Characteristics of Garlic of the Czech Origin

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Abstract

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We chose and evaluated the chemical characteristics of garlic of the Czech origin. The suggested quality indicators based on the measured values and the data from the literature were as follows: colour (white variety): L^* (brightness) > 90; firmness > 50 N (6-mm tip); pungency > 35μmol of pyruvate/g; moisture 55–70%; soluble solids > 30 °Brix; bulbs dimensions medium and large; the content of alliin > 2 g/kg.

Keywords: Czech garlic; quality indicators; morphology; pungency; sensory analysis

Garlic (*Allium sativum* L.), one of the oldest cultivated crops, is widely used around the world for its characteristic flavour as a seasoning or condiment. Garlic is a rich source of phytonutrients, hence contributing to treatment and prevention of a number of diseases, such as cancer, obesity, cardiovascular diseases, diabetes, hypercholesterolemia, hypertension, etc. (Lanzotti 2006; Pardo *et al.* 2007).

Biologically active substances of garlic can be divided into two main groups (Tenney 1995; Lanzotti 2006; Ma *et al.* 2011): (a) sulphur compounds and (b) sulphur-free active substances. Sulphur compounds, such as allicin, alliin, and ajoene, are a very important group of flavour compounds; the active substance of garlic, alliin, is sensorily inactive and biologically ineffective, after cell damage it is, however, enzymatically cleaved to form the characteristically smelly and effective garlic essential oil allicin. Allicin gives garlic its antibiotic properties and is responsible for garlic's strong odour; it is unstable (when exposed to air or at elevated temperatures) and degrades into various sulphur compounds, often volatile ones (monosulphides, disulphides and trisulphides), most of which contribute to the characteristic garlic odour. The group of sulphur-free active substances includes anthocyanins, flavonols, antibiotics garlicin, allistatin, adenosine, sapogenins, and saponins.

The current quality standard for garlic is defined by Commission Regulation (EC) No. 2288/97 laying down marketing standards for garlic. A high quality garlic bulb should be tight and firm to touch; clean, intact, with no fungus, visible germs, and off-flavours. However, the Regulation does not provide for other important quality parameters such as soluble solids, pungency and sensory characteristics, which are important from the aspect of consumer preferences.

The worldwide production of garlic is about 23.7 mil. t, with China being the biggest producer (19.2 mil. t), followed by India and Egypt (1.1 and 0.3. mil. t, respectively) (FAO 2011). Garlic growing in the Czech Republic has been traditional and quite important, with the annual production amounting to 8177 t (in 1997) (FAO 2011); and both

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experts and consumers have assessed positively the garlic originating from the Czech Republic based on its strong aroma and taste. In recent years, however, the amount of imported garlic has increased significantly, which has reduced domestic prices and affected farmhouse management, leading to a decline in domestic garlic production to 322 t/year (in 2011) (FAO 2011) contrary to the import of around 4000 t from abroad (mostly from China) (Ministerstvo zemědělství České republiky 2011).

To support consumer trust in local garlic: (a) proper labelling of the origin of garlic needs to be enforced more thoroughly; (b) objective data proving the physicochemical quality of Czech garlic cultivars need to be collected. The present study aims to determine relevant qualitative parameters for a set of garlic samples from different production areas in the Czech Republic, EU countries, and non-European countries and assess limits of the selected quality indicators of garlic to promote a local development strategy for garlic production.

MATERIAL AND METHODS

Plant material. A preliminary study was carried out with 10 selected garlic cultivars (harvest 2011) of defined origin from different production areas in the Czech Republic (i.e. sample I – cv. Benátčan, samples II and III – cv. Džambul), in the EU countries (sample IV – Slovak Republic, V – Spain, VI – Hungary, and VII – Italy), in non-Europe contries (sample VIII – China, sample IX – Argentina, and sample X – Egypt).

The study continued in the next year with the analysis of 22 samples of Czech origin (harvest 2012). The samples were provided by the company Česnek od pěstitele s.r.o (Prague, Czech Republic). The information about the varieties and production areas of the 22 samples are shown in Table 1.

All samples were provided by the company Česnek od pěstitele s.r.o. and they were stored for 2–3 months at 6.9 ± 1°C, 55% RH (relative humidity) in the dark in a refrigerator until they

Table 1. Characterization of garlic of the Czech origin (2012 harvest)

Sample No.	Locality	Variety	Moisture (%)	Pungency (μmol/g)	Alliin (g/kg)	Volatile compounds (g/kg)	Diallyl disulphide (%)	Sensory analysis (aroma pungency)
1	Zlonice	Krajová	55.0	15.5	3.5	0.5	86.0	2.7
2	Roudnice nad Labem	Sibiřák	60.3	51.1	6.6	1.2	71.1	3.5
3	Brozany nad Ohří	Krajová	60.5	36.9	5.2	0.1	71.4	3.4
4	Kostomlaty pod Řípem	Vekan	60.0	34.8	5.0	0.1	82.3	3.2
5	Louny	Krajová	61.3	50.4	5.8	0.1	80.6	3.5
6	Brozany nad Ohří	Krajová	59.5	44.1	4.6	0.1	76.6	3.5
7	Vratislavice nad Nisou	Vekan	61.9	28.0	4.3	<0.1	80.6	3.1
8	Šimonovice	Vekan	61.7	52.5	3.5	0.1	87.3	3.2
9	Znojmo	Krajová	59.9	62.7	6.4	0.1	77.3	3.5
10	Lkáň	Krajová	60.6	30.4	4.9	0.4	92.2	3.5
11	Trutnov	Krajová	68.6	28.3	3.3	0.7	85.7	3.0
12	Klobouky u Brna	Havran	66.0	42.9	6.6	0.7	74.4	3.6
13	Hrubá Vrba	Benátčan	60.8	36.9	5.5	0.3	88.9	3.5
14	Lipovec	Krajová	60.5	32.5	4.4	0.6	86.2	3.1
15	Drnovice	Krajová	62.7	35.7	3.2	0.7	80.9	3.0
16	Kameničná	Bjetin	61.6	30.0	2.9	0.2	84.8	2.9
17	Vojničky	Krajová	59.9	31.5	5.4	0.3	86.3	3.2
18	Troskotovice	Krajová	60.9	49.6	6.2	0.4	69.9	3.5
19	Praha	Vekan	61.5	52.7	5.7	0.6	67.9	3.6
20	Uherské Hradiště-Bílovice	Bjetin	59.0	43.7	5.9	0.1	79.8	3.5
21	Slaný	Goulurouse	65.6	30.2	3.8	0.6	78.7	3.0
22	Morkovice	Krajová	58.6	37.4	5.3	0.2	67.4	3.4

Krajová = local variety; (%) = relative representation of the content of volatile substances

were analysed. All bulbs were fresh and of the same maturity.

Methods. For the analysis, 0.5 kg of each sample was used. For each sample, 10 cloves were independently sampled from 5 bulbs (2 cloves from one bulb). Three independent measurements were performed for each parameter.

Clove weight. The garlic bulbs were separated into cloves, peeled by hand and weighed on an analytical balance (Kern ABJ; Unipro Alpha, Balingen, Germany).

Clove length, projected area and colour. The peeled garlic cloves were scanned (HP Scanjet 5470c scanner and HP PrecisionScan Pro 3.1 software; HP, Palo Alto, USA) and the final image was evaluated by NIS Elements AR 2.30 program (Nikon Instruments Inc., Tokyo, Japan). Clove length, colour, and projected area were analysed (Haciseferoğullari *et al.* 2005).

Firmness. Firmness was determined by measuring the compression force on the cloves until the breaking point. A ripper (Instron 4, Series 5544; Merlin Instron software; Instron Engineering Co, Bucks, UK) was used for the penetration analysis (6 mm flat tip, rate 10 mm/min) (Haciseferoğullari *et al.* 2005; Pardo *et al.* 2007).

Soluble solids. The peeled garlic cloves were pressed by a manual kitchen garlic press. The total soluble solid content (°Bx) was determined using a digital refractometer previously calibrated with distilled water (301-95; A. Krüss Optronic GmbH, Hamburg, Germany) (Pardo *et al.* 2007).

Moisture. 5 g of pressed garlic was mixed with sea sand and then dried at 105°C for 4 h (UFB 40 drying machine; Memmert, Büchenbach, Germany). After cooling, the sample was weighed on an analytical balance with the accuracy of 4 decimal places (Kern ABJ; Unipro Alpha, Baalingen, Germany) (Pardo *et al.* 2007).

Pungency (pyruvate analysis). 5 g of the pressed garlic was homogenised in a shaker (2 min) and adjusted to 1000 ml with distilled water. 2 ml of the filtrate and 1 ml of 2,4-dinitrophenylhydrazine (DNPH; 0.125 g/l) solution in 2M hydrochloric acid were pipetted into reaction tubes; for a blank sample 2 ml of distilled water and 1 ml of DNPH; for calibration 2 ml of the calibration solution of sodium pyruvate and 1 ml of DNPH. To determine the content of naturally occurring pyruvate and carbonyl compounds, which also react with DNPH, a control sample was prepared by heating 5 g of garlic in a microwave oven for 30 s at 650 W, the sample preparation described above followed. All tubes were homogenised (Grant-Bio PV-1 Vortex Mixer; Grant Instruments, Ltd., Cambridge, UK) and left to incubate in a water bath (37°C, 15 min; WB 22 water bath; Memmert GmbH and Co KG Büchenbach, Germany). Then 5 ml of 1.5M sodium hydroxide was added to the tubes and mixed. The pyruvate content was determined using a spectrophotometer at 420 nm (Thermo Spectronic Genesys 20; ThermoFisher Inc., Waltham, USA) (Schwimmer *et al.* 1961).

Determination of alliin content. About 10 g of the peeled garlic cloves was weighed in a tall 100-ml beaker. 50 ml of boiling distilled water was added to the sample and the mixture was boiled for 15 minutes. Then, the sample was cooled and homogenized for 3 min in a mixer (T18 Basic Ultra-Turrax; IKA Staufen, Baden-Wurttemberg, Germany). The homogenised sample was transferred into a 100 ml volumetric flask and adjusted with distilled water. Afterwards, 2 ml of the solution were centrifuged at 4°C at 16 000 rpm for 10 minutes. 1 ml of the supernatant was then pipetted into a 10 ml volumetric flask and adjusted with a mobile phase. After filtration through a PTFE 0.45 microfilter, the sample was injected into a liquid chromatograph (Agilent 1290 Infinity LC; Afilent Technologies, Inc., Santa Clara, USA). The chromatographic conditions were as follows Macherey-Nagel Nucleosil 100-5 NH_2 column (250 × 4.6 mm, 5 µm), flow rate 1 ml/min, mobile phase acetonitrile water (52/48 v/v), column temperature 30°C, detection DAD, wavelength 210 nm, external calibration, verification of the analyte by UV spectrum (Dethier *et al.* 2012).

Volatile compound analysis. The samples were peeled and cut into thin slices and immediately weighed into 10 ml vials (0.05 g) containing 5 ml of distilled water and 0.5 µl of an internal standard solution (benzyl methyl sulphide). The sample prepared in this way was placed into an autosampler and analysed by SPME-GC/MS (Agilent 7890A/5975C; Agilent Technologies, Inc., Santa Clara, USA). The chromatographic conditions were as follows: SPME fibre (50/30 µm, DVB/Carboxen/PDMS Stable Flex), pre-incubation time: 60 s, incubation temperature 30°C, extraction time 180 s, desorption time: 300 s; HP-5MS column (Agilent Technologies, Inc., Santa Clara, USA) 30 m × 0.25 mm × 0.25 µm; temperature program: 50°C for 3 min, 5°C/min at 210°C, run

time 35 min, inlet temperature 250°C, MS detector temperature 280°C, the carrier gas was helium at the flow rate of 1 ml/minute. Identification of volatile compounds was conducted by comparison with the mass spectra library (NIST). Quantification was performed using the internal standard (Clemente *et al.* 2011).

Sensory analysis. Sensory evaluation was performed by 10 panellists from the Department of Food Preservation (ICT Prague, Czech Republic). The panellists evaluated overall visual quality, aroma and taste using a 5-point system, where 1 was the worst and 5 the best rating (hedonic scoring; 5 = excellent, 4 = very good, 3 = good, 2 = fair, and 1 = poor).

The aroma was evaluated immediately after slicing the cloves. The taste was evaluated comparing toasts with rubbed garlic; water was used as a taste neutraliser (Pardo *et al.* 2007).

Sensory evaluation was performed twice a day. During one session, the panellists evaluated four samples.

Statistical analysis. The tests were carried out three times for each sample and the mean values are reported. The statistical analyses (ANOVA, cluster analysis, correlation matrix) were performed using the Statistica 8.0 statistics programme (StatSoft ČR, Prague, Czech Republic).

RESULTS AND DISCUSSION

Consumers prefer white bulbs (or other colours typical of the particular variety), cloves firm to touch, with high dry weight and soluble solids content (> 35% in both cases); characteristic pungency, typical external appearance and size (minimum diameter for the fresh market is about 4 cm) are required (Commission Regulation No. 2288/97 1997; USDA 1997). According to this, the relevant qualitative parameters were determined for our set of 10 garlic samples from different production areas (harvest 2011). The basic qualitative and morphological markers of garlic such as colour, size, firmness, soluble solids, moisture, pungency, alliin content and the content of characteristic volatile substances were analysed, together with sensory evaluation. This set was used for the optimisation and validation of methods, for the selection of suitable parameters and verification of the situation in the market. Based on these results, a wider study of a larger and consistent set of samples of the Czech origin harvested in 2012 was then conducted.

Basic qualitative parameters

The analysed morphological features (clove weight, length, projected area, and colour) are strongly affected by genetic and environmental factors, so they are perfect for evaluating individual bulbs and cultivars, but they can hardly be used for classification according to the origin (Table 2).

According to the literature, the colour of cloves of white garlic varieties ranges for L^* (lightness) value from 50.8 to 87.5, for a^* (redness), and b^* (yellowness) from –0.3 to 23.4 and from 4.9 to 14.7, respectively (Pardo *et al.* 2007). Our measured L^* values were higher for all samples (89.5–94.6), i.e. the samples were lighter: a^* and b^* ranged from –1.2 to 0.2 and from 2.8 to 10.8, respectively. Another significant characteristic of garlic is its adequate firmness, which can be objectively assessed by a penetration test. Our results ranged from 40.8 N to 69.4 N using a 6-mm tip. The data mentioned in the literature (Pardo *et al.* 2007) for Spanish garlic ranged from 62.5 N to 86.0 N for a 6.4-mm tip.

The values of soluble solids for our samples ranged from 31.6 °Brix to 38.7 °Brix. In comparison with data from the literature (Pardo *et al.* 2007), which range from 25.1 °Brix to 29.4 °Brix, our values were about 10 °Brix higher.

The values of moisture ranged from 62.5% to 68.7% and were comparable with data from the literature (Pardo *et al.* 2007), which range from 59.3% to 66.2%.

Active components

The pungency (pyruvate analysis) evaluation was based on the detection of pyruvate released by the enzyme alliinase after damage to the garlic tissue. In the literature, the values for garlic from Argentina were found to range from 52.3 µmol/g to 88.3 µmol/g (Poldma *et al.* 2011). Our data, which ranged from 7.6 µmol/g to 78.4 µmol/g, were lower in most cases.

The determined average content of alliin was 4.9, 5.2, and 8.2 g/kg for the Czech, European and non-European samples, respectively. The values ranged from 2.9 g/kg to 6.6 g/kg and

Table 2. Summary of the measured values for all garlic samples (2011 harvest)

Parameters	Czech samples (I–III)		European samples (IV–VII)		Non-European samples (VIII–X)	
	mean	SD	mean	SD	mean	SD
Clove weight (g)	6.4	3.2	5.4	2.7	4.7	1.5
Clove length (mm)	29.9	6.4	29.2	5.7	28.1	3.2
Projected area (mm^2)	493.3	194.2	449.0	160.3	413.1	90.4
Lightness L^*	91.1	0.8	90.8	0.8	91.5	2.8
Redness a^*	–0.4[a]	0.7	–0.6	0.6	–0.9[a]	0.2
Yellowness b^*	7.6	4.3	5.6[b]	3.2	9.3[b]	0.8
Firmness (6-mm tip) (N)	56.4	5.7	53.9	7.0	54.6	10.6
Soluble solids (°Brix)	34.9	2.5	35.5[b]	2.1	37.6[b]	0.4
Moisture (%)	66.4	1.3	65.6	1.9	66.4	1.8
Pungency (μmol/g)	47.0	22.9	39.5	24.7	25.8	13.0
Alliin (g/kg)	4.9[a]	1.2	5.2[b]	1.5	8.2[a,b]	2.8
Volatile compounds (g/kg)	0.4[a]	0.3	0.5[b]	0.6	1.8[a,b]	1.3

I–III – Czech Republic; IV – Slovak Republic, V – Spain, VI – Hungary, VII – Italy, VIII – China, IX – Argentina, X – Egypt; ANOVA analysis: [a]significant differences (at α = 0.05) between Czech and non-European samples; [b]significant differences (at α = 0.05) between European and non-European samples; SD – standard deviation

corresponded to the data from the literature, i.e. with the range of 1.6–11.4 g/kg (Kubec *et al.* 1999; Horníčková *et al.* 2010, 2011). The relationship between enzymatically produced pyruvate and the content of alliin was not very close (r = 0.73), but statistically significant at the 0.05 level.

The content of volatile substances was the most variable parameter, both among different samples and for the same garlic bulb (0.1–0.7 g/kg for the Czech samples, 0.1–2.3 g/kg for the European samples, and 0.2–4.3 g/kg for the non-European samples). Then the most abundant components, which represented more than 98% of the total in all samples, were identified. For quantification, the response factor was calculated only for diallyl disulphide (DADS), which accounted for about 80% of volatile components; for other compounds we assumed that the factor was the same to simplify the situation. No correlation between the volatiles and the content of other active components was found out, and the inconsistency in results which was recorded can be explained firstly by the impact of the experimental conditions, which resulted in poor reproducibility (RSD of the method ranged from 5% to 30%). Secondly, it is related to the fact that volatile compounds are the final degradation products, whose concentrations are affected by the concentration of alliin, the enzyme activity and the kinetics of the production of primary degradation products. And whereas some authors were able to find a close correlation between aliphatic sulphides and pungency determined by a pyruvate analysis (Alcala *et al.* 1998), other authors suggested that the formation of disulphides was unstable, increased with time and occurred after the formation of pyruvic acid (Schwimmer & Guadagni 1968). Thirdly, as Block (1992) and other authors already suggested, the majority of sulphur volatiles identified by the gas chromatography are thus artefacts produced during the isolation of the samples and decomposed at the high temperatures of the chromatographic system. On the other hand, the identity/structure of volatile sulphur compounds was relatively stable; the main components were diallyl disulphide (78%), (*Z*)-1-propenyl allyl disulphide (15%), allyl methyl disulphide (4%). Allyl mercaptane, allyl methyl sulphide, dimethyl disulphide, diallyl sulphide, methyl propyl disulphide, methyl propenyl disulphide and (*E*)-1-propenyl allyl disulphide were also identified in conformity with the literature (Clemente *et al.* 2011).

Sensory analysis

All garlic samples were sensorily analysed, the purpose was to determine whether the samples differ from each other sensorically. For the evaluation of aroma, taste and overall visual quality, hedonic evaluation was applied (verbal descriptive scale

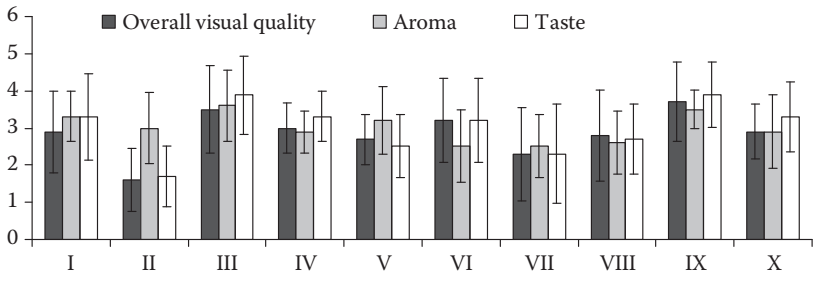

Figure 1. Hedonic sensory analysis of the garlic samples

I–III – Czech Republic; IV – Slovak Republic, V – Spain, VI – Hungary, VII – Italy, VIII – China, IX – Argentina, X – Egypt

completed grade: 5 – strong aroma and taste, excellent quality).

In the overall comparison of all partial sensory evaluations (Figure 1), the aroma of samples was average except of Czech Republic I and Czech Republic III, whose aroma was evaluated as strong. Two samples had weak taste (Czech Republic II and Italy). Surprisingly, probably due to the insufficient sensorial sensitivity of the panel, no correlation (r = 0.57) was obtained when enzymatically formed pyruvate was related to pungent taste.

Characterisation of samples of the Czech origin

To compare the obtained results (Table 2) with the properties of a consistent set of garlic samples of the Czech origin, 22 samples from 2012 harvest were analysed (Table 1). The group included 4 registered and 3 non-registered varieties; more than half of the samples (54.5%) were local varieties. Only the parameters directly linked to the characteristic flavour were determined (olfaction was evaluated according to the previous experience), i.e. alliin content (average content 4.9 g/kg), pungency (mean value 39 μmol/g), volatile compound content (0.4 g/kg), relative distribution of DADS (79.8%), and moisture content (61.2%). The measured values, except moisture, which was on average lower, correspond fully to the values of the samples from 2011 harvest.

The correlation between the analysed parameters related to pungency is summarised in Table 3. From these results it is evident that aroma pungency is influenced mostly by the concentration of alliin (r = 0.86) and pyruvate (r = 0.78). The absemce of correlation between the volatiles content and composition and the rest of the analysed parameters is remarkable, considering the reasons mentioned above, the methodology of determination of volatile compounds in our set is not appropriate for this purpose.

CONCLUSION

The suggested quality characteristics of garlic of any origin (including Czech) based on the measured values and data from the literature are as follows:
Colour (white variety): brightness (L^*) higher than 90
Firmness: higher than 50 N (6 mm tip)
Pungency: higher than 35 μmol/g
Moisture: 55–70%
Soluble solids: higher than 30 °Brix
Bulb dimensions: medium and large
Content of alliin: more than 2 g/kg

Table 3. Correlation of the analysed parameters with pungency

	Moisture (%)	Pungency (μmol/g)	Alliin (g/kg)	Volatile compounds (g/kg)	Diallyl disulphide (%)	Sensory analysis (aroma pungency)
Moisture (%)	1.00					
Pungency (μmol/g)	0.00	1.00				
Alliin (g/kg)	–0.17	**0.58**	1.00			
Volatile compounds (g/kg)	0.34	–0.04	0.06	1.00		
Diallyl disulphide (%)	0.02	–0.22	–0.51	–0.15	1.00	
Sensory analysis (aroma pungency)	–0.04	**0.78**	**0.86**	–0.08	–0.47	1.00

Bold values = significant correlation on the level α = 0.05; (%) = relative representation of the content of volatile substances

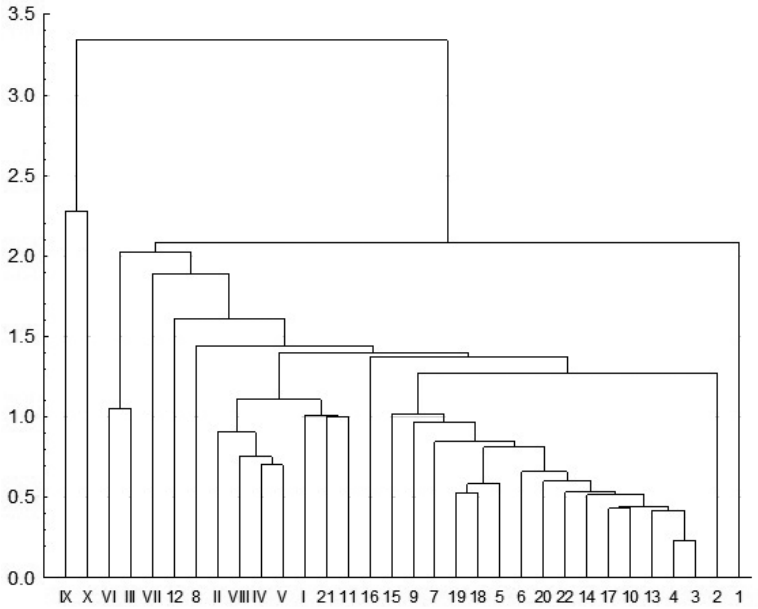

Figure 2. Dendrogram of all examined samples of garlic (variables involved: alliin, pungency, volatile compounds, and moisture)

Based on the multivariate analysis (Figure 2), no statistically significant differences among the samples of different geographic origin were found. It can be assumed that (a) the set of samples was too small to reflect a difference and (b) the values of the qualitative parameters were influenced to a larger extent by variety, year of harvest and conditions of growth than by geographic origin. The results of this study are planned to be confirmed by the analysis of a set of samples differing in the year of harvest and conditions of cultivation.

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