EFFECT OF DRYING TEMPERATURE OF *Passiflora edulis* RESIDUES ON RUMEN DEGRADATION KINETICS AND ENTERIC CH_4 AND CO_2 PRODUCTION[†]

[EFECTO DE LA TEMPERATURA DE SECADO DE LOS RESIDUOS DE *Passiflora edulis* SOBRE CINÉTICA DE DEGRADACIÓN RUMINAL Y PRODUCCIÓN DE CH_4 Y CO_2 ENTÉRICO]

M. Buenaño-Sánchez[1,2], M. Barros-Rodríguez[1,*], H. Zurita-Vásquez[1], L. Hidalgo[3], L. Pérez-Aldas[4] and D. Arias-Toro[5]

[1] Facultad de Ciencias Agropecuarias, Universidad Técnica de Ambato, Cantón Cevallos vía a Quero, sector el Tambo-la Universidad, 1801334, Cevallos, Tungurahua, Ecuador
Email: ma_barrosr@yahoo.es
[2] Instituto de Posgrado y Educación Continua, Escuela Superior Politécnica del Chimborazo, Riobamba, Riobamba, Ecuador
[3] Facultad de Administración de Empresas, Escuela Superior Politécnica del Chimborazo, Riobamba, Riobamba, Ecuador
[4] Facultal de Ciencias e Ingeniería en Alimentos, Universidad Técnica de Ambato, Ambato, Tungurahua, Ecuador
[5] Universidad Estatal de Bolívar, Dirección de Investigación y Vinculación, Guaranda, Ecuador
**Corresponding author*

SUMMARY

The aim of the present investigation was to evaluate the effect of the drying temperature on *Pasiflora edulis* husk residues on the degradation kinetics and enteric mitigation of CH_4 and CO_2. Four drying temperatures were evaluated: 60, 90, 120 and 150 °C. The digestibility of dry matter and organic matter showed differences ($P < 0.05$) between treatments, obtaining the higher digestion at 60, 90, 120 °C, in ranges between (75 and 68%) and (77 and 70%) of the DM and OM respectively. The rumen degradation of DM was observed that both in the soluble fraction (A), insoluble but potentially degradable fraction (B) and degradation rate in percentage per hour (*c*), it was higher at drying temperatures of 60, 90 and 120 ° C ($P < 0.05$). The rumen degradation kinetics of the NDF was obtained for both the soluble fraction (A), insoluble but potentially degradable fraction (B), the ruminal degradation rate (*c*) and effective degradation (ED) at the different passage rates (k) in *P. edulis* dried at 60 and 90 °C ($P < 0.05$). While for the degradation potential (A+B) the higher degradation was observed at 90 °C of drying temperature. Gas production [GV (mL gas)] was lower ($P<0.0001$) in *P. edulis* husks dried at 60, 90 and 120 °C with a difference of 147.8 mL gas/0.500g of fermented DM compared to *P. edulis* dried at 150 °C. However, the production of CH_4 and CO_2 [GV (mL CH_4); (mL CO_2)] was lower ($P=0.0002$; $P<0.0001$ respectively) in the dried *P. edulis* residue of 60 °C (24.9 mL CH_4 and 84.2 mLCO_2/0.500g of fermented DM respectively). It can be concluded that drying at high temperatures (150 °C) residues of *P. edulis* reduce rumen digestion and increase the production of greenhouse gases in ruminants.
Keywords: *Pasiflora edulis*; degradation; digestibility.

RESUMEN

El objetivo de la presente investigación fue evaluar el efecto de la temperatura de secado en los residuos de cascara de *Pasiflora edulis* sobre la cinética de degradación y mitigación de CH_4 y CO_2 entérico. Se evaluaron cuatro temperaturas de secado: 60, 90, 120 y 150 °C. La digestibilidad de la materia seca y materia orgánica mostró diferencias ($P<0.05$) entre tratamientos, obteniendo la mayor digestión a los 60, 90, 120 °C. Misma que bordeo entre el (75 y 68%) y (77 y 70 %) de la MS y MO respectivamente. La degradación ruminal de la MS se observó que tanto

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en la fracción soluble (A), fracción insoluble pero potencialmente degradable (B) y tasa de degradación en porcentaje por hora (*c*), fue mayor a temperaturas de secado de 60, 90 y 120 °C (P<0.05). La cinética de degradación ruminal de la FDN se obtuvo tanto para la fracción soluble (A), fracción insoluble pero potencialmente degradable (B), la tasa de degradación ruminal (*c*) y degradación efectiva (DE) a las diferentes tasas de pasaje (*k*) en la cascara de maracuyá secada a 60 y 90 °C (P<0.05). Mientras que para el potencial de degradación (A+B) se observó la mayor degradación a los 90 °C de temperatura de secado. La producción de gas [GV (mL gas)] fue menor (P<0.0001) en las cascaras secadas a 60, 90 y 120 °C con una diferencia de 147.8 mLgas/0.500g de MS Fermentable respecto a la cascara secada a 150 °C. Sin embargo, la producción de CH_4 y CO_2 [GV (mL CH_4); (mL CO_2)] fue menor (P=0.0002; P<0.0001 respectivamente) en el residuo de maracuyá secado de 60 °C (24.9 mL CH_4 y 84.2 mLCO_2/0.500g de MS Fermentable respectivamente). Se puede concluir que secar a altas temperaturas (150 °C) los residuos de maracuyá disminuyen la digestión ruminal e incrementa la producción de gases de efecto invernadero en los rumiantes.
Palabras clave: *Pasiflora edulis*; degradación; digestibilidad.

INTRODUCTION

The increase in greenhouse gas emissions in the atmosphere can cause serious problems to the environment. Methane (CH_4) is the second most important greenhouse gas after carbon dioxide (De Klein *et al*., 2008). In animal production systems, the most important source of methane emission is enteric fermentation, representing 30% of total CH_4 emissions (Shibata and Terrada, 2010). In tropical areas of Ecuador, ruminant feeding is based on direct grazing in monocultures of available grasses (Rangel-Quintos *et al*., 2016). Where forages contain low amounts of protein and high fiber, minimizing productivity in animals (Casey, 2006). Due, i) low fodder digestion and ii) energy expenditure in the form of methane expelled from the rumen. Same, ranging from 2 to 12% of the energy consumed per animal per day (Barros-Rodríguez *et al*., 2018).

Among the alternatives to correct these nutritional problems in ruminants in tropical areas have been incorporated into the diet, plants that contain bioactive compounds such as tannins and saponins to mitigate the production of methane produced in the rumen and increase productivity (Nieto *et al*., 2014). In this sense, Torres-Acosta *et al*. (2008); Bhatta *et al*. (2013); Ku-Vera *et al*. (2013) and Barros-Rodríguez *et al*. (2014) mention that plants that have secondary metabolites have great potential to reduce the production of gases from enteric fermentation, increase by-pass protein and reduce gastrointestinal nematodes. Also, crop by-products (agro-industrial waste) with low fiber concentration have been used, which have been shown to mitigate the gases producction in the rumen (Barros-Rodríguez *et al*., 2018). Among the agricultural by-products (waste) with great potential for ruminant feeding, it can mention the husk and pulp of *P. edulis* on fresh basis, which has been used for fattening bulls with promising results (Gaibor Bravo, 2013). However, the rapid decomposition of this residue does not allow storage for long periods, nor the incorporation in concentrated diets due to the low content of dry matter (13%). Hence the importance of this research, which aims to assess the effect of drying temperature on *P. edulis* residues on ruminal degradation kinetics and enteric CH_4 and CO_2 production

MATERIALS AND METHODS

Experiment Location

The trial was carried out at the Faculty of Agricultural Sciences of the Universidad Técnica de Ambato, Cevallos, Tungurahua, Ecuador.

Pasiflora edulis fruit agro-industrial residues and experimental treatments

The residues were obtained after the extraction of the pulp and seeds of *P. edulis* (50 kg fresh base). Immediately, the residue was transferred to the laboratory to perform the weighing on a fresh basis and assign to the following experimental treatments; the treatments consisted of drying the residues at 60 °C, 90 °C, 120 °C, and 150 °C temperature. The final drying time was established until the sample reached constant DM. After drying, the samples exposed to the different temperatures were ground in a hammer mill to a particle size of 2 mm. able 1 shows the chemical composition of Pasiflora edulis residue after heat treatment and the hours to reach constant DM. Table 1 shows the chemical composition of Pasiflora edulis residue after heat treatment and the hours to reach constant DM

in situ rumen degradation

The in situ rumen degradation of nutrients was estimated following the methodology of the nylon bag (0.42 µ) in the rumen described by (Orskov *et al*., 1980). For this, 6 bulls of approximately 4 years were used with an average weight of 550 kg provided with a fistula with permanent cannula in the rumen, the animals were fed a diet based on *Medicago sativa*,

Lolium perennial and water *ad libitum*. In each bull (n=6) a sachet containing 5g of each treatment was incubated at the following times (hours) 3, 6, 12, 24, 48 and 72h. At the end of 72 h the bags were removed, washed with running water and dried at 60 ° C. The waste will be stored in polyethylene bags at -4 °C until further analysis in the laboratory. The disappearance of nutrients was calculated as a proportion of the incubated and residual material. The data were adjusted to the equation: $Y = a + b (1 - e^{-ct})$ and the effective degradation was adjusted using the equation DE = a + [(b * c) / (c + k)] considering a rate of passage (k) of 0.02, 0.05 and 0.08% (Ørskov and McDonald, 1979; Prism 4, GraphPad Software, Inc. of San Diego, CA, USA.).

Gas, CH_4 and CO_2 production and *in vitro* digestibility

For these tests, the rumen content (liquid and solid fraction) was obtained separately from each bull (n=6). The ruminal content was collected before feeding in the morning and stored in plastic containers, transported to the laboratory for processing within the first hour of collection. Samples of *P. edulis* residue exposed to the different drying temperatures (60, 90, 120 and 150 °C) were incubated. For this, nitrogen rich media (artificial saliva) was prepared as described by Menke and Steingass (1988). Gas production was estimated using the methodology described by Theodorou *et al*. (1994) which consists of placing 0.500 mg of dry sample in glass bottles with a nominal capacity of 100 mL. In the bottles 60 ml of the inoculum (70:30 medium; artificial saliva/inoculum; rumen content) were incubated under constant flow of CO_2. The bottles were incubated between 39-40 °C.

The measurement of gas pressure and volume was taken manually at the following times 3, 6, 9, 12, 18, 24, 36, 48, 60, 72 and 96 hours after incubation with a pressure transducer (DO 9704, Delta OHM, Italy) and plastic syringes. The CH_4 and CO_2 production was quantified with a GAS Detection analyzer, model GX-6000, UK. For each drying temperature (treatments), 6 bottles were used in which the inoculum prepared by each bull (n=6) themselves that were considered as repetition was placed, and three additional bottles for each bull (inoculum) were used as blank. At the end of 96 h the data were adjusted to the monobasic equation $mLgas = GV (1 + (B/t)^{C}) -1$ described by Groot *et al*. (1996). Additionally, six more bottles for each drying temperature (treatment) were incubated until 48 hours to estimate the *in vitro* digestibility of the DM and OM by filtering the residues, and corrected with the residual DM of the bottles used as blank. And the gas, CH_4 and CO_2 production was estimated by 0.5 g of fermented DM

Experimental design and statistical analysis

A completely randomized design was used with 4 treatments (drying temperatures) and 6 repetitions (bulls: inoculums). The results of all the variables were subjected to an analysis of variance using the PROC GLM of SAS (SAS, 2002). And the comparison of the means was evaluated using the Tukey test.

Table 1. Chemical composition of passion fruit residue (*Passiflora edulis*) exposed to different drying temperatures (all values are expressed in % except where indicated otherwise) and hours to reach constant DM.

	passion fruit waste: Fresh base	Drying temperature in °C (Treatments)			
		60	90	120	150
DT (hours)*	0	75	30	12	4.5
DM	12.4				
OM	92.3				
CP		10.8	10.8	16.8	29.8
NDF		46.0	45.8	45.9	59.2
ADF		35.7	35.6	36.7	46.3
GE (kcal)		3,86	3,95	4,04	4,28
P		0,17	0,17	0,20	0,33
K		1,36	1,40	1,32	2,03
Ca		0,17	0,19	0,20	0,29
Mg		0,06	0,06	0,06	0,10
Cu (ppm)		5	3	2	3
Mn (ppm)		3	2	3	3
Zn (ppm)		9	7	7	10

* Drying time: hours it takes to reach constant DM

RESULTS

The highest digestibility of DM and OM (P=0.0001 and P=0.0001 respectively) was observed in passion fruit residues exposed to drying temperatures of 60, 90 and 120 °C with a digestion that ranged between DM: 68 to 75 and OM: 70 to 77% respectively (Table 2).

Regarding the rumen degradation kinetics of the DM, it was observed that both in the soluble fraction (A), insoluble but potentially degradable fraction (B) and degradation rate in percentage per hour (*c*), was higher in the treatments 60, 90 and 120 °C (P<0.05). Regarding the potential for ruminal degradation (A+B) and effective degradation (ED) at the different passage rates (k) in the rumen, it was higher (P<0.05) in treatments with drying temperatures of 60 and 90 °C respectively (Table 2, Figure 1 A).

The degradation of soluble fraction (A) of the OM was higher in the dried passion fruit peel at 60 °C (32%). However, in the insoluble but potentially degradable fraction (B) it was higher (P <0.05) at 90 °C drying (42%). However, the rate of ruminal degradation (*c*), degradation potential (A+B) and effective degradation (ED) at the different rates of passage through the rumen (k) showed greater degradation in the dried Pasiflora edulis husk a 60 and 90 °C (P<0.05, Table 2 and Figure 1 B).

Table 2. *In vitro* digestibility and parameters of *in situ* rumen degradation of dried *P. edulis* husk at different temperatures (all data are expressed in% except where otherwise stated)

	Treatments (drying temperature °C)				SEM	*P*
	60	90	120	150		
Digestibility						
DM	75.0a	68.2a	68.3a	31.0b	4.07	<0.0001
OM	77.4a	70.1a	70.4a	29.9b	4.35	<0.0001
Degradation DM						
A	39.4a	39.3a	32.7a	20.3b	2.96	<0.0001
B	32.4a	33.8a	34.1a	18.1b	2.89	<0.0001
c	0.148a	0.136a	0.106a	0.030b	0.0182	<0.0001
A+B	71.9a	73.2a	66.7b	38.5c	0.56	<0.0001
ED						
0.02 *k*	68.1a	68.7a	61.3b	31.2c	0.40	<0.0001
0.05 *k*	63.7a	63.9a	55.8b	27.2c	0.55	<0.0001
0.08 *k*	60.6a	60.5a	52.1b	25.4c	0.64	<0.0001
Degradation OM						
A	32.0a	28.7b	24.1c	16.9d	0.91	<0.0001
B	37.9b	42.0a	40.9ab	20.8c	1.13	<0.0001
c	0.157a	0.173a	0.115b	0.022c	0.0103	<0.0001
A+B	69.9a	70.8a	65.0b	37.7c	0.77	<0.0001
ED						
0.02 *k*	65.6a	66.4a	58.9b	27.5c	0.42	<0.0001
0.05 *k*	60.7a	61.3a	52.6b	23.1c	0.58	<0.0001
0.08 *k*	57.1a	57.4a	48.2	21.3c	0.68	<0.0001
Degradation NDF						
A	11.7a	11.5a	9.2b	2.0c	0.70	<0.0001
B	34.4a	36.7a	30.9b	15.3c	0.94	<0.0001
c	0.152a	0.125a	0.067b	0.030c	0.012	<0.0001
A+B	46.1b	48.3a	40.2c	17.4d	0.70	<0.0001
ED						
0.02 *k*	42.1a	43.0a	33.0b	11.4c	0.36	<0.0001
0.05 *k*	37.6a	37.5a	26.9b	7.9c	0.49	<0.0001
0.08 *k*	34.2a	33.7a	23.3b	6.3c	0.57	<0.0001

[a,b,c,d] Means with different letters between rows differ significantly (P <0.05). A: soluble fraction, B: insoluble but potentially degradable fraction, *c*: degradation rate in%/h, A+B: degradation potential, ED: effective degradation (k; rumen particle flow rate in %/h), SEM: standard error of the mean.

In relation to the rumen degradation kinetics of the DNF, it was obtained for both the soluble fraction (A), insoluble but potentially degradable fraction (B), the rate of ruminal degradation (*c*) and effective degradation (ED) at the different rates of passage (k) the greatest degradation in the shell of *P. edulis* dried at 60 and 90 °C (P<0.05). While for the degradation potential (A+B) the highest degradation was observed at 90 °C of drying temperature (P<0.0001, Table 2 and Figure 1 C).

The gas production [GV (mL gas)] shows differences between the drying temperatures of the *P. edulis* husk, with the lowest (P<0.0001) gas production in the dried husks at 60, 90 and 120 °C with a difference of 147.8 mLgas/0.500g of fermented DM with respect to the dried shell at 150 °C (Table 3 and Figure 2 A). However, the production of CH_4 and CO_2 [GV (mL CH_4); (mL CO_2)] was lower (P=0.0002; P<0.0001 respectively) in *Pasiflora edulis* residue dried at 60 °C (24.9 mL CH_4 and 84.2 mLCO_2/0.500g of DM fermented respectively: Table 3 and Figure 2 B and C).

DISCUSSION

Based on the results obtained from the *P. edulis* husk exposed to the different drying temperatures in both digestibility and rumen degradation, it could be related to the changes experienced by the passion fruit shell when subjected to the different drying temperatures, and may not affect the content of reserve polysaccharides, influencing, in turn, a greater digestion as observed in this work (Table 2). However, increasing the drying temperature to 150 °C considerably increases the FDN and FDA content (Table 1), perhaps due to the denaturation of the non-structural carbohydrates induced by the heat to which they are exposed (Yan *et al*., 2014; Khan *et al*., 2015), which minimizes the action of the microorganisms of the rumen towards the food. These results are consistent with those reported by Relling and Mattioli (2003).

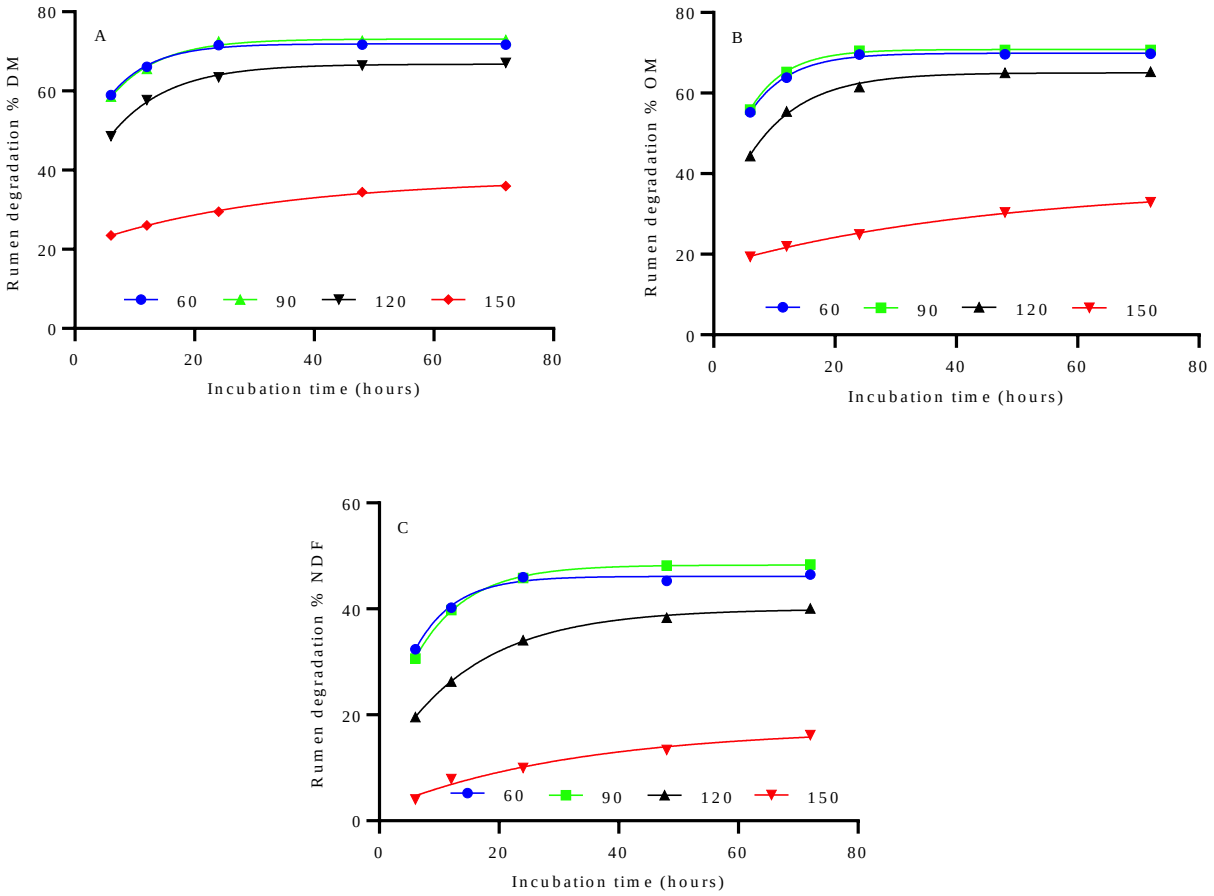

Figure 1. *In situ* rumen degradation kinetics of the DM (A), OM (B) and NDF (*c*) of the passion fruit peel (*P. edulis*) dried at different temperatures (60, 90, 120 and 150 °C)

With respect to the mitigation of the *in vitro* gas, CH_4 and CO_2 production, this could possibly be due to the better digestion that presented the dried residues at a lower temperature, causing greater action of the microorganisms of the rumen towards the soluble carbohydrates of the residue of *P. edulis* and probably increased propylene production in the rumen. On the other hand, the lower fiber content at a low drying temperature maximized fiber digestion (Table 2) and with it, the use of protein at the ruminal level, reduction of rumen methanogenesis (Blummel *et al*., 1997; Calsamiglia *et al*., 2010; Barros-Rodríguez *et al*., 2018). These results are consistent with those reported by Barros-Rodríguez *et al*. (2017).

CONCLUSION

Under the conditions of this study, it can be concluded that drying at temperatures above 90 °C the residues of *P. edulis* decreases ruminal digestion and increases the production of gas, methane and carbon dioxide in ruminants.

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Conflict of interest

The authors declare that they have no conflict of interest

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Table 3. *In vitro* gas, CH_4 and CO_2 production parameters (mL/0.500g of Fermented DM) of dried *P. edulis* husk at different temperatures.

	Treatments (drying temperature °C)				SEM	*P*
	60	90	120	150		
GV (mL gas)	300.4b	346.6b	301.0b	494.4a	19.94	<0.0001
B	13.3b	15.1b	18.7b	43.8a	6.62	0.0001
C	1.20a	1.13ab	1.02b	0.74c	0.044	<0.0001
GV (mL CH_4)	24.9b	31.4a	30.3a	29.7a	1.46	0.0002
B	24.8b	26.1b	33.0ab	41.8a	3.30	<0.0001
C	1.88a	1.73a	1.84a	1.88a	0.088	0.3360
GV (mL CO_2)	84.2c	108.5b	102.4b	146.9a	4.88	<0.0001
B	19.3b	21.6b	27.5b	48.0a	5.36	<0.0001
C	1.30a	1.30a	1.21ab	1.09b	0.061	0.0110

[a,b,c] Means with different letters between rows differ significantly (P <0.05). GV (mL/0.500g Fermented DM), B (gas production asymptote) and *C* (gas production rate (%/h) are the parameters of the equation: mL gas = GV $(1 + (B/t)^{C})^{-1}$ (Groot *et al*., 1996) SEM: standard error of the mean

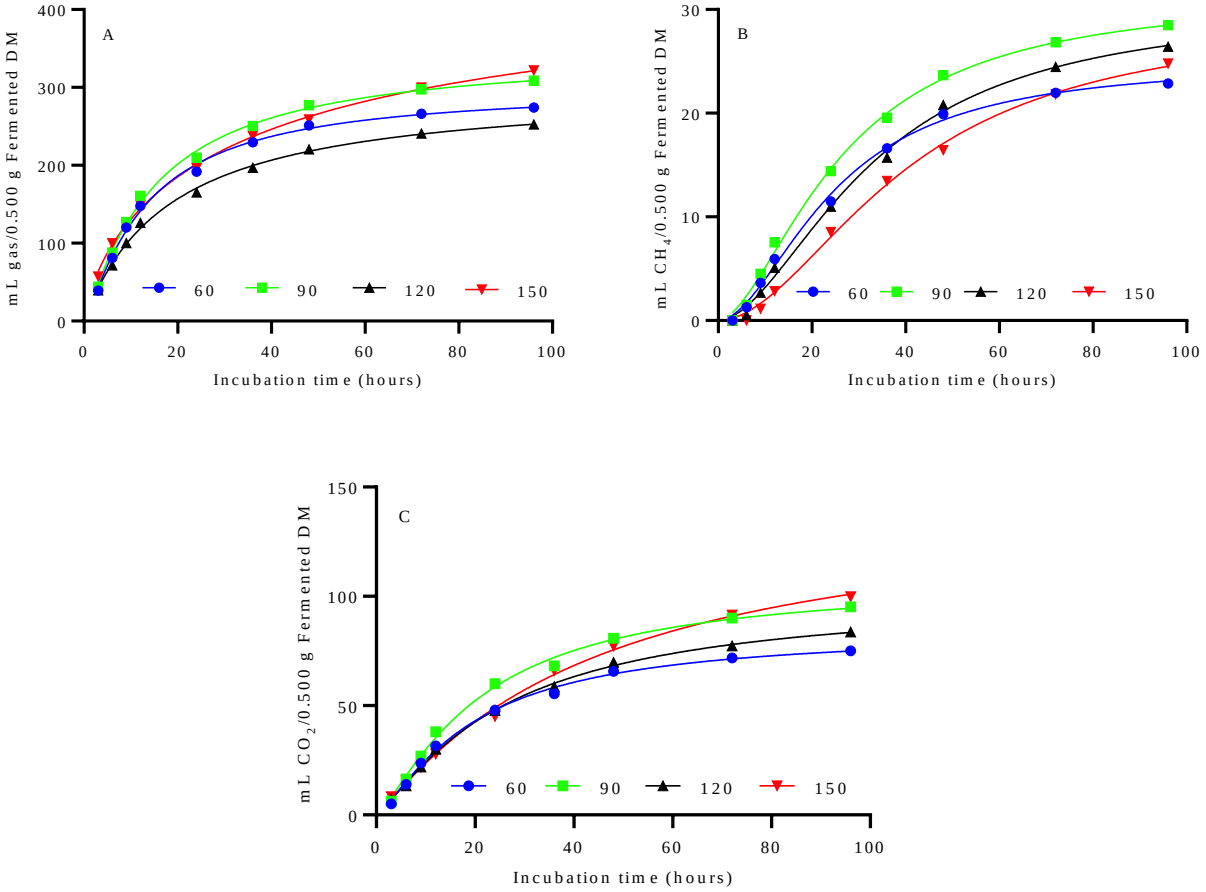

Figure 2. Gas production kinetics (A), CH_4 (B) and CO_2 (C) of the passion fruit peel (*P. edulis*) dried at different temperatures (60, 90, 120 and 150 °C).

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