American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 2 (5): 377-387, 2007
ISSN 1557-4571

Chemical Characterization and Evolution of Ascorbic Acid Concentration During Dehydration of Rosehip (*Rosa eglanteria*) Fruits

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Abstract: Rose hip fruits possess a high ascorbic acid content, which may partially degrade during dehydration in heated air. In this study, the chemical composition of the fruits was determined in order to study degradation of ascorbic acid as a function of drying temperature. The results indicated that, in effect, the content of this nutrient is reduced. The degradation mechanisms differed according to the drying temperature but the final ascorbic acid content was almost independent from such operating variable. The experimental evidence was used to calculate the degradation kinetic parameters. Though the extent of degradation was important, the final retention of ascorbic acid was considerable (42%) in view of the high initial content for this fruit.

Key words: Dehydration, rose hip fruits, ascorbic acid, degradation, kinetics

INTRODUCTION

The Rose hip, also known as Wild rose, is native of the European Mediterranean region and still grows there. The plant was introduced by European immigrants with ornamental purposes and is found to run wild in the Argentine Andean region, in the Chilean regions VII and X and in isolated zones of Peru particularly in the Department of Arequipa and Cusco.

Fruit coloration ranges from reddish orange to intense red according to the ripening degree, its size (diameter) varies from 12 to 15 mm and exhibits a fleshy pulp surrounding the seeds with epidermal hairs in its interior. This resource is scarcely used today, being restricted to production of flesh for jam manufacturing purposes and to produce relatively low-quality dehydrated fruits. In the San Carlos de Bariloche and El Bolsón Regions of Argentina, about 200 000 kg of jam are produced, starting from 300 metric tonnes of fresh fruit. While no official statistical data is available on rose hip dehydration, estimations indicate that about 700 tons of fresh fruit are processed annually by dehydration, out of a potential harvest of about 10 000 tons.

This fruit can be used to produce infusions, marmalades, jams, jellies and starting material for manufacturing cream soups, natural liquors, distilled beverages (spirits) and seed oil, among others. Most applications require dehydrated fruits, whose main purpose is to extend food shelf life by reducing water activity. This not only inhibits or reduces microbial growth but also hinders enzymatic activity (Fennema, 1996).

In dehydration, heat is applied under controlled conditions to eliminate most of the water present in the food by evaporation. Thus, it is a combined process of simultaneous heat and mass transfer between air and the dehydrating material, which induce organoleptic and nutritional quality changes in the food compared with the fresh produce.

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Rosehip fruits are characterised, among others, by its high L-ascorbic acid content, which varies from 500 to 2200 mg per 100 g fresh fruit, according to harvest date, ripening degree, prevailing weather conditions and production zone (for comparison, 100 mL of orange juice contain between 45 and 55 mg of Vitamin C).

The ascorbic acid, a constituent of Vitamin C, is however very sensitive to various degradation routes. Among the numerous factors affecting these reactions, the more relevant are temperature, salt and sugar concentration, pH, oxygen, enzymes, metal catalysts, the initial concentration of the acid and the ratio of ascorbic acid to its oxidised form, dehydroascorbic acid. All these factors are related with the processing technique used and product composition (Eison-Perchonok and Downes, 1982; Hsieh and Harris, 1987, 1993; Sahbaz and Somer, 1993; Jung *et al*., 1995). Taking into account the sugar content in rose hip fruits, its pH and presence of enzymes, acid ascorbic degradation is likely to occur.

The biological activity of Vitamin C in Humans is well-known so it must be preserved by paying special attention to operating conditions of the processes involved in the conservation methods. In dehydration of fruits with no previous chemical treatment, degradation kinetics of nutrients is related with drying rate. According to the literature Ochoa *et al*. (2002), the only operating variable affecting the drying rate of Rose hip fruits is air temperature.

The objective of the present study was (i) to measure the evolution of ascorbic acid during hot air dehydration of Rose hip fruits at various temperatures and (ii) to determine physical and chemical characteristics and kinetic parameters of the degradation reaction of L-ascorbic acid during the process, required for the design and optimisation of commercial dryers.

MATERIALS AND METHODS

Rosehips fruits were used which were harvested in the Region of El Bolsón, Province of Río Negro, Argentina. Fruits were stored in cold store at 4°C, until using.

Characterisation of Fruits

Fruits were characterised by applying the following techniques:

Moisture Content

The fruit samples were placed in vacuum oven set at 60°C, the vacuum being kept at 750 mm Hg. Heating was maintained until constant weight.

Ash Content

Determined according to AOAC (1990) Edit. 15ta.

Seeds Percentage

Seeds were extracted from a representative sample, cleaned and dehydrated until constant weight in a forced air oven set at 102°C.

Refractometric Soluble Solids

Obtained by following AOAC (1990) Edit. 15ta, method, by means of an ATAGO type 1, ABBE refractometer.

pH

For this variable, AOAC (1990) Edit. 15ta, method was employed. Measurements were done by utilising a model EA 940 ORION Ion Analyser fitted with a cat.:91-04, ORION combined pH electrode.

Glucose, Fructose and Sucrose

An enzymatic method (Boehringer Mannheim GmbH, 1995, was employed, with a Boehringer Mannheim Ref. 716-260 kit. A Metrolab UV-Visible spectrophotometer was utilised to identify the individual compounds.

Pectins

They were spectrophotometrically determined by the m-hydroxybiphenyl assay, Blumenkrantz and Asboe-Hansen (1973), as modified by Kintner and Van Buren (1982). Desesterification of pectic substances was conducted following the procedure suggested by McComb and McCready (1952). Spectrophotometric measurements were carried out at 520 nm and the results were obtained by comparison with a calibration curve.

Anthocyanins

The differential pH method, Wrolstad (1976) was utilised. The sample was prepared as suggested by Abers and Wrolstad (1979). Total anthocyanins were spectrophotometrically determined at 510 nm by using the molar absorptivity of Cyanidin-3-glucoside: 29600.

Carotenes

The sample processed by extraction several times with acetone at -18°C. Then with petroleum-ether to subsequently take the spectrophotometric reading at 460 nm. The molar absorptivity for red and yellow carotenes was used: 2000 (Davies, 1976).

Ascorbic Acid

Determinations were conducted by the 2,6 dichlorophenol-indophenol (DCPIP) method, Loeffler and Pointing (1942), as employed by Ochoa *et al*. (1999).

Colour Determination

Hunter tristimulus parameters L, a and b were measured by a Minolta Chroma CR-300 colour meter. The instrument was calibrated using a white ceramic plate (L = 95, 55; a = - 0.10 and b = + 2; 69). Two readings in the equatorial zone were taken per fruit and the results obtained are averages of at least twenty determinations (Ochoa *et al*., 1999).

Dehydration, Determination of Moisture Content and Ascorbic Acid in Partially Dehydrated Samples

Fresh fruit was selected and dehydrated in forced convection oven set at constant temperatures of 50, 60, 70 and 90±0.5°C, until reaching a residual moisture content of about 2% wet basis. Samples were weighed with a digital analytical balance. To this end, samples were removed at different times during dehydration, to determine moisture content and ascorbic acid using the methods mentioned above

RESULTS AND DISCUSSION

All results reported here are averages of triplicate determinations, except for those of Hunter surface colour. These, indicated earlier, are averages of 20 readings. Results were examined by Analysis of Variance (ANOVA), using the software Origin vs. 4.1 and the conclusions were confirmed by $LSD_{0.05}$ (Least Significant Difference) test. The values measured for characterising Rosehip fruits are presented in Table 1.

Table 1: Results obtained on the characterisation of Rose Hip fruits

Parameters	Content
Moisture content	47.7±0.7%
Seeds (g dry seed/100 g whole fruit)	15.2±0.2%
Ash	2.02±0.03%
pH	3.99±0.02
Acidity (Expressed as anhydrous citric acid)	3.10±0.07%
Refractometric soluble solids	16.2°Brix ±0.3
Ascorbic acid	1.141±0.09%
Pectins (Expressed as galacturonic acid)	4.90±0.14%
Sucrose	0.48±0.07%
Glucose	6.42±0.11%
Fructose	6.50±0.18%
Anthocyanins (Expressed as cyanidin-3-glucoside)	3.10 mg/100 g ±0.09
Colour (spectrophotometrical) (absorbance units at 460 nm)	1.44 A/100 g ±0.13
Carotenes (Expressed as β carotene)	4.26 mg/100 g ±0.33
Hunter L	34.06±3.69
Hunter a	33.13±4.03
Hunter b	13.53±2.51

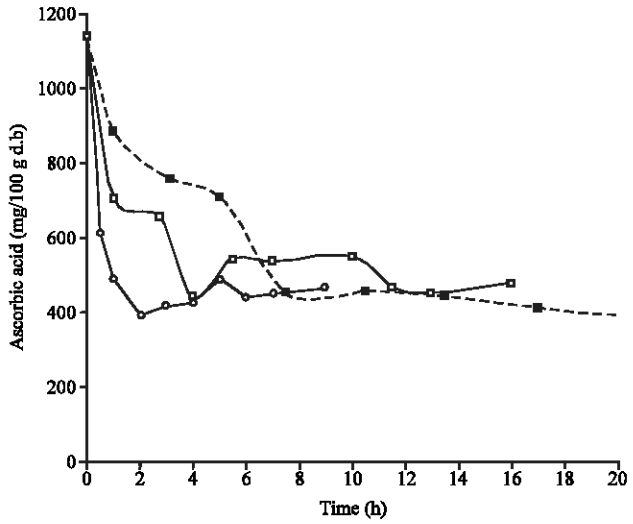

Fig. 1: Evolution of ascorbic acid content (mg/100 g sample, dry basis) in partially dehydrated Rose Hips as a function of drying time. At 60°C (■), 70°C (□) and 90°C (○)

Ascorbic acid degradation was studied along dehydration as a function of drying time or the normalized fruit moisture content, X/Xo (where X is the moisture content of samples at a given time and Xo their initial content). Results are presented in Fig. 1 and 2, respectively, at drying air temperatures of 60, 70 and 90°C. By statistical analysis of these results, the ascorbic acid concentration was found to be affected by both dehydration time and the residual moisture content of samples at the three temperatures tested. As observed in Fig. 1 and 2, ascorbic acid concentration was found to decrease considerably at the three temperatures studied, up to 5 to 6 h of drying, or normalized moisture contents between 1 and 0.5. For this range, ascorbic acid concentrations were significantly affected by dehydration temperature (ANOVA, confidence degree: 1 %). For lower values of X/Xo, in contrast, ascorbic acid concentrations converge and were not significantly affected by temperature.

Moreover, Fig. 3 shows the evolution of ascorbic acid content as a function of the normalized moisture content for drying temperatures of 50 and 90°C, so the same conclusions drawn for Fig. 2,

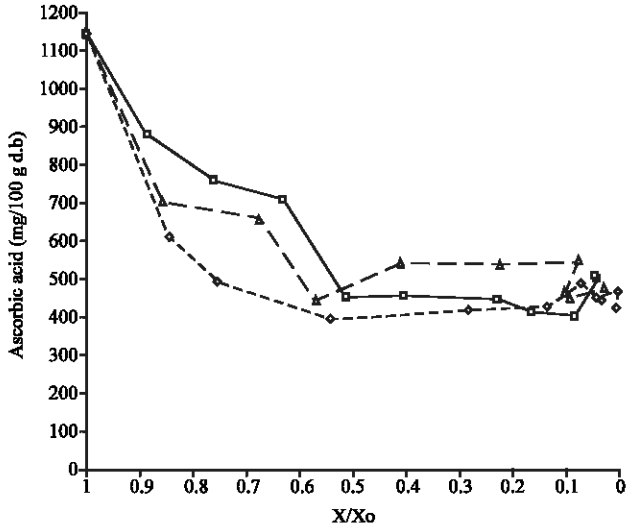

Fig. 2: Evolution of ascorbic acid content (mg/100 g sample, dry basis) in partially dehydrated Rose Hip as a function of the residual dimensionless moisture content, X/Xo. At 60°C (□), 70°C (Δ) and 90°C (◊)

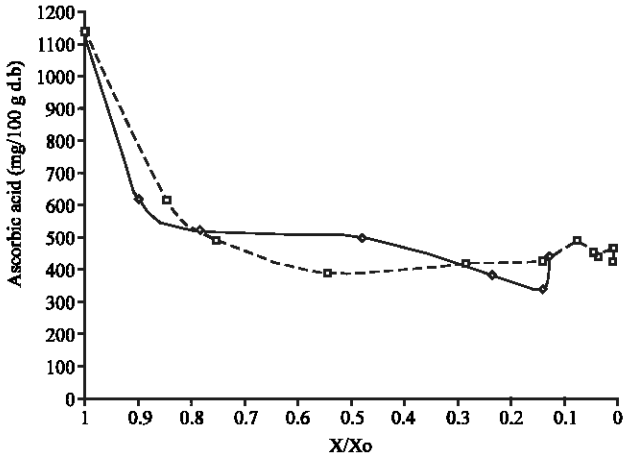

Fig. 3: Evolution of ascorbic acid content (mg/100 g sample, dry basis) in partially dehydrated Rose Hip as a function of the residual dimensionless moisture content, X/Xo, at 50 (◊) and 90°C (□)

would apply here as well. However, statistical analysis indicated that ascorbic acid values measured for 50°C do not differ from those determined for 90°C. For the low temperatures of 50°C, enzymatic degradation of ascorbic acid may be the main mechanism owing to the long exposures of fruits to high moisture conditions. In turn, at 60°C and higher temperatures, degradation may follow a predominantly oxidative route. Therefore, drying temperatures below 60°C may not be convenient, as they may allow sufficient time for enzymatic degradation to proceed under conditions where the oxidative mechanism of ascorbic acid destruction is of lower importance (Schwimmer, 1981).

The final content of ascorbic acid, seem even higher if the findings of Finholt *et al*. (1963) and Blang and Hajratwala (1972) are considered. These authors have indicated that ascorbic acid

degradation is maximum near its pK_1 (4.04 at 25°C), while the pH of Rose Hip as measured here were of 3.99±0.2, that is almost the pK, so the data measured here was collected in conditions close to the maximum degradation rate.

Several researchers have studied the effect of temperature on ascorbic acid degradation (Guerrant *et al*., 1945; Pope, 1972; Wanninger, 1972; Kramer, 1974; Vieira *et al*., 2000; Tudela *et al*., 2002; Uddin *et al*., 2002; Jinescu *et al*., 2004; Burdurlu *et al*., 2006; Nath *et al*., 2007) and they found the Arrhenius equation suitable to account for the effect of temperature on the ascorbic acid degradation rate in food systems.

The results obtained here agreed with previous literature (Heldman, 1975; Hoyem and Kvale, 1977; Labuza and Riboh, 1982; Ochoa *et al*., 1999; Vieira *et al*., 2000; Uddin *et al*., 2002; Frias and Oliveira, 2001; Burdurlu *et al*., 2006) on that the ascorbic acid degradation reaction follows a first-order kinetics for the temperature range usually found at commercial scale (60°C or higher). The reaction rate constant and the corresponding activation energy can be obtained by two methods: the Arrhenius model, usually referred to as the two-step method, or a procedure giving directly the activation energy, termed one-step method.

Arrhenius or Two Steps Method

The equation relating concentration, reaction rate constant and time is:

$$\frac{C}{Co} = \exp(-k\,t) \tag{1}$$

Where C_0 is the initial ascorbic acid concentration, k the reaction rate constant depending on temperature and C the ascorbic acid concentration at time t. For a first order reaction, data of ln(C/Co) plotted as a function of t would follow a linear behaviour whose slope is equal to (-k). For the results of this study, Eq. 1, as such, could not be used since it would require an accurate determination of the asymptotic ascorbic acid concentration, i.e., for X/Xo below 0.5. The rate constant can nevertheless be determined by applying the fractional conversion concept for an irreversible first order kinetics (Levenspiel, 1972; Steet and Tong, 1996; Avila and Silva, 1999; Vieira *et al*., 2000; Ochoa *et al*., 2001). The expression, a modified version of Eq. 1 is:

$$\frac{C - Ceq}{Co - Ceq} = \exp(-k' t) \tag{2}$$

Where Ceq is the asymptotic ascorbic acid concentration. Equation 2 allows to the rate constants to be obtained by regression of all concentration experimental data. Whilst the Ceq value is required for Eq. 2, the situation is less troublesome than with Eq. 1 since the determination of the time for which ascorbic acid concentration stabilises becomes unnecessary.

The values of k or k obtained with Eq. 1 or 2, in this study Eq. 2, is utilised for the Arrhenius relationship:

$$k = k_0 \exp(Ea/RT) \tag{3}$$

With which, plotting k vs. 1/T, Ea and k_0 can be determined. The symbol k_0 is the pre-exponential factor, Ea is the activation energy of the reaction, R is the gas constant (8.314 J mol^{-1} K^{-1}) and T, is the temperature in K

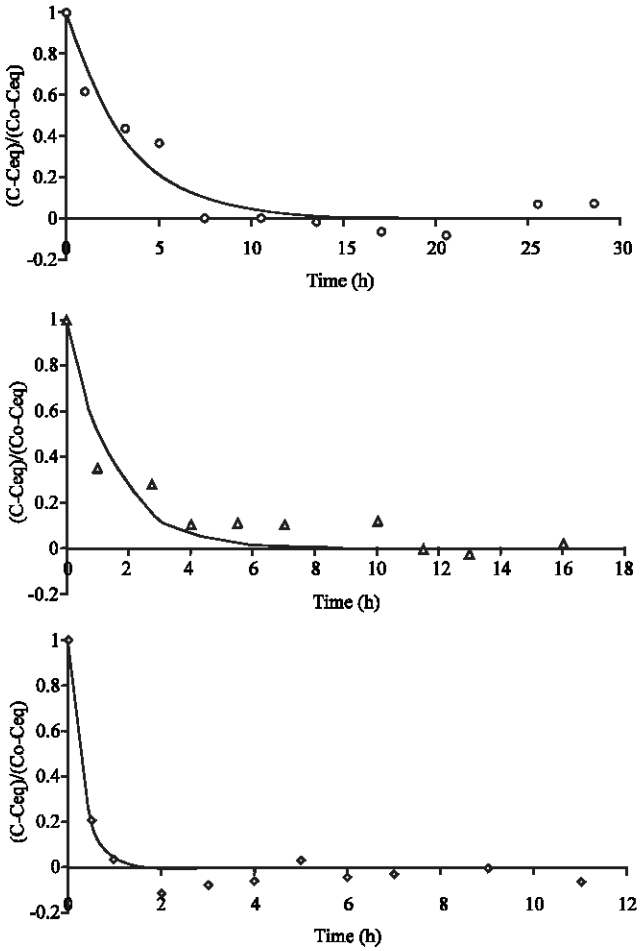

Fig. 4: Regression of experimental data of ascorbic acid concentration for fitting the reaction rate constant k (Ceq = 466.7 mg de ascorbic acid/100 g of sample). 60°C (○), 70°C (Δ) and 90°C (◇), _____ Estimated

One Step Method

When applying linear regression to determine the average value of the rate constant k using the two step procedure, the variability of each k generally results in a considerably large standard deviation in the Ea value and especially with wider confidence intervals, as a consequence of the lower number of degrees of freedom (Arabshahi and Lund, 1985).

A more suitable approach is to incorporate the rate constant variability by means of a weighted regression analysis proposed by Arabshahi and Lund (1985) named one step procedure, with the purpose of increasing the degrees of freedom:

$$C_{(t_{ij},T_i)} = Co_{(T_i)} \exp(-k_{(T_i)} t_{ij}) \quad (4)$$

Where $C_{(t_{ij},T_i)}$ is the concentration C for the time t_{ij} and temperature T_i and $Co_{(T_i)}$ is the initial concentration at time zero for the temperature T_i.

Table 2: Reaction rate constants and activation energy for ascorbic acid and corresponding regression coefficients (in brackets) at different temperatures

	k (h^{-1})				
Method	60°C	70°C	90°C	ko (h^{-1})	Ea (kJ mol^{-1})
Two-steps (k)	0.311±0.032 (0.952)	0.670±0.081 (0.940)	3.151±0.296 (0.973)	5.10 E+11	77.96±7.01 (0.999)
One step (k′)	---	---	---	1.10 E+11	79.04±4.12 (0.991)

By incorporating Eq. 3 (Arrhenius) into Eq. 4, it is possible to obtain:

$$C_{(t_{ij},T_i)} = Co_{(T_i)} \exp\left[-ko\ t_{ij} \exp\left(-Ea/R\ T_i\right)\right] \quad (5)$$

Equation 5 is a non-linear model with (N + 2) parameters: Co_1, Co_2, Co_3 ….Co_N, ko, Ea where N is the number of constant temperatures, T_1, T_2, T_3…..T_N, at which the concentration vs time data were obtained.

Figure 4 presents the regression obtained by means of non-linear analysis with Systat 5.02 software (SYSTAT, Inc., 1990-1993) using the criterion of maximising the regression coefficient R^2, obtained with Eq. 2 and the experimental data, using the value for *Ceq* that was obtained by linear regression of all experimental data of concentrations for X/Xo values lower than 0.5 ($R^2 = 0.991$), such us averaged final ascorbic acid concentration. Table 2 shows the values for k and k′ with the corresponding regression coefficients R^2. With the values for k and k′ and by applying Eq. 3, the values for the activation energy and pre-exponential factor were obtained which are also shown in Table 2.

Besides, by applying Eq. 5 in the same software, the values of Ea and ko for the one step method were determined and also listed in Table 2. Values for Ea, as fitted by the two methods result very similar: 77.96±7.01 kJ mol^{-1} for the two-step method and 79.04±4.12 kJ mol^{-1} for the one-step method, with high regression coefficients R^2. This results for Ea are very similar to published by Vieira *et al*. (2000) and Burdurlu *et al*. (2006). The value obtained by the one-step method was preferred because it presents a lower confidence interval. This confirms the conclusions drawn by Arabshahi and Lund (1985) and utilized, among others, by Ochoa *et al*. (2001).

CONCLUSIONS

There are numerous processes where moisture, temperature or both affect product quality and that is the case of dehydration, where the objective is to remove water. Therefore, the most adverse range of moisture contents must be traversed as soon as possible, since the rate of degradation reactions is a function of temperature and exposure time.

In Rose Hip fruits and in order to improve process operating conditions to retain most of the ascorbic acid content, it would be convenient to reach the X/Xo = 0.5 threshold as fast as possible since for lower normalized moisture contents, the ascorbic acid content seem to be independent of drying temperature. Therefore, drying temperatures below 60°C may not be convenient, as they may allow sufficient time for enzymatic degradation to proceed under conditions where the oxidative mechanism of ascorbic acid destruction is of lower importance.

The final ascorbic acid content was between 450 and 500 mg/100 g of sample, representing a retention of about 42% of its initial content, which is considerably higher compared with the concentration of other ascorbic acid-rich foods as citrus fruits.

The drying times for the whole fruits are very long, varying between 6 and 30 h for the temperatures of 90 and 50°C, respectively. Therefore, to improve ascorbic acid retention, process times must be reduced by means of pre-treatments in the fruits. Cutting the fruits into small pieces is not convenient because seed hardness quickly affects knives cutting edge. Thus, chemical treatments with alkaline solutions, or skin puncture with fine needles can be attempted.

The parameters of the reaction kinetics obtained in this work allow ascorbic acid degradation to be predicted in a drying simulation program based on the calculation of heat and mass transfer between fruits and air. Such application can be useful for design and optimisation of dryers for rose hip fruits.

NOMENCLATURE

C	Concentration of ascorbic acid (%)
$C_{(t_{ij},T_i)}$	Concentration of ascorbic acid at time t_{ij} and temperature T_i
Co	Initial concentration of ascorbic acid (%)
$Co_{(T_i)}$	Initial concentration at time zero for temperature T_i
Ceq	Equilibrium concentration of ascorbic acid (%)
Ea	Energy of activation (kJ mol^{-1})
k or k′	Reaction rate constant (h^{-1})
$k_{(T_i)}$	Reaction rate constant for temperature T_i
k_0	Reaction rate constant at infinite temperature or pre exponential factor (h^{-1})
N	Number of set of data concentrations versus time
R	Universal gas constant (kJ mol^{-1} K)
T	Absolute temperature (K)
X	Moisture content of samples at a given time, dry basis (kg/kg)
Xo	Initial moisture content of samples, dry basis (kg/kg)

ACKNOWLEDGMENTS

This study was funded by Universidad Nacional del Comahue, Facultad de Ingeniería, Asentamiento Universitario Villa Regina (FAIN I-125 Project) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, PIP 5511 Project) of Argentina. The authors appreciate the support given by CORFO Chubut and INTA AER El Bolsón. Author A. De Michelis is Member of CONICET.

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