Evaluation of quality during storage of apple leather

Natalia A. Quintero Ruiz, Silvana M. Demarchi, J. Facundo Massolo, Luis M. Rodoni, Sergio A. Giner

1. Introduction

Fruit leathers are dehydrated, restructured fruit-based products prepared by the acid - sugar - high methoxyl pectin gelation. They are eaten as candy or snacks, and presented as flexible strips or sheets. Due to their novel and attractive appearance, and because they do not normally require cold storage to avoid microbial growth, fruit leathers constitute a practical way to increase fruit solids consumption, especially for children and young people. In recent years, their popularity has increased: they are becoming an industrial product, evolving from their origins as a homemade snack. In addition to their nutritional value, fruit leathers are attractive as snacks for children due to their attractive appearance and their nutritional value and safety aspects (Heber, 2002; Khanna, 2002; Jaturonglumlert & Kiatsiriroat, 2010). Knowledge of the quality changes that occur during storage is essential for producers of fruit leather, the distribution chain and retail markets. However, the literature offers scarce information on the deteriorative changes that occur to apple leather on storage, and so the purpose of this work was (1) to evaluate the organoleptic as well as nutritional changes during storage at 20 °C of an apple leather prepared from a base formulation developed earlier (Leiva Díaz, Giannuzzi, & Giner, 2009), (2) to explore the effect of temperature, by means of accelerated storage tests (Labuza & Riboh, 1982).
2. Materials and methods

2.1. Materials

Ripe green apples (Malus domestica Borkh. L, cv. Granny Smith) and commercial sucrose were purchased in a local market in La Plata, Argentina. Citric acid, potassium metabisulphite and ethanol 96% were obtained from Quimica Anedra, while 2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), chlorogenic acid and potassium persulphate were provided by Sigma-Aldrich.

2.2. Apple leather formulation

The apple puree and leathers were prepared from a base formulation and procedure developed by Leiva Díaz et al. (2009). The main ingredients were (all expressed in % w/w): apple puree, prepared from green apples, 60.8; sucrose, 13.8; citric acid solution (concentration, 0.302 mol/l, similar to that in lemon juice), 2.3; distilled water, 23.1 and potassium metabisulphite (KMBS) 0.0057 (The control sample did not contain KMBS). Peeled apple cubes, were then thermally treated for 10 min in a food steamer to inactivate polyphenol oxidase (PPO) and also to impart an adequately soft golden colour and mechanical resistance to the leathers (possibly achieved by allowing time for pectins to diffuse out of the broken tissue and dissolve in the acid medium). A metabisulphite concentration of 173.7 mg/kg final product (based on the final product composition) was required to attain 100 mg/kg final product of SO2, which is only 10% of the limiting value accepted for dehydrated fruit products by the Codex Alimentarius (FAO-WHO, 2010).

An outline of the experimental work is presented in Fig. 1. Once the dehydration process was finished, gels were hermetically packaged for storage at 20 °C for 7 months. The packaging used was CRYOVAC M7340 (Sealed-Air, USA), a high-barrier, metallised-plastic material providing protection against light and with very low permeability to gases and water vapour.

2.3. Determination of moisture content

Samples of 7 g were kept in a Mettler LP 16 Moisture Analyzer set at 105 °C until reaching constant weight. This procedure follows the AOAC method 984.25 (AOAC, 1998).

2.4. Soluble solids content

The sample was prepared according to AOAC Method 932.12 (AOAC, 1998) and measurements were carried out in a Bellingham–Stanley R30-200 Abbé refractometer.

2.5. pH measurement

The sample was conditioned according to AOAC method 981.12 (AOAC, 1998). An Alpha PW-40 electrode, calibrated with buffer solutions of pH 4 and 7, was employed, which was connected to an Altronix TPA-V, pH meter with digital display.
2.6. Water activity determination

The water activity of samples was measured at 25 °C by the AOAC 978.18 hygrometric method (AOAC, 1998) in an AquaLab 3TE water activity meter (Decagon devices, Inc.).

2.7. Development of microorganisms

The number of colony forming units (moulds and yeasts) per gram of sample was determined in YGC agar (yeast glucose chloramphenicol) by immersion seeding on plates followed by incubation for 5 days at 25 °C (Camacho et al., 2009). Greensmith (1998) reported a value of 1000 CFU (yeast and moulds) per gram as the upper acceptable limit for dehydrated fruits.

2.8. Colour changes (Browning Index, BI)

A Konica-Minolta CR-400 tristimulus colorimeter was employed. Measured values were read in the \( L^* \), \( a^* \), \( b^* \) scale (CIELab), being converted firstly to \( X \) (red), \( Y \) (green), and \( Z \) (blue) coordinates (see Eqs. (1)–(3)), (Kang, 2006) and secondly to scale \( x \), \( y \), \( Y \) (Eqs. (4)–(6)). Browning index, was calculated by Eq.(7), specially suited for sugar-rich products (Buera, Lozano and Petriella, 1985/1986; Gonzales, Burin, & Buera, 1999). Reference values used in Eqs (1)–(3) were \( X_n = 91.97 \), \( Y_n = 93.8 \) and \( Z_n = 107.98 \), respectively.

\[
X = X_n \left( \frac{a^*}{500} + \frac{(L^* + 16)}{116} \right)^3 \tag{1}
\]

\[
Y = Y_n \left( \frac{(L^* + 16)}{116} \right)^3 \tag{2}
\]

\[
Z = Z_n \left( \frac{-b^*}{200} + \frac{(L^* + 16)}{116} \right)^3 \tag{3}
\]

\[
x = \frac{X}{(X + Y + Z)} \tag{4}
\]

\[
y = \frac{Y}{(X + Y + Z)} \tag{5}
\]

\[
Y = Y \tag{6}
\]

\[
BI = \frac{(x - 0.31)}{0.172} \times 100 \tag{7}
\]

2.9. Variation of the antioxidant activity

Determinations followed the ABTS\(^+\) radical cation decolorization assay method depicted in Fig. 2 (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993; Re et al., 1999). For a measurement to be considered valid, the final value of absorbance (i.e., after a reaction time of 6 min) must decrease by 20–80% from the base value of 0.7, which corresponds to ABTS\(^+\) in ethanolic solution. Determinations were carried out every month during storage, duplicate ethanolic extracts were prepared from the control and the sample with added KMBS. All extracts were kept in a freezer at −20 °C until the storage trial was complete and then all samples were analysed in the same laboratory session. The results are expressed as Chlorogenic acid equivalents (CAE), as this phenolic compound is dominant in apples (Wu et al., 2007). To this end, a calibration curve was prepared with standard solutions of chlorogenic acid of various concentrations.

2.10. Accelerated storage studies

In this type of test, samples are stored at a higher temperature to accelerate reactions of interest so that their changes can be measured in a much shorter period. The method also allows the determination of a \( Q_{10} \) value for each reaction which can be used to estimate the changes with time at lower storage temperatures.

![Fig. 2. Diagram showing experimental routes for the preparation of both sample and reactant (ABTS\(^+\) radical) in the antioxidant activity test.](image-url)
(Steele, 2004). By this method, changes in colour and antioxidant activity of apple leathers were measured during storage of packaged samples maintained at 30 °C in an air oven, over 35 days. Samples were removed for analysis every five days. Kinetic models were fitted to the experimental data thus collected as a function of time in order to determine the kinetic constants. These coefficients relate the quality parameter with its rate of change with time and enabled the calculation of the Q10 factors of both the browning index and antioxidant activity index.

The Q10 factor can be calculated as the ratio of kinetic constants (kc) for quality changes occurring at a given (reference) temperature (T) and 10 °C above this value (T + 10 °C) Eq. (8).

\[ Q_{10} = \frac{k_{cT+10}}{k_{cT}} \]  

(8)

The Q10 concept is based on kinetic constants determined by applying models of the type

\[ -\frac{dC}{dt} = k_C C^h \]  

(9)

where h is the order (0,1,2,…) of the kinetic model, and C the measured quality parameter Eq. (9) (Labuza & Schmidl, 1985).

In foods, experimental quality changes can usually be modelled with zero or first order models, which allow kinetic constants to be determined for every experimental temperature. Another measure of the effect of temperature on a quality parameter, is the Arrhenius activation Energy (Ea, J mol−1). This can be estimated from the value of Q10 Eq. (10) (Labuza & Schmidl, 1985).

\[ E_a = 2.303 \log_{10}(Q_{10}) R T_k(T_k + 10) \]  

(10)

Where, \( T_k \) is the absolute temperature in K.

2.11. Statistical analysis

Determinations on apple pectic gels were carried out in triplicate during storage. The statistical software OriginPro v 8.1 (Origin, 2009) was used for data analysis and model fitting. The statistical significance was assessed by one-way analysis of variance (ANOVA), as well as by the Tukey’s test \((p < 0.05)\), in order to detect significant differences of samples stored for several periods.

3. Results and discussion

3.1. Moisture and soluble solids content

The moisture content of apple leather after dehydration was 25% w/w or 0.333 kg water/kg dry matter and showed negligible change during storage. The soluble solids concentration, however, of about 75° Brix, was higher than the minimum value required for sugar-acid-high methoxyl pectin gelation (65° Brix) (Table 1.). The sum of the percentage moisture content on a wet basis and the soluble solids content in Brix, was almost 100% due to the extremely low proportion of low-solubility or insoluble components.

3.2. pH

The pH of the apple puree was 3.50, which dropped to 3.30 after addition of citric acid, and also showed negligible changes over the storage time.

3.3. Water activity

Water activity determinations were carried out in triplicate for each formulation and the average value for each formulation, which was substantially unaltered during storage, was 0.7. This value provides a margin of safety for the storage of acid foods at ambient temperatures (Welti-Chanes, Alzamora, López-Malo, and Tapia, 2000), because it would not only prevent growth of pathogenic microorganism but also would strongly inhibit growth of non-pathogenic fungi and yeasts as well. However, for this level of water activity, rates of non-enzymatic browning and enzymatic activity are not negligible and may even be considerable (Roos, 1995). As the PPO enzyme was inactivated in the preliminary thermal treatment, the organoleptic changes during storage would be more likely to occur due to non-enzymatic browning.

3.4. Growth of microorganisms

Despite the microbiologically safe characteristics of the fruit leathers (low pH and intermediate water activity), PCA counts were conducted to detect the possible presence of bacteria in the samples, for which negative results were found. In turn, yeast and mould counts were below the admissible levels in all the plates analysed during storage, being, for the control formulation, in the range of 50–100 CFU g−1 for yeasts and below 10 CFU g−1 for moulds. The samples with added preservative did not exhibit any detectable microbial growth over the seven-month storage period at 20 °C.

3.5. Colour changes

Measurements by the tristimulus colorimeter were conducted in triplicate. Average values of \( L^*, a^*, b^* \) for each sample were used in Eqs. (1)–(7), in order to calculate the browning index (Fig. 3). When evaluating the effect of time on each formulation separately, an ANOVA test was carried out and the results are shown in Fig. 3. Overall, the browning index increased with time (7-month storage) for both formulations. At a water activity value of 0.7 the fruit leathers were flexible, shiny and microbially stable, but led to conditions for high rates of Maillard browning (combination of reducing sugars with amino groups) and ascorbic acid oxidation reactions, both leading to the production of dark pigments (Sikorski, 2007). Browning index in the control leather was significantly higher than in the KMBS-added product \((p < 0.05)\) from the first up to the fifth month of storage, though there were no differences in months 6 and 7. Various kinetic models were fitted to the experimental data of browning index as a function of time in order to describe the variation of colour: zero order (Eq. (11)), first order (Eq. (12)) and logistic models (Eq. (13)) (Vaikousi, Koutousmanis, & Biliaderis, 2008)

\[ BI = B_{i0} + k_{ci} t \]  

(11)

\[ BI = B_{i1} \exp(k_{c1} t) \]  

(12)
to find an increase of 49.3% over a 14 day-storage at 5 °C. The change in browning index for the KMBS-added apple leather in this study was not significant (p < 0.05), essentially negligible over the first four months of the storage trial.

Therefore, browning development was considerably faster in the work by Picouet et al. (2009), possibly because of the higher concentration of reducing sugars in their sample which facilitated the development of Maillard reactions. Its higher vitamin C content may have also contributed, by undergoing oxidative browning. Furthermore, the higher initial browning index calculated for the samples studied by Picouet et al. (2009), may have contributed through a first-order kinetic mechanism, to a faster browning development.

3.6. Variations in antioxidant activity

Reduction of the ABTS⁺⁺ radical was between 20 and 80% in all cases, so dilution or concentration of the extracts was unnecessary. A linear expression was fitted to the calibration curve data with \( r^2 = 0.991 \). By rearranging the equation, the Chlorogenic acid equivalents (CAE) were expressed as a function of the percentage reduction of ABTS⁺⁺ absorbance (Eq. (14)).

\[
\text{CAE} = \frac{\% \text{Reduction of ABTS}^{++} - 2.685}{2435.8} 
\]

The results calculated as CAE can also be expressed as \( \mu \text{mol g}^{-1} \), by knowing the amount of dry matter necessary to prepare the ethanolic extract of the leather.

With regard to the preservative-added apple leather, the initial antioxidant activity was 0.0159 \( \mu \text{mol chlorogenic acid/g dry matter} \), which decreased by 15.9% over the entire storage period. However, results of the ANOVA statistical test with a confidence level of 95%, indicate that the variation was not significant. On the other hand, the antioxidant capacity of the control formulation (starting value of: 0.0162 \( \mu \text{mol chlorogenic acid/g dry matter} \) diminished by 47% over the storage period, being this decrease statistically significant. The results, normalised with the corresponding initial concentrations were plotted in Fig. 5 together with the corresponding predictions by a first-order model (Eq. (15)), which again proved to be more accurate than a zero order model.

\[
\text{CAE}_{i} = \exp(-k_{c}t) 
\]

where CAE is the chlorogenic acid equivalent at time \( t \), while CAE\(_{i} \) is the initial value. The resulting fitting parameters are listed in Table 3.

Concerning the preservative-added formulation, the scarce variation in antioxidant activity demonstrates the suitability of KMBS for protecting these micronutrients in the pectic gel. This effect was achieved with 100 mg/kg final product SO₂, which

---

Table 2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Model</th>
<th>Fitting parameters</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL(_{0})</td>
<td>BL(_{as})</td>
<td>( k_{c} )</td>
</tr>
<tr>
<td>Control</td>
<td>Zero-order</td>
<td>22.6 ± 3.6</td>
<td>4.86 ± 0.5(^a)</td>
</tr>
<tr>
<td></td>
<td>First-order</td>
<td>23.6 ± 2.1</td>
<td>0.13 ± 0.03(^b)</td>
</tr>
<tr>
<td></td>
<td>Logistic</td>
<td>29.3 ± 2.2</td>
<td>64.5 ± 9.7</td>
</tr>
<tr>
<td>Added with KMBS</td>
<td>Zero-order</td>
<td>18.9 ± 4.4</td>
<td>4.35 ± 1.05(^a)</td>
</tr>
<tr>
<td></td>
<td>First-order</td>
<td>19.6 ± 2.9</td>
<td>0.14 ± 0.03(^b)</td>
</tr>
<tr>
<td></td>
<td>Logistic</td>
<td>26.6 ± 1.7</td>
<td>53.8 ± 3.9</td>
</tr>
</tbody>
</table>

\( ^{a} \) value ± standard deviation.  
\( ^{b} \) units (month \(^{-1}\)).  
\( ^{c} \) units (browning index units month \(^{-1}\)).
represents only one tenth of the maximum admissible in dehydrated fruits (FAO-WHO, 2010). No published data for the storage of a similar food matrix (i.e., dehydrated apple leather) was found which could enable a comparison with the evolution of antioxidant activity measured here.

3.7. Accelerated storage studies

As indicated in materials and methods, accelerated studies allow the effect of storage temperature to be determined using a reference value \( T \) and a higher value, \( T + 10 \) °C. By assuming that the effect is similar between \( T \) and \( T + 10 \) and between \( T \) and \( T - 10 \) °C, an extrapolation can be carried out to estimate the deteriorative change that would occur over a longer period at a lower temperature, which, as such, is more difficult to assess experimentally. This procedure was employed both for browning index and antioxidant activity.

3.7.1. Colour changes (Browning Index, BI)

The change in colour was expressed as a browning index and plotted in Fig. 6 as a function of time during storage at 20 and 30 °C. A zero-order, first-order and logistic kinetic models were fitted to the experimental data by the least square method (Fig. 6). The most accurate was the logistic model (Table 4), which agrees with the results found for storage at 20 °C. Although the logistic model would be preferred for simulating the variation of quality parameters with time for each storage temperature, the effect of temperature was evaluated by coefficient \( Q_{10} \) which needs to be calculated as the ratio of kinetic constants of \( n \)-order type models, provided the \( n \)-order is the same for each quality parameter at the two temperatures (Vaikousi et al., 2008). On these grounds, the first-order kinetic model was chosen as it fitted the experimental data of browning index better (higher \( r^2 \)) than the zero-order model both at 20 and at 30 °C. Values of both kinetic constants are listed in Table 4. By using Eq. (8) and data of Table 5, the \( Q_{10} \) coefficient was calculated for the colour change in apple leathers to be 2.55. In turn, Eq. (10) was employed to estimate the Arrhenius activation energy, which resulted in \( E_a = 68.9 \) kJ mol\(^{-1}\). This preliminary value was within the published range of activation energies for colour changes of 68.0–80.8 kJ mol\(^{-1}\) by Toribio and Lozano (1984) for concentrated apple juice, and is also close to the value of 75.0 kJ mol\(^{-1}\) determined for colour variations in apple puree by Ibarz, Págán, and Garza (2000). The value of activation energy is a measure of the intensity of the temperature effect on a given parameter. Therefore, comparable values may indicate comparable quality decay mechanisms.

3.7.2. Antioxidant activity

The evolution of the normalised antioxidant activity with time for the KMBS added leather at storage temperatures of 20 and 30 °C was plotted in Fig. 7. In order to evaluate the effect of temperature, a first-order kinetic model (Eq. (15)), was also fitted to the normalised data of the KMBS-added formulation stored at 30 °C. The results of the fitting at 20 °C and 30 °C for both quality parameters were grouped together in Table 5. Oszmianski, Wolniak, Wojdylo, and Wawer (2008) worked with a red apple cultivar which was sliced and exposed to several alternative treatments. The sample, conventionally heated for 4 min at 90 °C without ascorbic acid was conventionally heated for 4 min at 90 °C without ascorbic acid.
Table 4
Results of the fitting of zero-order, first-order and logistic models to experimental data of browning index as a function of time for storage at 30 °C.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fitting coefficientsa</th>
<th>r²</th>
<th>s_y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-order</td>
<td>$B_{0}$ $B_{m}$ $k_{m}$ (month$^{-1}$) $t_{m}$ (month) $\beta$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First-order</td>
<td>29.3 ± 1.2 — 0.43 ± 0.08 —  — 0.878 1.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Logistic</td>
<td>30.7 ± 0.5 46.7 ± 1.7 26.5 ± 0.18 2.44 ± 0.97 0.982 0.72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values ± standard deviation.

Table 5
First order kinetic constants for the variation of browning index and normalised antioxidant activity as a function of time during storage of KMBS-added product at 20 and 30 °C.

<table>
<thead>
<tr>
<th>Quality parameter</th>
<th>Storage temperature</th>
<th>$k_{c}$ (day$^{-1}$)</th>
<th>r²</th>
<th>s_y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Browning index</td>
<td>20 °C</td>
<td>0.00473 ± 9.27 × 10$^{-4}$</td>
<td>0.789</td>
<td>5.69</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>0.012 ± 0.001</td>
<td>0.915</td>
<td>1.60</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>20 °C</td>
<td>0.00132 ± 1.81 × 10$^{-4}$</td>
<td>0.645</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>30 °C</td>
<td>0.02146 ± 1.85 × 10$^{-2}$</td>
<td>0.910</td>
<td>0.05</td>
</tr>
</tbody>
</table>

a Values ± standard deviation.

Addition (control), was then pureed, packaged and pasteurised at 90 °C for 20 min, then immediately cooled and placed in storage at 30 °C. Starting with a chlorogenic acid equivalent value of 1.77 × 10$^{-3}$ μmol Chlorogenic acid/g dry matter, its antioxidant activity at 6 months of storage was decreased by 63%. Returning to the results of the present work on apple leather, samples having activity at 6 months of storage was decreased by 63%. Returning to the oxidation activity at 20°C/C0

Lavelli (2009) presented sufficient data to allow us to estimate an Arrhenius activation energy of 339.6 kJ mol$^{-1}$ for this extremely sensitive quality parameter. With regard to changes in total antioxidant activities of freeze dried apple puree with $a_{m} = 0.7$ during storage, Lavelli (2009) presented sufficient data to allow us to estimate an $E_{a}$ of 116.7 kJ mol$^{-1}$ which, even if lower than the estimated value

found for apple leather in this work, shows the considerable influence of temperature, nature of the food matrix and the type of process on quality retention.

3.7.3. Estimation of the behaviour at a lower storage temperature

Labuza and Schmidl (1985) considered two reactions with different $Q_{10}$ values that cause quality losses in a given food. They indicated that the reaction with the higher $Q_{10}$ would be the most damaging at high temperatures, while that with the lower $Q_{10}$ would cause higher quality losses at lower temperatures. Results of the coefficient $Q_{10}$ for browning index and antioxidant activity calculated between 20 °C and 30 °C were employed to estimate the corresponding kinetic constants at a lower temperature of 10 °C. With them, the predicted behaviour of normalised browning index as a function of time for the three temperatures was calculated and plotted in Fig. 8. The corresponding results for the normalized antioxidant activity as a function of time are exhibited in Fig. 8. For the apple leathers, cautious quality limits were considered, in view of the experimental results determined in this work, so as to have

![Fig. 7. Normalised antioxidant activity of KMBS-added apple leather during storage at 20 °C (■) and 30 °C (○). Corresponding predicted trends (---- and ———) represent calculations by first-order model.](image1)

![Fig. 8. Predicted values as a function of time for storage of apple leather for (a) Normalised browning index at 10 °C (●), 20 °C (solid line) and 30 °C (—) and (b) Normalised antioxidant activity at 10 °C (●), 20 °C (solid line) and 30 °C (—).](image2)
a preliminary estimation of the allowable storage time. They are a normalised browning index = 2 (increase of the browning index by 100%) and a normalized antioxidant activity of 0.8 (decrease of the antioxidant activity by 20%). In Fig. 8, for temperatures of 10 and 20 °C, colour changes reach the limit faster than variations in antioxidant activity, while the opposite behaviour is predicted at 30 °C. For that reason, as far as the determination of shelf life of apple leathers is concerned, antioxidant activity should be chosen as the quality-determining parameter for temperatures above 20 °C, while the browning index should be the parameter to monitor in refrigerated storage.

If considered a functional food, apple leathers should be stored under refrigeration so as to minimize antioxidant activity losses.

4. Conclusions

A potassium metabisulphite (KMBS)-added formulation, satisfactorily maintained the quality characteristics of apple leathers without microbial development over a 7 month storage period.

The browning index, was observed to increase during storage at 20 °C. This increase was especially moderate in the KMBS-added leather. Zero- and first-order kinetic models were fitted to the data, as well as a three-parameter logistic model. The last provided the best fit, because it accounted better for the initial delay of the curve.

The antioxidant activity (AA), determined over storage and expressed as chlorogenic acid equivalents, decreased by 47% during the 7-month period at 20 °C, while at higher temperatures, antioxidant activity was observed to increase during storage at 30 °C. For that reason, as far as the determination of shelf life of apple leathers is concerned, antioxidant activity should be chosen as the quality-determining parameter for temperatures above 20 °C, while the browning index should be the parameter to monitor in refrigerated storage.

Acknowledgements

To Agencia Nacional de Promoción Científica y Tecnológica, Argentina, for funding provided to project PICT 2007-01088.

To Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet), Comisión de Investigaciones Científicas (CIC PBA) and Universidad Nacional de La Plata, for permanent support

References

