

### THE USE OF TARGETED ANALYSIS TO VERIFY THE GEOGRAPHICAL ORIGIN OF GROUND PAPRIKA

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#### ABSTRACT

This study investigated the use of targeted analysis to verify the geographic origin of ground paprika samples. In total, 36 samples were analyzed from all over the world (17 from Europe, 17 from Asia, one from North America, and one from Africa). Deliberately chosen methods that use equipment commonly available in control laboratories were used for the analysis. The samples were examined for total fat content, total protein content, sugar concentration, the concentration of selected elements, ASTA coloring, ash content, and pH of the extract. The results were processed using multivariate analysis, which showed that, based on these investigated parameters, it is possible to differentiate ground paprika samples from different regions. In addition to being able to separate samples originating from Asia from those originating from Europe, it was also possible to distinguish samples from Europe with protected geographical indications, such as paprika from Hungary, Spain, and Slovakia.

**Keywords:** paprika, geographical origin, targeted analysis, authentication, spice

#### INTRODUCTION

In its basic form, paprika is made up of ground dried bell peppers (*Capsicum annuum*). The name 'paprika' comes from the Hungarian word 'paprika', which is derived from the Latin word 'piper'. However, the pepper is native to South America. After originating in America, paprika was brought to Spain in the 16th century, mainly for use as a decorative plant before it became a popular spice. The commerce in paprika spread from the Iberian Peninsula through Africa and Asia, eventually reaching Central Europe via the Ottoman-controlled Balkans (Govindarajan, 1986). Paprika has many uses in food processing, including as a food colorant, a source of pungency, flavor, and texture (Berke and Shieh, 2012). The world production of sweet paprika spice is about 200 thousand Mt annually. Although worldwide paprika production is relatively stable, the global paprika market grows yearly and was valued at USD 433 million in 2018 (Govindarajan and Salzer, 1985; GVR, 2019). High demand and a developed paprika trade are the main reasons that paprika is often an attractive target for adulteration. Food adulteration occurs mainly through changes in the food's composition or false information on the product's label (Hong et al., 2017). In the case of paprika, foreign substances such as brick dust, tomato skins, or synthetic dyes are mixed into the paprika powder to increase the weight or improve the product's basic properties. Mixtures of higher and lower-quality spices are also often marketed. Misleading the consumer by providing false information on the packaging is most often caused by incorrect indication of the origin of the paprika or misuse of the protected designation. In recent years, several studies have addressed verifying the geographical origin of paprika (Brunner et al., 2010; Monago-Marana et al. 2021; Fiamegos et al., 2021; Barbosa et al., 2020). However, specific methods that are not available in routine control laboratories, such as isotope ratio inductively coupled plasma mass spectrometry or ultrahigh-performance liquid chromatography tandem mass spectrometry, have been developed to detect paprika adulteration. Ceto et al. (2020) used high-performance liquid chromatography with an ultraviolet detector for the non-targeted analysis of paprika samples and drew attention to the lack of low-cost analytical methods applicable to assess the authenticity of paprika. The aim of the present study is to verify whether targeted analysis of paprika using well-known chemical and readily available instrumental methods can be helpful in determining the geographical origin of paprika.

#### MATERIAL AND METHODS

##### Samples

Paprika samples were purchased from the Czech sales network or delivered by Worlée NaturProdukte GmbH (Hamburg, Germany). A total of 37 samples from 10 countries were analyzed in this study (Table 1). Sample processing for individual chemical analysis is described in detail in the previous pilot study

(Štursa et al., 2018). All samples were mineralized in a microwave oven in the presence of 5 ml of nitric acid and 2 ml of hydrogen peroxide prior to analysis on an optical emission spectrometer with inductively coupled plasma. Sample weight for microwave decomposition was 100 mg. To extract the sugars, 1 gram of the sample was stirred for 30 minutes with 10 ml of hot 50% ethanol solution. After pouring the extract into a volumetric flask, a repeated 30-minute extraction of the sample was performed with another 10 ml of hot 50% ethanol solution. Before analysis using instrumental techniques, the sample was filtered through a nylon syringe filter with a pore size of 0.45 µm.

##### Chemical and instrumental analysis

The following standardized methods were used for the analysis: ISO 7540:2020 (total lipids content), ISO 1871:2009 (total proteins content), ISO 7541:2020 (determination of the extractable colour), ISO 10523:2008, ISO 763:2003 (ash content) and ISO 1842:1991 (determination of pH). Instruments used during this study includes: spectrophotometer Helios Gamma (Spectronic Unicam, USA), pH meter with combined SenTix electrode (WTW, Germany), Kjeldahl digester (Kjeldatherm, Gerhard, Germany), steam distillation apparatus (Vapodest, Gerhard, Germany), Soxhlet extractor (Soxtherm, Gerhard, Germany), microwave oven (Milestone 1200, Milestone, Italy), muffle furnace (LAC LE 0511, LAC, Czech republic) and hot air oven (Memmert UFE550, Memmert, Germany). Analysis of glucose, fructose and sucrose was performed on high performance chromatograph with evaporative light scattering detector (Agilent Infinity 1260, Agilent Technologies, USA) according to the method described in Štursa et al. (2018). This method uses 5µm hydrophilic polymeric gel particles with an aminopropyl functional groups as a stationary phase and a mixture of acetonitrile and water (75:25 volume ratio) as a mobile phase. The concentration of individual elements was determined using an optical emission spectrometer with inductively coupled plasma (Ultima 2, Horiba Scientific, France) according to the method described by Diviš et al. (2015). All analyzes were performed in triplicate. Mineral content, the content of glucose, fructose, sucrose, TLC and TPC were expressed as dry weight.

##### Statistical analysis

All experimental data were statistically processed using software XLstat (Addinsoft, USA). Principal component analysis was used to reduce the entire dataset to a small number of principal components and for visualization of data. The heat map and cluster analysis were created in the ClustVist application (Metsalu and Vilo, 2015).

**Table 1** List of paprika samples investigated in this study.

Country of origin	Region of origin	PDO	Sample
Marocco	Rabat-Salé-Kénitra	no	AF-MAR
China	Gansu Sheng	no	A-CHN.1
China	Guizhou Sheng	no	A-CHN.2
China	Jiangsu	no	A-CHN.3
China	unknown	no	A-CHN.4
China	Anhui Sheng	no	A-CHN.5
China	Jiangsu	no	A-CHN.6
India	Andhra Pradesh	no	A-IND.1
India	unknown	no	A-IND.2
India	unknown	no	A-IND.3
India	Karnataka	no	A-IND.4
India	Karnataka	no	A-IND.4
India	Gujarat	no	A-IND.5
India	Rajasthan	no	A-IND.6
India	Maharashtra	no	A-IND.7
India	unknown	no	A-IND.8
Uzbekistan	unknown	no	A-UZB
USA	California	no	AM-USA
Turkey	Malatya	no	A-TUR.1
Turkey	unknown	no	A-TUR.2
Spain	La Vera	yes	E-ESP.1
Spain	La Vera	yes	E-ESP.2
Spain	La Vera	yes	E-ESP.3
Spain	Murcia	yes	E-ESP.4
Spain	unknown	no	E-ESP.5
Hungary	Kalocsa	yes	E-HUN.1
Hungary	Kalocsa	yes	E-HUN.2
Hungary	unknown	no	E-HUN.3
Hungary	unknown	no	E-HUN.4
Hungary	unknown	no	E-HUN.5
Hungary	Szeged	yes	E-HUN.6
Hungary	Szeged	yes	E-HUN.7
Slovakia	unknown	no	E-SVK.1
Slovakia	Danubian lowland	yes	E-SVK.2
Slovakia	Danubian lowland	yes	E-SVK.3
Bulgaria	unknown	no	E-BUL
Romania	Transylvania	no	E-ROM

## RESULTS AND DISCUSSION

### Moisture, ash, pH of the extract and ASTA value

Increased moisture in paprika leads to loss of flavor and promotes clumping and mold growth, resulting in deterioration. Moisture content among the analyzed samples ranged from 7.3 to 18.4% ( $\pm 0.1\%$ ). The average moisture content of paprika is 11.2%, according to the American nutrition database (ANDB). The moisture content of most samples corresponds to the tabulated values. However, higher moisture was found in samples from China (12.7-18.4%), Uzbekistan (18.1%), and Bulgaria (15.0%). The ash content indicates the amount of inorganic compounds in paprika. Excessive ash content may suggest adulteration with foreign substances. The ash content of the analyzed samples varied from 4.7 to 8.3% ( $\pm 0.2\%$ ), which is consistent with the results of Lee *et al.* (2017) and Zaki *et al.* (2013). The pH value of the paprika extract can provide information on the content of organic acids in the paprika, which is related to the quality and degree of ripeness of the raw material for powdered paprika production. The pH of the paprika extract ranged from 4.13 to 5.28 ( $\pm 0.05$ ), similar to the analyses performed by Lee *et al.* (2017) and Zaki *et al.* (2013). The ASTA value indicates the total content of carotenoid pigments in paprika and is a significant factor in determining the final price of the product. The addition of artificial dyes often adulterates paprika. The ASTA value of paprika samples analyzed in this study varied between 43 and 186 ( $\pm 5$ ). In general, high ASTA values were typical for Hungarian and Indian paprika, while samples from China showed below-average ASTA values. On average, the ASTA value of the paprika samples examined in this study

matched the results published by other authors (Zaki *et al.*, 2013; Molnár *et al.*, 2018).

### Total lipid and protein content

The fat content is an important parameter that can reveal the artificial treatment or adulteration of paprika. Paprika is a good source of oleoresin, primarily used as a coloring or flavoring in food products. Once this oleoresin is removed from paprika, the remaining defatted product has limited use and a low price (Galvin-King *et al.*, 2020). This lower-quality product is often used in culinary applications where the color of the product is not the essential parameter, or it can be mixed with another type of paprika, which is considered adulteration. As shown in Table 2, below-average fat content was found in all paprika samples from China and the sample from Uzbekistan. Given that all these samples also showed a low ASTA value (Table 2), it is likely that they were defatted and can be classified as spent paprika. The other samples had a fat content in the range of 6.3-17.5% ( $\pm 0.3\%$ ), corresponding to the average value of 12.9% indicated by ANDB. The protein content in paprika powder depends on the variety of processed *Capsicum annum* plant, the technique used to grow this plant, and the climatic conditions of the locality where the plant is grown. The total protein content ranged between 11.9 and 22.0% ( $\pm 0.1\%$ ). Higher protein content was found in the group of samples originating in India, while in other samples, the protein content corresponded to the average tabulated value of 14.1% in the ANDB (Table 2).

### Fructose, glucose and sucrose content

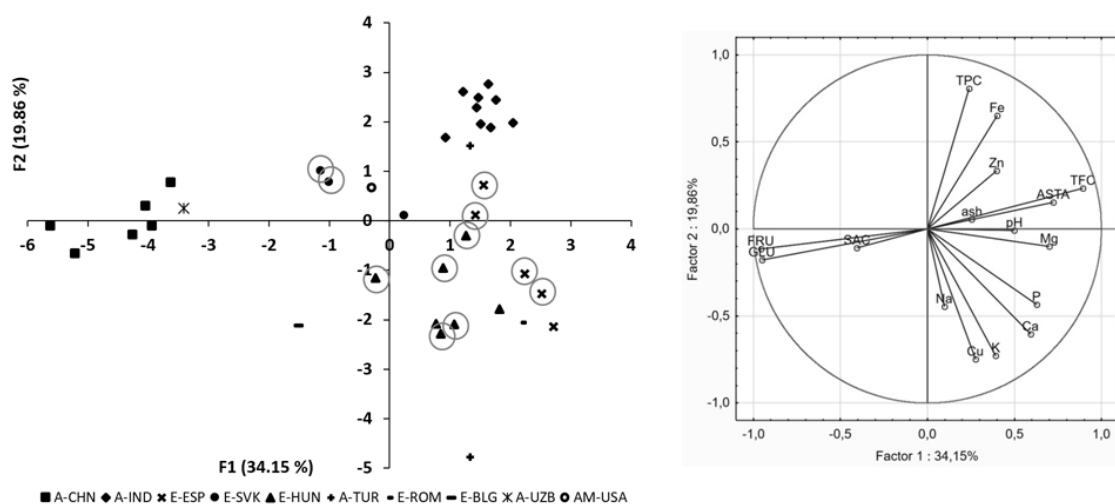
The concentration of sugars depends on the variety of grown plants and the degree of maturity of harvested fruits. It can also be related to climatic conditions in the cultivation site, and, as a result, it can reflect the geographical origin of paprika unless artificially sweetened. The average concentration of individual sugars in the examined paprika samples ranged from 0.11 to 126 mg/g (Table 2) and decreased in the order of glucose > fructose > sucrose. From the results shown in the Table 2 it can be seen that paprika samples originating from India showed lower than average fructose concentration. At the same time, above-average glucose content was found in paprika samples from China. Obtained results complied with data published in nutrition tables (Finglas *et al.*, 2015).

### Content of macro and trace elements

Determining the mineral content of a sample is one of the practical tools for verifying the geographical origin of spices (D'Archivio *et al.*, 2014; Ahmad *et al.*, 2020; DiDonato *et al.*, 2023). The results of content determination of the analyzed macro elements and trace elements are shown in Table 3. Among the macro elements, potassium was the most abundant in the ground paprika samples (average concentration 18.9 mg/g). The other macro elements in the investigated samples had a similar content pattern,  $P > Mg \sim Ca > Na$ , and their concentration was approximately ten times lower than potassium. One sample from Turkey contained an extremely high sodium concentration compared to the other samples (18.2 mg/g compared to an average content of 0.55 mg/g), which was (according to the manufacturer's data) caused by adding salt to the ground paprika. Of the monitored microelements, iron was the most represented in the paprika samples (average concentration 0.17 mg/g). In contrast, the concentration of copper and zinc was in the order of tens of  $\mu\text{g/g}$ . Obtained results complied with data published in nutrition tables (Finglas *et al.*, 2015) and with studies published by DiDonato *et al.* 2023, Zhang *et al.* 2019 and Tokailoglu *et al.* 2019.

### Multivariate analysis of obtained data

By applying PCA to amounts of 16 analytical variables and 37 objects, two factors were extracted, explaining 54.01 % of the variance of the initial data set. From the planar projection of these two principal components F1 and F2 it can be observed that paprika samples were separated into three main clusters (Figure 1). Paprika samples from China were projected at the interface of the first and third quadrants, samples from India were projected in the second quadrant, and samples originating from Europe (except for samples from Slovakia) were projected in the fourth quadrant. With the help of multivariate analysis, it was also possible to distinguish paprika samples originating from Europe and bearing a protected geographical indication. These samples originating from Hungary, Slovakia and Spain are circled in figure 1 and form three separate clusters. The relationship between all the components and the good separation of individual samples by country of origin is shown in Figure 2.



**Figure 1** Projection of the PCA score of paprika samples into a 2-D factor plane of principal components F1 and F2 and loading plots of variables defined by principal components F1 and F2.

**Table 2** Basic chemical characterisation of paprika samples investigated in this study.

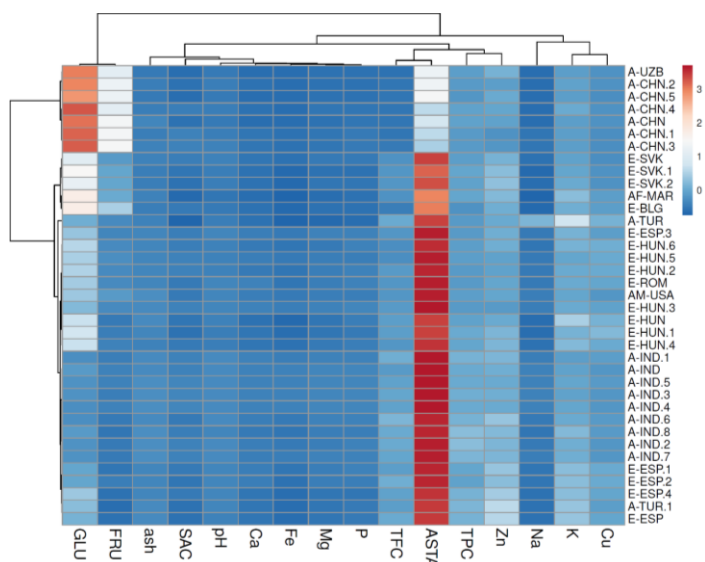
		TFC	TPC	GLU	FRU	SAC	ASTA	ash	pH
CHN	Min	2.05	13.5	105	47.9	0.84	34	4.69	4.61
	Max	3.10	15.5	126	67.6	5.55	64	8.28	4.93
	Av.	2.53	14.8	113	59.4	3.05	45	5.54	4.76
IND	Min	12.6	16.1	5.33	0.11	1.08	103	5.02	4.92
	Max	17.5	22.0	11.5	0.98	2.81	186	6.47	5.41
	Av.	16.2	20.0	8.25	0.15	1.95	144	5.94	5.15
ESP	Min	11.0	13.7	14.7	0.56	0.99	83	4.96	4.73
	Max	16.9	16.3	31.5	4.67	2.91	148	6.93	5.31
	Av.	13.4	14.7	19.8	2.03	1.63	109	5.65	5.09
HUN	Min	8.18	12.1	28.2	3.16	2.02	84	5.45	4.59
	Max	13.9	17.7	41.4	8.94	4.43	172	7.04	5.14
	Av.	11.8	15.0	34.5	6.24	2.92	132	6.16	4.91
SVK	Min	10.6	15.2	46.5	13.7	2.13	95	5.50	4.88
	Max	12.0	15.9	53.2	16.7	3.42	124	5.70	5.02
	Av.	11.3	15.5	50.2	15.6	2.87	106	5.60	4.97
TUR	Min	11.0	11.9	15.9	0.11	0.34	82	6.21	5.18
	Max	15.1	16.9	18.5	8.41	1.09	86	6.30	5.36
	Av.	13.1	14.4	17.2	4.26	0.81	84	6.26	5.27
USA*	-	12.1	14.8	32.4	13.5	0.73	149	7.44	4.55
BLG*	-	6.33	12.9	68.4	32.6	5.02	105	5.76	4.93
ROM*	-	10.9	13.1	32.4	7.43	1.45	136	5.81	5.13
UZB*	-	2.06	17.9	117	55.7	1.17	62	5.06	4.88
WORLD**	Av.	11.1	16.1	42.6	15.9	2.27	110	5.90	4.97

**Legend:** TFC – total fat content (%), TPC – total protein content (%), GLU – glucose (mg/g), FRU – fructose (mg/g), SAC – sucrose (mg/g), CHN – China, IND – India, ESP – Spain, HUN – Hungary, SVK – Slovakia, TUR – Turkey, USA – United States of America, BLG – Bulgaria, ROM – Romania, UZB – Uzbekistan, WORLD – data for all analysed samples, Av. – average, \* n=1, \*\* n=37

**Table 3** Content of selected elements in paprika samples investigated in this study.

		Ca*	Na*	K*	Mg*	P*	Fe*	Cu**	Zn**
CHN	Min	0.68	0.28	15.5	1.67	1.76	0.10	9.40	11.7
	Max	1.26	0.95	17.6	1.88	2.26	0.19	11.9	21.3
	Av.	0.93	0.65	16.4	1.76	2.04	0.14	11.1	15.8
IND	Min	1.09	0.42	11.8	2.06	2.24	0.17	9.24	18.9
	Max	2.30	0.77	19.3	2.46	3.03	0.30	12.4	21.9
	Av.	1.41	0.58	16.6	2.29	2.68	0.24	10.3	20.7
ESP	Min	1.73	0.44	18.3	2.06	2.26	0.11	13.1	20.8
	Max	3.21	0.81	23.0	3.10	4.07	0.25	16.6	26.3
	Av.	2.64	0.66	21.6	2.51	3.32	0.17	15.1	22.9
HUN	Min	1.24	0.30	18.1	1.60	2.71	0.10	16.4	14.2
	Max	2.88	0.68	23.2	2.73	4.14	0.28	23.3	21.5
	Av.	1.91	0.43	21.1	2.27	3.62	0.16	19.5	17.9
SVK	Min	1.15	0.24	15.8	1.78	2.41	0.11	9.67	21.4
	Max	2.00	0.35	17.6	2.68	2.87	0.16	11.1	23.9
	Av.	1.52	0.27	16.7	2.09	2.64	0.13	10.3	22.5
TUR	Min	2.25	0.49	20.7	1.76	2.04	0.08	10.9	15.8
	Max	3.37	18.2	29.8	2.00	2.35	0.33	16.9	26.3
	Av.	2.81	9.35	25.2	1.88	2.20	0.20	13.9	21.1
USA <sup>a</sup>	-	1.60	1.06	17.2	1.84	2.34	0.16	10.6	17.1
BLG <sup>a</sup>	-	1.98	0.41	21.4	1.99	3.02	0.07	16.5	21.2
ROM <sup>a</sup>	-	3.52	0.84	19.3	2.94	3.79	0.20	17.6	17.6
UZB <sup>a</sup>	-	1.16	0.29	18.2	1.57	2.25	0.10	13.6	25.3
WORLD <sup>b</sup>	Av.	1.76	1.02	18.9	2.17	2.83	0.17	13.5	19.8

**Legend:** CHN – China, IND – India, ESP – Spain, HUN – Hungary, SVK – Slovakia, TUR – Turkey, USA – United States of America, BLG – Bulgaria, ROM – Romania, UZB – Uzbekistan, WORLD – data for all analysed samples, Av. – average <sup>a</sup> n=1, <sup>b</sup> n=37, \*mg/g, \*\*µg/g



**Figure 2** Heatmap representation of 16 monitored variables in 37 paprika samples. Annotations on top of the heatmap show clustering of the samples.

**CONCLUSION**

While untargeted analysis is currently receiving quite a lot of attention, targeted analysis is neglected when verifying the geographical origin of spices. This study shows that targeted analysis with the application of well-known chemical and instrumental methods is suitable for ascertaining the geographical origin of ground sweet paprika spice. By application of this approach, it was possible to separate samples originating from Europe and Asia reliably. Based on the processing of all obtained data, it was also possible to separate samples from Europe and bear a protected geographical indication of origin. The success of using any procedure for verifying the geographical origin of spices is greatly influenced by available and verified information on the sample's origin and a sufficient number of analyzed samples. For this reason, it would be appropriate for producers or suppliers of spices to cooperate more closely with research organizations so that it would be possible to create a reliable and as simple as possible methodology for verifying the geographical origin of spices. Such a methodology would help fair manufacturers and suppliers consolidate their market position and prevent frequent misleading of consumers.

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