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

2 ***Lactobacillus plantarum***

3

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18   **ABSTRACT**

19

20   Whole oat flour obtained by hammer milling was fermented with *L. plantarum* along

21   with white flour and bran in order to compare the suitability of these substrates for the

22   production of a probiotic beverage. The three substrates show a viable cell concentration

23   at the end of fermentation above the minimum required in a probiotic product. The

24   highest cell concentration was observed in white flour (9.16 Log_{10} CFU/mL) and the

25   lowest in the bran sample (8.17 Log_{10} CFU/mL).

26

27   **Keywords:** *Lactobacillus plantarum*, probiotic, fermentation, oat flour, unstructured

28   mathematical model.

29

30   **INTRODUCTION**

31

32   There has been a recent increase in the use of dietary components that help to maintain,

33   or even improve, the gut micro flora balance. Previous studies have shown that cereals

34   are good substrate for the proliferation of probiotic lactic acid bacteria and could also be

35   used as prebiotics and symbiotic (Charalampopoulos, Pandiella, Wang, & Webb, 2002).

36   Oat grains are packed with nutrients and impart valuable health benefits. They contain

37   biologically active ingredients like dietary and functional fibres that are part of the bran

38   and germ of the grain. Some of these parts of the grain are removed in conventional flour

39   milling to produce white oat flour. Whole grains would have added health benefits by

40   maximising the intake of fibre (Nyman, Siljestroem, Pedersen, Bach Knudsen, Asp,

41   Johansson, & Eggum, 1984).

42

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).

50

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

3

65 reduction grindings by a pair of smooth rolls, separated the groats pearled for 20 s into six

66 flour fractions (white flour) and four bran fractions (bran) (Wang, Koutinas, & Campbell,

67 2007). The overall flour extraction rate reached 51% of the original groat weight. The

68 weight ratio of the bran fractions together with the pearlings, 49%, satisfied one of the

69 four criteria in the definition for oat bran by the AACC (not more than 50% of the

70 starting material; Fulcher & Miller, 1993).

71

72 **Fermentation monitoring**

73 *Microorganism and inocula*

74 *L. plantarum* (NCIMB 8826) originally isolated from human intestine was used for the

75 fermentation of the oat sample and the strain was stored on slopes of MRS at 4°C.

76

77 To obtain sufficient cells for parallel experiments each inoculum was proliferated from

78 the slopes twice in universal bottles containing 20 mL MRS suspension. After 48 h, 0.5

79 mL of the broth from the first incubation were transferred into freshly sterilized MRS

80 suspension to propagate for another 24 h.

81

82 *Fermentation procedures*

83 Shake-flask fermentations were performed in duplicate using 500 mL screw-capped glass

84 bottles. In all fermentations, 5% w/v suspensions of the different fractions were prepared

85 and autoclaved at 121°C for 15 min. Bottles were inoculated with a 2% v/v of lactic acid

86 bacteria and incubated at 150 rpm and 37°C for 30 h.   Samples were regularly taken for

4

87 total cell counting and centrifuged fermented media (10 min, 4500 rpm) were stored at -

88 20°C for later analysis.

89

Cell enumeration

91 Viable cells were enumerated using the method of Miles and Misra (Collins, 1984).

92 Decimal dilutions of fermentation broths were prepared using sterile Ringer's solution.

93 12 µL were dropped onto 3-4 day old MRS agar plates and then incubated at 37°C for 2-3

94 days. Viable cell counts were calculated as $\log_{10}$ colony forming units per mL. Dilutions

95 with less than 10 or more than 130 colonies were discarded.

96

Chemical analyses

98 Total dietary fibre, soluble fibre and insoluble fibre were determined according to the

99 method of Prosky & al. (1992). β-glucan was measured by the McCleary & Codd method

100 (McCleary & Codd, 1991) using an assay kit from Megazyme. The concentration of

101 soluble free amino nitrogen (FAN) during fermentation was assayed by the EBC-

102 ninhydrine colorimetric method (European-Brewery-Convention, 1973). The protein

103 content was calculated by multiplying the total Kjeldahl nitrogen by a factor of 6.25.

104 Total reducing sugar (TRS) was assayed by the dinitrosalicylic acid method (Miller,

105 1959), and the concentration of lactic acid was obtained using an analytical kit from

106 Megazyme.

107

108 **Mathematical models**

109 In order to describe and compared the kinetics of the lactic acid bacteria on the oat flour

110 media, an unstructured mathematical model was used (Vázquez & Murado, 2008;

111 Vázquez & Murado, 2008). The variables fitted by this approach were the biomass

112 concentration (X: as $\log_{10}C$, being C the colony forming units per mL), the lactic acid

113 concentration (L), the total the reducing sugars (S) and the free amino nitrogen (N). The

114 definition and units of the model parameters and variables are shown in table 1.

115
$$X = \frac{X_m}{1 + \exp\left[2 + \frac{4 \cdot v_{mx}}{X_m} \cdot (\lambda_x - t)\right]} \tag{1}$$

116
$$L = \frac{L_m}{1 + \exp\left[2 + \frac{4 \cdot v_{ml}}{L_m} \cdot (\lambda_l - t)\right]} \tag{2}$$

117
$$S = S_0 + \frac{X_0}{Y_{x/s}} - \frac{1}{Y_{x/s}} \cdot \frac{X_m}{1 + \left(\frac{X_m}{X_0} - 1\right) \cdot e^{-\frac{4 \cdot v_{mx}}{X_m} \cdot t}} - \frac{m_s \cdot X_m^2}{4 \cdot v_{mx}} \cdot \ln\left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mx} \cdot t}{X_m}} - 1\right) + X_m}{X_m}\right] \tag{3}$$

118
$$N = N_0 + \frac{X_0}{Y_{x/n}} - \frac{1}{Y_{x/n}} \cdot \frac{X_m}{1 + \left(\frac{X_m}{X_0} - 1\right) \cdot e^{-\frac{4 \cdot v_{mx}}{X_m} \cdot t}} - \frac{m_n \cdot X_m^2}{4 \cdot v_{mx}} \cdot \ln\left[\frac{X_0 \cdot \left(e^{\frac{4 \cdot v_{mx} \cdot t}{X_m}} - 1\right) + X_m}{X_m}\right] \tag{4}$$

119

120 **Numerical and statistical methods**

121 Fitting procedures and parametric estimations calculated from the results were carried out

122 by minimisation of the sum of quadratic differences between observed and model-

123 predicted values, using the non linear least-squares (quasi-Newton) method provided by

124 the macro 'Solver' of the Microsoft Excel XP spreadsheet. Statistica 6.0 software

125   (StatSoft, Inc. 2001) was used to evaluate the significance of the estimated parameters by

126   fitting the experimental values to the proposed mathematical models, and the consistency

127   of these equations.

128

129   **RESULTS**

130

131   The characterisation of the different oat samples is shown in table 2. Figure 1 shows the

132   growth of *L. plantarum* and the chemical changes during fermentation in the three media

133   with time. The numerical values of the kinetic parameters obtained from fitting the

134   experimental data to the unstructured mathematical models, as well as the statistical

135   analysis of the equations and parameters validation are summarised in the table 3.

136   According to these results, the medium prepared from white flour led to the highest

137   maximum biomass production and the maximum growth rate (v_{mx}). Whole flour

138   produced a slightly lower cell concentration, and in bran a maximum cell concentration

139   one Log_{10} CFU/mL below white flour was achieved. The numerical values of the

140   parameter $-\lambda_x-$ are not shown in table 3 because they were negatives (not realistic) and

141   therefore not useful for comparative purposes.

142

143   The highest concentration ($p < 0.05$) and maximum production rate of lactic acid (v_{ml})

144   was also obtained in white flour (1.2 g/L and 0.09 g L^{-1} h^{-1} respectively). The evolution of

145   pH decreased accordingly to this organic acid formation. The final TRS concentrations

146   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,

147   respectively. The sugar consumption was adequately described by the proposed model (3)

7

148 (see statistical analysis in table 3). The maximum value of $Y_{x/s}$ was obtained in whole

149 flour followed white flour and bran. In all three fermentation FAN decreases after a small

150 initial rise in the first 4 h of fermentation. For the whole flour broth, FAN increases from

151 74.86 mg/L to 80.44 mg/L and then decreases to 36.42 mg/L after 30 hours. Similar

152 trends were observed for the white flour and bran media.

153

154 **DISCUSSION**

155

156 Due to the complexity of the cereal substrates used, the main compositional changes that

157 were monitored to justify their fermentability were FAN and sugars. Research studies

158 using semi-defined synthetic media have identified these compounds as the most crucial

159 factors for LAB growth (Bethin & Villadsen, 1996; Taillandier, Gilis, Portugal, Laforce,

160 & Strehaiano, 1996; Loubiere, Cocaign-Bousquet, Matos, Goma, & Lindley, 1997). The

161 fractions used had a maximum particle size of 850 μm (the aperture size of the sieve in

162 the hammer mill was 850 μm). The broths prepared from them are homogeneous liquid

163 media with some non-fermentable insoluble bran particles in suspension. It is then

164 possible to compare the different cultures and to fit the numerical data to the

165 mathematical models defined in the materials and methods section.

166

167 The growth of LAB in the whole flour was comparable with the results obtained from

168 previous workers in oat and other cereals. *L. plantarum* has also been reported to grow

169 well in wheat and barley without the need for additional nutrients, where along with other

170 LAB it is used for the industrial production of lactic acid (Hofendahl & Hahn-Hagerdal,

171   1997). Patel & al. (2004) reported a maximum growth of *L .plantarum* in malt, barley and

172   wheat of 9.15 Log_{10} CFU/mL, 8.46 Log_{10} CFU/mL and 8.39 Log_{10} CFU/mL respectively.

173   The maximum cell concentrations in whole and white flours were 8.97 Log_{10} CFU/mL

174   and 9.16 Log_{10} CFU/mL respectively, comparable with these results.

175

176   LAB are able to assimilate nitrogen in both inorganic and organic forms, although the

177   availability of amino acids is critical for the growth of fastidious bacteria such as

178   lactobacilli (Plessis, Dicks, Vescovo, Torriani, & Dellaglio, 1996; Vescovo, Torriani,

179   Dellaglio, & Bottazi, 1993). The nitrogen uptake in the fermentations was monitored by

180   measuring FAN. Though the FAN concentration decreased over the course of the

181   fermentations, small increments were observed in the stationary phase of growth.

182

183   The white flour showed the maximum cell concentration, which is probably due to the

184   fact that does not contain bran and non-fermentable outer layers. The bran fraction

185   obtained by this method will contain most of the outer non-fermentable layers of the

186   grain but also fermentable fractions, which justifies the considerably high value or cell

187   concentrations obtained. All values exceed the level suggested by Sanders & Huis in 't

188   Veld (1999) for a probiotic product formulation (10^6 CFU/g or CFU/mL).

189

190   In summary, the three substrates studied demonstrate their capability to support a

191   probiotic fermentation by human L. plantarum. The highest cell populations were

192   obtaind for white oat flour. According to our results the bran fraction, usually discarded

193 by the flour milling industry, could be feremented with a probiotic microorganism. This

194 could lead to the development of novel probiotic beverages.

195

196 **REFERENCES**

197

198 Axelsson, L. (1998). Lactic acid bacteria: classification and physiology. Lactic Acid Bacteria:

199 Microbiological and Functional Aspects. Ed., Salminen, S., Von Wright, A., New York, Marcel

200 Dekker 1-72.

201

202 Bethin, S., & Villadsen, J. (1996). Amino acids utilization by *Lactococcus lactis* subsp. *cremoris*

203 FD1 during growth on yeast extract or casein peptone. *Journal of Applied Biotechnology, 80,* 65-

204 72.

205

206 Charalampopoulos, D., Pandiella, S.S., Wang, R., & Webb, C. (2002). Application of cereals and

207 cereal components in functional foods: A review. *International Journal of Food Microbiology,*

208 *79,* 131-141.

209

210 Collins, C.H. (1984). Microbiological methods, Ed: Butterworths, London.

211

212 European-Brewery-Convention. (1973). Ninhydrin method for determination of free a-amino

213 nitrogen. *Journal of Institute Brewing, 79,* 37-41.

214

215 Fulcher, G.R., & Miller, S.S. (1993). Structure of oat bran and distribution of dietary fiber

216 components. *In: Oat Bran.* St. Paul, MN, USA, American Association of Cereal Chemists.

217

218   Hofendahl, K., & Hahn-Hagerdal, B. (1997). L-lactic acid production from whole wheat flour

219   hydrolysates using strains of lactobacilli and lactococci. *Enzyme and Microbial Technology, 20,*

220   301-307.

221

222   Loubiere, P.M., Cocaign-Bousquet, M.M., Matos, J., Goma, G., & Lindley, N.D. (1997).

223   Influence of end-products inhibition and nutrient limitations on the growth of *Lactococcus lactis*.

224   *Journal of Applied Microbiology, 82,* 95-100.

225

226   McCleary, B.V., & Codd, R. (1991). Measurement of (1-3) (1-4) beta glucan in barley and oats:

227   A streamlined enzymatic procedure. *Journal of Science Food and Agriculture, 55,* 303-312.

228

229   Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar.

230   *Analytical Chemistry, 31,* 426-428.

231

232   Mousia, Z., & Pandiella, S.S. (2004). Debranning advantage. *World Grain*, March (www.World-

233   Grain.com).

234

235   Nyman, M., Siljestroem, M., Pedersen, B., Bach Knudsen, K.E., Asp, N.G., Johansson, C.G, &

236   Eggum, O. (1984). Dietary fiber content and composition in six cereals at different extraction

237   rates. *Cereal Chemistry, 61,* 14-19.

238

239   Patel, H.M., Pandiella, S.S., Wang, R.H., & Webb, C. (2004). Influence of malt, wheat and barley

240   extracts on the bile tolerance of selected strains of lactobacilli. *Food Microbiology, 21,* 83-89.

241

242   Plesis, E., Dicks, L., Vescovo, M., Torriani, S., & Dellaglio, F. (1996). *Lactobacillus acidophilus*

243   and related species: a review. *Annali di Microbiologie ed Enzimologia,* 46, 319-340.

244

245 Prosky, L., Asp, N.G., Schweizer, T., DeVries J., & Furda, I. (1992). Determination of insoluble

246 and soluble dietary fibre in foods and food products: Collaborative study. *J.-A. Official Analytical*

247 *Chemistry 75,* 360-367.

248

249 Saarela, M., Mogensen, G., Fonden, R., Matto, J., & Mattila-Sandholm, T. (2000). Probiotic

250 bacteria: safety, technological and functional properties. *Journal of Biotechnology, 84,* 197-215.

251

252 Sanders, M.E., & Huis in 't Veld, J.H.J. (1999). Bringing a probiotic-containing functional food to

253 the market: Microbiological, product, regulatory and labelling issues. *Antonie Van Leewenhoek,*

254 *76,* 293-315.

255

256 Taillandier, P., Gilis, F., Portugal, F.R., Laforce, P., & Strehaiano, P. (1996). Influence of

257 medium composition, pH and temperature on the growth and viability of Lactobacillus

258 acidophilus. *Biotechnology Letters, 18,* 775-780.

259

260 Vázquez, J.A., & Murado, M.A. (2008). Mathematical tools for objective comparison of

261 microbial cultures. Application to evaluation of 15 peptones for lactic acid bacteria productions.

262 *Biochemical Engineering Journal*, *39,* 276-287.

263

264 Vázquez, J.A., & Murado, M.A. (2008). Unstructured mathematical model for biomass, lactic

265 acid and bacteriocin productions by lactic acid bacteria in batch fermentation. *Journal of*

266 *Chemical Technology and Biotechnology, 83,* 91-96.

267

268   Vescovo, M., Torriani, S., Dellaglio, F., & Bottazzi, V. (1993). Basic characteristics, ecology and

269   application of *Lactobacillus plantarum*: a review. *Annali di Microbiologie ed Enzimologia, 43,*

270   261-284.

271

272   Wang, R., Koutinas, A.A., & Campbell, G.M. (2007). Dry processing of oats- Application of dry

273   milling. *Journal of Food Engineering, 82,* 559-567.

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275

276 **FIGURE CAPTIONS**

277

278 **Figure 1.** Fermentation of *Lactobacillus plantarum* in the oat samples. Continuous lines

279 represent the mathematical models used to fit experimental data represented by points. ○,

280 White Flour; ●, Bran; ▲, Whole Flour. L: Lactic acid; TRS: Total reducing sugars; N:

281 Nitrogen.

282

283

284 **TABLE CAPTIONS**

285

286 **Table 1**. Notation used with units.

287

288 **Table 2**. Characterisation of the oat samples used (mean ± standard deviation for n=3).

289

290 **Table 3.** Parametric estimations corresponding to the kinetic models (1-4), applied to the

291 cultures of *L. plantarum* in the oat samples. CI: confidence intervals ($\alpha = 0.05$). *F*: F-

292 Fisher test (df_1 = model degrees freedom and df_2 = error degrees freedom). r = correlation

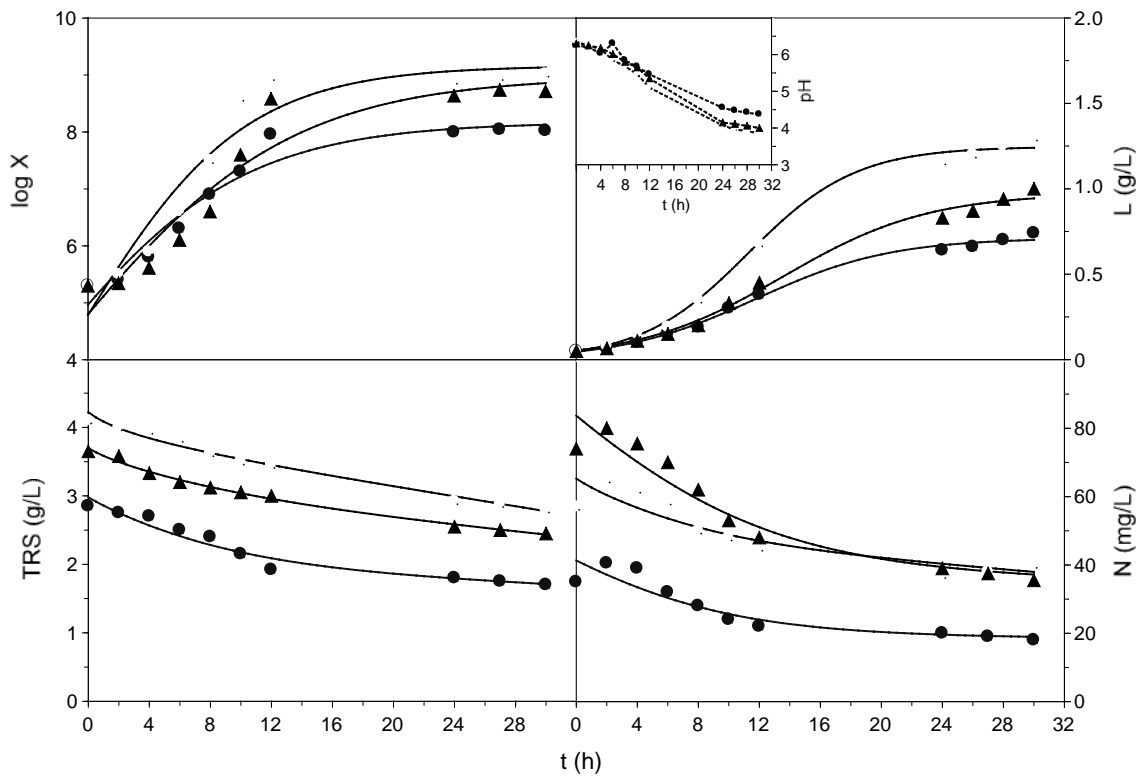293 coefficient between observed and predicted data. NS = Not Significant.

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297

298

FIGURE 1

15

TABLE 1

X :	Biomass as logaritm 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)
v_{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
v_{ml} :	Maximum lactic acid production rate, g L^{-1} 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, g (sugar) L^{-1} [$\log_{10}$ (CFU/mL)] $^{-1}$ 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, mg (nitrogen) L^{-1} [$\log_{10}$ (CFU/mL)] $^{-1}$ h^{-1}

TABLE 2

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

TABLE 3

	OAT SAMPLES		
VARIABLES	Whole flour	White flour	Bran
GROWTH (X)	values $\pm$ CI	values $\pm$ CI	values $\pm$ CI
X_m	8.973 ± 0.910	9.161 ± 0.703	8.172 ± 0.469
X_0	4.800 ± 0.120	4.785 ± 0.121	4.966 ± 0.101
v_{mx}	0.314 ± 0.173	0.431 ± 0.198	0.324 ± 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 ± 0.086	1.246 ± 0.048	0.711 ± 0.038
v_{ml}	0.051 ± 0.012	0.088 ± 0.017	0.040 ± 0.008
λ_l	4.032 ± 1.539	4.207 ± 1.088	2.950 ± 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 ± 0.079	4.143 ± 0.092	2.944 ± 0.183
$Y_{x/s}$	11.789 ± 1.674	9.248 ± 4.489	3.393 ± 1.845
m_s	0.002 ± 0.000	0.002 ± 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 ± 8.442	65.292 ± 7.993	40.495 ± 6.381
$Y_{x/n}$	0.096 ± 0.079	0.326 (NS)	0.176 ± 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