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Shaping food systems towards improved nutrition: a case study on Tuscan Bread Protected Designation of Origin

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Materials S1. Chemical analysis.

The chemical analysis entailed the following steps:

1. In laboratory sourdough bread production by using suitable flour and according to the Protected Designation of Origin specification

The sourdough utilized was refreshed on a daily basis by following the methodology generally adopted in Tuscan bakeries. Soft wheat flour type 0 was utilized (Giambastiani mill, Lucca, Italy, 'Consortium of Promotion and Protection of Tuscan Sourdough Bread'), water (60% of used flour) and a share (30% of flour) of sourdough coming from the previous baking. According to the methodology described in the EU Regulation for Protected Designation of Origin 'Pane Toscano' sourdough consist of a portion of dough from a previous preparation which, kept in a suitable environment, undergoes a gradual process of fermentation and acidification. When suitably refreshed this sourdough, called 'the starter' (biga), is able to initiate the rising process when combined with new dough.

In detail, at each cycle of refresh, the following amounts of the involved components are used to prepare the starter (biga): 1 kg of soft wheat flour type 0, at least 500 ml of sterilized water, at least 200 g of sourdough coming from the previous back-sloping process. After the mixing, an aliquot of the biga is stored in controlled conditions for at least 8 hours to be utilized in the refresh process of following day, while the remaining portion is maintained at room temperature for at least 2.30 hours before being cooked.

In the laboratory tests, the following amounts of the three involved components were used in order to refresh the sourdough utilized: sourdough coming from the previous back-sloping process (75 g), soft wheat flour type 0 (250 g) and sterilized water (150 g). These ingredients were mixed for 20 min in a kneading machine. A sample (50 g) of this dough was collected to be analysed, while the remaining aliquot was further divided into two different portions, the first (150 g) was stored inside a temperature controlled cell for about 24 hours to be utilized in the refresh process of following day, while a part (200 g) of the remaining portion was maintained at 30 °C for 4 hours before to be cooked (30 min at 230 °C) inside an automatic oven to produce the wished bread. Three different values of storage temperatures (13 °C, 19 °C and 27 °C) were tested, while, for every temperature analyzed, the refresh procedure and the related sample collection was carried on for about two weeks.

2. Chemical characterization: sourdough and cooked bread

Concentrations of the main fermentative metabolites (ethanol, D/L – lactic acid) produced in the sourdough during the storage time and in the bread samples after cooking, were determined by using specific Enzymic Kits (Megazyme), after pre-extraction with HCl 0.1N.

3. Sensorial analysis of bread (crust and crumb)

Descriptive analyses was used to determine the sensory profiles of the bread samples. A panel of trained assessors evaluated bread samples 2 hours after to be taken out of the oven. The crumb separately from bread crust were tasted to better identify the specific contribution of these two bread fractions.

A 20 g portion of each sample, including 10g of crust and 10g of crumb, was presented to assessors in 3-digit coded glasses covered with a glass cover; 10 min intervals were allowed between each sample. All samples were assessed in duplicate. For evaluation, each assessor was

provided with filtered water and un-salted crackers and asked to cleanse their palate between tastings. In addition, assessors received a list of attributes that included definitions to aid in their assessments. Sample attributes were scored on unstructured 100 mm line scales labeled from low at 5 mm to high at 95 mm intervals. For each attribute, ratings on the unstructured line scale were measured geometrically to produce intensity values.

4. Statistical analysis

To evaluate the statistical significance of the data obtained, the chemical and sensorial evaluations were performed in duplicate. Statistical analysis of data was performed by one-way ANOVA (CoStat, Cohort 6.0), and means separation by the Tukey's HSD test at $P \leq 0.05$ of significance.