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In vitro gas production kinetics of whole citrus fruits*

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ABSTRACT

The *in vitro* gas production technique was used to evaluate the fermentation kinetics of 125 whole citrus fruits samples (WCF). Six models were tested to describe the fermentation characteristics of this product characterized by high sugar and pectin and low protein content: 487, 195 and 64.7 g/kg dry matter, respectively. The models were: exponential with and without lag (EXP_0 and EXP_L, respectively); generalized Mitscherlich ($GEXP_L$); and one, dual and three pool logistic models ($OLOG_L$, $DLOG_L$ and $TLOG_L$, respectively). EXP_0 had the highest residual standard deviation (RSD=9.0 ml), the lowest being for the $TLOG_L$ (5.93 ml). $TLOG_L$ model obtained the best values fitting individual curves with a mean prediction error of 0.25 ml. Significant correlations were observed between the three different fractions of the $TLOG_L$ model and the chemical composition: quickly fermentable fraction with sugars (+0.43; $P<0.001$), high fermentable fraction with pectins (+0.23; $P<0.05$) and slow fermentable fraction with fibres (+0.38 and +0.34 for NDF and ADF, respectively; $P<0.01$), which could indicate the presence of three main fractions in WCF with different fermentation rates.

KEY WORDS: *in vitro*, fermentation, kinetics, gas, citrus, sugars, pectins, fibre, ruminants

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INTRODUCTION

World citrus production generates many residues (citrus pulp, unmarketable fruit, etc.) that constitute a great environmental problem for the production areas. As an example, the Valencia region (eastern Iberian Peninsula) produces about 4 million tonnes of citrus per year, generating citrus pulp but also whole citrus fruit (WCF) residues. This waste consists of fruits which do not meet the requirements for fresh products (2%; unmarketable fruits) and a percentage of the total citrus production (maximum 5% of commercialized fruits; European Union Regulation-2200/96), which is withdrawn from the market in order to maintain the price (surplus, imposed by the European Union). Animal nutrition, and specifically small ruminants, could contribute to solving the environmental problem posed by its elimination. Moreover, high nutrient by-products are recently welcome because they are available for use as livestock feeds at competitive prices.

Until now, there have not been many studies related to the use of whole citrus fruits in livestock nutrition, although citrus pulp has been extensively studied and widely used. WCF should behave in a similar way to the citrus pulp, but as higher amounts of sugars, easily rumen fermentable are present, a higher risk of acidosis problems could also be expected (Bacha, 2002), especially if the whole citrus are fed as a concentrate complement. In addition, the high variability of WCF must be considered, due to: the different species of citrus, the great amount of different varieties within each group, the collection stage, or the geographical differences. For these reasons, widely varying nutritional value and ruminal fermentation patterns could be expected.

Mathematical description of gas production profiles allows analysis of data and comparison of substrates or fermentation environment characteristics, and can provide useful information concerning the substrate composition and the fermentability of soluble and slowly fermentable components of the substrate. Therefore, an adequate tool to test these possible differences in the fermentation pattern could be the gas production technique, which allows evaluation of feed digestibility and fermentation kinetics by fitting mathematical models to the gas measurements (Groot et al., 1996). The diversity of gas production profiles and the relationship between the gas production kinetics and the extent of degradation in the rumen requires the application of an appropriate model to describe the curves and link the profile to ruminal degradation.

The aim of this work was to study the gas production kinetics of WCF, by fitting different mathematical models to the WCF gas production curve, in order to find the model which could best describe the degradation characteristics, and could give a better understanding of the ruminal degradation of this by-product.

MATERIAL AND METHODS

Samples collection and preparation

One hundred and twenty five fresh samples of whole citrus fruit (WCF) were obtained from cooperatives located at different areas of Valencia region, at three different periods of time: November-December 2002, January-February 2003, and March 2003. In each period, every citrus species available in the selected cooperatives was collected. The set consisted of 51 sweet oranges, 61 mandarins, 10 lemons and 3 grapefruits from surplus or unmarketable fruits. Once in the laboratory, the fresh WCF samples were ground up and frozen (-20ºC) until analysis. A part of each frozen sample was freeze-dried and ground to pass through a 1 mm sieve in a Wiley mill for the *in vitro* gas production analysis.

In vitro gas production measurements

Ruminal liquor was obtained from three adult Manchega sheep with ruminal fistulae. Experimental procedures were developed according to the Committee on Animal Use and Care guidelines at the Polytechnic University of Valencia, and following the codes of practice for animals used in experimental works set out by the EU (2003). The animals were fed once a day with a rich citrus pulp diet at maintenance level, kg: ensiled citrus pulp 0.7, wheat grain 0.2, soyabean meal 0.05, pelleted lucerne hay 0.3 and barley straw 0.2, on a fresh basis. Ruminal liquor of each sheep was obtained just before the morning feeding, transported to the laboratory and immediately strained through four layers of cheese cloth and mixed into an Erlenmeyer flask (the donor animals' rumen fluid had in average a pH of 6.89, 7.38 mmol N-NH_3/l and 53.26 mmol of total volatile fatty acids (VFA)/l). The squeezed rumen fluid was mixed with Menke and Steingass (1988) buffer solution (consisting of a mixture of mineral salts in a bicarbonate buffering solution: NH_4HCO_3 4 g and $NaHCO_3$ 35 g per l of distilled water) in a 1:4 (v/v) ratio at 39ºC under continuous flow with CO_2.

The samples were incubated in duplicate in two incubation trials (four replicates per sample), 0.5 g of ground freeze-dried WCF being accurately weighed into 125 ml bottles (Laboratorios Ovejero S.A., León, Spain). Fifty ml of buffered rumen fluid was added to each bottle under CO_2 flow. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39ºC.

The pressure transducer technique (Theodorou et al., 1994) was applied, the gas measurements being recorded after 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36 and 48 h of incubation using a pressure transducer (Delta OHM, Padova, Italy).

Afterwards, all the bottles were uncapped, the pH was measured immediately and the fermentation was stopped by swirling the bottles on ice.

For VFA analysis, 800 µl of the bottles supernatant was transferred to Eppendorf® vials and mixed with 80 µl of preservative solution (5% H_3PO_4 and 1% Cl_2Hg in ultra-pure water) and 100 µl of 4-methyl-valeric acid. The Eppendorf® vials were kept frozen at -80ºC until analysis. After that, the content of the bottles was transferred to previously weighed filter crucibles. The incubation residue was washed with 50 ml hot distilled water, dried at 50ºC for 48 h and weighed again to calculate the apparent *in vitro* dry matter digestibility (dMD_{iv}) as done by Carro and Ranilla (2003).

Analytical procedures

Samples were analysed for DM, ash, crude protein (CP) and crude fibre (CF) according to the AOAC methods (2003). Neutral- (NDF) and acid- (ADF) detergent fibre analyses were carried out according to Van Soest et al. (1991), with a thermo-stable amylase pre-treatment. Total soluble sugars were determined using the Fehling reagent, following the method described by Matissek et al. (1998). Pectins were extracted from the citrus samples with ethanol (Yu et al., 1996), the galacturonic acid content in extracts was analysed by the m-hydroxydiphenyl method (Kintner and Van Buren, 1982), the pectin content being calculated as the galacturonic acid content multiplied by 3 (Garleb et al., 1991).

The main VFA, acetic, propionic and butyric acids, were determined by gas chromatography, using 4-methyl-valeric acid (Fluka, Germany) as the internal standard, being identified by comparing their retention times with the ones obtained with VFA standard 46975-U from Supelco® (PA, USA). The samples were defrosted and centrifuged for 10 min (3000 rpm), then were filtered through a cellulose filter (0.45 µm) and 250 µl of them were transferred to the injection vials. Two µl of each sample were injected into the gas chromatograph (FISONS 8000 series, Milano-Italy) equipped with an automatic injector AS800. The column used was a BD-FFAP 30 m × 0.25 × 0.25 mm (J. and W. Scientific, USA). The temperature of the injector and the detector were maintained at 220 and 225ºC, respectively.

Curve fitting

In order to describe and interpret the fermentation kinetics of WCF samples, several models, frequently found in the bibliography to describe the fermentation kinetics of many raw materials, were tested: the exponential model was as described by Ørskov and McDonald (1979); the exponential model including the

lag time, by McDonald (1981); generalized Mitscherlich by France et al. (1993) and the one, dual and three pool logistic models, Schofield et al. (1994) and Pell et al. (1998). These equations will enable us to distinguish the different fractions of product fermentation: water-soluble fraction, non-soluble fermentable fraction and non-fermentable fraction, as well as the gas production fractional rates of each fraction.

These models were fitted to the cumulative gas production, determined by summation of the regression-corrected gas volumes (individual measurements) after subtractions of gas which accumulated in the correspondent control culture:

1. Exponential model (EXP_0): $Y = B\,[1- \exp(- C*t)]$;
2. Exponential model including the lag time (EXP_L): $Y = B\,[1- \exp(-C*(t - L))]$

where: Y (ml) is the gas volume at time t, B (ml) is the maximum volume at $t = \infty$, C is the fractional degradation rate (h^{-1}) and L is the lag time (h).

These models describe simple first-order reaction kinetics without or with a lag phase, respectively, and the rate of gas volume change is assumed to be proportional to the substrate level but independent of microbial mass, Schofield et al. (1994);

3. Generalized Mitscherlich ($GEXP_L$): $Y = B\,[1 - \exp(- K_1*(t - L) - K_2*(t^{1/2} - L^{1/2}))]$

where: K_1 and K_2 parameters allow the determination of the fractional rate of degradation $\mu = K_1 + K_2/(2*t^{1/2})$, which it is postulated to vary with time (France et al., 2000);

4. One pool logistic model ($OLOG_L$: $Y = B_1\,(1+\exp(2 - 4*C_1*(t - L)))^{-1}$;
5. Dual pool logistic model ($DLOG_L$) : $Y = B_1\,(1+\exp(2 - 4*C_1*(t - L)))^{-1} + B_2\,(1 + \exp(2 - 4*C_2*(t - L)))^{-1}$

The logistic models assume the rate of gas production is proportional both to the current microbial mass and to the substrate level, and where C_1 and C_2 are the specific rates of degradation of B_1 and B_2 fractions, respectively, comparable to the fractional rates of the EXP_L equation (Schofield et al., 1994; Pell et al., 1998).

Taking into account the citrus nature and the possible existence of different fractions, testing included;

6. Three-phasic logistic equation ($TLOG_L$): $Y = B_1\,(1+\exp(2 - 4*C_1*(t - L)))^{-1} + B_2\,(1+\exp(2 - 4*C_2*(t - L)))^{-1} + B_3\,(1+\exp(2 -4*C_3*(t - L)))^{-1}$.

Usually these fractions are defined as the water-soluble fraction (B_1), non-soluble fraction (B_2) and microbial turnover (B_3) (Cone et al., 1997), their respective rates of degradation being C_1, C_2 and C_3. However, due to the high diversity of fermentative fractions in citrus fruits, we were not completely sure about the last assumption.

Statistical analysis

The equations were fitted to the global and individual gas production curves by a non-linear regression procedure (PROC NLMIXED) of SAS (1990), providing least-squares estimates of parameters B, C and L. Residuals, defined as the values of the differences between the predicted and observed gas production values, were calculated for each individual curve, obtaining the residual mean and the standard deviation for each model. The Bayesian information criterion (BIC), mean prediction error (MPE) and the residual standard deviation (RSD; calculated as the root square of MPE) were obtained.

The main criteria initially used to compare models were the BIC, RSD and the coefficients of determination (R^2) of the models obtained from the global gas production, and, subsequently, the relative size of the MPE and RSD for the individual curves. The suitability of the models to predict individual gas production curves was also evaluated through the proportion of predictions with acceptable fitting, following the criteria: "Good" (RSD<2), "Fair" (2 >RSD<4) and "Poor" (RSD>4).

Finally, to test the relationship between the parameter values of the most adequate models and the chemical composition and fermentation characteristics of the analysed samples, simple correlation coefficients were obtained using the PROC CORR of SAS (1990).

RESULTS

The WCF (Table 1) had a high water content (about 85%), being its dry matter rich in sugar content (49%). The WCF was poor in crude protein (6.5% DM) and fibre (ADF=7.4% DM). Another important feature of this product was its high pectin content (19.5% DM). The high variability in sugar, pectin and fibre contents of WCF samples leads us to suppose a different fermentative behaviour, confirmed by the high variability shown for the VFA concentration after fermentation (CV about 43%).

Fitting the models to the global gas production. Figure 1 shows the gas production profile of three citrus samples: sweet orange, mandarin and lemon. The plateau of curves was reached at 48 h. For each of the six models evaluated, the fitting and parameters obtained from the global gas production of the WCF samples incubated are presented in Table 2. The coefficients of determination

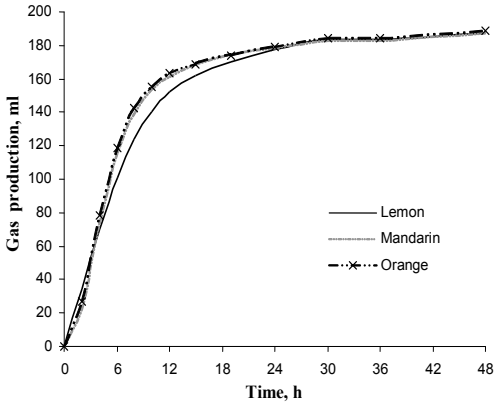

Figure 1. Gas production profile of three citrus samples: orange, mandarin and lemon incubated with buffered rumen fluid

Table 1. Chemical composition of whole citrus fruits samples (g/kg DM), and pH, *in vitro* dry matter digestibility (%) and volatile fatty acids (mmol/100 ml) after incubation (48 h)

Variable	n	Mean	SD	Minimum	Maximum	CV, %
Chemical composition						
dry matter, g/kg	125	158	19.5	116	258	12.3
ash	125	31.4	5.48	19.9	66.0	14.4
crude protein	125	64.7	12.8	33.0	103.0	22.4
crude fibre	125	28.6	14.0	3.6	69.0	49.0
NDF	125	109	34.1	43	206	31.4
ADF	125	73.7	29.4	20.0	156.0	39.9
sugars	124	487	84.9	166.5	654	17.4
pectin	125	195	53.7	60.2	343	27.5
Rumen fermentation parameters						
pH	124	6.39	0.22	4.82	6.60	3.4
dDM_{iv}	125	96.4	1.61	91.6	99.7	1.7
VFA, mmol/100 ml	100	5.49	2.37	2.62	11.30	43.1
molar proportions						
acetate	100	0.57	0.06	0.44	0.74	11.3
propionate	100	0.25	0.04	0.17	0.36	14.3
butyrate	100	0.18	0.05	0.05	0.29	27.8

NDF - neutral detergent fibre; ADF - acid detergent fibre; dDM_{iv} - apparent *in vitro* dry matter digestibility; VFA - acetate+propionate+butyrate

Table 2. Parameters, determination coefficient (R^2), residual standard deviation (RSD) and Bayesian information criterion (BIC) for the mean gas production curve of whole citrus fruits fitted to the models: EXP_0, EXP_L, $GEXP_L$, $OLOG_L$, $DLOG_L$ and $TLOG_L$ after *in vitro* incubation

Models	B_1 ml	B_2 ml	B_3 ml	C_1 h^{-1}	C_2 h^{-1}	C_3 h^{-1}	K_1 $h^{-1/2}$	K_2 $h^{-1/2}$	L h	R^2	RSD	BIC
EXP_0	188.6			0.1440						0.9781	8.998	10723
EXP_L	184.3			0.1896					1.1813	0.9903	5.972	10752
$GEXP_L$	185.2						0.1527	0.1510	1.2950	0.9904	5.945	9406.8
$OLOG_L$	178.1			0.1134					0.8181	0.9741	9.782	10827
$DLOG_L$	120.9	64.33		0.2035	0.0553				1.6673	0.9888	6.448	8747.4
$TLOG_L$	69.36	85.51	34.10	0.3144	0.1152	0.0326			1.8990	0.9905	5.927	8597.7

B - fermentable fraction/s (B_1, B_2 and B_3); C - fractional/specific fermentable rate/s (C_1, C_2 and C_3); K_1, K_2 - constants of the fractional rate of degradation, $\mu = K_1 + K_2/(2*t^{1/2})$; L - Lag; EXP_0 - exponential model without lag time; EXP_L - exponential model with lag time; $GEXP_L$ - generalized Mitscherlich; $OLOG_L$,$DLOG_L$, $TLOG_L$ - one, dual and three pool logistic models

(R^2) were high for all the models (R^2>0.97). The EXP_0 and $OLOG_L$ models presented the highest RSD (8.99 and 9.78 ml, respectively) and BIC values (10723 and 10827, respectively). The inclusion of the lag time (EXP_L equation) improved the RSD (5.972 ml), but not the BIC value (10752). The introduction of additional parameters in the $GEXP_L$ model only improved slightly the BIC value (9407). The $DLOG_L$ model only showed a better BIC (8747) than the EXP_L, but worse R^2 and RSD values. Finally, the $TLOG_L$ model had the best fit, presenting the lowest RSD (5.93) and BIC (8598) values. However, it could be due to a mathematical artefact, if no biological explanation can be provided, although the BIC is calculated considering the number of parameters used.

The residuals for EXP_L, $DLOG_L$ and $TLOG_L$ are showed in Figure 2. The EXP_L and $DLOG_L$ models presented a similar gas production pattern overestimating the gas production from 12 to 30 h of incubation and underestimating it after. However, the $TLOG_L$ model overcame theses problems, showing a good fit in the first stage of fermentation, as well as in the asymptotic phase. In any case, a well-knowing of models fitting to the individual curves would be interesting to discriminate between them.

Fitting of models to the individual gas production curves. The goodness of the models to fit individual curves, using the MPE and their individual RSD values, is presented in Table 3. Main of individual RSD values for the $OLOG_L$ and EXP_0 models were greater than 4 ml. Although the $GEXP_L$ model had a good fit to the global curves, it was worse for the individuals, having 70% of them a RSD value greater than 4 ml. The EXP_L model, with a MPE about 14 ml, had RSD values lower than 4 ml for more than 62% of the curves.

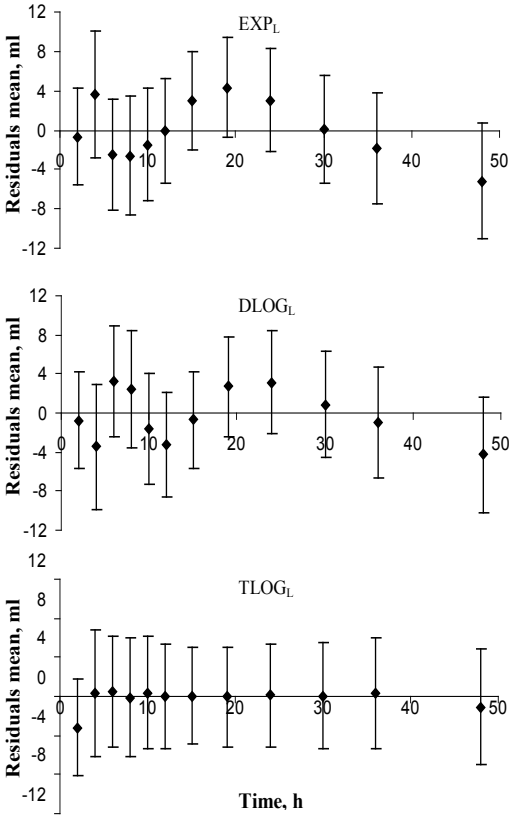

Figure 2. Residuals obtained as the difference between the predicted and the observed values for the exponential (EXP_L), dual pool logistic ($DLOG_L$) and three pool logistic ($TLOG_L$) models fitted to whole citrus fruits *in vitro* gas production

Table 3. Model goodness in fitting individual gas production curves of whole citrus fruits (n=118)

Models	MPE[1], ml	RSD[3]<2		2<RSD<4		RSD > 4	
	mean ± SE[2]	n	%	n	%	n	%
EXP_0	80.39 ± 2.20	0	0	5	4.3	112	95.7
EXP_L	13.71 ± 0.48	4	3.4	75	64.1	38	32.5
$GEXP_L$	26.18 ± 1.43	2	1.7	33	28.2	82	70.1
$OLOG_L$	88.22 ± 1.13	0	0	0	0	118	100
$DLOG_L$	8.15 ± 0.25	5	4.2	113	95.8	0	0
$TLOG_L$	0.25 ± 0.02	118	100	0	0	0	0

[1] MPE - mean prediction error; [2] SE - standard error; [3] RSD - calculated as the root of the MPE; abreviations of the models see Table 2

Nevertheless, the $DLOG_L$ and $TLOG_L$ models were those which showed the best fit to individual curves. $DLOG_L$ had an MPE value around 8 ml and 100% of the individual RSD lower than 4 ml, while $TLOG_L$ had a MPE of 0.25 ml and the 100% of the individual RSD lower than 2 ml.

Considering the fitting ability to the global and individual curves of the models, all of them were discarded with the exception of the EXP_L (simple and moderate fit) and $TLOG_L$ (complex and good fit).

Correlation between chemical composition and fermentation products with fermentation kinetics parameters. The special nature of the citrus samples, with a high number of carbohydrates with different fermentative patterns (sugars, pectins, hemicelluloses, cellulose), could justify the use of the $TLOG_L$ model, but only the introduction of more parameters in the model could explain the improvement shown in the fit. Therefore, the biological meaning of the model parameters, from the correlation between the fermentation kinetics parameters, pH or VFA production after the *in vitro* incubation and the chemical composition of WCF samples, was evaluated for the EXP_L and $TLOG_L$ models (Table 4). The lag time was highly and positively correlated with the sugar content of the samples in both models ($r=+0.68$ and $+0.54$, respectively; $P<0.001$), and negatively correlated with pectins and the fibre fractions. For the EXP_L model, B_1 was only slightly negatively correlated with the sugar content, and the C_1 correlation matrix was similar to that observed for the lag time. The higher fermentation rate (C_1) observed, the higher was the VFA production.

In the case of the $TLOG_L$ model, sugars were positively correlated with B_1 and negatively with B_2 and B_3 ($P<0.001$). The fermentation rate of B_1 (C_1) was only correlated with the protein availability ($r=+0.19$; $P<0.05$). B_2 was positively correlated to the pectin content of the samples ($r=+0.23$; $P<0.05$) and its fermentative rate C_2 was to the sugar content. On the other hand, B_3 fraction was positively correlated to fibre content (NDF: $r=+0.38$; $P<0.001$; ADF:$+0.34$; $P<0.01$), as well as its fermentation rate C_3 (NDF: $r=+0.27$; $P<0.05$ and ADF: $+0.25$; $P<0.001$). The higher the B_1 fraction, the higher was the VFA production, especially correlated to butyrate. However, B_2 and B_3, positively correlated with the pectins and fibres, were negatively correlated with VFA production, especially with butyrate ($r=-0.46$; $P<0.001$).

DISCUSSION

In vitro studies on individual components have shown that the rates of fermentation differ widely. Soluble sugars and pectin ferment more quickly than most starch presentation and much more quickly than cellulose and hemicellulose

Table 4. Correlation matrix (r=Pearson coefficient) between the chemical composition, pH or volatile fatty acids and the fermentation kinetics parameters of the exponential model (EXP_L) and the three pool logistic model ($TLOG_L$) of the whole citrus fruits

Item	EXP_L			$TLOG_L$						
	B_1	C_1	L	B_1	C_1	B_2	C_2	B_3	C_3	L
Chemical composition										
crude protein					0.19*			-0.19*		
NDF		-0.34**	-0.41***	-0.20*				0.38***	0.27*	-0.29*
ADF		-0.25*	-0.33**					0.34**	0.25***	-0.25*
sugars	-0.23*	0.61***	0.68***	0.43***		-0.36***	0.26*	-0.39***		0.54***
pectin		-0.34**	-0.37***	-0.29*		0.23*		0.27*		-0.27*
Fermentative parameters										
pH					0.20*		0.18*			
VFA	-0.36**	0. 43*		0.27*		-0.31**		-0.24*		-0.22*
acetate	-0.32*	0.33*	-0.22*		-0.21*	-0.25*				-0.34**
propionate	-0.34**	0.52***		0.21*		-0.25*		-0.27*		
butyrate	-0.39***	0.52***		0.51***		-0.46***		-0.46***		

abbreviations see Tables 1 and 2; *P<0.05; **P<0.01; ***P<0.001

Table 5. Mean fermentation kinetic parameters for oranges, mandarins and lemons fitted to EXP_L and $TLOG_L$ models

Model	Citrus species	B_1, ml	B_2, ml	B_3, ml	C_1, h^{-1}	C_2, h^{-1}	C_3, h^{-1}	L, h	R^2	RSD	BIC
EXP_L	Orange	185.5	-	-	0.1909	-	-	1.2294	0.9115	5.779	4500.9
	Mandarin	182.8	-	-	0.1956	-	-	1.2376	0.9916	5.545	5218.1
	Lemon	187.4	-	-	0.1492	-	-	0.4749	0.9887	6.631	722.4
$TLOG_L$	Orange	68.88	87.39	33.99	0.3149	0.1171	0.0321	1.9528	0.9917	5.630	3424.7
	Mandarin	71.43	83.26	32.51	0.3208	0.1161	0.0331	1.9499	0.9919	5.465	3993.6
	Lemon	55.43	93.00	42.67	0.3179	0.1014	0.0322	1.3545	0.9858	7.234	675.5

B - fermentable fraction/s (B_1, B_2 and B_3); C - fractional/specific fermentation rate/s (C_1, C_2 and C_3); L - Lag

(Sniffen et al., 1992). The multi-pool kinetic analysis of gas curves also predicts up to three pools with varying sizes, digestion rates and (sometimes) lag terms. The relationship, if any, between these mathematically derived pools and the chemical fractions in the WCF seems to be of interest. One way to test the validity of theses assignments is to examine mixtures of simple homogeneous substrates as reported by Schofield et al. (1994), and to determine the biological meaning of the different fractions by analysing the correlation between the kinetic parameters and the chemical composition of the substrate. For this reason, as we will see below, the results obtained in the present work have been compared with those given by authors who tested individual components of the foods (Schofiel et al., 1994; Stefanon et al., 1996).

The values for B_1 and C_1 EXP_L parameters (184 ml and 0.19 h^{-1}, respectively) were consistent with those obtained by Getachew et al. (2004) for beet pulp (B_1=163.1 ml and C_1=0.10 h^{-1}), whose fibre characteristics are close to citrus fibre. Megías et al. (2002) fitted the EXP_L model to *in vitro* gas production of some by-products (stem broccoli, fresh artichoke, lemon and orange peel), obtaining similar RSD values to those obtained in the present study for WCF, but C_1 values for these by-products were much lower (0.033-0.053 h^{-1}) than that obtained for WCF. These differences seem to be related to the lower sugar and the higher NDF content of these by-products, taking into account that these are the main factors correlated with C_1 value (+0.61 and -0.34, respectively). However, the different rumen fluid source used (goats) must be also considered. The C_1 EXP_L value was intermediate to the values obtained by Schofield et al. (1994) with this same model for a highly degradable substrate and cellulose (0.22 and 0.09 h^{-1}, respectively), perhaps indicating the presence of different fermentable carbohydrates in the WCF with different fractional fermentation rates. The lag was highly and positively correlated with the sugar content for both models (r= +0.54 to +0.68). This could be explained by the relationship between the lag phenomena and the solubilization, although it is impossible to separate the lag from the initial solubilization if no measurements of the latter were taken at time 0 (Mertens, 1993). The B_1 and C_1 EXP_L parameters had little biological meaning: B_1 was only negatively correlated with sugar content and the fermentative activity (VFA), but was not positively related to other fermentative fractions, and C_1 was highly and positively correlated with the sugar content and negatively with pectins and the fibre fractions. On the other hand, the three B_1, B_2 and B_3 $TLOG_L$ fractions were positively correlated with the sugar (r=+0.43), pectin (r=+0.23) and fibre content (NDF: r=+0.38), respectively; the three most important components of WCF samples. The specific rate of B_1 fraction (C_1) depended on the CP content and the pH reached after incubation. These correlations could be explained considering that the fermentation of easily fermentable carbohydrates in the rumen depends on

the availability of protein for microbial yield, and as the citrus samples are poor in protein, it could be a limiting factor for microbial growth in the initial stages of fermentation. The fermentation rate of the fraction related to pectins (C_2) was correlated with the sugar content and the pH, while the fermentation rate of the fraction related to fibres (C_3) was also correlated to the NDF and ADF content. Moreover, the value for C_2 specific rate obtained by Stefanon et al. (1996) for the insoluble fraction of lucerne (0.038 h^{-1}) was comparable to the C_3 $TLOG_L$ (0.033 h^{-1}) values obtained for the WCF. These results could indicate that it is likely possible to distinguish three fermentable fractions for the WCF *in vitro* incubated with rumen fluid, which were fermented at three different specific rates. Therefore, it could be hypothesized that B_1 $TLOG_L$ fraction would be related with the content in soluble carbohydrates, B_2 fraction with the pectin content and B_3 fraction with the fibre content. Each fraction being fermented at different specific fermentation rates: high (0.31 h^{-1}) for the soluble carbohydrates and limited by the protein availability for microbial growth, medium for the pectins (0.12 h^{-1}) and low for the fibre (0.03 h^{-1}). The correlations between the fermentation kinetics parameters and the chemical composition of WCF could make the feature of these fractions stand out. However, further studies with these isolated fractions (sugars, pectins and NDF) should be carried out. The existence of three main fractions degraded at three specific fermentation rates could be in same extend confirmed by the mean fermentation kinetics parameters obtained for each type of citrus: sweet oranges, mandarins and lemons (Table 5). As it can be seen, the B_1 EXP_L fraction was almost the same for all the citrus types (about 185 ml), but the fractional fermentation rate was not the same for the three types of citrus (0.15 h^{-1} for the lemons vs 0.19 h^{-1} for the oranges and mandarins). This could be explained by the possible existence of only one fraction fermented at different rates. And as C_1 is positively correlated with sugars (Table 4), and lemons had a much lower amount of sugars, the fractional fermentation rate (C_1) was also lower. The EXP_L model could also explain the differences in the lag between lemons and the rest (0.48 vs 1.23 h, respectively) because, as mentioned above, the lag was positively correlated with the sugars. However, this model makes difficult to explain the existence of different fermentable fractions: easily fermentable soluble carbohydrates, highly fermentable cell wall components and slowly fermentable fibres.

In the opposite, the $TLOG_L$ model was more open to recognize the different fractions of this product because it gives the possibility of three fractions with three specific fermentation rates. As occurs for the EXP_L model, the lag was higher for sweet oranges and mandarins than for the lemons. The B_1, B_2 and B_3 parameters were different for the different types of citrus, but the specific fermentation rate for each fraction was very similar regardless of the citrus type. Therefore, lemons would have a lower B_1 value than the other two citrus sorts due to its lower sugar

content, but higher B_2 and B_3 fractions by their greater content on pectins and fibre. In addition, the fact that each specific fermentation rate was almost the same for the different types of citrus would explain that each fraction was the same for all of them. Silva et al. (1997) also reported that slowly ruminally degradable fraction of DM (0.029 h^{-1}) did not differ among fresh lemon, sweet oranges peel or sweet orange peel silage. Therefore, the $TLOG_L$ model could be an interesting tool for an adequate description of the fermentation kinetics of whole citrus fruits, and could be used in practice for a better understanding of the nutritive value and ruminal behaviour of this product when used in ruminant feeding.

CONCLUSIONS

It was possible to fit all the models tested to the *in vitro* gas production profile of the whole citrus fruits (WCF). The study of the different models' kinetics parameters showed the possible existence of different fractions in the WCF samples which are fermented at different rates. The three-phasic logistic equation model was very interesting, because the fermentation fractions of this model are highly correlated with the main fractions of whole citrus fruits. The fermentative behaviour of the WCF will depend on the amount in these three main fractions: soluble carbohydrates, highly fermentable cell wall components and slowly fermentable carbohydrates.

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