Influence of grain quality, semolinas and baker's yeast on bread made from old landraces and modern genotypes of Sicilian durum wheat

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ABSTRACT

Several studies showed that products made with ancient wheat genotypes have beneficial health properties compared to those obtained with modern wheat varieties, even though the mechanisms responsible for the positive effects are not clear. Ancient durum wheat genotypes are being currently used for the production of pasta, bread and other typical bakery products but the consumption is strictly local. In this work 15 genotypes of *Triticum turgidum* subsp. *durum*, including 10 ancient and 5 modern, were characterized for their technological traits through the determination of different parameters: protein content, dry gluten, gluten index, yellow index, ash, P/L, W and G. In addition, the baking aptitude of all genotypes was evaluated. All semolinas were subjected to leavening by commercial baker's yeast and the experimental breads were subjected to the qualitative characterization (weight loss, height, firmness, colour, volatile organic compounds, image and sensory analysis). The results obtained showed that protein content of grains and semolinas was higher in ancient rather than modern genotypes. Dry gluten ranged from 6.7% of the modern variety Simeto to 13.6% of the ancient genotype Scorsonera. Great differences were found for the yellow index which reached the highest value in Saragolla variety. The P/L and W ratios were significantly higher for the modern genotypes. On average, weight loss was about 14 g, while bread height varied significantly between the trials. Bread consistency varied between 12.6 and 31.3 N. Differences were observed for the yellow of the crumb (higher for modern genotypes) and for the redness of the crust (higher for ancient genotypes). The sensory evaluation displayed a high variability among the breads from the 10 ancient genotypes, while the control breads received scores closed to those of the modern genotypes. This study revealed that the modern durum wheat varieties showed a certain uniformity of behaviour, while the ancient genotypes exhibited a great variability of the final attributes of breads.

Keywords: Sicilian ancient landraces; baker's yeast; semolinas; *Triticum durum*; volatile organic compounds.

1. Introduction

The history of Sicily, the biggest island in the Mediterranean Sea, is strictly linked to durum wheat (*Triticum turgidum* subsp. *durum*) cultivation. Thus, the bread made with durum wheat semolina represents one of the main products of the Sicilian gastronomic tradition, with a homemade production of more than 50 different bread types widespread throughout the region (Costanzo, Liberto, & Russo, 2001).

The majority of traditional bread types produced in Sicily are prepared from re-milled semolina from ancient durum wheat genotypes. These are represented by the landraces and the varieties grown in Sicily (and in general in Southern Italy) in the late 19th century and the first half of the 20th century, when they were quickly replaced by new improved genotypes (the so-called "modern" varieties), genetically uniform, better suited to intensive cultivation, higher yielding and with a superior technological quality (De Vita et al., 2007). Fortunately, a number of ancient genotypes have been cultivated in Sicily (although mostly in very small acreages) during that transition period or preserved in *ex situ* collections, avoiding their extinction.

In the last decade, the landraces and the ancient varieties of durum wheat have gained new attention, presumably thanks to the increased public awareness of environmental issues and the increased consumers' demand for genuine and traditional foods, including typical breads (Giunta et al., 2020). Regarding the first aspect, the ancient genotypes have been proven to be particularly suited (often more than the modern varieties) to the organic or low-input agricultural systems typical of marginal areas (Ruisi et al., 2015), where they might represent a resource to increase economic revenues from food systems. Concerning the second aspect, the products obtained from the ancient durum wheat genotypes are generally perceived by consumers to be more "natural" and safer than those obtained from the modern varieties (Di Francesco et al., 2020). Furthermore, consumers often attribute these products peculiar to organoleptic, nutritional and health-promoting

properties. This perception has been confirmed, to some extent, in some studies. For instance, Vita et al. (2016) found qualitative and quantitative differences between landraces and modern varieties of durum wheat for volatile organic compounds, thus suggesting that the aromatic profiles of both kernels and wholemeal flour can be successfully used to differentiate wheat genotypes. Di Loreto et al. (2018) reported higher total phenolic acid compounds and antioxidant activity in ancient genotypes rather than modern durum wheat genotypes. Similarly, Dinelli et al. (2009) observed diverse qualitative phytochemical profiles by analysing the whole grains of ancient and modern durum wheat genotypes, with a considerable higher number of phenolic compounds (specifically phenolic acids, flavonoids, tannins and other aromatic molecules with a strong antioxidant capacity) in the ancient genotypes. However, according to Di Francesco et al. (2020), comparison of nutritional and nutraceutical value for ancient and modern durum wheat genotypes is still controversial, indicating a need for further researches.

The adoption of the Mediterranean diet, Intangible Cultural Heritage of Humanity, by a continuous growing number of persons has encouraged the consumption of semolina bread. In Italy, this phenomenon has determined the massive increase of utilization of durum wheat for bread making (Alfonzo et al., 2017; Gaglio et al., 2020a) and the start of breeding programs to select new varieties with defining bread making aptitudes (De Vita et al., 2010).

Proteins of wheat kernels influence the technological quality of the resulting semolinas and, consequently, their potential for processing into different products, such as pasta, bread and other bakery products (Samaan, El-Khayat, Manthey, Fuller, & Brennan, 2006). Gliadin and glutenin are particularly important proteins, because, after hydration and mechanical action, form gluten that is responsible for the viscoelastic properties of wheat doughs (Troccoli, Borrelli, De Vita, Fares, & Di Fonzo, 2000).

In Italy, bread is the product obtained from total or partial baking of a leavened dough prepared with wheat flour (milled), water and a leavening agent, with or without salt (sodium chloride)

addition (D.P.R. 502/1998). Bread production is quite simple, but in reality bread is the result of several complex reactions and its organoleptic characteristics (taste, flavour, aroma and texture) that are particularly influenced by raw materials, technology applied and baking conditions (Hansen, & Schieberle, 2005). Regarding bread making technology, the leavening process is particularly relevant to the final quality for acceptability. To this purpose, the biological leavening most commonly applied worldwide in bread making is carried out by baker's yeasts with *Saccharomyces cerevisiae* being the main species (Jenson, 1998).

Straight-dough is one of the mostly applied method for bread making. In this process all ingredients and baker's yeast are mixed together into a one-step production and *S. cerevisiae* is used as the sole leavening agent (Jayaram et al., 2013) responsible for the production of carbon dioxide gas, which is trapped in the dough matrix (Maloney, & Foy, 2003). Although sourdough technology is reported to be the best strategy to generate aroma compounds in breads (Corona et al., 2016), the role of yeasts in bread making is not limited to gas production, since they produce several metabolites that might influence bread aroma and flavour (Alfonzo et al., 2021).

During baking, the form of the dough and its porous structure are stabilized due to gluten denaturation and loss of extensibility. The increasing baking temperature is responsible for the biochemical modifications, especially Maillard reaction and caramelization, from which derive most of the final bread characteristics such as flavour, crust colour and crispiness (Purlis, 2010).

The present work was aimed to characterise the physicochemical properties of several durum wheat genotypes, including modern varieties and ancient Sicilian landraces, and to evaluate their technological performances in bread-making performed with baker's yeast as leavening agent. Fermented doughs as well as the resulting breads were analysed for several quality parameters.

2. Materials and methods

2.1. Wheat genotypes and milling process

113 Fifteen genotypes of durum wheat different for morphological, agronomic and quality traits were
114 used in this study. These 15 varieties included 10 “ancient” and five “modern” genotypes (Table 1).
115 Within the first group, nine were landraces collected from Sicilian farmers and one (Senatore
116 Cappelli) was a pure line selected from the Tunisian landrace Jenah Rhetifah and released in 1915.
117 All ancient genotypes were widely grown in Southern Italy (particularly in Sicily) in the 19th
118 century and the first half of the 20th century. Some genotypes like Perciasacchi, Russello, Timilia
119 and Senatore Cappelli have been recently rediscovered by scientists, farmers and consumers in
120 order to produce breads with peculiar nutritional and health-promoting characteristics, as well as
121 unique organoleptic properties (Di Loreto et al., 2018). The five modern genotypes were all pure
122 lines, released from 1970 to 2004; some of them are among the most spread durum wheat cultivars
123 grown today in Southern Italy.
124 All the accessions used in this study were grown in open field during the 2013–2014 growing
125 season at the farm Pietranera, located about 30 km north of Agrigento, Italy (37°30’N, 13°31’E;
126 178 m asl). The field experiment was set up in a randomized complete block design with three
127 replications, each plot being 9 m^2 (8 rows, 6.0 m long, about 0.19 m apart). At maturity (June
128 2014), grain was harvested from each plot using a plot combine and three grain samples per
129 genotype (one from each replication) were taken. Each sample was then divided into two parts. One
130 part was used to measure some grain quality traits (1000-kernel weight, test weight, and protein
131 content); the other part was milled to semolina (400-600 µm) by means of the Bühler MLU 202
132 experimental mill (Bühler, Uzwil, Switzerland) according to the AACC method 26-21A (AACC,
133 2000).
134
135 *2.2. Determination of grain and semolina quality*
136 For each of the genotypes, 1000-kernel weight, test weight and protein content were measured on
137 the grain. Thousand-kernel weight was estimated by weighing two sets of 250 kernels from each

plot and multiplying the mean weight by four. Test weight was determined by means of the humidimeter TM NG (Tripette and Renaud – Chopin, Villeneuve-la-Garenne, France). The nitrogen (N) content of whole grain was determined according to the Dumas method (AACC method 46-30; AACC, 2000) by means of the automatic N-analyser DuMaster D-480 (Buchi Labortechnik, Flawil, Switzerland); the conversion factor for calculating the protein content from the N content was 5.7. Semolinas of each genotype, together with a commercial semolina (CTR; Mulini Gaspare Salvia, Partinico, Italy) were analysed for determination of ash and moisture contents by the AACC methods 08-01 and 44-15, respectively (AACC, 2000). Yellow index was determined by means of the reflectance colorimeter Chroma Meter CR-300 (Konica Minolta Sensing, Osaka, Japan). The protein content of semolinas was determined according to the AACC method 39-11 (AACC, 2000). Dry gluten and gluten index were determined by means of the Glutomatic System (Perten Instruments, Hägersten, Sweden) according to the AACC method 38-12 (AACC, 2000). Alveograph parameters (P, L, W and G) were determined by means of the Chopin Alveograph (CHOPIN Technologies, Villeneuve-la-Garenne Cedex, France) according to the AACC method 54-30 (AACC, 2000). In the alveogram, P is the height of the peak and represents the maximum overpressure needed to blow the dough bubble, which is an indicator of the dough tenacity; L is the length of the alveogram up to the point of bubble rupture (i.e. the time required to break it), which is an indicator of the dough extensibility; W is the area under the pressure-time curve and represents the deformation energy, that is the work necessary to inflate the bubble to the point of rupture, which is an indicator of the dough strength; and G is the square root of the volume of air necessary to inflate the bubble to the point of rupture, which is an indicator of the dough swelling. All measurements on both grains and semolinas were made in three replicates per genotype.

2.3. Dough production and analyses

For all the genotypes, doughs of 300 g were prepared as reported by Alfonzo et al. (2016). Prior to mixing, sterile water was used for each trial to suspend 3 g of fresh baker's yeast (La Parisienne, AB Mauri Italy S.p.A., Casteggio, Italy) containing *S. cerevisiae* cells (concentration of cells > 7 Log CFU/g corresponding to 1% w/w of dough weight). All dough ingredients were manually mixed into 1 L-volume sterile glass beaker by means of a sterile spoon under a flow laminar hood. One hundred grams of each dough were weighted into rectangular stainless steel pans as reported by Alfonzo et al. (2016). The remaining 200 g of each dough were placed into beakers and covered by parafilm. Both dough aliquots of each trial were incubated at 25°C for 2 h. The trials were carried out in duplicate and repeated after two weeks.

The fermentation of the doughs was determined by pH, total titratable acidity (TTA) and development of yeasts. The pH and TTA values misured in terms of mL of NaOH/10 g of dough were measured as reported by Francesca et al. (2019). Yeast numbers expressed as colony forming units (CFU/g) were investigated by plate count as follows: 10 g of each dough were suspended into 90 mL of Ringer's solution (Sigma-Aldrich, Milan, Italy), homogenized by stomacher as reported above and serially diluted. Yeasts were spread-plated onto yeast potato dextrose (YPD) agar (Oxoid, Milan, Italy), incubated aerobically at 25°C for 72 h (Alfonzo et al., 2016). In order to evaluate the dominance of yeasts over other microbial populations, total mesophilic microorganisms (TMM) were also investigated by spread-plating onto plate count agar (PCA; Oxoid, Milan, Italy) and the Petri dishes incubated aerobically at 30°C for 72 h (Alfonzo et al., 2016). The samples were analysed at T_0 (zero time, when yeast inoculum occurred) and after 2 h.

2.4. Baking process and analyses of breads

The baking of the leavened doughs was carried out using the Air-o-steam (Electrolux, Pordenone, Italy) industrial oven by applying a 3-step cooking program consisting of 1 min at 190°C, 8 min at 180°C with 70% relative humidity (RH) and 10 min at 185°C with 20% RH. This cooking

187 procedure was repeated for each replicate for two independent productions carried out in two
188 consecutive weeks.
189 At the end of baking, the breads were left cool at ambient temperature for 30 min and subjected to
190 the evaluation of quality parameters, including weight loss, bread height, firmness and colour of
191 crust and crumb. Weight loss was calculated as weight difference of the bread before and after
192 baking using the analytical balance GP1200-G (Sartorius Lab Instruments GmbH & Co. KG,
193 Goettingen, Germany). Bread height was determined through digital precision caliper 841-2518 (RS
194 Components S.r.l., Sesto San Giovanni, Italy) (Schober, Messerschmidt, Bean, Park, & Arendt,
195 2005). Colour was measured according to the method described by Settanni et al. (2013) by means
196 of a colorimeter (Chroma Meter CR-400C, Minolta, Osaka, Japan). Crumb firmness was
197 determined as reported by Corsetti et al. (2000) by means of the Instron-5564 (Instron Corp.,
198 Canton, MA). Single slices of 25mm in thickness were placed under a 38.1 mm diameter cylindrical
199 probe and bread was compressed to 40% of the original height.
200 Bread image analysis included calculation of void fraction, cell density and mean cell area, as
201 reported by Settanni et al. (2013).
202 The volatile organic compounds (VOCs) emitted by each sample consisting of bread crust and
203 crumb were analysed applying the solid phase micro-extraction (SPME) isolation technique as
204 described by Corona et al. (2016) and the identification of the compounds occurred as described by
205 Settanni et al. (2013).
206 All determinations on breads were performed in triplicate.
207
208 *2.5. Sensory evaluation of breads*
209 All breads were analysed for their sensory traits. A descriptive panel of 11 tasters composed of six
210 women and five men in the age range 26-60 years old were specifically trained for bread attribute
211 evaluation. The panellists were asked to judge 23 descriptors including crust colour, crust thickness,

crumb colour, porosity, alveolation, alveolation uniformity, odour intensity, bread odour, yeast odour, sourdough odour, unpleasant odour, aroma intensity, bread aroma, yeast aroma, sourdough aroma, unpleasant aroma, salty, acid, bitter, taste persistency, adhesiveness in mouth, crispness and the overall assessment (Alfonzo et al., 2016). The analysis was performed following the guidelines of the ISO 13299 (2003). The judges scored the level of each attribute with a mark on a 6-point scale (0 = extremely low; 5 = extremely high).

2.6. Statistical and explorative multivariate analyses

ANOVA test was applied to identify significant differences among quality characteristics of grains and semolinas, characteristics of doughs and bread. The post-hoc Tukey's method was applied for pairwise comparison of all data. Statistical significance was attributed to $P < 0.05$.

The results of pH and TTA measurements at 0 h and 2 h were evaluated by t-Student test at 5% of significance level.

Multiple factor analysis (MFA) was performed on the data matrix consisted of 16 rows (trials) × 44 columns (44 variables, including eight quality characteristics of semolinas, 20 sensory analysis, eight VOC and eight characteristics of breads) to explore the correlation between variables and different trials, as well as discrimination among the trials. Data of the 44 variables were transformed by standardized (n-1) before performing MFA analysis. Agglomerative hierarchical cluster analysis (AHCA) was also performed on the same data matrixes MFA to explore the variations and similarities of the trials in relation to the characteristics of semolinas, sensory analysis, VOCs and characteristics of breads.

In order to graphically represent the concentrations of VOCs, a heat map clustered analysis (HMCA), based on hierarchical dendrogram with heat map plot, was employed to represent the individual content values contained in the data matrix as colours. The heat map was generated using ascendant hierarchical clustering based on Ward's method and Euclidian distance at 0.25

interquartile range to show the similarities between VOCs and dough obtained from different wheat genotypes. The relative values of VOC concentrations were depicted by colour intensity from grey (lowest concentration) to brown (highest concentration). Heat map analysis of the volatile levels was performed using the autoscaled data (Gaglio et al., 2017). Statistical data processing and graphic construction were performed with the XLStat software version 2020.3.1 (Addinsoft, New York, USA) for excel.

3. Results and discussion

3.1. Characteristics of grains and semolinas

The quality characteristics of grains are reported in Table 2. The results of 1000-kernel weight showed an average of 46.5 g, but it varied greatly among genotypes, ranging from 38.4 g (Biancuccia) to 65.0 g (Perciasacchi). Similarly, great differences were observed among genotypes for test weight: the lowest and highest values were 73.1 and 84.3 kg hl^{-1} registered for the landrace Aziziah and the modern variety Creso, respectively. Thousand-kernel weight and test weight are positively correlated with semolina yield (Troccoli et al., 2000). Thus, high values are desirable to influence positively market grade and price. To this purpose, the work of De Vita et al. (2010) who analysed a large set of durum wheat genotypes (including landraces, modern varieties and advanced breeding lines), covering more than 100 years of breeding activity, is particularly useful. The Sicilian genotype Perciasacchi was characterised by the highest 1000-kernel weight 65 g. Based on morphological traits, Perciasacchi has been recently classified as *T. turgidum* subsp. *turanicum* rather than *T. turgidum* subsp. *durum* by Ficco et al. (2019) similarly to the Khorasan variety, for which the high kernel weight is widely documented (Grausgruber et al., 2005).

In this work, a great variation was also observed for grain protein content which ranged from 11.4–16.6 g $100g^{-1}$). The average values of ancient genotypes (14.5 g $100g^{-1}$) showed higher values than the modern genotypes (12.4 g $100g^{-1}$). This result might be imputable to the negative relationship

between grain yield, markedly higher in the modern genotypes, and grain protein content (Giambalvo et al., 2010; Ruisi et al., 2015), suggesting that an undesired decline in grain protein content occurred because of successful breeding for higher grain yields (De Vita et al., 2007).

Data regarding the main qualitative characteristics of semolina are reported in Table 3. The protein content of semolinas was on average slightly lower than that of whole grains. Dry gluten ranged from 6.7 g $100g^{-1}$ (Simeto) to 13.6 g $100g^{-1}$ (Scorsonera), with the ancient genotypes showing markedly higher average gluten contents (10.7 g 100 g^{-1}) than the modern genotypes (7.9 g 100 g^{-1}). Gluten index was always higher in the modern (range 84–91) than the ancient genotypes (range 35-69). The quality of gluten of the ancient genotypes was not at the same level of that evaluated for the modern genotypes. This evidence is consistent with the findings of other authors (Troccoli et al., 2000; De Vita et al., 2007) who evidenced how during the second half of the 20^{th} century, Italian breeders focused mainly on selection of varieties with superior grain quality ―in addition, of course, to a higher yield potential― in order to improve pasta quality. On the other hand, the lack of a relationship, even a negative one, between protein content and gluten index has been reported for durum wheat (De Santis et al., 2017). Indeed, according to Giunta et al. (2020), it has to be pointed out that grain quality depends not only on the protein content, but also on the allelic composition of glutenins (elasticity) and gliadins (viscosity) (i.e. the endosperm storage proteins, major components of gluten) and on their ratio, which together largely determine the viscoelastic behaviour of the dough and, hence, its technological performances. Interestingly, in the present study a certain variability was observed for gluten index within the group of the ancient genotypes in the range 35–69, suggesting the possible valorisation of these genotypes by using their semolinas to obtain different types of products (bread, pasta, baked goods, etc.).

Great differences were detected for the yellow index that varied from 11.4 to 27.0 for Realforte rosso and Saragolla, respectively. Ash content ranged from 0.5% to 1.0% for Realforte rosso,

286 Biancuccia and Perciasacchi, respectively; no appreciable differences were observed between
287 ancient and modern genotypes.
288 The alveograph parameters also showed a high variability among the genotypes analysed. The P/L
289 ratio (i.e. the ratio between tenacity and extensibility of the dough) ranged from 0.5 (Tripolino) to
290 2.6 (Simeto). The values of this rheological parameter recorded for the modern genotypes were on
291 average higher than those showed by the ancient genotypes. In particular, the last group displayed a
292 higher internal variability, ranging from 0.5 to 2.3 (Scorsonera). The parameter W, which indicates
293 the strength of the dough, varied significantly among the genotypes analysed, with values ranging
294 from 45×10^{-4} J (Russello) to 250×10^{-4} J (Creso). Again, the ancient genotypes showed on average
295 significantly lower values of W than the modern ones (97×10^{-4} J *vs* 234×10^{-4} J). The P/L ratio and
296 the W index both exhibited a wide variability, being on average higher in the modern genotypes
297 than in the ancient ones (1.9 *vs* 1.2 for P/L and 234 *vs* 97 for W, respectively). This is the result of
298 the breeding aimed to select varieties that best meet the quality requirements of pasta industry (i.e. a
299 tenacious and inelastic gluten, suitable for the pasta making technologies commonly adopted on an
300 industrial scale). On the other hand, more balanced P/L ratios and the lower W values of the ancient
301 genotypes, would suggest their preferential use for baking, since their gluten is not excessively
302 tenacious or strong (high strength has indeed a tendency to tenacious gluten and imparts reduced
303 extensibility of the dough) (Edwards et al., 2007), favouring dough workability and a greater
304 swelling during the leavening phase.
305
306 *3.2. Fermentation process*
307 Dough leavening was followed by the evolution of the acidification parameters and yeast cell
308 densities (Table 4). The initial pH of all doughs produced from semolinas of ancient landraces were
309 between 6.0 and 6.1, while, with the exception of T11 (Iride) that displayed a pH of 6.2, almost all
310 doughs prepared from semolinas of modern genotypes were in the range 5.8 – 5.9. At the end of

311 fermentation, pH slightly decreased for all doughs and the highest drop (0.5 pH) was registered for
312 the trial T4 carried out with Realforte rosso. A significant pH decrease was observed in almost all
313 trials after 2 h except for T3 (Biancuccia), T6 (Scorsonera), T9 (Bidì) and T14 (Saragolla). A
314 significant increase in TTA values was observed after 2 h for all trials except T4 (Realforte rosso).
315 Even though pH and TTA were inversely correlated, the increase of TTA was not proportional to
316 the pH drop at the same extent for all trials; e.g. the highest TTA increase (4.75 ml NaOH 0.1 N)
317 was registered for the trial T10 (Senatore Cappelli) whose pH decrease was barely 0.2, on the
318 contrary, trial T4 (Realforte rosso) which showed the highest pH drop (0.5) displayed only 0.5 ml
319 NaOH 0.1 N of TTA increase. Moreover, pH and TTA values at 0 h were statistically different
320 between ancient and modern genotypes, while at 2 h of fermentation no statistically significant
321 differences were observed. The values of pH were different from those commonly found in yeasted
322 doughs from soft wheat flour which generally ranged from 5.3-5.7 (Gaglio et al., 2019; Liguori et
323 al., 2020). In particular, the final pH and TTA (4.3-6.3 mL of 0.1 N NaOH/10 g of dough) values
324 were higher and this finding could be imputable to the different particle size distribution between *T.*
325 *aestivum* and *T. turgidum* L. ssp. *durum* wheat (Stoddard, 1999). The different texture of the
326 endosperm of soft and durum cultivars affects consistently their milling and the resulting products,
327 flour and semolina, respectively, in terms of particles obtained (Pauly, Pareyt, Fierens, Delcour,
328 2013). As matter of fact, flour is finer than semolina (Posner, 2000), thus, the differences in particle
329 size between the two products indicate a different contact surface for the fermenting
330 microorganisms with a consequent less utilization of carbohydrates and a final pH of semolina
331 doughs higher than those registered for flour doughs. Similar behaviours were observed when the
332 fermentation was operated by lactic acid bacteria rather than yeasts (Gaglio et al., 2018; Francesca
333 et al., 2019). The decrease of pH was correlated to the increase of TTA in all doughs. However,
334 when the TTA levels registered for semolina trials are compared to those displayed by soft wheat
335 flour (Gaglio et al., 2019; Liguori et al., 2020) they are unexpectedly higher even though pH values

in semolina doughs were higher. Gaglio et al. (2019) explained this observation with the higher buffering capacity of semolina rather than flour due to the higher protein content. In fact, soft wheat cultivars have been bred to yield flour containing less protein (about 8 to 11%) than durum wheats (up to 14% protein) (Delcour et al., 2012).

Regarding the microbial levels, at the starting time as well as after 2 h of fermentation, the number of colonies detected on YPDA were higher than those found on PCA, because the last medium is not specific for yeast growth. Cell densities increased on both media during leavening, although the increase was quite limited due to the high levels of yeast inoculums (7.5 – 8.2 Log CFU/g). The highest increase of yeast numbers were displayed by the trials T7 (Perciasacchi) and T8 (Aziziah). No statistically significant differences between ancient and modern genotypes were found on both PCA and YPDA after 2 h of fermentation. Yeast cell densities were comparable to those reported for flour doughs (Gaglio et al., 2019; Liguori et al., 2020), indicating that all semolinas allowed the development of the fermenting agents and determined a standard biological leavening.

3.3. Evaluation of bread characteristics

After baking, the breads were evaluated for several parameters to investigate on the suitability of ancient and modern genotypes of durum wheat cultivated in Sicily not only for pasta production (Subira et al., 2014), but also for bread making. The results are shown in Table 5. Significant differences were found for the weight loss of the breads among the 16 trials followed. The highest weight loss values were observed in T11 (Iride) with 83.9 g, while the lowest weight loss was recorded in T4 (Realforte rosso) with 87.1 g. The weight loss of all other trials were intermediate to T4 and T11. On average, the breads released 14 g of water during baking. On the contrary, the height of the breads varied significantly among the trials with values in the range 2.5 and 3.5 cm. Surprisingly, the lowest height (2.5 cm) was recorded for the trials CTR and T12 (Creso), basically both carried out with modern genotypes, even though the commercial semolina included 30%

361 ancient landraces, while the highest increase in bread height was shown by the trial T5 (Tripolino).
362 This parameter is strictly related to the rheological characteristics of doughs, in particular to P/L and
363 W index. Indeed, low P/L values indicate a high extensibility of the doughs which together with low
364 W alveograph indexes determine the production of breads characterised by a consistent volume [the
365 height of the breads is linearly and directly proportional to volume (Corona et al., 2016)], soft and
366 spongy crumb that represent the desirable quality attributes in bread (Ponzio, Ferrero, & Puppo,
367 2013). According to Pasqualone et al. (2004), for bread making purposes, the P/L ratio should not
368 exceed 2, with the optimum value in the range 0.4 - 0.8. In the present study, the P/L ratios were
369 always below this threshold for the ancient genotypes (with the exception of Scorsonera), falling in
370 some cases into the optimal range. On the other hand, the modern genotypes, by presenting higher
371 P/L ratios, showed a lower suitability for bread making purposes. With this regard, the breads from
372 the ancient landrace Tripolino, characterised by a low P/L ratio (0.5) and a low strength (W =
373 68×10^{-4} J), reached a final height much higher than that registered for the modern genotype Creso
374 that showed a high tenacity (a parameter opposite to extensibility) and strength (González-Torralba,
375 Arazuri, Jarén, & Arregui, 2013). Low width/height ratio is well appreciated and indicates a certain
376 bread quality, since a higher width/height ratio suggests more spread and flat pieces (Ponzio et al.,
377 2013). In our study, all breads were baked into stainless steel pans of the dimensions indicated by
378 the AACC, thus, the height of the breads provided a direct indication of the bread making
379 performances of the different semolinas analysed.
380 The firmness ranged between 12.6 and 31.3 N with the lowest levels found for the trial T8 (Aziziah)
381 and the highest for T3 (Biancuccia). In particular, the firmness of the breads of the trials T5
382 (Tripolino), T7 (Perciasacchi), T8 (Aziziah) and T10 (Senatore Cappelli) was comparable to that
383 observed for the trials carried out with the modern genotypes and with CTR trial. The firmness of
384 the breads is indirectly correlated with their height (Chin, Tan, Yusof, & Rahman, 2009).
385 Comparing our data with those of works carried out on flour breads, firmness values of all semolina

breads were characterised by higher values (Gaglio et al., 2019, 2020b; Liguori et al., 2020). In particular, firmness values of CTR were superimposable to those registered with other yeasted breads processed from commercial semolina (Alfonzo et al., 2020). Texture analysis revealed also that firmness of the final breads produced in study was highly variable and that the majority of breads obtained from semolinas of the ancient genotypes were characterised by a higher firmness than those obtained from semolinas of the modern genotypes. However, the firmness of the breads produced with Aziziah, Vertola and Saragolla semolina (trials T8, T13 and T14) was different from those obtained with Biancuccia (T3), while all other trials, including commercial semolina (CTR), showed similar values. Similarly, to height, also firmness was related to the rheological properties of doughs; in particular, bread firmness was directly proportional to dough tenacity. In general, semolinas with high W values generated firmer breads with a low level of spongy crumb.

With regard to the colour parameters, significant differences were found for all trials for all three values (L*, a* and b*) in both crust and crumb. T14 (Saragolla) recorded the highest values of b* (29.4 in the crust and 41.0 in the crumb), while T6 (Scorsonera), recorded the lowest L* value (45.3) in the crust and registered the highest (67.9) in the crumb. This situation was also observed in the crust where T6 showed the highest value for a* (17.2). The lowest values for b* was instead observed for T4 (Realforte rosso; 16.2 in the crumb) and T6 (Scorsonera, 31.4 in the crust). In particular, the values b* of crumb were linearly correlated (r=0.89) with the value of yellow index of semolinas and were higher in the modern than the ancient genotypes. An opposite trend was registered for the parameter a* of the crust which was higher for the trials carried out with semolinas from ancient genotypes. Colour of breads, distinct per crust and crumb, undoubtedly indicated that yellowness of crumb was higher for the breads processed from modern genotypes, while redness of crust was higher for the trials carried out with ancient landrace semolinas. These results were quite expected, since the increase of crumb colour intensity was one of the objectives of the breeding programs on durum wheat grains (Clarke et al., 1998), because colour is highly

411 appreciated by consumers (Boukid et al., 2020) and, consequently, requested by the transformation
412 industry. Regarding the increase of crumb yellowness of breads in comparison to semolinas (almost
413 15%), it has to be linked to the better reflection of the incident light of crumb (Kruger and Reed,
414 1988) and also to Maillard reaction that enhances this parameter, even though the influence of
415 Maillard reaction and caramelization of sugars on colour formation are more typical of the crust
416 (Purlis, 2010). A higher degree of redness in ancient *vs* modern genotypes has been also registered
417 within *Triticum aestivum* L. ssp. *aestivum* (Boukid et al., 2020). However, the presence of high
418 yellow index values in Saragolla (T14) could be attributed to the high concentrations of carotenoids
419 that influence the colour of the flour and, therefore, also the colour of the final breads (Henteschel
420 et al., 2002). However, some authors claim that the colour of flour is determined not only by the
421 carotenoid content, but also by the size of the flour particles (Hildago, Fongonaro & Brandolini,
422 2014).
423 Image analysis (Fig. 1) revealed significant differences among the trials for all three parameters
424 considered (void fraction, cell density and mean cell area). The highest void fraction values were
425 registered for the trial CTR (58.1 %) and significant differences were observed in all other breads.
426 The lowest values were obtained in T6 (Scorsonera) and T13 (Vertola; 40.2%). Regarding cell
427 density, the values were between 34.4 (T8, Aziziah) and 59.7 (T3, Biancuccia), with statistically
428 significant differences between the different trials. Regarding mean cell area, the highest values
429 were observed in CTR and T12 (Creso; 0.6 mm^2), in all other trials this parameter was in the range
430 0.3-0.5 mm^2. All breads obtained from ancient landraces showed more numerous alveoli than
431 modern genotype breads. The same analysis was used to differentiate the yeasted breads processed
432 from ancient genotypes, including Senatore Cappelli, Russello and Timilia, and modern genotypes,
433 including Iride and Simeto, by Gallo et al. (2010). Those authors reported that the morphological
434 parameters were significantly different between the two groups but did not provide single data for a

deep comparison. A high variability among ancient genotypes of soft wheat in terms of number of pores and their dimensions was also registered by Boukid et al. (2020).

The breads produced with semolinas from ancient and modern genotypes of durum wheat analysed in this study emitted a total of 49 VOCs, including 15 esters, 13 alcohols, 7 aldehydes, 6 acids, 4 aromatic hydrocarbons, 2 ketones, 1 lactone and phenol (Fig. 2). The compounds found at the highest levels in all breads were toluene (41.08 – 62.77 %) among aromatic hydrocarbons and phenylethyl alcohol (4.21 – 23.35 %) and 3-methyl-1-butanol (8.59 – 16.22 %) among alcohols. The heat map clearly showed a high degree of variability among the breads which is directly imputable to the genotypes used for semolina production. To this purpose, VOC analysis allowed to group the breads based on wheat genotypes into five main clusters (group 1: T1, T2, T3 and CTR; group 2: T4, T6 and T7; group 3: T8, T11 and T12; group 4: T5, T13 and T15; group 5: T9, T10 and T14). Within ancient landraces, the highest similarity was observed among Realforte rosso, Scorsonera and Perciasacchi, but also among Timilia, Russello and Biancuccia the level of similarity was consistent. Regarding the modern genotypes, Vertola, Simeto and Saragolla clustered together, while Iride and Creso were grouped with the old landrace Aziziah. The breads obtained with the commercial semolina clustered together with those from semolinas of the ancient genotypes Tripolino, Senatore Cappelli and Bidì with the last two landraces very closed to each other. The differences found among the breads depend on the wheat genotypes (Vita et al., 2016). In order to differentiate ancient landraces and modern genotypes, Vita et al. (2016) determined the profiles of VOCs of wholemeal semolinas. The authors detected a total of 32 VOCs. However, other authors evidence some differences in VOCs from wholemeal and refined semolinas (Ficco et al., 2017). From the direct comparison of the VOCs from wholemeal semolinas (Vita et al., 2016) with the VOCs emitted from the breads produced in this study, it is clear that some compounds, including toluene and other minor aromatic hydrocarbons such as styrene, and phenylethyl alcohol originate from the raw materials, while 3-methyl-1-butanol was generated during leavening,

because it is known as “fermented” flavour in breads (Salim-ur-Rehman, Paterson, & Piggott, 2006). Due to their volatility, some VOCs detected in semolinas are no more found in breads, because of the baking process, but in general the higher number of VOCs found in breads is undoubtedly due to the fermentation process. To this purpose, it has to be noticed that when the same raw materials were processed by sourdough fermentation rather than baker’s yeast a lower number of VOCs was detected (Alfonzo et al., 2016). Raimondi et al. (2017) reported that the addition of bakers' yeast during the processing of the sweet leavened baked product “Colomba” increased the concentration of aldehydes, ketones and alcohols and decreased that of acids and esters. Ficco et al. (2017) confirmed these findings for semolina breads produced from ancient landraces and modern genotypes showing that the commercial brewing yeast (corresponding to baker’s yeast) generated more alcohols and aldehydes than sourdough. In general, the leavening agent exerts a greater impact than the type of wheat flour on the profile of bread VOCs (Makhoul et al., 2015) and a similar result should be expected for semolina breads.

3.4. Sensory characteristics of breads

When testing the suitability of raw materials for bread production, a sensory evaluation is of paramount importance. For this reason, all breads were analysed for their main attributes by a group of judges. Fig. 3 shows the results of the sensory evaluation of the breads. Statistical significance differences were observed for all bread attributes judged except for the descriptors “unpleasant aroma”, “unpleasant odour”, “sourdough aroma” and “sourdough odour”. A high variability among the 10 ancient genotypes was observed for crust colour (0.8 – 3.1) with Scorsonera being the darkest, while all modern genotypes resulted quite similar (1.0 – 1.6). A similar trend was observed also for the other parameters, for which the breads obtained from the ancient genotypes were scored differently while those from the modern genotypes were highly similar. In general, the control breads received scores close to those of the modern genotypes for the different attributes.

485 Sourdough odour and aroma, as well as unpleasant odour and aroma were not perceived by the
486 majority of judges. Bitter, salty and acid sensations were at very low levels in all breads. Regarding
487 the overall assessment, that is a general evaluation based on the scores of the other attributes
488 (Gaglio et al., 2019), the panellists gave a quite homogenous judgment within modern genotypes
489 from 2.1 (Iride and Creso) and 2.3 (Vertola and Saragolla), while their scores varied consistently
490 among the ancient genotypes from Bidì (1.6) and Scorsonera (2.7) which resulted to be the most
491 appreciated breads. The final scores were quite different among ancient genotype trials, while a
492 similar appreciation was obtained by the modern genotypes and control breads. Also, Raffo et al.
493 (2003) reported that the sensory profile of breads was scarcely affected by the modern genotypes of
494 durum wheat. The results of the present study almost confirmed the sensory evaluation reported by
495 Alfonzo et al. (2016) who used the same 15 durum wheat genotypes to produce sourdough breads.
496 In both works the most appreciated breads were those processed from the old landrace Scorsonera
497 semolina. Thus, sensory analysis showed that the semolinas analysed in this study show a similar
498 aptitude to bread making independently on the biological leavening agent (baker's yeast and
499 sourdough starter) used.
500
501 *3.5. Discrimination of trials based on their quality characteristics of semolinas and breads,VOC*
502 *and sensory analysis*
503 The quality characteristics of semolinas, sensory analysis, VOCs and characteristics of breads were
504 determined, and their correlations were explored by MFA. This analysis led to the identification of
505 four Factors with eigen-values higher than 1, indicating that the total number of variables (44) for
506 the 16 trials could be grouped into only four factors which explained 63.67% of the total variance.
507 The association between the variables and the MFA factor is indicated by the contribution and cos^2
508 value. The firmness, cell density, dry gluten, gluten index, protein content, W, and alcohols were
509 associated to F1. Overall assessment, void fraction, taste persistence and crust colour were

associated to F2. Crispness, weight loss, bitter and height were associated to F3. The variables acid mean cell area, yeast (aroma), ash, esters and P/L were associated to F4. As shown in Fig. 4a and b, the two-dimension model of MFA of variables explained 40% of the total variance, with F1 and F2 accounting for 24.65 and 15.35%, respectively. The variables loading plot of MFA (Fig. 4a) showed that 16 variables were located in the first quadrant, six in the second quadrant, nine in the third quadrant and 13 in the fourth quadrant. Fig. 4b shows that the trials were grouped into three clusters. However, both MFA observation plot (Fig. 4b) and AHC dendrogram (Fig. 4c) showed that the CTR grouped with trials T1-T5 (Timilia, Russello, Biancuccia, Realforte rosso and Tripolino), T7 (Perciasacchi), T9 (Bidì) and T10 (Senatore Cappelli). Interestingly, the trial T6 (Scorsonera) did not cluster with the most representative group of ancient genotypes. In addition, modern genotypes T11-T14 (Iride, Creso, Vertola, Saragolla and Simeto) represented a different cluster and trial T8 (Aziziah; ancient genotype) merged into this group.

4. Conclusions

This study revealed that the modern durum wheat genotypes showed, in general, a certain uniformity of behaviour, giving rise to rather homogeneous semolinas. In contrast, ancient wheat genotypes exhibited a large variability for several traits. The production of experimental breads made it possible to evaluate the baking attitude of the two groups (ancient and modern) genotypes of Sicilian durum wheat. Like first transformation (production of semolina), the modern genotypes showed a great homogeneity of the second transformation products (breads). Some differences have been found among the ancient genotypes, but all them showed baking attitudes. After regular fermentation and baking, the experimental breads showed different characteristics in relation to the semolina. On the whole, the breads obtained with semolina from ancient genotypes showed a more pleasant appearance, with a more attractive crust colour. The joint analysis of the sensory data, the different aromatic profiles and the characteristics of the processed product revealed a certain

uniformity among modern genotypes, while the ancient genotypes were highly diversified. This great diversity existing among the ancient Sicilian durum wheat landraces in terms of quality and sensory parameters (as well as agronomic parameters) certainly represents a heritage to be conserved, preserved and enhanced.

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Table 1. Wheat genotypes.

Trials	Genotypes	Year of release	Group	Plant stature	Heading time	Pedigree[a]
T1	Timilia	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T2	Russello	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T3	Biancuccia	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T4	Realforte rosso	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T5	Tripolino	1920–1925	Ancient	Mid-Tall	Mid-Early	North-African selection from Palestinian landraces
T6	Scorsonera	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T7	Perciasacchi	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T8	Aziziah	1920–1925	Ancient	Mid-Tall	Mid-Early	North-African selection from Palestinian landraces
T9	Bidì	–	Ancient	Tall	Late	Indigenous landrace from Sicily
T10	Senatore Cappelli	1915	Ancient	Tall	Late	Italian selection from the North-African landrace Jean Rhetifah
T11	Iride	1996	Modern	Short	Early	Altar 84/Ares sib
T12	Creso	1974	Modern	Short	Late	Cpb 144/[(Yt54-N10-B) Cp 63 Tc]
T13	Vertola	2003	Modern	Short	Early	Italian selection from a North American hybrid population
T14	Saragolla	2004	Modern	Short	Early	Iride/Line PSB 0114
T15	Simeto	1988	Modern	Short	Early	Capeiti8/Valnova

[a]The majority of information on pedigree has been obtained from GRIS database (http://wheatpedigree.net/; Accessed 05.06.2020).

Table 2. Main quality characteristics of grains.

Trials	1000-kernel weight (g)	Test weight (kg hl^{-1})	Protein content (g $100g^{-1}$)
Ancient			
T1	41.2±0.4^{bc}	78.8±0.4^{c}	15.1±0.4^{g}
T2	44.2±0.3^{d}	80.3±0.5^{de}	14.5±0.4f
T3	38.4±0.4^{a}	80.7±0.1^{ef}	16.6±0.2i
T4	40.4±0.2^{b}	80.1±0.5^{d}	13.7±0.1^{c}
T5	42.4±0.1^{c}	81.0±0.5^{fg}	12.9±0.3^{c}
T6	48.2±0.4^{e}	80.6±1.0^{def}	16.3±0.1^{h}
T7	65.0±0.6^{h}	77.7±1.2^{b}	14.4±0.2^{f}
T8	41.6±0.5^{bc}	73.1±0.4^{a}	12.7±0.4^{c}
T9	49.2±0.5^{e}	82.0±1.5^{hi}	13.8±0.2^{c}
T10	54.0±0.4^{g}	82.0±1.2^{hi}	14.5±0.3^{f}
Mean ± SD	46.5±8.1	79.6±2.65	14.5±1.3
Modern			
T11	41.8±0.2^{bc}	79.2±1.0^{c}	11.9±0.3^{b}
T12	49.6±0.4^{ef}	84.3±0.3^{j}	13.6±0.4^{c}
T13	49.6±0.5^{ef}	81.7±0.5^{h}	13.3±0.1^{d}
T14	41.6±0.4^{bc}	82.3±1.0^{i}	11.7±0.1^{b}
T15	50.8±0.5^{f}	81.5±0.6^{gh}	11.4±0.1^{a}
Mean ± SD	46.7±4.57	81.8±1.83	12.4±1.0
Mean ± SD	46.5±6.92	80.3±2.56	13.8±1.5
Statistical significance	**	*	**
Ancient vs. Modern	N.S.	**	***

For each genotype, values are the mean ± standard deviation (SD) of three replicates.
Abbreviations: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; N.S., not significant.
Data within a column followed by the same letter are not significantly different according to Tukey's test.

Table 3. Main quality characteristics of semolinas.

Trials	Protein content† (g 100g^{-1})	Dry gluten† (g 100g^{-1})	Gluten index	Yellow index (b*)	Ash (%)	P/L	W (10^{-4} J)	G (mm)
Ancient								
T1	14.4±0.3^{g}	12.7±1.0^{i}	60.0±3.1^{e}	13.9±0.3^{b}	0.8±0.0^{c}	1.0±0.0^{abc}	111.0±6.4^{cde}	19.0±1.7^{ij}
T2	13.4±0.4^{f}	12.1±0.4^{h}	35.0±0.6^{a}	21.3±0.3^{e}	0.7±0.0^{b}	1.3±0.2^{bcde}	45.0±1.5^{a}	13.0±0.8^{b}
T3	15.5±0.2^{h}	12.4±1.1^{hi}	59.0±3.4^{de}	19.4±0.4^{c}	1.0±0.0^{e}	1.0±0.4^{abc}	136.0±8.3^{f}	19.1±1.6^{ij}
T4	13.2±0.4^{ef}	11.4±0.9^{g}	48.0±1.3^{c}	11.4±0.3^{a}	0.5±0.1^{a}	1.6±0.4^{cdef}	92.0±9.6^{bc}	15.4±2.6^{d}
T5	11.2±0.2^{b}	7.9±0.4^{b}	69.0±4.4^{f}	21.4±0.5^{e}	0.8±0.0^{c}	0.5±0.2^{a}	68.0±2.9^{ab}	19.1±2.1^{ij}
T6	15.7±0.3^{h}	13.6±1.3^{j}	39.0±1.4^{b}	24.4±0.4^{h}	0.9±0.0^{d}	2.3±0.3^{gf}	61.0±9.7^{a}	11.3±0.5^{a}
T7	13.2±0.2^{ef}	9.7±0.8^{de}	59.0±1.9^{de}	26.5±0.7^{i}	1.0±0.0^{e}	1.1±0.1^{abc}	110.0±6.1^{cde}	17.7±0.9^{gh}
T8	11.2±0.5^{b}	7.6±0.7^{b}	67.0±0.9^{f}	20.8±0.2^{de}	0.8±0.0^{c}	0.7±0.1^{ab}	97.0±5.3^{cd}	19.5±1.1^{j}
T9	12.9±0.4^{de}	9.4±1.1^{ce}	56.0±0.6^{d}	21.6±0.8^{e}	0.9±0.0^{d}	1.4±0.2^{bcde}	120.0±4.6^{def}	17.0±0.9^{f}
T10	13.3±0.2^{f}	10.6±0.9^{f}	61.0±9.4^{e}	23.3±0.5^{fg}	0.8±0.0^{c}	1.1±0.1^{abc}	127.0±3.4^{ef}	18.9±1.0^{i}
Mean ± SD	13.4±1.5	10.7±2.1	55.0±11.2	20.4±4.6	0.8±0.1	1.2±0.5	97.0±30.1	17.0±2.9
Modern								
T11	11.4±0.2^{b}	7.8±0.8^{b}	84.0±3.8^{g}	22.7±0.5^{f}	0.8±0.0^{c}	1.7±0.2^{cdef}	230.0±7.3^{gh}	18.2±0.8^{h}
T12	12.2±0.2^{c}	9.0±1.4^{c}	88.0±2.5^{h}	20.4±0.4^{d}	0.9±0.1^{d}	2.0±0.2^{efg}	250.0±12.8^{h}	18.8±1.0^{i}
T13	12.6±0.4^{d}	9.3±1.0^{cd}	91.0±1.4^{h}	23.7±0.9^{gh}	0.8±0.0^{c}	1.2±0.1^{abcd}	240.0±7.2^{h}	21.3±0.7^{k}
T14	11.2±0.1^{b}	6.7±0.5^{a}	91.0±2.2^{h}	27.0±0.9^{i}	0.7±0.0^{b}	1.9±0.3^{defg}	244.0±10.9^{h}	17.6±1.2^{g}
T15	10.8±0.3^{a}	7.0±0.8^{a}	90.0±0.7^{h}	24.4±0.7^{h}	0.7±0.0^{b}	2.6±0.4^{g}	207.0±10.4^{g}	14.7±1.8^{c}
Mean ± SD	11.7±0.7	7.9±1.5	89.0±2.9	23.6±2.4	0.8±0.1	1.9±0.5	234.0±16.8	18.1±2.4
CTR	12.9±0.4^{de}	9.8±0.1^{e}	89.0±0.6^{h}	18.7±0.1^{c}	0.5±0.0^{a}	2.5±0.0^{g}	226.0^{gh} ± 2.9	16.2±0.9^{e}
Mean ± SD	12.8±1.5	9.8±2.2	68.0±19.0	21.3±4.1	0.8±0.1	1.5±0.6	148.0 ± 72.4	17.3±2.6
Statistical significance	*	***	***	***	*	***	***	**
Ancient vs. Modern	***	***	***	*	N.S.	***	***	N.S.

† Expressed on dry matter basis.

For each genotype, values are the mean±standard deviation (SD) of three replicates.

P/L, W, and G are the parameters obtained from the Alveograph test: P/L, tenacity/extensibility ratio; W, strength; G, swelling.

CTR, control trial.

***, P < 0.001; **, P < 0.01; *, P < 0.05; N.S., not significant.

Data within a column followed by the same letter are not significantly different according to Tukey's test.

Table 4. Characteristics of doughs.

Trials	pH		*t*-test (α=0.05)	TTA (mL of 0.1 N NaOH/10 g of dough)		*t*-test (α=0.05)	Microbiological counts (Log CFU/g)			
	0 h	2h	*p*-value	0 h	2h	*p*-value	PCA (0 h)	YPDA (0 h)	PCA (2 h)	YPDA (2 h)
Ancient										
T1	6.0±0.0^{bc}	5.8±0.0^{bc}	0.0160	3.5±0.1^{cd}	5.5±0.9^{bcde}	0.0158	6.7±0.8^{a}	7.6±0.3^{ab}	7.8±0.0^{a}	7.9±0.1^{a}
T2	6.1±0.0^{c}	5.8±0.1^{bc}	0.0058	3.8±0.6^{d}	6.2±0.2^{e}	0.0026	6.9±0.2^{a}	7.6±0.1^{ab}	7.9±0.0^{a}	8.0±0.1^{a}
T3	6.1±0.0^{c}	6.0±0.0^{d}	0.2451	2.7±0.1^{c}	4.4±0.2^{ab}	0.0002	6.6±0.0^{a}	7.5±0.1^{a}	7.9±0.1^{a}	8.0±0.2^{a}
T4	6.1±0.0^{c}	5.6±0.0^{a}	0.0000	3.8±0.9^{d}	4.3±0.1^{a}	0.3716	7.0±0.6^{a}	7.6±0.2^{ab}	7.8±0.3^{a}	7.9±0.0^{a}
T5	6.1±0.1^{c}	5.7±0.1^{ab}	0.0009	3.1±0.2^{cd}	4.6±0.2^{abc}	0.0009	7.2±0.0^{a}	7.7±0.1^{ab}	8.1±0.1^{a}	8.2±0.4^{a}
T6	6.1±0.0^{c}	5.9±0.1^{cd}	0.1004	3.7±0.1^{d}	4.9±0.1^{abcd}	0.0000	7.5±0.1^{a}	7.5±0.0^{a}	7.6±0.6^{a}	7.8±0.3^{a}
T7	6.1±0.1^{c}	5.8±0.1^{bc}	0.0363	1.8±0.1^{b}	5.8±0.2^{de}	0.0000	7.5±0.1^{a}	7.7±0.5^{ab}	7.6±0.7^{a}	8.4±0.7^{a}
T8	6.0±0.0^{bc}	5.9±0.0^{cd}	0.0073	1.8±0.1^{b}	5.7±0.2^{cde}	0.0000	6.9±0.5^{a}	8.1±0.2^{ab}	7.2±1.0^{a}	8.3±0.2^{a}
T9	6.0±0.2^{bc}	5.8±0.0^{bc}	0.1778	1.5±0.1^{ab}	5.7±0.0^{cde}	0.0009	7.7±0.1^{a}	8.0±0.2^{ab}	7.8±0.3^{a}	8.1±0.1^{a}
T10	6.0±0.0^{bc}	5.8±0.0^{bc}	0.0000	1.5±0.1^{ab}	6.3±0.9^{e}	0.0000	7.7±0.1^{a}	8.0±0.1^{ab}	8.0±0.1^{a}	8.5±0.4^{a}
Mean±SD	6.1±0.1	5.8±0.1		2.7±1.0	5.3±0.7		7.2±0.4	7.7±0.2	7.8±0.3	8.1±0.2
Modern										
T11	6.1±0.0^{c}	5.8±0.0^{bc}	0.0001	1.4±0.3^{ab}	5.8±0.1^{de}	0.0001	6.9±0.5^{a}	8.1±0.1^{ab}	8.2±0.3^{a}	8.4±0.0^{a}
T12	5.8±0.0^{a}	5.7±0.0^{ab}	0.0007	0.9±0.1^{a}	4.5±0.4^{ab}	0.0001	7.7±0.2^{a}	8.0±0.2^{ab}	7.8±0.0^{a}	8.2±0.4^{a}
T13	5.8±0.0^{a}	5.7±0.0^{ab}	0.0055	1.3±0.1^{ab}	4.4±0.1^{ab}	0.0000	6.8±0.3^{a}	8.2±0.1^{b}	8.2±0.1^{a}	8.5±0.0^{a}
T14	5.8±0.0^{a}	5.7±0.0^{ab}	0.2761	1.3±0.1^{ab}	4.8±0.2^{abcd}	0.0000	6.9±0.5^{a}	8.2±0.0^{b}	8.2±0.1^{a}	8.3±0.1^{a}
T15	5.9±0.0^{ab}	5.7±0.0^{ab}	0.0008	1.4±0.1^{ab}	4.7±0.1^{abcd}	0.0000	7.1±0.9^{a}	7.9±0.3^{ab}	7.7±0.1^{a}	8.1±0.0^{a}
Mean±SD	5.9±0.1	5.7±0.0		1.3±0.2	4.8±0.6		7.1±0.4	8.1±0.1	8.0±0.2	8.3±0.2
CTR	5.8±0.0^{a}	5.7±0.0^{ab}	0.0001	1.2±0.1^{ab}	5.3±0.4^{abcde}	0.0000	7.0±0.2^{a}	7.9±0.4^{ab}	7.9±0.4^{a}	8.0±0.3^{a}
Mean±SD	6.0±0.1	5.8±0.1		2.2±1.1	5.2±0.7		7.1±0.4	7.9±0.2	7.9±0.3	8.1±0.3
Statistical significance	***	***		***	***		N.S.	**	N.S.	N.S.
Ancient vs. Modern	**	N.S.		*	N.S.		N.S.	**	N.S.	N.S.

Results indicate mean values ± SD of four plate counts (carried out in duplicate for two independent productions).

Abbreviations: CTR, control trial; TTA, titratable acitidy; PCA, plate count agar for mesophilic microorganisms; YPDA, yeast peptone dextrose agar for yeast; P value: P value: ***, $P < 0.001$; **, $P < 0.01$; N.S., not significant.
Data within a column followed by the same letter are not significantly different according to Tukey's test.

Table 5. Characteristics of breads.

Trials	Weight loss (g)	Height (cm)	Firmness (N)	Crumb colour			Crust colour			Imagine analysis		
				L^*	a^*	b^*	L^*	a^*	b^*	Void fraction (%)	Cell density ($n.cm^2$)	mean cell area (mm^2)
Ancient												
T1	86.6±0.1^{ab}	3.2±0.2^{bcd}	23.4±2.3^{ab}	61.6±1.6^{c}	-0.7±0.8^{i}	19.1±0.5^{b}	63.5±4.7^{ef}	8.1±3.7^{e}	34.8±2.4^{c}	54.5±0.2^{c}	51.0±0.2^{g}	0.4±0.0^{ab}
T2	85.5±0.7^{ab}	3.0±0.1^{abcd}	25.9±5.7^{ab}	60.7±3.2^{b}	-1.7±0.3^{g}	22.0±0.5^{d}	58.5±3.7^{c}	12.2±3.7^{i}	37.5±1.0^{ef}	46.1±0.2^{b}	52.3±0.3^{h}	0.4±0.0^{ab}
T3	86.1±0.1^{ab}	3.2±0.1^{bcd}	31.3±9.0^{b}	64.2±1.8^{hij}	-0.7±0.8^{i}	21.7±1.0^{cd}	53.7±5.5^{b}	13.7±4.8^{k}	33.9±1.6^{b}	46.5±0.1^{b}	59.7±0.3^{i}	0.3±0.0^{a}
T4	87.1±0.5^{b}	3.2±0.1^{bcd}	23.4±5.0^{ab}	64.1±1.8^{ghi}	-1.7±0.1^{g}	16.2±0.4^{a}	67.4±3.5^{i}	7.4±2.0^{cd}	35.1±1.1^{c}	56.2±0.2^{d}	51.1±0.1^{g}	0.5±0.1^{bc}
T5	86.7±1.0^{ab}	3.5±0.1^{d}	19.2±3.8^{ab}	63.7±2.3^{ef}	-3.0±0.3^{a}	24.7±0.4^{f}	66.2±4.5^{h}	8.0±3.3^{de}	39.9±0.4^{i}	57.5±0.2^{e}	46.2±0.2^{de}	0.5±0.0^{bc}
T6	85.5±1.6^{ab}	3.0±0.1^{abcd}	26.0±6.2^{ab}	67.9±1.7^{l}	-2.3±0.1^{e}	26.0±1.5^{g}	45.3±4.7^{a}	17.2±2.6^{l}	31.4±5.5^{a}	40.2±0.2^{a}	51.2±0.2^{g}	0.4±0.0^{ab}
T7	86.1±0.8^{ab}	3.0±0.3^{abcd}	20.3±2.3^{ab}	63.8±2.5^{efg}	-2.5±0.3^{d}	27.8±0.8^{h}	58.0±5.0^{c}	13.0±3.6^{j}	38.7±1.7^{h}	57.2±0.1^{e}	46.8±0.2^{e}	0.3±0.1^{a}
T8	87.0±0.4^{ab}	3.4±0.1^{cd}	12.6±0.5^{a}	59.8±2.6^{a}	-2.7±0.1^{c}	24.7±1.3^{f}	67.5±3.6^{i}	6.5±3.3^{b}	38.7±2.2^{h}	46.5±0.1^{b}	34.4±0.3^{a}	0.5±0.1^{bc}
T9	86.0±1.1^{ab}	2.7±0.1^{ab}	21.7±2.8^{ab}	64.0±3.6^{fgh}	-2.1±0.2^{f}	25.2±0.7^{f}	61.1±2.8^{d}	10.2±2.9^{g}	37.5±1.3^{ef}	57.5±0.2^{e}	48.5±0.4^{f}	0.3±0.1^{a}
T10	86.9±0.2^{ab}	2.7±0.1^{ab}	19.3±4.0^{ab}	61.7±1.2^{c}	-2.1±0.2^{f}	23.4±0.7^{e}	60.3±3.5^{d}	9.2±3.1^{f}	35.9±1.1^{d}	57.2±0.1^{e}	46.5±0.3^{e}	0.4±0.0^{ab}
Mean±SD	86.4±0.6	3.1±0.3	22.3±0.5	63.2±2.3	-2.0±0.8	23.1±3.4	60.2±6.9	10.6±3.4	36.3±2.6	51.9±6.4	48.8±6.4	04±0.1
Modern												
T11	83.9±1.2^{a}	2.9±0.1^{abc}	19.8±3.5^{ab}	64.5±1.7^{j}	-2.8±0.2^{bc}	25.2±0.8^{f}	63.0±3.7^{e}	7.2±3.7^{c}	37.5±0.8^{ef}	57.3±0.1^{e}	46.4±0.2^{e}	0.5±0.1^{bc}
T12	84.7±1.8^{ab}	2.5±0.4^{a}	18.5±5.3^{ab}	63.5±2.8^{e}	-1.3±0.5^{h}	21.2±1.5^{c}	61.1±4.4^{d}	10.5±3.7^{gh}	37.1±0.7^{e}	56.2±0.1^{d}	45.5±0.2^{d}	0.6±0.1^{c}
T13	86.3±1.3^{ab}	3.0±0.1^{abcd}	12.8±2.6^{a}	64.4±2.9^{ij}	-2.5±0.2^{d}	24.7±1.0^{f}	64.7±4.4^{g}	8.2±4.8^{e}	38.9±2.4^{h}	40.2±0.2^{a}	34.7±0.2^{a}	0.5±0.1^{bc}
T14	85.6±1.3^{ab}	3.0±0.1^{abcd}	14.9±2.5^{a}	62.1±2.0^{d}	-2.9±0.2^{ab}	29.4±0.7^{i}	66.8±2.7^{hi}	5.2±4.5^{a}	41.0±3.2^{j}	46.1±0.3^{b}	37.6±0.2^{b}	0.5±0.0^{bc}
T15	85.9±1.1^{ab}	2.8±0.1^{ab}	17.7±3.6^{ab}	64.5±1.7^{j}	-1.8±0.1^{g}	27.2±0.6^{h}	64.5±5.2^{fg}	6.9±5.0^{bc}	38.1±1.7^{g}	54.5±0.3^{c}	43.6±0.2^{c}	0.5±0.1^{bc}
Mean±SD	85.3±1.0	2.8±0.2	16.7±2.8	63.8±1.0	-2.3±0.7	25.5±3.1	64.0±2.1	7.6±1.9	38.5±1.5	50.9±7.4	41.6±5.1	0.5±0.0
CTR	85.8±1.1^{ab}	2.5±0.3^{a}	19.4±9.2^{ab}	66.0±3.5^{k}	-1.2±0.3^{h}	26.0±1.4^{g}	61.3±6.6^{d}	10.9±4.4^{h}	37.8±3.9^{fg}	58.1±0.1^{f}	46.5±0.3^{e}	0.6±0.1^{c}
Mean±SD	86.0±0.8	2.8±0.2	20.4±4.9	63.5±2.0	-2.0±0.7	24.0±3.4	61.4±5.7	9.7±3.2	37.1±2.4	52.0±6.5	46.4±6.6	0.5±0.1

Statistical significance	*	***	**	**	**	***	***	***	**	***	***	***
Ancient vs. Modern	*	N.S.	*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	*	**

Abbreviations: CTR, control trial; P value: ***, P < 0.001; **, P < 0.01; *, P < 0.05, N.S., not significant.
Results indicate mean values ±SD of four determinations (carried out in duplicate for two independent productions).
Data within a column followed by the same letter are not significantly different according to Tukey's test.

Fig. 1. Digital images (15 x 15 mm crumb area) converted to grey-level image (8 bit) of bread samples and relative binary image (left) obtained applying the Otsu's threshold algorithm in order to calculate void fraction, cell andmean cell area: (a) Biancuccia; (b) Simeto; (c) Control.

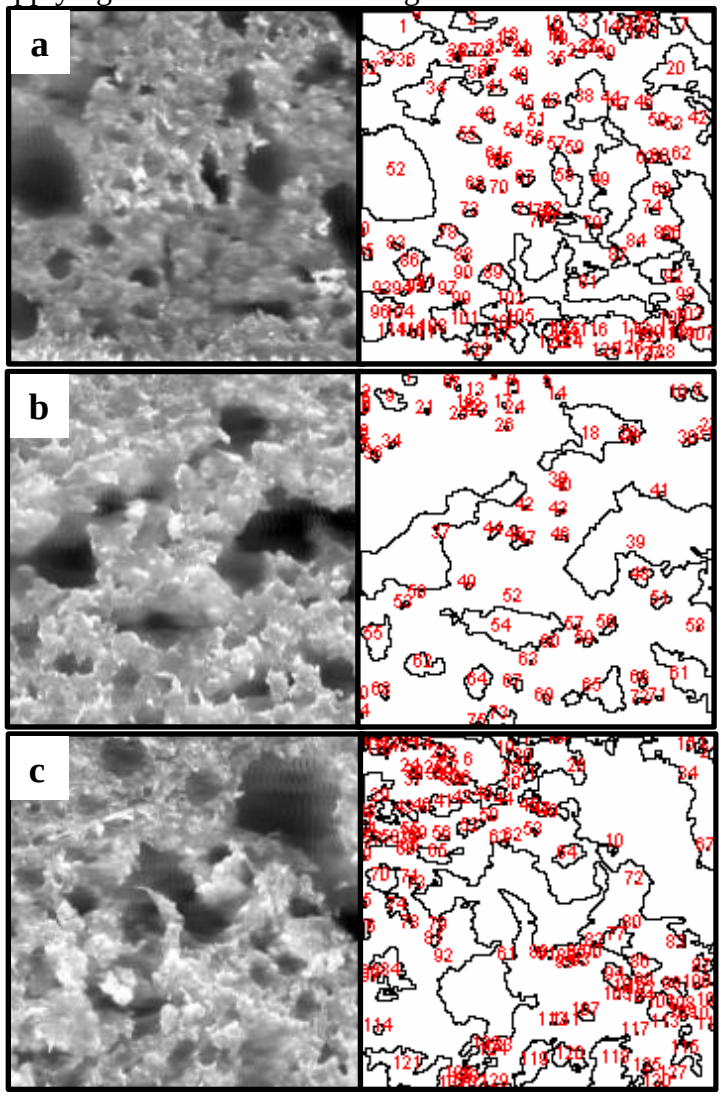

Fig. 2. Distribution of the volatile organic compounds emitted from bread expressed as relative peak areas (peak area of each compound/total area) × l00. The hierarchical dendrogram is based on the values of VOCs. The heat map plot depicts the relative percentage of each compound within each bread. Abbreviations: T1, Timilia; T2, Russello; T3, Biancuccia; T4, Realforte rosso; T5, Tripolino; T6, Scorsonera; T7, Perciasacchi; T8, Aziziah; T9, Bidì; T10, Senatore Cappelli; T11, Iride; T12, Creso; T13, Vertola; T14, Saragolla; T15, Simeto; CTR, control. Colour scale: ■, 0-20; ■, 21-40; ■, 41-60; ■, 61-80; ■,81-100.

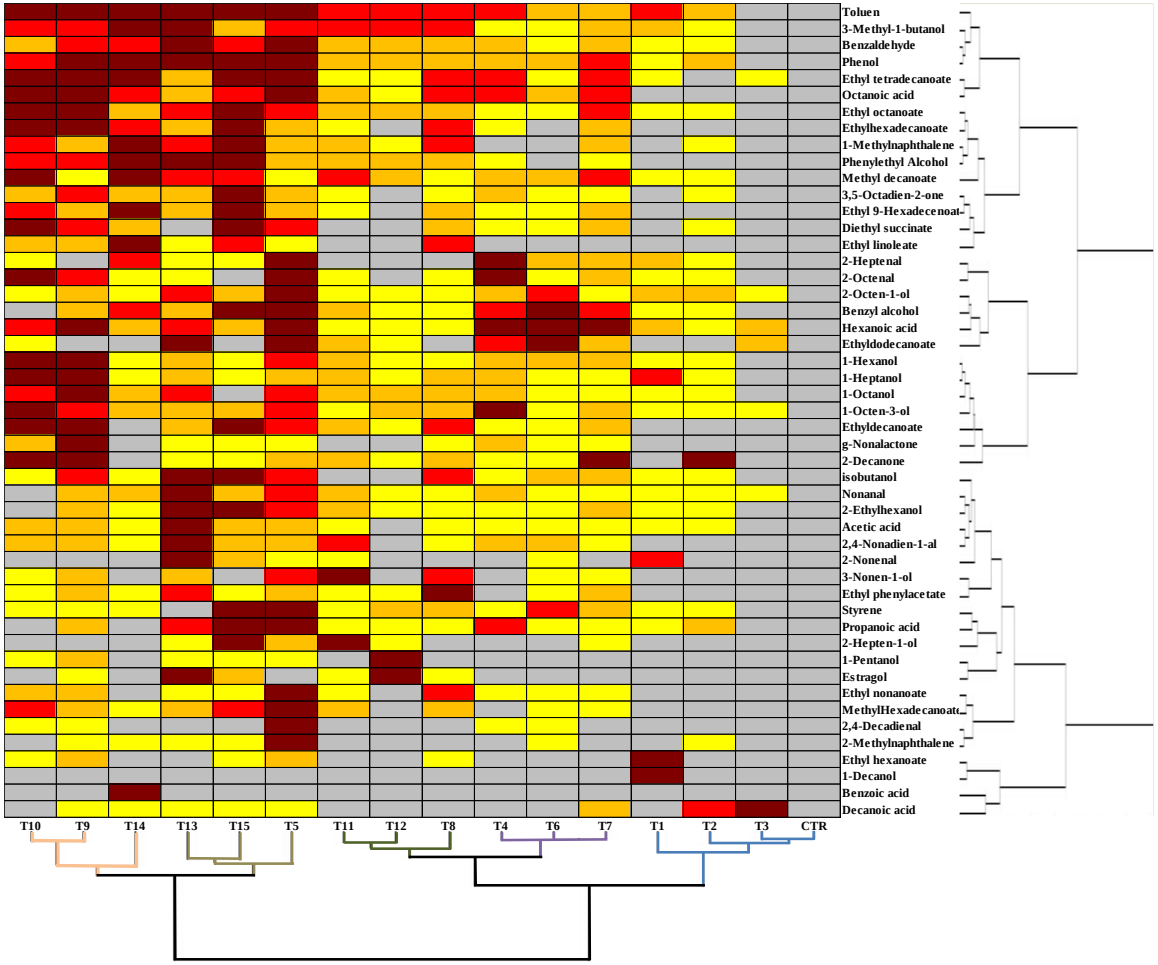

Fig. 3. Spider chart representation of bread sensory characteristics. Abbreviations: N.S., not significant; P value: *, P < 0.05; **, P < 0.01; ***, P < 0.001.

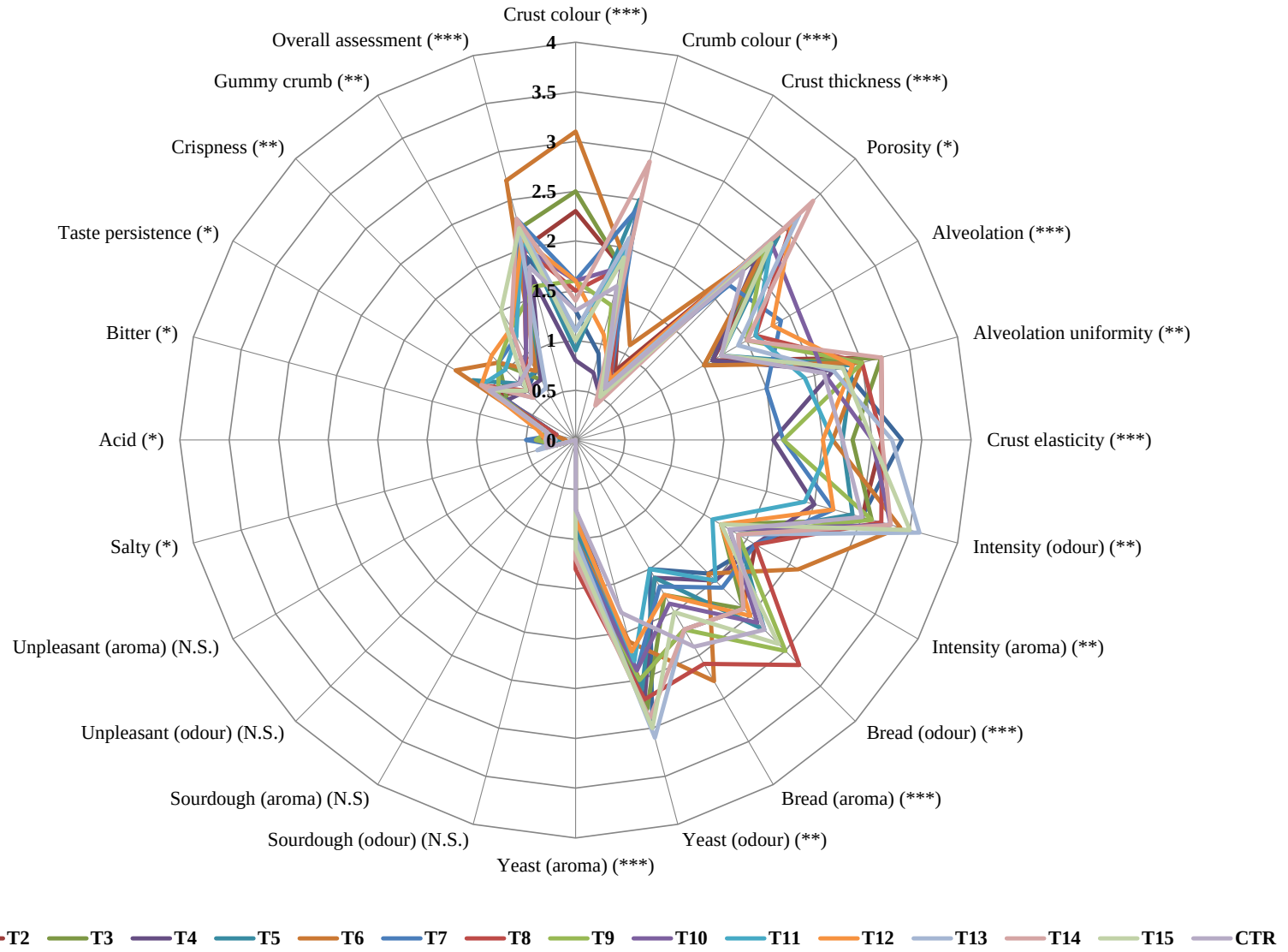

Fig. 4. Correlations of the quality characteristics of semolinas, sensory analysis, VOC, characteristics of breads and discrimination among different trials. (a) Variable loading plot of MFA: ■ characteristics of bread, ■ quality characteristics of semolina, ■ sensory analysis, ■ VOC; (b) sample scores of MFA analysis; (c) AHC dendrogram of trials based on their dissimilarity.