Quick determination of compounds contained in caraway (*Carum carvi* L.) by a method usable in agricultural practice

Lucie Horackova[1], Helena Pluhackova[1], Marta Bradacova[1], Barbora Kudlackova[2]
[1]Department of Crop Science, Breeding and Plant Medicine
Mendel University in Brno
Zemedelska 1, 613 00 Brno
[2]Institute of Analytical Chemisty of the Czech Academy of Sciences v.v.i.
Veveri 967/97, 602 00 Brno
CZECH REPUBLIC

lucie.vagnerova@mendelu.cz

Abstract: Caraway is a very important agricultural commodity whose quality is determined by parameters such as dry matter, essential oil content and composition, especially the ratio of its two major components – carvone and limonene. Appropriate method for their analysis is given in the Český lékopis (2017); however, this method is rather time-consuming, costly and demands large quantity of sample. The use of a NIR spectrometer could be a viable alternative; it is much faster and cheaper, as can be clearly seen from the comparison of both methods in this paper. In the time aspect, it's saving from many hours to a few minutes. Newly presented method could potentially be more accessible to agricultural companies who need quick quality verification of their product before taking it to the market from the viewpoint of final product quality – not just the quantity, which, in most cases, is nowadays a current state of practice.

Key Words: caraway, essential oil, carvone, limonene, quick method

INTRODUCTION

Caraway (*Carum carvi* L.) is one of the most important commodities belonging to the MAP group (medicinal plants, aromatic plants and spice) grown in the Czech Republic. It belongs to the *Apiaceae* family. Caraway grows as a wild plant in Europe and West Asia; cultivated cultivars can be found in autumn-sown or, more often, biennial form. According to history of cultivation caraway was one of the first plants cultivated in ancient times used both as spice and as a medicinal plant (Bailer et al. 2001). The achenes contain 1–6% of essential oil which gives it its characteristic aroma and taste. Essential oil contains up to 30 different components; however, major components – carvone and limonene – take about 95% of the total essential oil amount (Aćimović et al. 2015). Acetaldehyde, furfural, carveol, pinene, thujone, camphene, phelandrene and other compounds are also present on the essential oil. In addition to the essential oil the achenes contain also 13–21% oil, 25–35% crude protein, 13–19% fiber and 9–13% water (Azza et al. 2010).

As for the use, caraway is considered to be a very versatile plant. Caraway fruits, the achenes, are widely used in food, distillery and meat industries for their pleasant but intense taste and smell. They also found application in the production of spice mixtures, beverages, both alcoholic or non-alcoholic, various bakery products, ice cream, confectionery, pickles, meat, cheese etc. Its antibacterial and fungicidal effects are significant and often used in veterinary and human medicine, especially bioactive effects of certain components that work as anticancer, antioxidant, antimicrobial, antidiabetic, anti-ulcerogenic, antihyperglycemic and hypolipidemic agents (Azza et al. 2010, Aćimović et al. 2015, Sachan et al. 2016). In cattle breeding, caraway is considered to be a beneficial component of feed mixtures. It helps to increase milk production, increases the overall palatability of the feed mixture and, last but not least, it reduces flatulence. Caraway essential oil can be used in agriculture as an effective germination inhibitor for stored potatoes (Azza et al. 2010, Seidler-Łożykowska et al. 2013).

The determination of essential oil content in caraway achenes is subject to the Český lékopis (2017). The standard method, steam distillation, uses the volatility of essential oils that are extracted and removed from the sample by water vapour and after cooling condensed again as a liquid.

The mixture of volatile essential oil and steam is cooled and trapped in a pear-shaped flask in the distillation apparatus. The extract can be moved from there to calibrated capillary to directly measure the volume of distilled essential oil and stored in a vial for further analysis (Hay and Watermann 1993). However, even if the procedure is followed precisely, significant differences can occur for the same sample analysed by different laboratories. The differences can vary from a few hundredths of a percent up to tenths of a percent, which is no longer negligible. The procedures used in individual laboratories are not a problem, human factor is: there are differences in sample grinding (particle size), used instrumentation used and workers attitude and skills (Prugar et al. 2008). Similar results were obtained by Smallfield et al. (2001) who proved that just the use of two types of distillation apparatus gives almost identical results, while the quality of sample grinding and total distillation time had serious impact on the quantity and composition of the essential oils. Standard Czech Pharmacopoeia reference method is not advantageous for growers, especially economically. The use of spectroscopy would provide a rapid non-destructive method for both qualitative and quantitative determination of contained compounds to growers and plant breeders.

NIR is a physical, quick, non-destructive method that requires none or minimum sample preparation. It is widely used in the food and feed industry, human nutrition and also in textile, pharmaceutical and petrochemical industries to determine the material quality (Chen et al. 2008, Gaspardo et al. 2012). The method is used to determine qualitative and quantitative parameters from both chemical and physical viewpoint (Blažek et al. 2005). The advantage of this method is also the fact that several parameters can be measured at the same time. To be measurable, the sample must contain chemical bonds N-H, C-H, S-H and O-H, and the amount of measured component should be > 1 g/kg (Míka et al. 2008). NIR operates in the spectral range 700–2500 nm, i.e. between visible and medium infra-red radiation. Water, fat, carbohydrates and proteins are the basic components detected using the NT-NIR spectrometer in agricultural commodities. The method is based on correlation in the determination of the physico-chemical properties of known sample measured by reference method and on the reflectance or transmittance of light at different wavelengths in the NIR region. Thus, the principle is measuring the change and loss of radiation emitted by the instrument after contact with a given sample (Bradáčová et al. 2014).

MATERIAL AND METHODS

The aim of the experiment was to compare two methods for the determination of quantitative and qualitative parameters of caraway (*Carum carvi* L.) in terms of their time demands. Quantitative and qualitative parameters of interest were: dry matter, total content of the essential oil and representation of major components (carvone, limonene) in caraway essential oil. A quantity of four samples was selected to illustrate the time demand, as this is the number of samples that could be distilled in the laboratory at the same time in terms of space intensity.

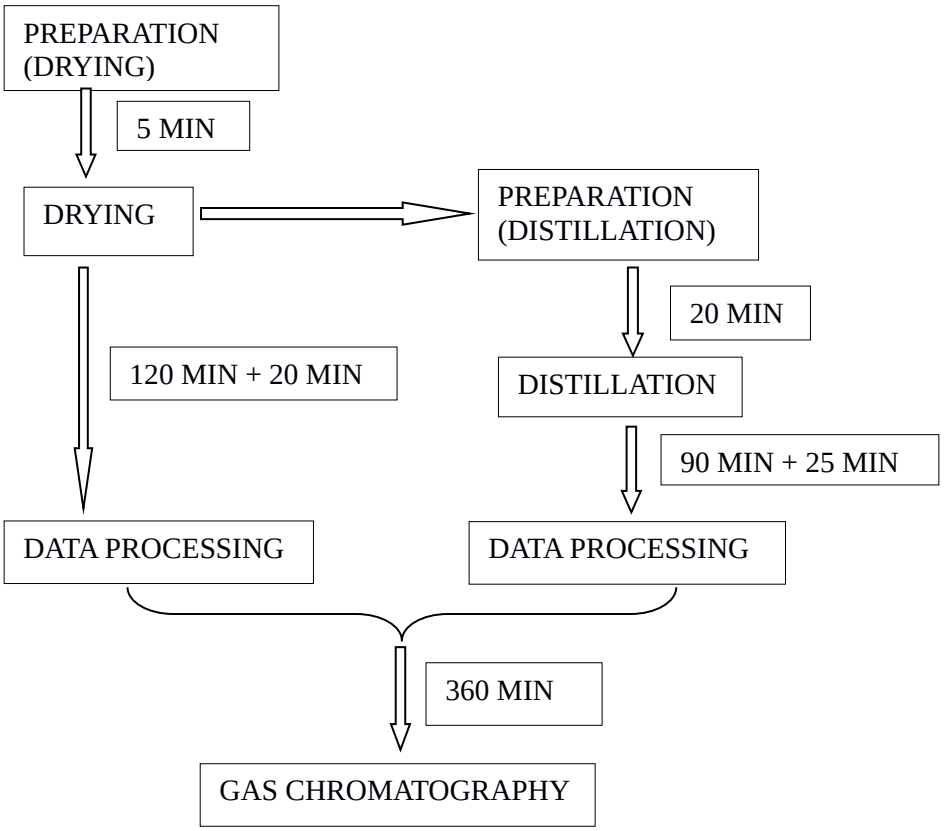
The first method is the reference method for the analysis of caraway achenes given in the Český lékopis (2017). The analyses were carried out partly at the Department of Crop Science, Breeding and Plant Medicine, Faculty of AgriSciences, Mendel University in Brno (MENDELU) and partly at the Institute of Analytical Chemistry, Czech Academy of Sciences (IACH). The determination of dry matter and total content of the essential oil was performed at the Department of Crop Science, Breeding and Plant Medicine and subsequent evaluation of the representation of main components in caraway essential oil was done at the IACH. The first step of the method is sample grinding using a shredder mill. The sample thus prepared is weighed on analytical scales into pre-weighed dryers. The dryers with the sample go to pre-heated drying oven, they are dried at 130 °C for 2h and then put into a desiccator. Cool dryers are weighed and % dry matter is computed. The sample used for this determination is already destructed and must be discarded. Another part of the ground sample is used for steam distillation. The distillation can start during the drying process and be performed simultaneously. The sample can be ground together for both dry matter determination and steam distillation, if the initial sample weight is big enough. For steam distillation, 10g of ground sample is weighted, transferred quantitatively into the distillation flask, 200 ml of distilled water is added, as well as boiling chips to prevent secret boiling and the distillation apparatus is assembled. Steam distillation is carried out using hot plate for 90 minutes. After the given time the heating is turned off. When the boiling stops, the amount of condensed essential oil is determined from the apparatus scale,

the essential oil is transferred into pre-labelled vial and placed in the freezer. Now the sample is prepared for gas chromatography analysis. Assuming that there is enough sample, the distillation is performed in two replicates. The time required for this procedure is 145 minutes when determining the dry matter and the amount of essential oil at the same time.

After the steam distillation, gas chromatography analysis follows, performed at the IACH. Gas chromatograph Trace GC with flame ionization detector (ThermoFinnigan) was used for the analysis of major components of caraway essential oil: carvone and limonene. GC separation was carried out on a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm). 1 µl of ethanol-diluted extract was injected to the column in on-column mode at injection temperature 40 °C. The temperature program was set as following: T1 = 40 °C for 1 min, 20 °C/min to T2 = 280 °C for 5 min. The helium carrier gas flow rate was 1 ml/min, the detector temperature was 280 °C. Five calibration solutions of 2.5, 5, 10, 15 and 20 µl/ml, resp., of carvone and limonene were prepared for calibration. Each sample was analysed twice, so with 40 minutes per sample and one GC data acquisition, the total duration was 80 minutes (sample preparation and data processing included). The whole procedure is shown in Figure 1.

The other method is sample analysis using the FT-NIR Nicolet Antaris II DR instrument and evaluating given parameters by the means of the Omnic 8 programme. The procedure is as follows: turning on the computer and the instrument, opening the program, preparing the measurement: filling the cells with plant material. It takes about 3 minutes including the description input to the computer.

Figure 1 Graphical representation of the time consumption of the method of determining the quality of caraway seeds given by the Český lékopis 2017



RESULTS AND DISCUSSION

As clearly seen from Material and Methods, where the whole procedure of analysis is described in detail including the average time consumption, the reference method requires about 505 minutes (8 hours and 25 minutes) for the analysis of samples. NIR spectroscopy technology is much more favourable and convenient. Our results are in good accordance with findings of other authors.

For example, Teye et al. (2013) use NIR for identifying the origin or quality of cocoa beans and Gaspardo et al. (2012) uses it in his work on detection of fumonisins in corn meal.

The difference in time demand is enormous, even when only the "net time" is counted. Another important factor is the fact that the use of reference method in our case required the cooperation with another institution, the Institute of Analytical Chemistry, Czech Academy of Sciences. Financial demands of both procedures also need to be taken into account, as well as the material demands. FT-NIR not only takes less time, it is also less financially and materially demanding, because it is a non-destructive method. It finds a lot of use especially in plant breeding and related fields, where the amount of sample available for analysis can be relatively limited. It is also necessary to take into account that in the case of reference analytical methods carried out in the laboratory the work is not only a matter of "net time". Preparatory work must be taken into account as well. This includes the washing of laboratory glass, cleaning the distillation apparatus, preparation of sample for the distillation (assembly of distillation apparatus, time from turning on the heat plate to the moment when the boiling starts), transport of the samples from the Department of Crop Science, Breeding and Plant Medicine to the IACH etc. Of course, the NIR spectroscopy method also includes preparatory work (time to turn on the computer, background measurements that are done every hour etc.), but these matters only take a few minutes.

This paper only deals with comparison of time consumption of the measurement itself. At the conclusion, it is of course necessary to emphasize the fact that in order to be able to perform such fast and undemanding analyses on the NIR spectrometer, it is necessary to create calibration models, according to which the samples are subsequently determined. From a financial point of view, it is worth highlighting the initial high investment to purchase the necessary instrumentation.

CONCLUSION

From the viewpoint of the agricultural practice and public experts, the FT-NIR method has great potential to be used for direct, rapid analyses, especially for the quality determination of the final product before it is sold, in a wide range of sectors and fields of interest in agriculture and food industry. Nowadays, caraway trade is done mainly on the frameworks of quantity, not quality, which is a very important parameter not only for this commodity. Given its low time, financial and material demands, the method would be available to almost any farmer who would be interested in knowing the quality of their products.

ACKNOWLEDGEMENTS

The research was financially supported by the TA CR TE02000177 „Centre for innovative use and strengthening of competitiveness of Czech brewery raw materials and products ".

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