THE EFFECT OF DIFFERENT COMMERCIAL SOURDOUGHS ON THE QUALITY OF GLUTEN FREE BREADS

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Original Research Article
UDC 664.654.1 : 664.236
664.64.016.8

SUMMARY
The search for solutions to alleviate technological and nutritional defects in gluten free (GF) breads is a major research topic in the area of food technology. Up to now, the strategies used only contemplated the design of complex matrices by adding ingredients and additives, raising the cost of these products. Nevertheless, the strategy of exploiting the sourdough benefits has been scarcely explored. The possible influence of sourdough on bread quality might reduce the need for additives. In this study, the impact of different commercial sourdough on the structural and quality behaviour of rice wholemeal breads. Breads obtained did not show significant differences in relation to specific volume, moisture and water activity. The addition of sourdough resulted in more acidic bread crumbs and the consequent increase in titratable acidity (TTA), resulting in harder crumbs and irregular crumb grain of the sourdough breads. On the other hand, there were no significant differences in the nutritional composition of the breads. Overall, commercial sourdoughs changed the textural characteristics of the GF breads, without affecting the specific volume.

Keywords: gluten free, sourdough, bread, quality, staling

INTRODUCTION
Nowadays, the presence of gluten-free products (GF) in the market is growing due to the increasing demand, not only for people with medical needs, but also consumers who include this type of products as part of their lifestyle. This trend has led to the search for GF products with similar sensory and nutritional characteristics to traditional cereal based products. Nevertheless, the development of good quality GF products continues to be a challenge for food scientists and technologists. In fact, GF products, currently available on the market, still have low technological and nutritional quality (Mariotti et al., 2013). Specifically, GF bakery products have lower texture, lower volume, poor colour and short shelf-life.
(Gallagher et al., 2004; Matos and Rosell, 2012), as well as poor nutritional composition (Matos and Rosell, 2011). Different studies focused on improving the technological and sensory quality of GF breads describe complex formulations needed to overcome the negative impact of the absence of gluten (Matos and Rosell, 2011). These formulations combine diverse additives in order to mimic the viscoelastic properties of gluten (Masure et al., 2016). However, the addition of these ingredients supposes an increase of the final product. In addition, the current trend of consumers is directed towards healthy products with good aroma and taste, good texture and a long shelf-life, made in a "natural" way without the addition of artificial additives. In this sense, the use of sourdough has a long tradition associated with artisan baking. Nevertheless, the use of sourdoughs, beyond market trends, plays an important role in the preparation of breads, improving the technological, nutritional, organoleptic and maintenance shelf-life properties of breads (Moroni et al., 2009). Thus, the positive influence of the sourdough could be exploited to reduce the need for additives.

The aim of this research was to evaluate the effect of different commercial sourdoughs on the technological and nutritional properties of GF breads. For that purpose, four GF sourdoughs, obtained from different raw sources, were tested. Breads obtained were evaluated regarding physicochemical analysis, hydration properties, crumb microstructure, crumb texture, and nutritional analysis.

**MATERIALS AND METHODS**

**Materials**

Four commercially dehydrated available sourdoughs, coming from different flour origin, were used in this study. Corn (MM1), rice (MM2), buckwheat - organic quality (MM3) and quinoa – organic quality (MM4) sourdoughs were obtained from Böcker (Minden, Germany); rice flour was purchased from La Meta (Lerida, Spain); bran rice was provided by Arrocera Antonio Tomás; dry yeast by DHW Europa; salt by local market; and hydroxypropylmethylcellulose (HPMC) Methocel K4M by Dow Wolf Cellulosics GmbH, USA.

**Baking procedure**

Control breads (PC) were prepared using 90% rice flour and 10% rice bran, 110% water, 1.5% salt, 3% yeast and 1% HPMC. The samples were prepared by adding 5% sourdough. To keep a constant flour/bran ratio, the percentage of added sourdough to the samples was replaced by the equal amount of flour/bran mixture in control dough formulation. Mixing was carried out in a Vimar 18-2 spiral kneader (Barcelona, Spain) at speed 1 for 5 minutes. The batter was scaled to 100 g into baking tins and placed into a proofer (Lezo, Spain) for 30 min at 35 °C and a relative humidity of 85%. The breads were baked for 35 min at 180 °C in a Eurofours oven (Gommegnies, France), injecting steam for the first 5 seconds. After
baking, bread loaves were removed from the tins and cooled at room temperature for 1 h. Baking was performed on 2 different days (2 independent trials) and 3 loaves were prepared for each bread type at each baking trial.

**Bread physicochemical analysis**

Bread moisture content was determined following the ICC Standard Methods 110/1 (ICC, 1994). Weight loss during baking was assessed by weighing the pans before and after baking. Bread volume was determined by the rapeseed displacement method. The specific volume was calculated as the ratio between the volume of the bread and its weight. Water activity of samples was measured using an Aqua Lab Series 3 (Decagon Devices, Pullman, USA) at 22 °C. The pH of the crumbs was measured using a suspension of crumb according to a standard method (Getreideforschung, 1954). The total titratable acidity (TTA) of bread samples was also determined. A suspension of 10 g of sample in 90 mL of water was homogenized together using a Polytron Ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, USA) for 0.5 min at 14,000 rpm and titrated with 0.1 N NaOH to pH 8.5. These measurements were carried out in three breads of each batch.

The color of the bread crumbs was measured at three diverse locations by using a Minolta colorimeter (Chromameter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate ($L^* = 96.9; a^* = -0.04; b^* = 1.84$). The color was recorded using CIE-$L^*a^*b^*$ uniform color space (CIE-Lab) where $L^*$ indicates lightness, $a^*$ indicates hue on a green ($-$) to red ($+$) axis, and $b^*$ indicates hue on a blue ($-$) to yellow ($+$) axis. Data from three slices per bread were averaged.

An image analysis system was used to analyze the bread crumb structure. Images of the GF bread slice (10-mm thick) were captured using a flatbed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP scanjet G3110, Hewlett-Packard, USA). The images were scanned full scale at 600 pixels per inch, analyzed in levels of gray (eight bits, readout 0–255) and captured in tiff format for each measurement. A 15 x 15 mm square field of view (FOV) was evaluated for each image. This FOV captured the majority of the crumb area of each slice. The image analysis was carried out using Image J software (UTHSCSA Image Tool software). Threshold was assessed applying the Otsu’s algorithm according to Gonzales-Barron and Butler (2006). Data derived from the crumb structure analysis included: number of cells or alveoli, average cells area and cell circularity, and were used for comparing purposes among different samples.

Crumb hardness, chewiness, cohesiveness, springiness and resilience were evaluated using a Texture Profile Analyser (TA.XT.Plus Stable Microsistsems, UK) with a 5 kg load cell, which compresses the bread crumb with a 25 mm aluminum cylindrical probe. Bread samples were sliced into 10 mm slices and analyzed with a test speed of 2 mm/s and a trigger force of 5 g to compress the middle of the bread crumb to 50% of its original height at a crosshead speed of 1 mm/s and applying 30 s gap between compressions. The measurement with the various parameters was conducted on the day of baking.
Chemical composition
The chemical composition of the samples was determined according to ICC corresponding standard methods (ICC, 1994) namely, fat (ICC 136), proteins (N x 6.25) (ICC 105/2) and ash (ICC 104/1). Total carbohydrates were determined by difference from 100 g minus the sum of moisture content, protein, ash and fat expressed in grams/100 g FAO (2003). For the estimation of dietary fibre, samples were finally powdered to pass through a sieve of 250 μm. Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents were determined following the AACC method (AACC.32-07, 1995). Determinations were done in duplicate for obtaining mean values.

Statistical analysis
The data reported are the mean of replicates and expressed as a mean ± standard deviation. Statistical analyses were carried out with Fisher’s least significant differences test with a significance level of 0.05. Pearson correlation coefficient (r) and p-value were used to indicate correlations and their significance using Statgraphics Centurion XV software (Bitstream, Cambridge, N). The correlation coefficient was classified in different levels of correlation: perfect (|r| = 1.0), strong (0.80 ≤ |r| ≤ 1.0), moderate (0.50 ≤ |r| ≤ 0.80), weak (0.10 ≤ |r| ≤ 0.50), and very weak (almost none) correlation (|r| ≤ 0.10).

RESULTS AND DISCUSSION
The effect of sourdough on the quality parameters of gluten free breads based on rice flour
To determine the role of the different sourdoughs in GF breads, several commercial dehydrated sourdoughs from different sources were selected and a 90:10 mixture of rice flour: rice bran was used as the formulation bases. The statistical analysis indicated that the sourdough addition significantly influenced the weight loss of breads (p <0.001), but did not prompt a significant effect on the specific volume and water activity parameters (Table 1). GF samples displayed specific volume values that ranged from 1.47 to 1.78 mL/g. These results are in agreement with literature (Gujral and Rosell, 2004a). The moisture content values obtained for control and sourdough samples showed values of 41.16 – 43.64%. The moisture content values obtained in this study are rather high compared to those found in different commercial GF breads marketed in Spain (Matos and Rosell, 2011). Similar trend was observed in water activity parameter. Likely, the moisture retention, as well as the high water activity might be attributed to the high water holding capacity of the incorporated hydrocolloids and the addition of rice bran to the formulation, as suggested by Wang et al. (2002) and Guarda et al. (2004). The incorporation of sourdough significantly affected the weight loss parameter. Samples MM3 and MM4 did not show a significant
difference with the PC bread, while MM1 and MM2 samples had a significantly lower weight loss than PC. As expected, sourdough addition resulted in more acidic crumbs and an increase in TTA value (Table 1), showing a strong negative correlation between both parameters \((r = -0.8444, p = 0.0021)\). Control bread displayed the highest pH followed by bread obtained from MM4> MM1 and MM3> MM2. The highest TTA value was noted in the MM2 bread followed by MM3, MM1, MM4 and PC bread. These results confirm the formation of different proportions of metabolites in the different sourdough used (Arendt et al., 2007).

Results from the crumb colour parameters are presented in Table 1. The \(a^*\) and \(b^*\) values for crumb colour showed significant \((p < 0.05)\) differences among the different sourdough added breads, but no difference was observed for \(L^*\) value. Nevertheless, lightness of bread crumbs was higher than those reported in the literature for rice flour GF breads, likely due to the rice bran added (Phimolsiripol et al., 2012). Regarding \(a^*\) and \(b^*\) values, all samples showed positive values, indicating hue on red and yellow axis for all samples evaluated. The obtained results were similar to those reported in the literature for GF breads based on rice flour with bran rice added (Phimolsiripol et al., 2012). In addition, great variation derived from the different sourdough added in each formulation was observed. An increased of \(a^*\) value was observed in all samples, except in the case of MM1 sample. \(b^*\) parameter also maintained a similar pattern, while it remained constant for MM3 and MM4 samples. The results suggest that developed colour was the result of the interaction of ingredients, the addition of 5% of sourdough being enough to influence these parameters.

### Table 1 Different quality characteristics of the control and sourdough samples

<table>
<thead>
<tr>
<th></th>
<th>Specific Volume (mL/g)</th>
<th>Weight loss (g)</th>
<th>Moisture (%)</th>
<th>Water activity</th>
<th>pH</th>
<th>TTA (mL)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>1.72 ± 0.06</td>
<td>29.09 ± 0.08</td>
<td>41.16 ± 1.15</td>
<td>0.968 ± 0.000</td>
<td>6.09 ± 0.031</td>
<td>3.90 ± 0.051</td>
<td>73.30 ± 1.63</td>
<td>3.44 ± 0.042</td>
<td></td>
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<tr>
<td>MM1</td>
<td>1.47 ± 0.16</td>
<td>25.88 ± 1.25</td>
<td>43.64 ± 0.28</td>
<td>0.970 ± 0.001</td>
<td>5.70 ± 0.034</td>
<td>4.97 ± 0.050</td>
<td>71.95 ± 0.33</td>
<td>0.25 ± 0.012</td>
<td></td>
</tr>
<tr>
<td>MM2</td>
<td>1.67 ± 0.00</td>
<td>25.31 ± 0.99</td>
<td>42.58 ± 0.24</td>
<td>0.968 ± 0.002</td>
<td>5.43 ± 0.024</td>
<td>6.30 ± 0.144</td>
<td>70.75 ± 0.36</td>
<td>0.56 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>MM3</td>
<td>1.58 ± 0.00</td>
<td>27.38 ± 0.44</td>
<td>42.20 ± 0.21</td>
<td>0.968 ± 0.002</td>
<td>5.64 ± 0.012</td>
<td>6.37 ± 0.144</td>
<td>70.17 ± 2.30</td>
<td>1.44 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>MM4</td>
<td>1.78 ± 0.09</td>
<td>28.97 ± 0.35</td>
<td>42.84 ± 1.07</td>
<td>0.969 ± 0.007</td>
<td>5.92 ± 0.026</td>
<td>4.77 ± 0.240</td>
<td>72.53 ± 1.38</td>
<td>0.74 ± 0.067</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0848</td>
<td>0.0128</td>
<td>0.1284</td>
<td>0.9784</td>
<td>0.0000</td>
<td>0.3076</td>
<td>0.0000</td>
<td>0.0196</td>
<td></td>
</tr>
</tbody>
</table>

Parameters from the image analysis of the control and sourdough bread crumbs (Figure 1) displayed a large variability among crumb bread structures (Table 2). PC, MM3 and MM4 samples exhibited similar cells or alveoli number value, whereas lower values for alveoli number were seen for MM1 and MM2. Moore et al. (2006) reported values of this parameter in GF breads ranged from 15 to 20 cells/cm². Nevertheless, our results showed high values for this parameter. McCarthy et al. (2005) observed that the number of cells/cm² increases as HPMC and water increase. In this study, no direct correlation between the cell number and bread moisture was found. However, the weight loss displayed a strong positive correlation with this parameter \((r = 0.9977, p = 0.0432)\). In contrast, samples MM1 and MM2 showed lower average cell area (mm²) than the other samples, likely due...
to the coalescence of many gas cells into one large cell. No significant differences were observed for circularity values. Sample crumbs had circularity values ranging from 0.60 to 0.65, indicating a fairly uniform shape.

Figure 1 Digital images of control and sourdough bread crumb samples (15 x 15 mm field of view of breads)

Crumb hardness, chewiness, cohesiveness, springiness and resilience parameters determine the bread acceptability in consumers (Matos and Rosell, 2011). Sourdough addition had a significant effect on hardness and chewiness parameters (Table 2), but not on the cohesiveness, springiness and resilience parameters. The hardness obtained for control sample showed values of 4.88 ± 0.34 N. GF products are mainly composed of carbohydrates, resulting in crumbs with a high hardness. Matos and Rosell (2012) reported hardness values of GFB crumb higher than 10N. However, the hardness values observed for the control sample in this study showed lower values that those reported in the literature. Likely, low hardness might be ascribed to rice bran added, as suggested by Phimolsiripol et al. (2012). This value was significantly increased after the MM1 and MM2 sourdough addition. Hayman et al. (1998) related the coalescence of gas cells during the early stages of cooking with excessive hardening of cell walls. On the other hand, Moroni et al. (2011) observed a strengthening of the starch gel after the acidification of the buckwheat doughs. The pH results obtained, as well as the image analysis of the crumbs suggest that both phenomena could be occurring in MM1 and MM2 samples, which could explain the increase in hardness of the crumbs.

Table 2 Analysis of crumb microstructure and texture

<table>
<thead>
<tr>
<th></th>
<th>Number of alveoli/cm²</th>
<th>Total area alveoli mm²/cm²</th>
<th>Hardness (N)</th>
<th>Springiness</th>
<th>Chewiness (N)</th>
<th>Cohesiveness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>32 ± 5</td>
<td>0.900 ± 0.128</td>
<td>4.88 ± 0.34</td>
<td>0.92 ± 0.01</td>
<td>3.13 ± 0.23</td>
<td>0.71 ± 0.02</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>MM1</td>
<td>24 ± 6</td>
<td>1.127 ± 0.156</td>
<td>4.87 ± 0.46</td>
<td>0.95 ± 0.01</td>
<td>5.47 ± 0.27</td>
<td>0.68 ± 0.01</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>MM2</td>
<td>21 ± 3</td>
<td>1.189 ± 0.086</td>
<td>7.56 ± 0.38</td>
<td>0.94 ± 0.02</td>
<td>5.08 ± 0.19</td>
<td>0.71 ± 0.02</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td>MM3</td>
<td>29 ± 3</td>
<td>0.818 ± 0.077</td>
<td>7.20 ± 1.26</td>
<td>0.96 ± 0.01</td>
<td>4.82 ± 0.72</td>
<td>0.69 ± 0.01</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>MM4</td>
<td>28 ± 6</td>
<td>0.830 ± 0.036</td>
<td>5.40 ± 0.82</td>
<td>0.97 ± 0.01</td>
<td>3.71 ± 0.28</td>
<td>0.72 ± 0.05</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>P-value MM</td>
<td>0.0400</td>
<td>0.0064</td>
<td>0.0388</td>
<td>0.4011</td>
<td>0.0388</td>
<td>0.4961</td>
<td>0.4327</td>
</tr>
</tbody>
</table>

Chewiness characterizes the time required masticating a bread piece prior to swallow. Low chewing values means easy break of the bread in the mouth (Matos and Rosell, 2011). Chewiness varied from 3.13 to 5.47 N, observing a significant effect with the sourdough addition. Following the trends displayed up to the
moment, PC and MM4 followed by MM3 samples displayed the lowest values for this parameter, while MM2 and MM1 showed the highest one (Table 2).

Regarding cohesiveness, springiness and resilience, no significant difference was observed. The extent to which a material can be deformed before it ruptures, reflecting the internal cohesion of the material is cohesiveness. High cohesiveness is desirable in breads because it forms a bolus rather than disintegrates during mastication, while low cohesiveness means bread has an increased susceptibility to fracture or crumble (Onyango et al., 2011). A 0.70 value for this parameter was observed for all the samples (Table 2). Similar results about the impact of sourdough on buckwheat breads were reported by Moroni et al. (2011) in their study. In agreement with data reported in the literature (Matos and Rosell, 2012), samples showed springiness values around 0.95. The low springiness value indicates brittleness and reflects the tendency of bread to crumble when sliced (McCarthy et al., 2005). The resilience is related to the capacity of the crumb to adapt to the compression and recovery. The results obtained in this study were 0.33 for this parameter.

**The effect of sourdough on the chemical composition of gluten free breads based on rice flour**

The chemical composition of control and sourdough breads is summarized in Table 3. No significant difference in chemical composition between control and sourdough breads ($p > 0.05$) was observed, with the exception of dietary fibre values (Table 3). As expected, the major component of the different samples were carbohydrates (Matos and Rosell, 2011), while the minor component were the minerals. The results suggest that sourdough did not significantly influence the nutritional composition parameters. However, GF breads obtained showed higher nutritional contribution than GF breads with complex formulations (Matos and Rosell, 2011). A plausible explanation for the high nutritional contribution would be the rice bran used in the recipe. De Delahaye and Peña (2009) reported that the rice bran added in wheat-based formulations provides a contribution of 16% protein, 8.5% minerals and 20% fat.

**Table 3 Chemical composition, expressed as g/100 g of wet matter, of the control and sourdough breads**

<table>
<thead>
<tr>
<th></th>
<th>Protein (%)</th>
<th>Ash (%)</th>
<th>Fat (%)</th>
<th>Carbohydrate (%)</th>
<th>Total dietary fibre (%)</th>
<th>Insoluble dietary fibre (%)</th>
<th>Soluble dietary fibre (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>5.33 ± 0.10</td>
<td>1.02 ± 0.02</td>
<td>1.38 ± 0.22</td>
<td>51.09 ± 0.78</td>
<td>0.70 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.09</td>
<td>0.49 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM1</td>
<td>5.17 ± 0.05</td>
<td>1.06 ± 0.01</td>
<td>1.16 ± 0.18</td>
<td>48.94 ± 0.53</td>
<td>2.19 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.15</td>
<td>1.53 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM2</td>
<td>5.21 ± 0.03</td>
<td>1.07 ± 0.01</td>
<td>1.22 ± 0.27</td>
<td>49.77 ± 0.02</td>
<td>1.74 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53 ± 0.14</td>
<td>1.21 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM3</td>
<td>5.42 ± 0.03</td>
<td>1.01 ± 0.01</td>
<td>1.31 ± 0.09</td>
<td>50.13 ± 0.16</td>
<td>1.66 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.21</td>
<td>1.07 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MM4</td>
<td>5.33 ± 0.19</td>
<td>1.04 ± 0.02</td>
<td>1.19 ± 0.03</td>
<td>49.62 ± 0.82</td>
<td>1.23 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.17</td>
<td>0.72 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
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The fibre composition of the different breads revealed the influence of the addition of the different sourdoughs (Table 3). TDF ranged from 0.70 to 2.19 g/100 g,
showing that sourdough breads contained more of dietary fibre than PC bread. That effect was due to the SDF content, which resulted in an increase of the TDF content. In general, all bread samples showed higher amount of SDF than IDF fraction. A direct correlation was observed with the composition of the sourdoughs described by the supplier’s datasheets. Thus, the commercial sourdoughs contain SDF in their composition, which might improve the content of SDF, contributing to the increase in the level of TDF.

CONCLUSIONS
The results obtained allow concluding that the technological characteristics and nutritional properties of GF breads are notably influenced by the sourdough addition. The commercial sourdough addition decreased the pH of the crumb of the breads and this effect was dependent on the flour used in the manufacture of the sourdough. The highest acidification was obtained with MM2 obtained from rice flour. The texture of the bread crumbs was affected after the commercial sourdough addition, observing an increase in hardness caused by the addition of MM1 and MM2 (both from cereal flours). Nutritionally, the sourdoughs did not modify the chemical composition of the GF breads, except for the soluble fibre content that increased with the presence of sourdough, and consequently the amount of total fibre. This increase was attributed to the possible presence of compounds in the sourdough with soluble fibre functionality.

The overall analysis of the results showed a remarkable discrimination between the control and sourdough samples. The greater effect was caused by MM2 sourdough from rice flour. Therefore, commercial sourdoughs are a suitable alternative to modify the technological characteristics of GF breads, obtaining more acidic products and modified crumb textures. The commercial sourdoughs studied contributed to increase the soluble and total fibre content of the GF breads.

ACKNOWLEDGEMENT
Authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project AGL2014-52928-C2-1-R) and the European Regional Development Fund (FEDER). Y. Benavent-Gil would like to thank predoctoral fellowship from Spanish Ministry of Economy and Competitiveness.

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