC. The error bars are the confidence intervals (α=0.05; n=2).

Figure 3. Cell concentration during growth of *Lactobacillus* strains (●: *L. plantarum*, ■: *L. reuteri*, ▲: *L. acidophilus*) in different indigestible components of oat fractions (A: 1-3% pearling, B: 3-4.5% pearling, C: whole oat flour, D: oat bran) at 37ºC. The error bars are the confidence intervals (α=0.05; n=2).

FIGURE 1

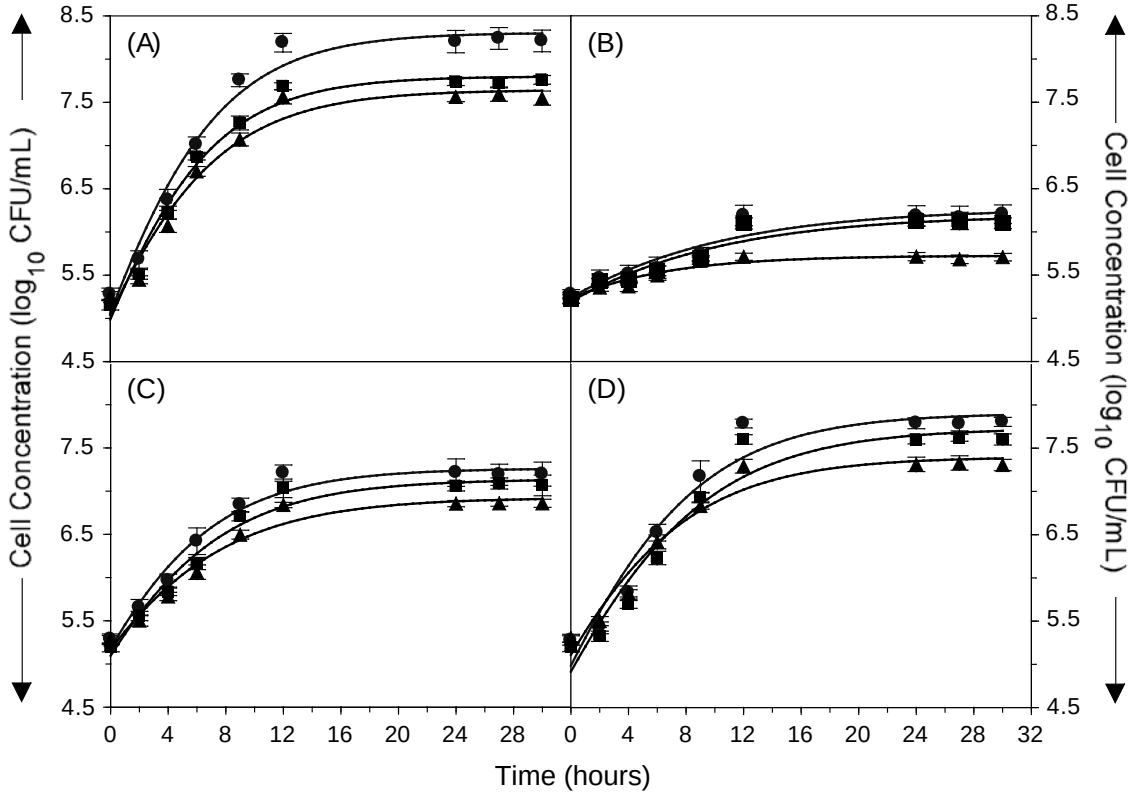
(A)
(B)
(C)
(D)
Cell Concentration (log_{10} CFU/mL)
Cell Concentration (log_{10} CFU/mL)
Time (hours)
8.5
7.5
6.5
5.5
4.5
0
4
8
12
16
20
24
28
32

FIGURE 2

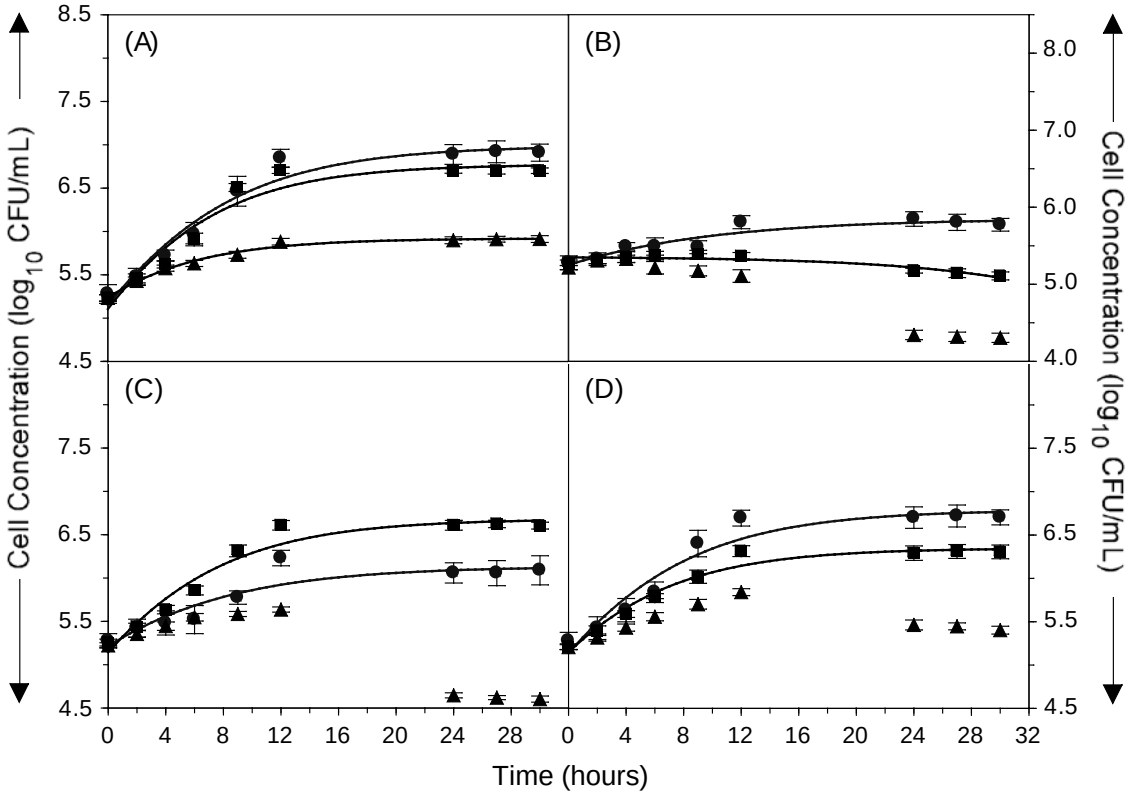
(A)
(B)
(C)
(D)
Cell Concentration (log_{10} CFU/mL)
Cell Concentration (log_{10} CFU/mL)
Time (hours)
8.5
7.5
6.5
5.5
4.5
8.0
7.0
6.0
5.0
4.0
0
4
8
12
16
20
24
28
32

FIGURE 3

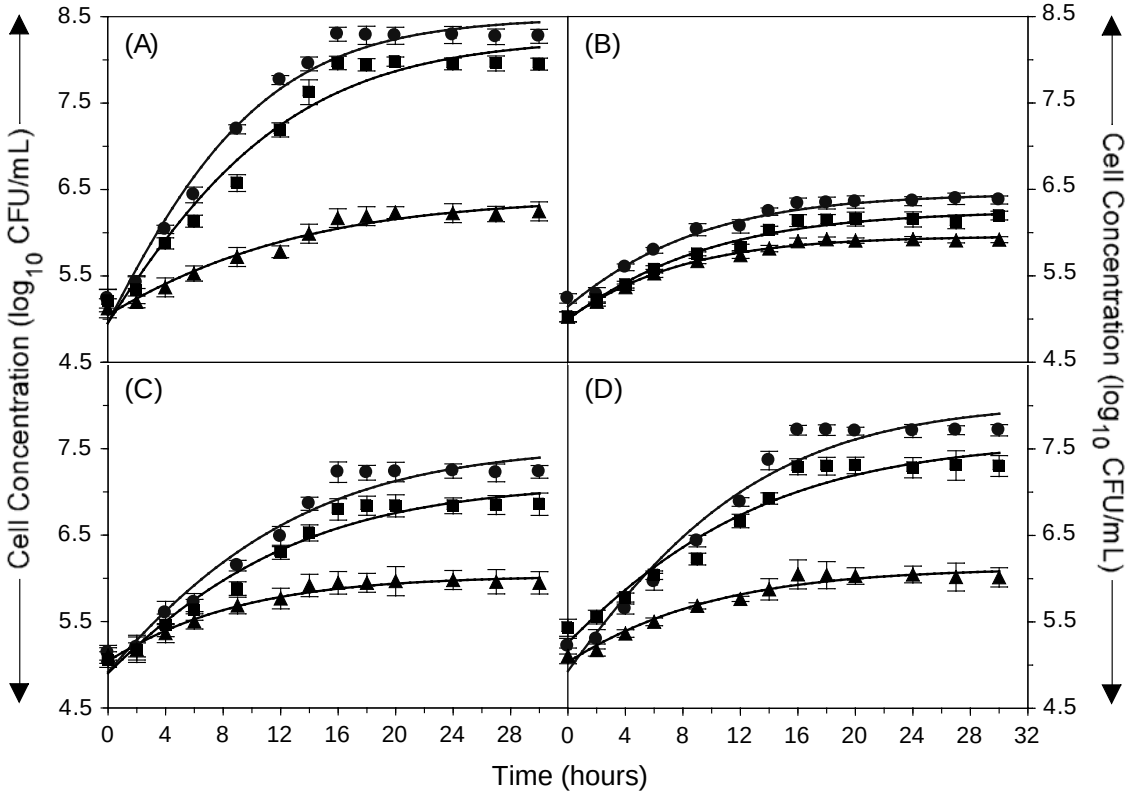
(A)
(B)
(C)
(D)
Cell Concentration (log_{10} CFU/mL)
Cell Concentration (log_{10} CFU/mL)
Time (hours)
8.5
7.5
6.5
5.5
4.5
0
4
8
12
16
20
24
28
32

TABLE 1

Oat Sample	Chemical Composition (%)					
	Moisture	Protein	Total dietary Fiber	Soluble Fiber	Insoluble Fiber	β-Glucan
1-3% Pearling Fraction	11.24	9.09	32.34	14.56	17.46	7.43
3-4.5% Pearling Fraction	12.45	10.81	7.23	2.83	4.31	2.12
Whole Oat Flour	11.91	15.31	12.82	5.93	6.66	4.05
Oat Bran	11.31	12.76	17.42	7.43	7.96	5.06

TABLE 2

Soluble Fractions	Strains	X_m (value ± CI)	v_m (value ± CI)	*F*-Fisher (df_1=3, df_2=6; α=0.05)	r
1-3 % pearling	*L. plantarum*	8.306 ± 0.277	0.448 ± 0.130	4371.91	0.9901
	L. reuteri	7.801 ± 0.215	0.431 ± 0.114	6404.51	0.9918
	L. acidophilus	7.643 ± 0.273	0.386 ± 0.137	4079.19	0.9856
3-4.5 % pearling	*L. plantarum*	6.269 ± 0.291	0.170 ± 0.137	7037.42	0.9606
	L. reuteri	6.195 ± 0.255	0.170 ± 0.125	8638.06	0.9665
	L. acidophilus	5.724 ± 0.092	0.230 ± 0.149	29050.25	0.9630
Whole Flour	*L. plantarum*	7.263 ± 0.194	0.351 ± 0.112	7782.47	0.9889
	L. reuteri	7.137 ± 0.203	0.320 ± 0.106	7342.28	0.9883
	L. acidophilus	6.924 ± 0.205	0.289 ± 0.111	7476.00	0.9852
Bran	*L. plantarum*	7.906 ± 0.410	0.352 ± 0.158	2145.75	0.9783
	L. reuteri	7.731 ± 0.438	0.321 ± 0.155	1963.55	0.9759
	L. acidophilus	7.399 ± 0.285	0.326 ± 0.133	4045.22	0.9825

TABLE 3

Insoluble Fractions	Strains	X_m (value ± CI)	v_m (value ± CI)	*F*-Fisher (df_1=3, df_2=6; α=0.05)	r
1-3 % pearling	*L. plantarum*	6.988 ± 0.249	0.263 ± 0.113	5883.99	0.9829
	L. reuteri	6.773 ± 0.258	0.288 ± 0.148	4417.34	0.9735
	L. acidophilus	5.920 ± 0.047	0.258 ± 0.063	105898.90	0.9942
3-4.5 % pearling	*L. plantarum*	5.850 ± 0.204	0.156 (NS)	13012.86	0.9431
	L. reuteri	-	-	-	-
	L. acidophilus	-	-	-	-
Whole Flour	*L. plantarum*	6.133 ± 0.288	0.204 ± 0.203	4369.24	0.9278
	L. reuteri	6.680 ± 0.224	0.261 ± 0.124	6358.35	0.9787
	L. acidophilus	-	-	-	-
Bran	*L. plantarum*	6.789 ± 0.278	0.258 ± 0.139	4443.41	0.9734
	L. reuteri	6.348 ± 0.138	0.258 ± 0.100	14678.43	0.9856
	L. acidophilus	-	-	-	-

TABLE 4

Indigestible Fractions	Strains	X_m (value ± CI)	v_m (value ± CI)	*F*-Fisher (df_1=3, df_2=10; α=0.05)	r
1-3 % pearling	*L. plantarum*	8.489 ± 0.277	0.335 ± 0.075	7438.43	0.9902
	L. reuteri	8.257 ± 0.402	0.269 ± 0.080	4918.01	0.9839
	L. acidophilus	6.410 ± 0.212	0.151 ± 0.053	25084.49	0.9859
3-4.5 % pearling	*L. plantarum*	6.451 ± 0.104	0.216 ± 0.054	39888.52	0.9907
	L. reuteri	6.249 ± 0.101	0.195 ± 0.046	47266.28	0.9919
	L. acidophilus	5.958 ± 0.041	0.219 ± 0.033	176117.4	0.9965
Whole Flour	*L. plantarum*	7.534 ± 0.422	0.210 ± 0.075	5590.47	0.9801
	L. reuteri	7.105 ± 0.326	0.192 ± 0.064	8991.67	0.9837
	L. acidophilus	6.028 ± 0.074	0.193 ± 0.045	74648.57	0.9921
Bran	*L. plantarum*	8.041 ± 0.452	0.242 ± 0.081	4503.46	0.9810
	L. reuteri	7.604 ± 0.422	0.197 ± 0.077	6962.12	0.9787
	L. acidophilus	6.122 ± 0.123	0.181 ± 0.059	34961.02	0.9859