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Research Article

Comparing Performances of Microscopy and GC-MS Analysis in Cereal Flour Authentication

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Abstract

The aim of this work was to employ light microscopic analysis of cereal starch granules in order to evaluate the possibility of cereal species authentication and differentiation according to appropriate botanical origin. Additionally, the goal was to compare the obtained results with newly published approach for cereal flour authentication using gas chromatography - mass spectrometry (GC-MS) instrument with multivariate data analysis in order to compare performances of the traditional and newly developed methods. The analyzed genotypes of the following cereal species: wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), triticale (*Triticosecale* Wittm.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and corn (*Zea mays* L.), were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia. Grain samples were milled into flour, starch suspensions were extracted from each sample and analyzed using a light microscope. The obtained light micrographs clearly demonstrated that starch characteristics of oat and corn samples could visually easily be differentiated from those obtained from the rest of the small grain species: wheat, rye, triticale and barely. These plant species contain similar starch granules (size, shape and size distribution), which is indicating that light microscopy is not a method able to discriminate them based on botanical origin. The results obtained are completely in agreement with those obtained by lipid profiling in flour samples of the same cereal plant species utilizing a GC-MS instrument with various multivariate data analysis tools, indicating that using both approaches it is possible to clearly distinguish the samples of gluten-free corn flour, from the samples of gluten-containing small grain flour, and among small grains to distinguish the samples of oat flour. Flour samples of other small grains demonstrate strong similarities.

Keywords: Cereal Flour; Corn; Small Grains; Light Microscopy; Starch

Abbreviation

GC-MS: Gas Chromatography - Mass Spectrometry

Introduction

Fraud procedures on the food market are known from the Antique period [1,2]. Consumer interest in the safety, authenticity and quality of food products is constantly increasing [3]. Authenticity is related to truthfulness, so a food product can be said to be authentic if it was not subject to any fraud [4].

With an annual production of more than 2 billion tons, cereals represent the most important food crop in the world [5]. Bakery products play a very important role in human nutrition with regard to their total consumption on a global scale. In addition to macronutrients, such as starch and dietary fiber, they contain micronutrients, such as vitamins, minerals and antioxidants [6,7]. The application of various non-wheat types of flour in bread production is a recent trend in the development of the formulation of a wide range of gluten-free products, due to the high incidence of celiac disease in the world's population [7].

An analysis of the authenticity of cereal products is necessary in order to determine the accuracy of the food product label. This would avoid the unfair economic gain of some food manufacturers and prevent the consumption of certain cereals, whose proteins are toxic to the body of sensitive and allergic individuals. The regulations of the European Union and the Codex Alimentarius Commission require a compulsory declaration of ingredients that can cause intolerance and allergic reactions. This applies in particular to cereals containing gluten, such as wheat (including bread wheat, durum wheat and spelt), rye, barley, oats and their hybrids, as well as those products derived from these plant species [8-10]. Thus, identification of the presence of these botanical species of cereals in products can be characterized as very significant [11]. Various techniques have been used for the confirmation of cereal flour authenticity, including spectroscopy (NIR, MIR, fluorescence, NMR, ICP-OES, ICP-MS, hyperspectral imaging), isotopic analysis, chromatography, PCR and real-time PCR. Chromatographic and PCR techniques are among the most important methods used in cereal flour authentication and adulteration [11-19].

Aim of the Study

The aim of this study was to analyze starch characteristics of different cereal species in order to investigate the possibility of cereal flour authentication using a light microscope. Additionally, these results were compared with the results obtained using a GC-MS system with multivariate analysis tools in order to compare performances of the traditional and newly developed methods.

Materials and Methods

All analyzed cereal samples (21 small grain genotypes and 19 samples of various genotypes of corn) were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia, and labeled as follows: wheat (W); barley (B); oat (O); rye (R); triticale (T) and corn (C), table 1. All cereal grains were grown in the same year and at the same experimental field, thus enabling a comparison independent from differences in environmental conditions. About 10 g of cereal grain samples were milled into flour using a laboratory mill (Falling number 3100, Knifettec 1095, Slovakia). The obtained flour had the following characteristics: particles of diameter > 500 μm (0 - 10%); particles > 210 but < 500 μm (25 - 40%), and particles < 210 μm (75 - 50%). The starch granules of obtained cereal flour samples listed in table 1 were analyzed using a light microscope. The amount of 2g of each sample of cereal flour was weighed using an analytical balance. The flour was introduced into a 50 mL glass beaker, after which, while stirring with a glass rod, a few drops of distilled water were added to form a thick dough. Distilled water was then used to rinse starch granules from a dough made of each flour sample. Glass beakers of 100 mL were used to collect rinsed water, which contained dissolved starch granules. 100 mL glass beakers were filled to the calibration mark. The starch solutions of all analyzed samples of cereals were left to precipitate for 24 hours in the refrigerator (at 4°C). The precipitated granules, obtained using this procedure, were then analyzed using a light microscope (Leika Imaging Systems, Cambridge, England) at a magnification of 400 times. The micrographs of all samples were created using the Zoom Browser EX program and mean values of starch granule diameters were determined for each cereal species.

Cereal species	Investigated genotypes
Wheat (W)	6 samples: Rapsodija, Evropa 90, Milijana, Nataša, Venera, Durumko
Barley (B)	9 samples: Novosadski 525, NS Pinon, NS Zitos, Atlas, Somborac, Rudnik, NS Marko, Golijat, NS Mile
Oats (O)	3 samples: Dunav, Jadar, Sedef
Triticale (T)	2 samples: NS Karnak, NS Trifun
Rye (R)	1 sample: NS Savo
Corn (C)	19 samples: Genotypes C1-C19

Table 1: Samples of cereal species analyzed in this study.

Results and Discussion

Figure 1 shows starch granule micrographs of randomly chosen genotypes of an each cereal species analyzed (samples: Evropa 90 (W), NS Mile (B), Jadar (O), NS Trifun (T), NS Savo (R), and corn genotype C12), obtained by light microscopy.

The general size and shape of starch granules from different sources can be observed with this technique. It is obvious that between the following samples of small grain species: wheat, barley, rye and triticale, clear differences in shape, size and size distribution of starch granules cannot be observed by the application of this method. In these samples two groups of starch granules can be observed: (i) large granules, round to elliptical shaped, about 30 μm in diameter; and (ii) a multitude of significantly smaller granules, round shaped, about 5 μm in diameter. It was observed that the mutual similarity of starch granules among genotypes belonging to small grain species is much higher compared to the similarity among wheat, corn, rise and potato flour samples reported in literature, using the same method [20]. The increased similarity of starch granules among all samples of small grains is probably a result of the strong botanical relationships among these cereal species. On the other hand, in the sample of corn starch round to angular and polygonal shaped granules are observed with approximately uniform size, and a medium diameter of about 15 μm. Shapes and dimensions of starch granules of oat genotype represent a clear exception, because they differ from corn sample, but also clearly differ from the other samples of small grains. Oat starch granules are more uniform in size, polygonal shaped and smaller in diameter (about 10 μm) compared to starch granules of other small grain species.

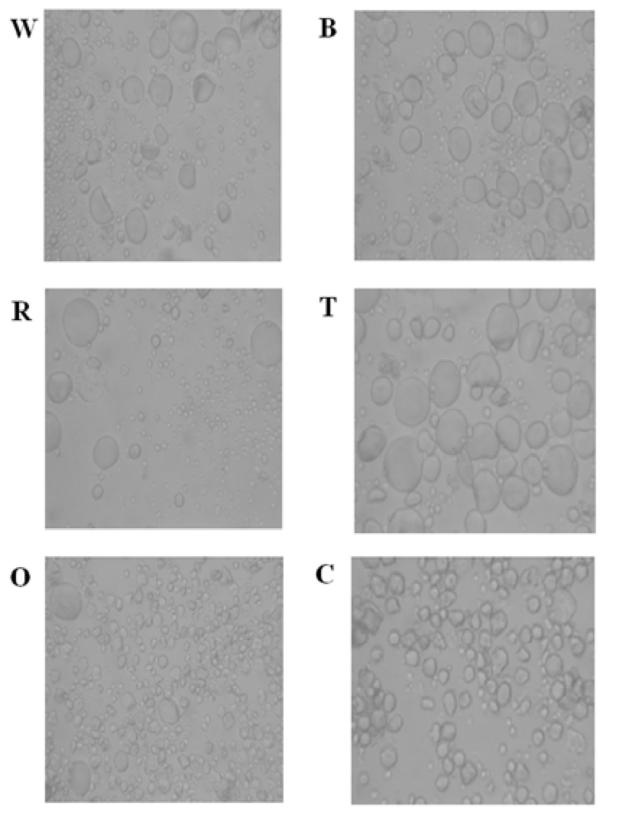

Figure 1: Light micrographs of analyzed cereal species showing the characteristics of starch granules (W: Wheat; B: Barley; R: Rye; T: Triticale; O: Oat; C: Corn).

The observed possibility to differentiate gluten-free corn flour samples from samples of gluten containing small grains, as well as to differentiate flour samples of oat from other samples of small grain species is in a complete accordance with the results obtained using a newly developed approach for cereal flour authentication, using gas chromatography - mass spectrometry with various multivatiate data analysis tools, such as hierarchical cluster analysis and principle coordinate analysis [21]. The microscopy itself can be characterized as a rapid and simple technique with good performances in differentiation corn, oat and small grain flours (wheat, rye, triticale, and barley).

Conclusion

Shapes and sizes of starch granules extracted from flour samples of various cereal species can be easily evaluated using light microscopy. Starch granule characteristics of the samples of gluten-free corn flour can be differentiated from starch granules extracted from gluten-containing flour samples of small grains. However, in the group of small grains, the samples of oat flour demonstrate high level of differences from the other samples of small grains: wheat, rye, triticale and barley. These results could be brought into relationship with the results published by Pastor., *et al.* [21], wherein the same manner of differentiation was observed on the same group of samples, but using highly sophisticated analytical equipment, gas chromatography - mass spectrometry combined with multivariate data analysis tools.

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Volume 2 Issue 5 May 2018