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## Quality aspects in open-pollinated onion varieties from Western Europe

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### Summary

Commercial onion breeders limit their selection criteria by focusing almost exclusively on conventional farming. This raises the demand for certain well known varieties, but lowers the general diversity available on the mainstream market. A way to maintain biodiversity is to preserve old open-pollinated varieties. Through their distinct aroma and flavor, these plants are again drawing the interest of farmers and consumers alike, making them a viable alternative to commercial varieties. To assess yield and quality aspects of West-European open-pollinated onions, we have evaluated nine varieties and compared them against two of their commercial, well-established counterparts. The study included onion production on the field in South-West Germany, evaluation of the quality and flavor parameters, as well as a trained sensory taste panel. Results showed high diversity in yield and chemical properties of the studied onion varieties, where two varieties in particular, stood out significantly. Compared to the control, the variety “Birnförmige” performed best and demonstrated high concentrations of fructan and pyruvic acid, both known to have curative and medicinal properties. On the other end of the spectrum, the variety “Jaune des Cévennes” demonstrated low dry matter content, low concentration of enzymatically-produced pyruvic acid and a high bolting percentage. The study also confirmed the link between individual quality components in onion bulbs, including the significantly negative correlation between minerals (such as calcium and magnesium) and fructan.

**Keywords:** *Allium cepa* L., open-pollinated varieties, yield, quality compounds, sensory taste

### Introduction

According to the Food and Agriculture Organization (FAO), onion (*Allium cepa* L.) is, after tomato and watermelon, the third most cultivated vegetable by production quantity in the world, with a total of 92 million tons produced in 2014 (FAOSTAT, 2017). Onions are highly valued for their unique flavor and their nutritional attributes including high amounts of vitamins, minerals and trace elements. These characteristics and in particular the following three compounds have been the focus of onion-related research worldwide: substances derived from flavor- and aroma-inducing sulfur compounds, powerful antioxidative flavonoids, including quercetin and anthocyanin, as well as fructo-oligosaccharides and fructans. The last two act as prebiotics, since they cannot be digested in the upper intestine and serve in the caeco-colon as an important source of energy for the local microbiota (BREWSTER, 2008; GRIFFITHS et al., 2002). On average, organic foods, *i.a.* onions, can contain higher levels of these healthy compounds, especially higher concentrations of antioxidants, aroma and flavor than conventionally produced foods as confirmed in many studies (REN et al., 2017A; REN et al., 2017B; WOESE et al., 1997).

It is difficult to trace the exact origin of onions to a single source. Over thousands of years, onions have adapted to different climates, temperatures and photoperiods, creating a wide range of varieties

and landraces (BREWSTER, 2008). Old open-pollinated varieties and landraces in particular have been valued for their yield stability and resistance against adverse conditions. Their production capacity, however, is less than that of modern cultivars (ZEVEN, 1998). With the modernization of agriculture and discovery of cytoplasmic male sterility (CMS) in the onion variety Italian Red, farmers have increasingly focused on growing hybrids and abandoned traditional landraces and open-pollinated varieties, leading to genetic erosion (CRAMER, 2001; PETROPOULOS et al., 2015). In contrast to old varieties, modern onion hybrids are mainly bred to perform well in conventional systems that rely on chemical inputs. These varieties in turn do not match the standards of the organic sector where organic farmers do not apply synthetic pesticides and fertilizers, nor is the breeding methodology of CMS hybrids compatible with the principles set forth by the world umbrella organization for organic farming IFOAM (OSMAN et al., 2008). In order to optimize organic farming systems, new varieties are required that fit the growing conditions in organic agriculture (LAMMERTS VAN BUEREN et al., 2002; OSMAN et al., 2008). Under unfavorable conditions landraces and old open-pollinated varieties may be able to compete and even outperform modern varieties, therefore offering organic farmers new opportunities to cope with plant biotic and abiotic stresses, as well as create base populations that can be used to generate new varieties (ZEVEN, 1998).

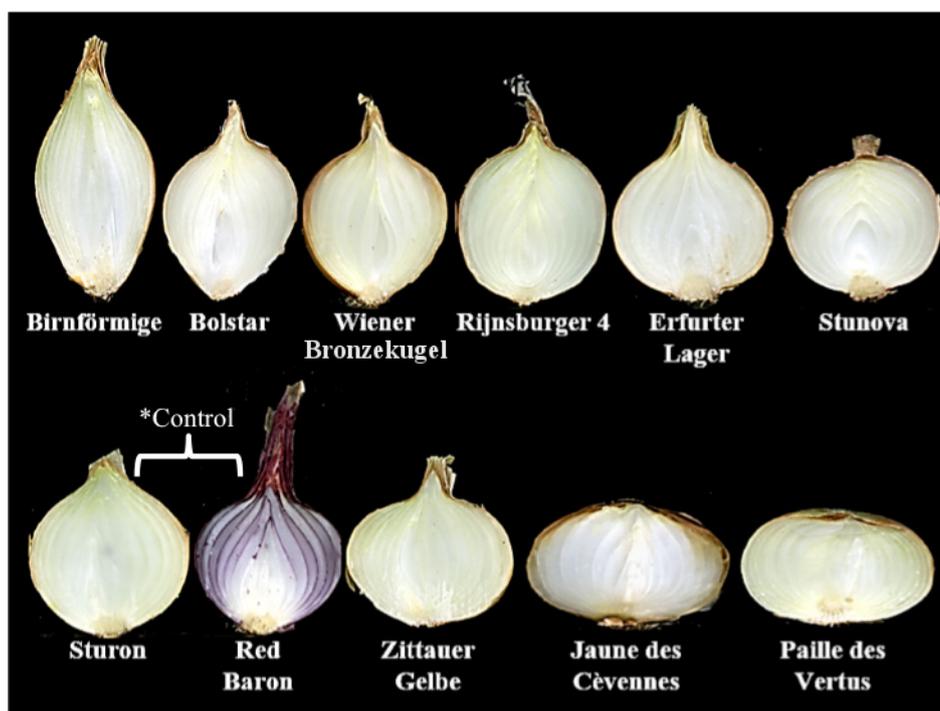
During the past few decades, many public and private initiatives started to collect, characterize and maintain genetic resources for different vegetable and cereal species around the world. In Europe, in particular, the Netherlands, Italy, Spain and Greece have already contributed with studies of genetic diversity and quality on their own old onion varieties (LIGUORI et al., 2017; PETROPOULOS et al., 2015; RIVERA et al., 2016) but in Germany and many neighboring countries, such studies are still scarce. In order to provide new insights about the quality aspects of old onion varieties, the aim of this study was to characterize the morphological, agricultural and phytochemical characteristics of nine old open-pollinated varieties grown under organic conditions and compare them with two well established open pollinating onion varieties marketed in West-Europe (“Red Baron” and “Sturon”), focusing on their nutritional compounds. The results compiled by this study should enable us to answer the following questions: (i) How big are the differences and potential of quality parameters among the varieties? (ii) Is there a link between the quality components of the studied onion varieties? (iii) Which varieties are better suited for organic farming in South-Germany?

### Materials and methods

#### Plant material

A total of eleven onion varieties were used (Fig. 1, Tab. 1). Nine of these were West-European open-pollinated onion varieties provided by the organization Kultursaat e.V., whereas the two additional commercial open-pollinated cultivars “Red Baron” and “Sturon” that were used as control. They were selected based on their quality and because they are widely used in commercial organic farming in the study area.

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**Fig. 1:** Longitudinal section of open-pollinated onion varieties used in the field trial.

**Tab. 1:** Variety, skin color, country of origin or seed provider and market availability of analyzed open-pollinated onions.

Variety	Abbreviation	Skin color	Seed Provider / Origin	Availability
Jaune des Cévennes	JdC	Yellow	Kultursaar e.V. / France	Limited
Stunova	Stu	Yellow	Kultursaar e.V. / Germany	Limited
Sturon*	Str	Yellow	Bingheimer Saatgut / Germany	Common
Bolstar	Bol	Yellow	Kultursaar e.V. / The Netherlands	Limited
Wiener Bronzekugel	WBr	Yellow	Kultursaar e.V. / Austria	Limited
Rijnsburger 4	Rij4	Yellow	Kultursaar e.V. / The Netherlands	Limited
Birnförmige	Bif	Yellow	Kultursaar e.V. / Germany	Limited
Erfurter Lager	ErL	Yellow	Kultursaar e.V. / Germany	Limited
Zittauer Gelbe	ZtG	Yellow	Kultursaar e.V. / Germany	Limited
Paille des Vertus	PdV	Yellow	Kultursaar e.V. / France	Limited
Red Baron*	RB	Red	Bejo / The Netherlands	Common

\* Control varieties

### Field experiment

The field trial was conducted in 2015, with seeds of eleven onion varieties sown in a controlled environment in the greenhouse during the last week of February. After six weeks, seedlings were transplanted to the experimental station for organic farming "Kleinhohenheim", University of Hohenheim (48°43'54"N, 9°12'5"E Stuttgart, South-West Germany). Climate data can be found in Supplementary Fig. S1. The experiment was set up in a randomized block design with 4 replicates and an individual plot size of 3.2 × 1.5 m. Each experimental plot (one variety per plot) had three rows and a total of 90 plants per quadrat meter. Similar to the field experiment of REENTS et al. (2007), a distance of 0.375 m was maintained between rows and 0.20 m between plants in the row. The rotation on the experimental field included the following precrops: 1<sup>st</sup> year grass-clover (high share of red clover), 2<sup>nd</sup> year cabbage, 3<sup>rd</sup> year summer

wheat, 4<sup>th</sup> year onions (year of the experiment).  $N_{min}$  measurements were done before transplanting in April (0 - 60 cm 36 kg N/ha). During all plant growth stages, hand-weeding was carried out to combat weed. The onions were harvested when the foliage leaves senesced at the end of the first growing season. Each onion variety were lifted by hand after 60% of each plot top-down. For optimum storage quality, onions were cured in an indoor space for three weeks. After this, onions were stored for approximately four weeks until all samples were processed. During this period, observation of diseases and disorders in the onion bulbs was conducted. Yield and onion bulb size were determined prior to laboratory work. An onion size sorting machine was used to sort onion as < 30 mm, 30 - 45 mm, 45 - 55 mm, > 55 mm (Amazon BK3 sorting machine, AMAZONEN-Werke H. Dreyer GmbH & Co. KG, Hansberger, Germany).

### Morphological features

After harvest, yield determination, and size sorting, nine individual onions (30 - 55 mm) from each plot were gathered for further analysis. Prior to phytochemical composition analysis, data of bulb length and diameter, dry weight, number and color of tunic, firmness and total soluble solids (TSS) of edible flesh were collected. Bulb diameter was measured using a caliper at the widest point of the bulbs and the statistical average of nine samples was used as variety diameter. Bulb length was obtained by measuring the average vertical length. Bulb firmness was determined in degree Shore with a durometer (Shore Instrument & Manufacturing Company, Inc., Jamaica, NY). Three single-point durometer readings were measured equatorially on each bulb to calculate the average durometer firmness.

### Processing of samples

Four replicates of each variety composed of nine bulbs were used for sample processing. The outer dry scales were removed and the onions were cut longitudinally. One part of the sample was bulked and homogenized in a 1:1 vol (w/v) of double-distilled water (ddH<sub>2</sub>O) using a mixer (BÜCHI Mixer B-400, BÜCHI Labortechnik AG, Flawil, Switzerland). The homogenates were then filtered using folded paper filter (520 A ½ 185 mm, Schleicher & Schuell, Dassel, Germany) and the clear supernatants were stored at -20 °C for further pyruvic acid analysis. A second part of the sample was chopped and freeze-dried. The resulting lyophilized onions were then ground into powder. The samples were stored at -20 °C prior to extraction of phenolic compounds. The third part of the sample was homogenized solely and stored for the analysis of sugar, soluble solids, and dry matter content.

### Determination of dry matter content

20 g of homogenized samples were dried in an oven with air circulation, first at 70 °C for 42 h and then at 105 °C for 3 h. Every determination was made in triplicate.

### Determination of soluble solids content (TSS)

The soluble solids content (in °Brix) was measured at 22 °C using the Bausch and Lomb Abbe 3L refractometer (Bausch and Lomb Incorporated, Rochester, NY) on the supernatant obtained from 1 g of raw homogenate by centrifugation for 10 min at 10000 rpm

### Determination of non-structural carbohydrates

Non-structural carbohydrates were analyzed based on the Official Analytical Chemists (AOAC) method and the Megazyme fructan-assay kit using p-hydroxybenzoic acid hydrazine (PAHBAH) with some modifications (MCCLEARY et al., 2000). For the preparation of the standard curves, stock solutions containing 0, 0.1, 0.25, 0.5, 0.75 and 1 mg mL<sup>-1</sup> of glucose were prepared. For reducing sugar analysis, samples of 50 µL of diluted extract (1 mL onion juice with 4 mL ddH<sub>2</sub>O) were mixed with 200 µL ddH<sub>2</sub>O and 2.5 mL of PAHBAH solution, heated for 4 min at 95 °C, diluted immediately with 5 mL ddH<sub>2</sub>O and mixed. For sucrose determination, 50 µL of diluted extract were mixed with 100 µL sucrase solution (enzyme) and 100 µL sodium maleate buffer (100 mM, pH 6.5), heated at 40 °C for 30 min, diluted with 2.5 mL PAHBAH solution, heated again for 4 min at 95 °C and finally mixed with 5 mL ddH<sub>2</sub>O. For hydrolysis and measurement of fructan concentration, samples of 1 mL of undiluted extract were mixed with 3 mL ddH<sub>2</sub>O and 1 mL 1M HCl. The mixture was placed in a water bath at 95 °C for 15 min and then 1 mL of the hydrolysate was mixed with 1 mL of ddH<sub>2</sub>O. For fructan measurement, 50 µL of the diluted hydrolysate was added to 200 µL ddH<sub>2</sub>O and 2.5 mL PAHBAH solution. After 4 min

at 95 °C this was mixed with 5 mL of ddH<sub>2</sub>O. Absorbance was read against blank at 415 nm using spectrophotometer SPECORD 50 (Analytic Jena AG, Jena, Germany).

### Determination of pyruvic acids

Due to the high correlation between both, volatile sulfur flavor components in onions are measured by a simple indirect method of determining enzymatically-produced pyruvic acid concentration in disrupted onion tissue (SCHWIMMER and WESTON, 1961). Enzymatically produced pyruvic acid concentration in the filtrated juice was measured using dinitrophenylhydrazine (DNPH) reagent according to the improved method of ANTHON and BARRETT (2003) and background pyruvic acid after deactivation of allinase by heating onion tissues in a microwave according to the method of YOO and PIKE (2001). A spectrophotometric assay was carried out using 25 µL of filtrate with 1 mL of ddH<sub>2</sub>O and 1 mL of DNPH solution (12.5 mg DNPH / 50 mL 1 M HCl). The reaction mixture was placed in a water bath at 37 °C for 10 min and then 1 mL of 1.5 M NaOH was added. The absorbance was measured at 515 nm. The standard curve was prepared from a series of sodium pyruvate (0, 1, 2, 4, 6, 8 mM). The concentration of enzymatically produced pyruvic acid was calculated by subtracting the background concentration of pyruvic acid from the total pyruvic acid concentration. The results were expressed as µmol g<sup>-1</sup> fresh weight (FW).

Selection of onion samples for the taste-panel assessment was assigned based on pyruvic acid results according the classification of CROWTHER et al. (2005). Onions with a pyruvic acid level below 4.0 µmol g<sup>-1</sup> FW were classified as sweet, those with a level between 4.0 and 7.0 µmol g<sup>-1</sup> FW as mild, and onions with levels more than 7.0 µmol g<sup>-1</sup> FW were classified as pungent.

### Determination of total phenolic content (TPC)

Phenolic compounds were extracted according to SANTAS et al. (2008), with some modifications. Aliquots of 0.15 g of freeze-dried onion powder were added to 1.5 mL ethanol:water (75:25 v/v). After 30 min with 900 rpm magnetic stirring at room temperature and 20 min sonification in an ultrasonic bath, the extract was centrifuged at 3000 rpm for 15 min. The whole extraction procedure was repeated twice with magnetic stirring for 45 and 90 min, respectively. The pooled supernatant fractions were stored at -20 °C in the dark for further analyses.

Total phenolic content was determined in triplicate using the Folin-Ciocalteu spectrophotometric method as follows: 200 µL of the diluted ethanolic onion extract (100 µL ethanol + 100 µL extract 1:2 v/v) was mixed with 1.5 mL diluted Folin-Ciocalteu reagent (dilution 1:10 reagent / water v/v). Samples were mixed properly and allowed to stand for 10 min at room temperature (20 °C). Then 1.5 mL of a 2% sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 120 min and the absorbance was measured at 765 nm against the blank, containing 75% ethanol instead of the sample extract. The standard curve was prepared from a series of gallic acid (GAE) standards (0, 20, 50, 80, 100, 120, 200 mg/L) and the results were expressed as mg GAE g<sup>-1</sup> dry weight (DW).

### Determination of antioxidant activity

The radical scavenging activity of the extracts was carried out according to BRAND-WILLIAMS et al. (1995) using the '2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method. The DPPH reagent was prepared by sonication of 0.1 mM of DPPH in 75% ethanol for 1 h in an ultrasonic bath. 200 µL of the diluted ethanolic onion extract was mixed with 2 mL of DPPH reagent and incubated

for 60 min in the dark at room temperature. Absorbance was read at 515 nm against the blank, containing 75% ethanol instead of the sample extract. The standard curve was prepared from a series of trolox standard solutions (0, 50, 100, 200, 300  $\mu\text{M}$ ) and the results were expressed as  $\mu\text{mol}$  Trolox Equivalent (TE)  $\text{g}^{-1}$  DW.

#### Determination of mineral elements and ash content

Mineral concentrations (K, Mg, and Ca) were determined using 300 mg of freeze-dried onion bulb material solubilized in 10 mL of 69% nitric acid by microwave digestion at 190 °C for 25 min (MARS 5; CEM Cooperation, Matthews, NC, USA). The solution was filtered and diluted to a final volume of 100 mL. Cations were measured using an atomic absorption spectrometer (3300 series; Thermo Fisher Scientific, Dreieich, Germany).

To determine the ash, approximately 100 mg of freeze-dried onion bulb material was placed into a ceramic crucible. Samples were placed into a muffle furnace for at least 7 h at 550 °C. After that, the samples were cooled in a desiccator and weighed to determine ash percentage.

#### Pungency taste-panel assessment

Taste-panel was conducted by twelve members of the Institute of Crop Science (Viticulture). Three samples were used for the triangle and ranking test. One sample was an onion variety classified as “sweet” with significant low levels of enzymatically produced pyruvic acid and the other two samples were the same variety, classified as “pungent” with very high levels of enzymatically produced pyruvic acid. For the triangle tests, panelists were asked to ingest a similar amount of the homogenized samples and then identify the deviating sample. Through the ranking test the panelists were then asked to assign scores to the samples by ranking them from lowest to highest for flavor intensity, with the rating: 1, mild; 2, slightly pungent; 3, pungent. Data was evaluated using the expanded tables for multiple comparison procedures in the analysis of ranked data from NEWELL and MACFARLANE (1987).

#### Statistical analysis

One-way ANOVA (Analysis Of Variance) followed by post-hoc Tukey HSD (Honestly Significant Difference) were used for comparing multiple treatments. Differences were considered as significant when  $p < 0.05$ .

Correlation matrix and principal component analysis (PCA) were used to determine the relationship between the flavor/quality attributes and provide a graphical description of the varieties characteristics. PCA was applied to the centralized and standardized values of all eleven varieties. Standardization and centralization was necessary because of the different magnitudes and weight of the values. All analyses were performed using R (R DEVELOPMENT CORE TEAM, 2016).

## Results

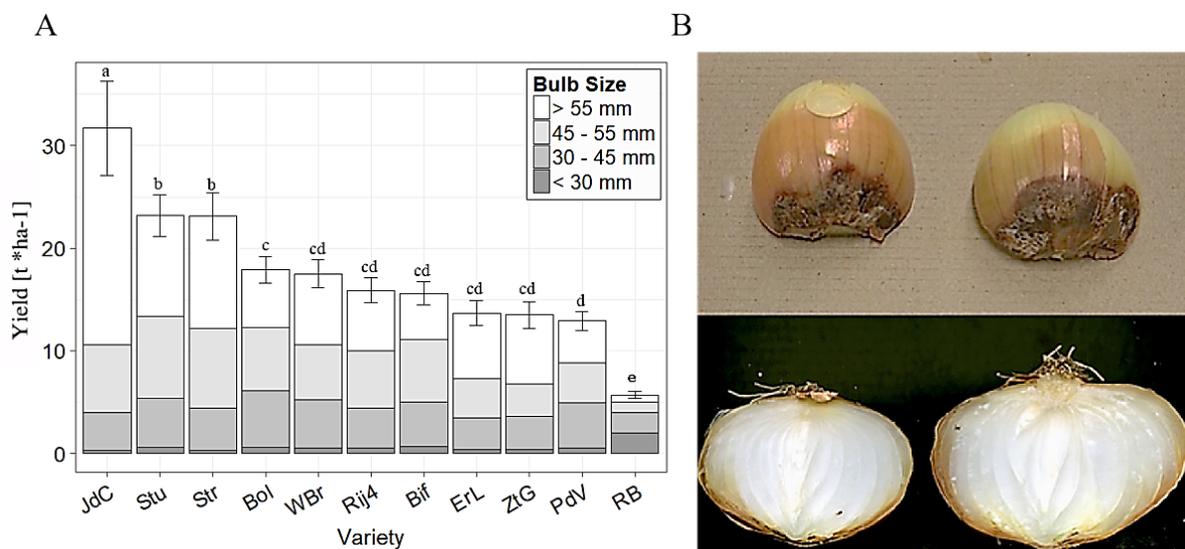
#### Onion Yield and Agronomic Traits

Mean yields of the best performing “Jaune des Cévennes” and the least performing “Red Baron” varieties were 33.3 and 5.52  $\text{t} \cdot \text{ha}^{-1}$  respectively (Fig. 2A). Jaune des Cévennes and the other studied varieties developed bulbs generally larger than 30 mm diameter, with an exception of Red Baron, which mostly yielded smaller onion bulbs. The highest yield was obtained for Jaune des Cévennes, but >14% was not marketable because of the high bolting percentage. The shortest time to achieve physiological maturity (158 days) was observed with Birnförmige, while Jaune des Cévennes, Red Baron, Bolstar, Erfurter Lager and Wiener Bronzekugel required more than 175 days from sowing to physiological maturity (Tab. 2).

Diseases and disorders were observed only in the variety Jaune des Cévennes. Symptoms of sour skin and neck rot were detected with a high number of bulbs in this variety (Fig. 2B).

#### Morphological Traits of the Bulbs

The morphology of the bulbs was significantly different between the varieties for the following traits: dry weight, diameter, height, number of tunics, firmness and total soluble solids.



**Fig. 2:** **A:** Onion total yield and bulb size. Stacked bar graph in which the overall height of the bar indicates the total yield, data are mean  $\pm$  SE,  $n = 4$ , letters indicate significant differences among the varieties determined by “Post-hoc” test ( $p < 0.05$ ). The height of each segment (coded as shown in the figure) represents the amount of bulbs sorted by size in a variety. Jaune des cévennes (JdC), Stunova (Stu), Sturon (Str), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (ErL), Zitaue Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB). **B:** Diseases and disorders observed during curing and storage of onion bulbs (Jaune des Cévennes).

Significant differences were found among the onion varieties in their mean diameter and height. Birnförmige is an oval, pear-shaped variety (Fig. 1), and for that reason its height is with 66 cm significantly bigger than that of the other onion varieties. In contrast, because of their flat shape, both, Jaune des Cévennes with 61.8 cm and Paille des Vertus with 60.2 cm, have notably bigger diameters compared to other varieties (Tab. 3).

In terms of dry matter, total soluble solids, number of tunics, and firmness Jaune des Cévennes showed the lowest significant values compared to the other varieties. On the other side of the spectrum, Birnförmige and Paille des Vertus showed the highest content of dry matter, TSS, as well as good bulb firmness (Tab. 3).

### Quality Traits

Bulb total sugar concentration ranged from 68.3 (Jaune des Cévennes) to 150.7 mg g<sup>-1</sup> FW (Birnförmige). Total sugar (Fig. 3A) consisted

mainly of fructans (Fig. 3B), where concentrations ranged between 7.8 (Jaune des Cévennes) and 135.4 mg g<sup>-1</sup> FW (Birnförmige), sucrose concentrations (Fig. 3D) ranged between 1.9 (Jaune des Cévennes) and 13.5 mg g<sup>-1</sup> FW (Sturon), reducing sugar concentrations (Fig. 3C) ranged between 8.7 (Birnförmige) and 58.3 mg g<sup>-1</sup> FW (Jaune des Cévennes).

Fig. 3E shows the results of antioxidant activity by the commonly used method DPPH- in ethanol solvents. The variety “Red Baron” had the highest value of antioxidant activity (13.7 μmol TE g<sup>-1</sup> DW) and “Sturon” the lowest activity (4.8 μmol TE g<sup>-1</sup> DW).

Total phenolic concentrations (Fig. 3F) varied in the range of 2.8 mg GAE g<sup>-1</sup> DW to 6.9 mg GAE g<sup>-1</sup> DW for the variety “Jaune des Cévennes” and “Red Baron” respectively.

Enzymatically produced pyruvic acid of the entries (Fig. 4A) ranged between 2.9 (Jaune des Cévennes) and 7.4 μmoles g<sup>-1</sup> FW (Birnförmige). Jaune des Cévennes was the only variety which could be categorized as “sweet”, according to its pyruvic acid level results.

**Tab. 2:** Agronomic parameters of the varieties.

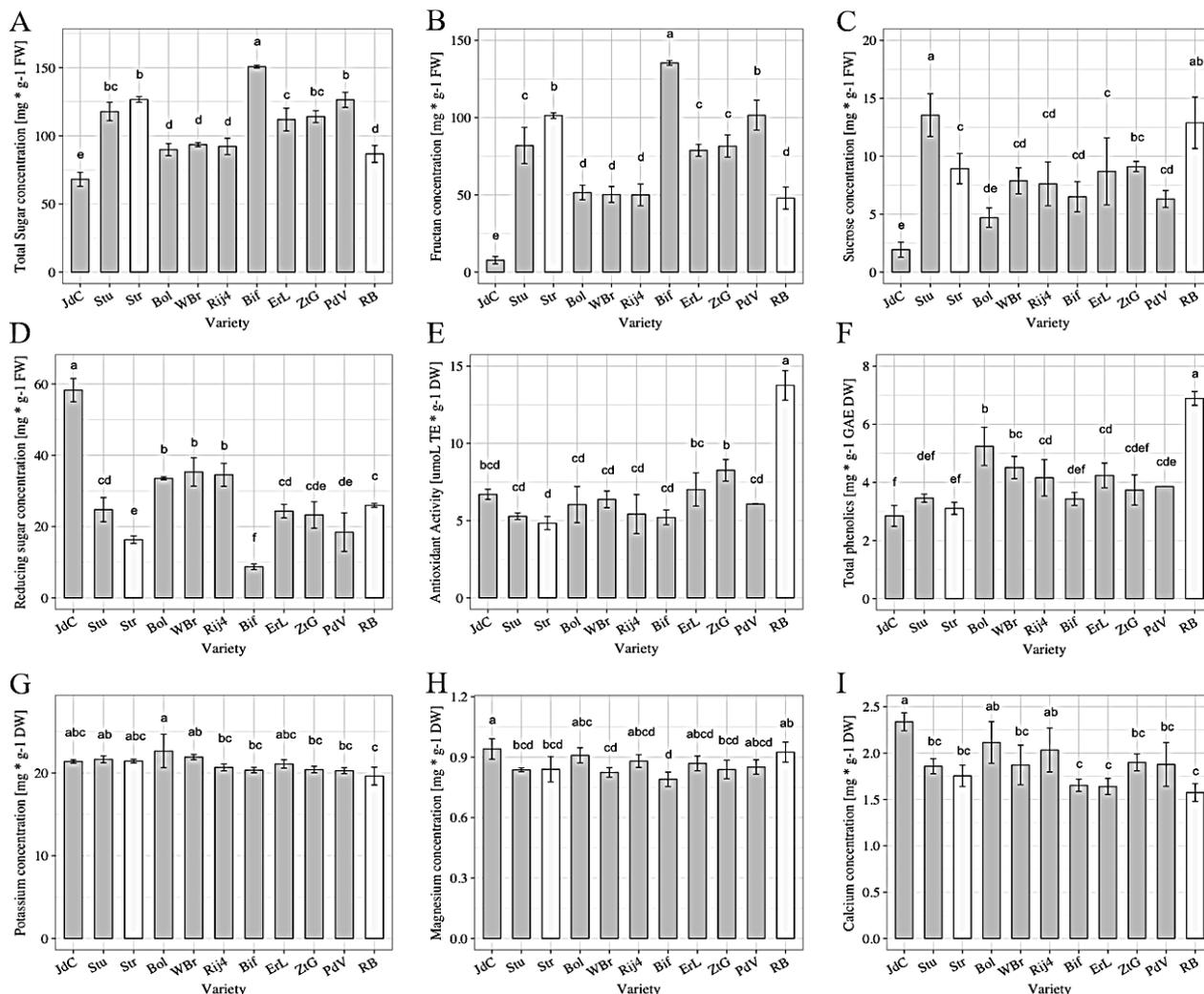
Variety	Number of Bulbs / Plot	Bolting (%)	Days to physiological maturity after sowing
JdC	184.2 ± 30.9 ab	14.1 ± 5.1 a	177.5 ± 1.7 a
Stu	187.0 ± 53.0 ab	0.11 ± 0.2 b	164.2 ± 3.9 bc
Str*	176.0 ± 17.6 ab	0.00 ± 0.0 b	161.0 ± 6.0 c
Bol	191.7 ± 38.3 a	0.11 ± 0.2 b	178.5 ± 6.8 a
WBr	158.0 ± 11.16 ab	1.28 ± 0.6 b	182.0 ± 3.5 a
Rij4	140.5 ± 45.2 abc	0.00 ± 0.0 b	175.2 ± 3.8 ab
Bif	148.7 ± 21.6 abc	0.00 ± 0.0 b	158.0 ± 0.0 c
ErL	113.7 ± 7.2 bc	0.69 ± 0.9 b	178.5 ± 6.5 a
ZtG	118.7 ± 24.4 abc	0.00 ± 0.0 b	161.0 ± 0.0 c
PdV	136.5 ± 39.1 abc	0.14 ± 0.3 b	165.5 ± 9.0 bc
RB*	76.75 ± 25.8 c	0.00 ± 0.0 b	185.2 ± 1.5 a

Data are mean ± SE, n = 4. Significant test by Tukey’s HSD (p < 0.05) are indicated by different letters. Jaune des Cévennes (JdC), Stunova (Stu), Sturon (Str), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (ErL), Zitauer Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB). \*Control varieties.

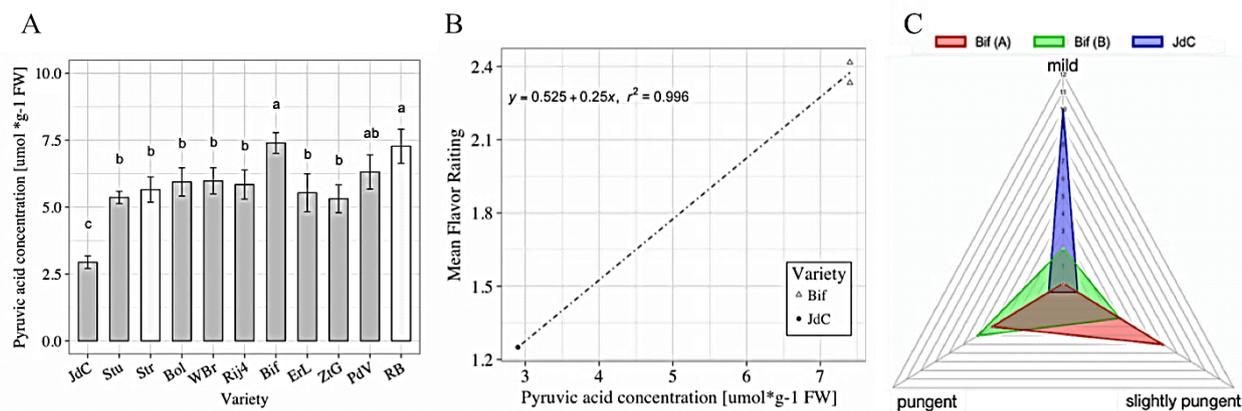
**Tab. 3:** Morphological parameters of onion bulbs.

Variety	Dry matter (%)	Diameter (mm)	Height (cm)	Number of tunics	Firmness (Shore)	TSS (°Brix)
JdC	9.2 ± 0.4 g	61.8 ± 1.5 a	48.6 ± 1.4 de	1.4 ± 0.1 d	84.4 ± 1.0 b	8.2 ± 0.3 g
Stu	14.3 ± 0.8 d	58.4 ± 1.6 abc	55.7 ± 1.4 bc	2.0 ± 0.1 ab	91.0 ± 1.7 a	12.7 ± 0.6 cd
Str*	15.7 ± 0.2 bc	58.5 ± 1.0 abc	55.6 ± 0.7 bc	2.0 ± 0.1 ab	90.3 ± 2.4 a	14.2 ± 0.3 b
Bol	11.9 ± 0.4 ef	57.8 ± 0.4 bc	53.6 ± 1.7 bc	2.0 ± 0.1 ab	93.3 ± 1.3 a	11.0 ± 0.1 ef
WBr	12.6 ± 0.1 e	55.4 ± 0.8 cd	57.0 ± 2.9 b	2.1 ± 0.2 ab	92.7 ± 0.7 a	12.0 ± 0.2 de
Rij4	12.5 ± 0.6 e	58.5 ± 1.4 abc	52.8 ± 0.8 c	2.1 ± 0.1 ab	90.0 ± 0.8 a	11.9 ± 0.7 de
Bif	18.1 ± 0.1 a	48.8 ± 1.1 e	66.0 ± 1.6 a	2.0 ± 0.0 ab	90.2 ± 1.2 a	16.4 ± 0.3 a
ErL	14.2 ± 0.3 d	59.0 ± 0.8 ab	52.7 ± 2.8 cd	1.8 ± 0.2 bc	93.8 ± 1.2 a	13.3 ± 0.3 bc
ZtG	14.9 ± 0.6 cd	58.0 ± 0.7 bc	46.3 ± 1.2 e	2.2 ± 0.2 a	91.9 ± 0.4 a	13.4 ± 0.4 bc
PdV	16.9 ± 0.8 ab	60.2 ± 1.1 ab	35.6 ± 0.7 f	1.7 ± 0.0 cd	93.1 ± 2.1 a	15.6 ± 0.6 a
RB*	11.1 ± 0.5 f	53.2 ± 3.2 d	54.0 ± 1.6 bc	2.1 ± 0.1 ab	90.8 ± 4.2 a	10.7 ± 0.6 f

Data are mean ± SE, n = 4. Significant test by Tukey’s HSD (p < 0.05) are indicated by different letters. TSS: Total soluble solids. Jaune des Cévennes (JdC), Stunova (Stu), Sturon (Str), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (ErL), Zitauer Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB). \*Control varieties.



**Fig. 3:** Quality traits. **A:** Total sugar concentration, **B:** fructans, **C:** sucrose, **D:** reducing sugar as well as **E:** antioxidant activity, **F:** total phenolics, **G:** potassium, **H:** magnesium and, **I:** and calcium of the studied onion varieties. Data are mean  $\pm$  SE,  $n = 4$ . Significant test by Tukey's HSD ( $p < 0.05$ ) are indicated by different letters. Control varieties Sturion and Red Baron are highlighted as blank barplots. Jaune des Cévennes (JdC), Stunova (Stu), Sturion (Str), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (Erl), Zitauer Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB). DW = dry weight; FW = fresh weight; GAE = gallic acid equivalent; TE = Trolox Equivalent.



**Fig. 4:** Pungency of onions. **A:** Enzymatically produced pyruvic acid. Data are mean  $\pm$  SE,  $n = 4$ . Significant test by Tukey's HSD ( $p < 0.05$ ) are indicated by different letters. Control varieties Sturion and Red Baron are highlighted as blank barplots. Jaune des Cévennes (JdC), Stunova (Stu), Sturion (Str), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (Erl), Zitauer Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB). FW = fresh weight. **B:** Relationship between pungency perception and enzymatically produced pyruvic acid by two onion varieties Birnförmige (Bif), and Jaune des Cévennes (JdC). Regression coefficients are significantly different from zero ( $p < 0.05$ ). Flavor rating scale: 1 = mild, 2 = slightly pungent, 3 = pungent. **C:** Radar chart of the sensory analysis, the samples of the onion varieties Birnförmige: Bif A (red), Bif B (green) and Jaune des Cévennes: JdC (blue), were categorized as mild, slightly pungent and pungent as described in the radar chart.

Most varieties fall into the category “mild”, except Birnförmige and Red Baron, which were categorized as “pungent”.

Depending on the enzymatically produced pyruvic acid concentrations, two varieties were selected for the pungency sensory taste panel. Fresh homogenates of the variety Jaune des Cévennes (sweet) and double samples of Birnförmige (pungent) were used for the triangle and ranking test. Results of the triangle test showed that nine of the twelve panelists identified the variety “Jaune des Cévennes” as the deviating sample. By averaging scores (1, mild; 2, slightly pungent; 3, pungent) given during the ranking test, Fig. 4B shows a significant high linear correlation ( $r = 0.99$ ) between pyruvic acid levels and pungency taste-panel assessment. Fig. 4C shows the number of panelists (12) and their assignment of categories for flavor intensity during the ranking test. Ten from twelve members assessed the variety Jaune des Cévennes with the score 1, mild, while the double sample of the variety Birnförmige were assessed by the majority of the panelists either as 2, slightly pungent; or as 3, pungent.

Results of bulb mineral contents are shown in Fig. 3 G - I. Potassium, calcium and magnesium concentrations ranged between 19.6 (Red Baron) to 22.6  $\text{mg g}^{-1}$  (Bolstar), 1.5 (Red Baron) to 2.3  $\text{mg g}^{-1}$  (Jaune des Cévennes), and 0.79 (Birnförmige) to 0.94  $\text{mg g}^{-1}$  DW (Jaune des Cévennes) respectively.

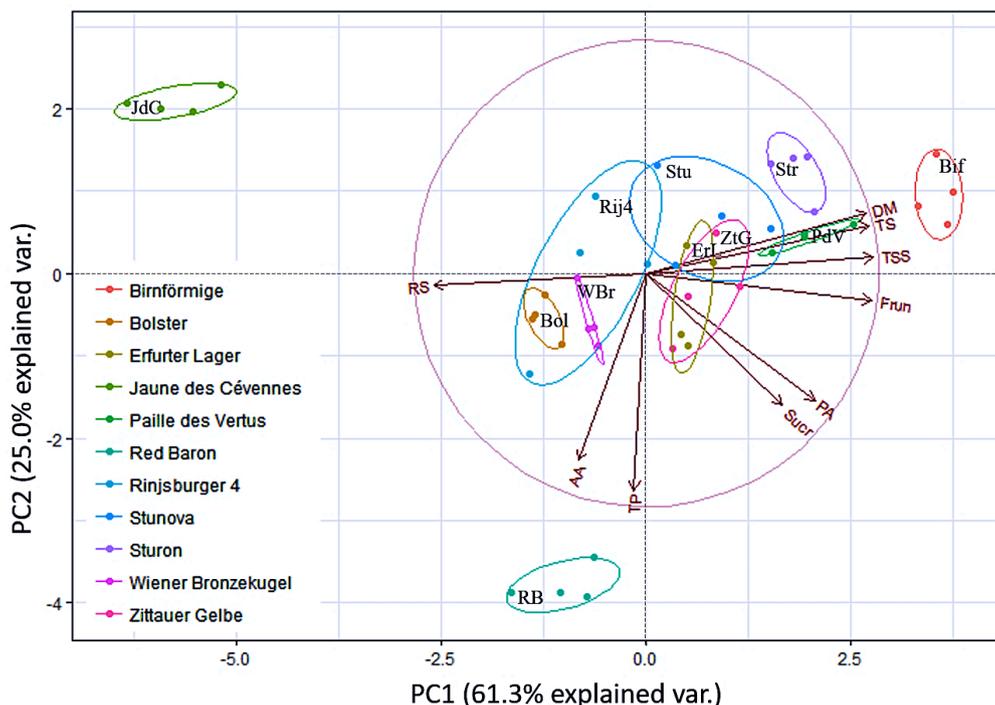
The principal component analysis (Fig. 5) summarizes information relating to all quality traits, *i.e.*, dry matter content, TSS, fructans, enzymatically produced pyruvic acid, total phenolics, antioxidant activity and reducing sugars. PC1 explained 61.3% of the total variance in the data set, while PC2 explained 25.0%. The correlation circle groups the most correlated varieties (Stunova, Sturon, Bolster, Wiener Bronzekugel, Rijnsburger 4, Erfurter Lager, Zittauer Gelbe, Paille des Vertus). Only three varieties, Birnförmige, Red Baron and Jaune des Cévennes, lie outside the circle, showing significantly different quality characteristics than the rest. The location of Birnförmige in the upper right-hand quadrant can be explained by its

high values of dry matter, TSS, fructans, pyruvic acid and sucrose. In strong contrast, Jaune des Cévennes is on the negative side of PC1, which is a result of overall higher reducing sugar concentration and lower concentration of fructans, dry matter content, TSS and pyruvic acid. Red Baron, which is located in the lower quadrant of the plot is characterized by its high total phenolics and antioxidant activity levels.

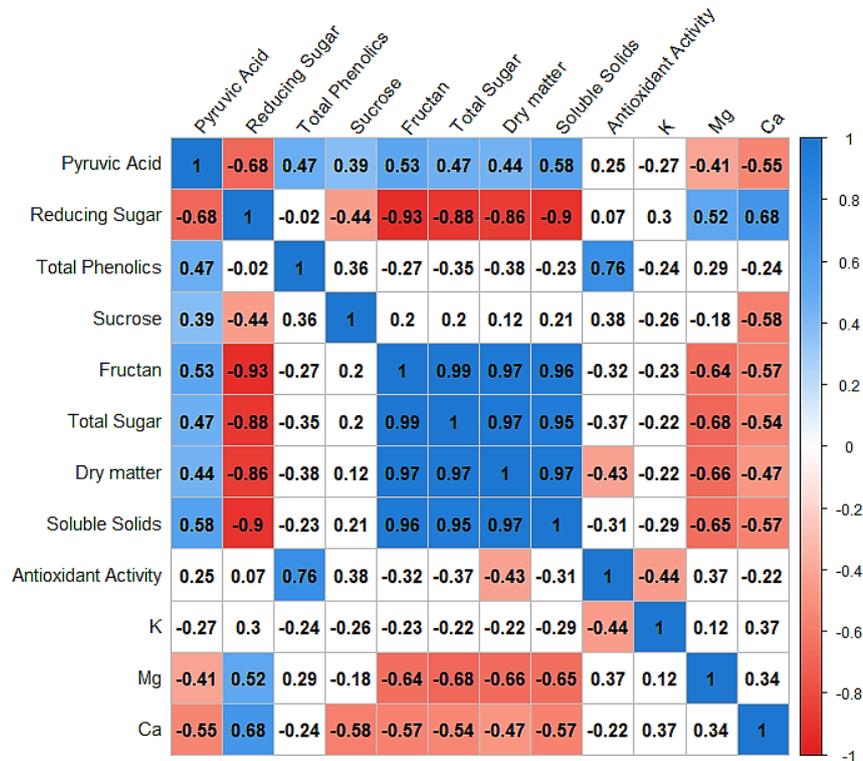
The correlation matrix based on all quality trait data (Fig. 6) shows a high significant positive correlation ( $r > 0.95$ ) between fructans, dry matter content and TSS. High significant negative correlation was observed between reducing sugar, fructans, dry matter content and TSS ( $r > 0.85$ ). Pyruvic acid had a positive correlation with sugar, except reducing sugars, total phenolics and antioxidant activity. On the other hand, enzymatically produced pyruvic acid correlated negatively with potassium, magnesium, and calcium ( $r = -0.27, -0.41, \text{ and } -0.55$ , respectively). A negative correlation could be also found by comparing fructans, dry matter content and TSS with potassium, magnesium, and calcium ( $r < -0.45$ ). The element concentrations of calcium and magnesium showed a significantly positive correlation with reducing sugars ( $r > 0.52$ ).

## Discussion

Screening onion landraces and open-pollinated cultivars in Western Europe is an effective way of collecting material for future breeding programs. Onion varieties with high yield stability, high concentration of nutritional compounds and good flavor are in high demand. This study collected a comprehensive amount of data, from onion production on the field up to the evaluation of the quality and flavor parameters, including a sensory taste panel. Quality, as well as morphological and yield differences between the varieties indicate that the selected samples used in this study could represent a valuable source of information for breeding purposes. The characterization



**Fig. 5:** Principal component analysis (PCA), describing the relationship among varieties depending on their quality traits. Quality traits: DM = Dry Matter, TS = Total Sugar, TSS = Total soluble solids, Frun = Fructan, PA = enzymatically produced pyruvic acid, Sucr = Sucrose, TP = Total Phenolics, AA = Antioxidant Activity, RS = Reducing Sugar. Onion varieties are grouped by colors as shown in the figure. Points represent the biological replications of each variety  $n = 4$ . Jaune des cévennes (JdC), Sturon (Str), Stunova (Stu), Bolstar (Bol), Wiener Bronzekugel (WBr), Rijnsburger 4 (Rij4), Birnförmige (Bif), Erfurter Lager (Erl), Zittauer Gelbe (ZtG), Paille des Vertus (PdV), Red Baron (RB).



**Fig. 6:** Correlation matrix of the most important quality traits in the studied onion varieties. Correlations with p-value < 0.01 are considered as significant and are highlighted: Red = negative correlation, Blue = positive correlation. Non significant coefficient values are not highlighted.

of “traditional varieties” provides valuable information in order to preserve and maintain the local genetic variability and therefore act against genetic erosion.

#### How big are the differences and potential of quality parameters among the varieties?

The study places particular emphasis on differences in distinct quality parameters of open-pollinated varieties by analyzing the dry matter content, total soluble solids (TSS), non-structural carbohydrates, enzymatically produced pyruvic acid and total phenolics. The resulting data showed significant differences between all onion varieties, but the largest deviation was identified with three varieties, in particular, Red Baron, Birnförmige and Jaune des Cévennes, where results varied significantly outside of the correlation circle as shown in Fig. 5.

Flavor and quality of onions are determined by the concentration and interaction between sulfur compounds and carbohydrates (McCALLUM et al., 2010; VAGEN and SLIMESTAD, 2008). Both of these compounds were responsible for the significant differences in quality found among the eleven varieties. In the variety “Birnförmige”, high concentration of pyruvic acid and fructan, as well as high dry matter content were observed, meeting the “dehydrated onion” criteria. By contrast, Jaune des Cévennes showed lower pungency, lower dry matter content and higher concentration of reducing sugars, which are distinctive characteristics of a “sweet onion” (MALLOR et al., 2011). “Sweet onions” are also well-known for their lower storability due to higher water content (SUZUKI and CUTCLIFFE, 1989), which is also a primary reason for their rapid degradation. Moreover, pathogen symptoms were found solely in Jaune des Cévennes after a few weeks of postharvest storage.

Early studies already confirmed the positive high correlation between mean pungency rating and enzymatically produced pyruvic acid (CROWTHER et al., 2005; WALL and CORGAN, 1992). Differences

in the pyruvic acid concentration among the varieties were also detected through our pungency taste panel. These were determined by tasting the two opposing onion varieties. The mildness of Jaune des Cévennes against the pungency of Birnförmige was clearly perceived by the test persons, giving a high correlation between enzymatically produced pyruvic acid and taste pungency ( $r^2 = 0.99$ ).

Within the eleven studied onion varieties, Red Baron stands out significantly due to elevated total phenolics concentration and almost 3-fold higher difference of antioxidant activity levels *e.g.* compared to the yellow variety “Sturon”. Similar studies with various onion varieties have demonstrated that red onions had higher total phenolics concentrations and antioxidant activity levels than yellow and white varieties, because of anthocyanins present only in red onions (LEE et al., 2015; LISANTI et al., 2016; SHARMA et al., 2015). At least 25 different anthocyanins have been reported for red onions, representing approximately 10% of the total flavonoid content (FULEKI, 1971; SLIMESTAD et al., 2007).

#### Is there a link between the quality components of the studied onion varieties?

A study of correlation between all analyzed quality parameters was carried out to discover the link between individual quality components in fresh onion bulbs. The high positive correlations between fructans, total sugar and TSS revealed that the non-digestible carbohydrates are the main carbohydrate fraction, contributing to more than 60% of the total sugar concentration, in almost all onion varieties, except for Jaune des Cévennes. This variety demonstrated an opposite behavior with high levels of reducing sugar (glucose, fructose) and lower levels of fructans and dry matter. This positive correlation confirms the results of a study of JAIME et al. (2001), showing a very high positive correlation between fructans, TSS, total sugar and dry matter ( $r = 0.97$ ) and a high negative correlation between reducing sugar, TSS, total sugar, and dry matter ( $r = 0.86$ ).

It has been speculated that the more pungent the onion, the higher the potential of health benefits, because of the strong concentration of the volatile sulfur compounds in these varieties (LEE et al., 2015). In our results, however, we found that the most pungent onion “Birnförmige” did not have the highest antioxidant levels. This confirms the results of LEE et al. (2015), who observed only a low positive correlation between antioxidant activity levels and enzymatically produced pyruvic acid ( $r = 0.25$ ). This could be due to phenolic compounds, which were found to be the primarily responsible for the antioxidant activity. The positive correlation between total phenolics and antioxidant activity is already known from previous studies (ASAMI et al., 2003; CHENG et al., 2013; CHU et al., 2002). In our study we were also able to confirm that phenolics represent a considerable part of the total antioxidant activity ( $r = 0.76$ ).

The elemental composition of the edible onion parts has not been thoroughly studied so far. Nevertheless, there are studies demonstrating a correlation among minerals and trace elements (RODRÍGUEZ GALDÓN et al., 2008) but no information is available regarding the correlation between minerals and other quality compounds in onions. Interestingly, significant positive correlations were found between calcium, magnesium and reducing sugar ( $r = 0.68, 0.52$  respectively) and significant negative correlations between calcium, magnesium and fructans ( $r = -0.57, -0.64$  respectively). A significant negative correlation could also be confirmed between calcium, magnesium and pyruvic acid, which would mean that obtaining sweeter onion through calcium or magnesium fertilization might be a possibility.

#### Which varieties are better suited for organic farming in South-Germany?

Our results demonstrated that, with the exception of the variety “Jaune des Cévennes”, most of the researched varieties adapt well to the climate conditions of South West Germany. For example, the variety “Birnförmige” produced an acceptable yield with onions free from bolting and diseases, good firmness, high concentration of fructan, pyruvic acid, dry matter and sucrose in less than 160 days. “Jaune des Cévennes”, despite the high yields, exhibited high bolting percentages and a large number of unmarketable onions, with high risk of diseases and disorders. As ZEVEN (1998) mentioned in his article: “Landraces are a population which naturally developed in a certain region under the influence of regionally prevailing conditions of climate, soil management, without or with little mass selection”. This allows us to speculate why the old French variety “Jaune des Cévennes”, which comes from the mountainous region of The Cévennes (elevation 1700 m) and different climate conditions, may be better suited for planting on higher altitude terraces than the region of Stuttgart (elevation 245 m) in the South-West of Germany. Through the study a clear trend was established where most of the varieties and old open-pollinated cultivars from South-Germany performed similar or better compared to the commercial cultivars (Sturon and Red Baron) in both quality parameters and yield, therefore demonstrating the potential to provide an important contribution to the regional organic farming system.

#### Conclusions

The focus of this study is the analytical characterization of local open-pollinated onion types, while comparing them against commercial, well-established varieties and examining their agricultural, morphological and nutritional traits.

Qualitative and quantitative analytical differences that were detected among the varieties allowed a clear discrimination and identifications of the cultivars particularly suitable for organic farming based on their agricultural and quality performances. High variability and correlations found in the examined traits point out that these onion

varieties could be well-suited candidates for future studies. Yield value from the old open-pollinated varieties were significantly higher than control varieties Sturon and Red Baron, indicating that the maximum yield potential of the control varieties has not yet been reached and an optimization of their yield could be attained through breeding programs. Among the 11 varieties, Birnförmige is the most promising in terms of quality, aroma and flavor. In contrast, the cultivar Jaune de Cévennes showed poor quality and small concentrations of sugar and pyruvic acid.

Nonetheless, further study of molecular and metabolome characterization should be conducted. Morphological, analytical and agricultural research accompanied with molecular and metabolome characterization will result in broader dataset, able to clarify the genetic variability within and among the varieties.

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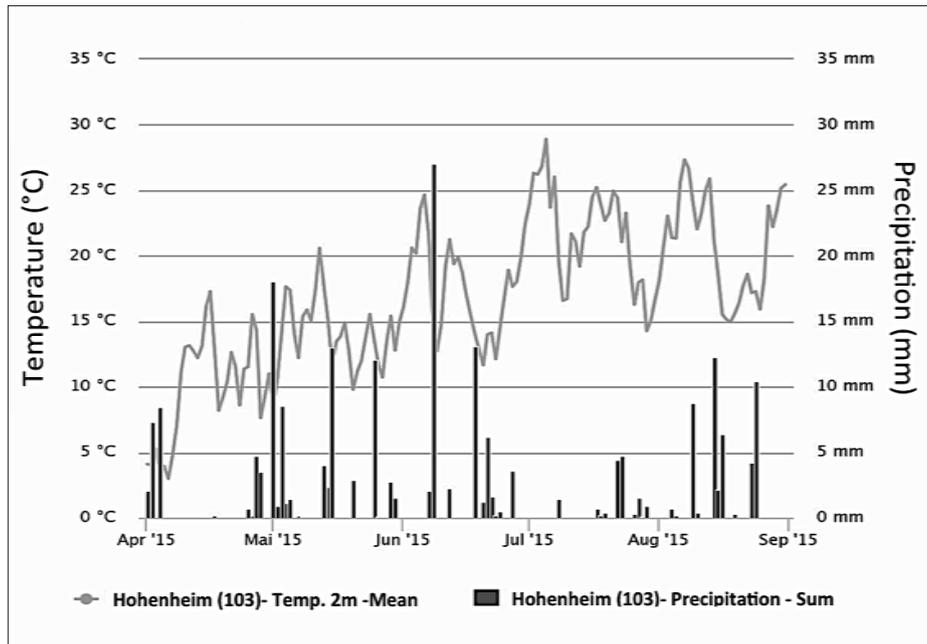
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## Supplementary material



**Fig. S1:** Daily mean precipitation and temperature over the main onion growing season in Kleinhohenheim, Stuttgart, Germany. Source: Agrarmeteorologie Baden-Württemberg.