The importance of a good diet is recognised by experts and consumers alike, and phytochemicals have been identified as one key ingredient. Scientists throughout the world are striving to determine the nature, role and significance of phytochemicals in diets, whole foods and supplements, in order to identify and exploit commercial opportunities that will offer health benefits for the consumer.

Biologically-active Phytochemicals in Food, the proceedings of an international conference organised by the Food Chemistry Division of FECS to celebrate its 25th anniversary, looks at the biosynthesis and significance of phytochemicals, their analysis and biological activity (with especial attention on their anti-oxidant properties). It also discusses the influence of structure and processing on bioavailabilty, and hence, efficacy.

Presenting state-of-the-art international research, this book will be welcomed by food chemists, researchers from other disciplines representing the extended food chain, as well as representatives of industry, regulatory bodies and consumer organisations.

ISBN 0-85404-806-5

biologically-active phytochemicals in food

edited by W.PFANNHAUSER, G.R.FENWICK and S. KHOKHAR

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DETECTION OF THE MAJOR COMPONENTS OF CAPSICUM OLEORESIN AND ZINGERONE by HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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1 INTRODUCTION

Capsicum is a substance that surrounds the hot pepper seeds as a protective mechanism to keep animals from eating the seeds. The oily residue of extracted red peppers is normally referred to as Capsicum Oleoresin. It is used as a flavouring additive in many dishes and is also an important ingredient in cough medicines and topical ointments. Capsicum oleoresin is the active ingredient of a pepper spray which is used as a non lethal control/ defensive tool. It contains a complex mixture of closely related amides also called capsaicinoids : capsaicin (C), dihydrocapsaicin (DHC), nordihydrocapsaicin (NDC), homocapsaicin (HC) and homodihydrocapsaicin (HDC) (figure 1).[1]

Ginger is a rhizome of Zingiber officinale Roscoe, a plant cultivated in many tropical and subtropical countries. Ginger is used as a spice in Asian cooking and in Chinese traditional medicine.[2] The constituents for the pungent taste of ginger are a homologous series of phenolic ketones, known as gingerols, shogaols (dehydration products of gingerols) and zingerone (Z) (retro-aldol degradation of gingerols).[3,4] Zingerone also known as zingiberone is a flavouring agent used in root beer, ginger ale, chewing gum, candies, ice creams and baked goods.[5]

Various methods for the detection of capsicum or the capsaicinoids and zingerone are described in literature using HPLC with UV or fluorescence detection.[4,6,7,8,9] Also GC-MS methods are reported.[10] Because of the thermal lability and low volatility GC-MS requires complicated derivatization techniques. An LC-MS method is reported.[11]
The advantage in using tandem mass spectrometry lies in a better specificity and better sensitivity. No methods have been reported in which ESI and APCI combined with tandem mass spectrometric detection are used for determination of the capsaicinoids or zingerone. This paper describes the preliminary work of identification and detection with LC-MSMS of five capsaicinoids in a Capsaicin Oleoresin preparation and the confirmation of the structural relation with zingerone.

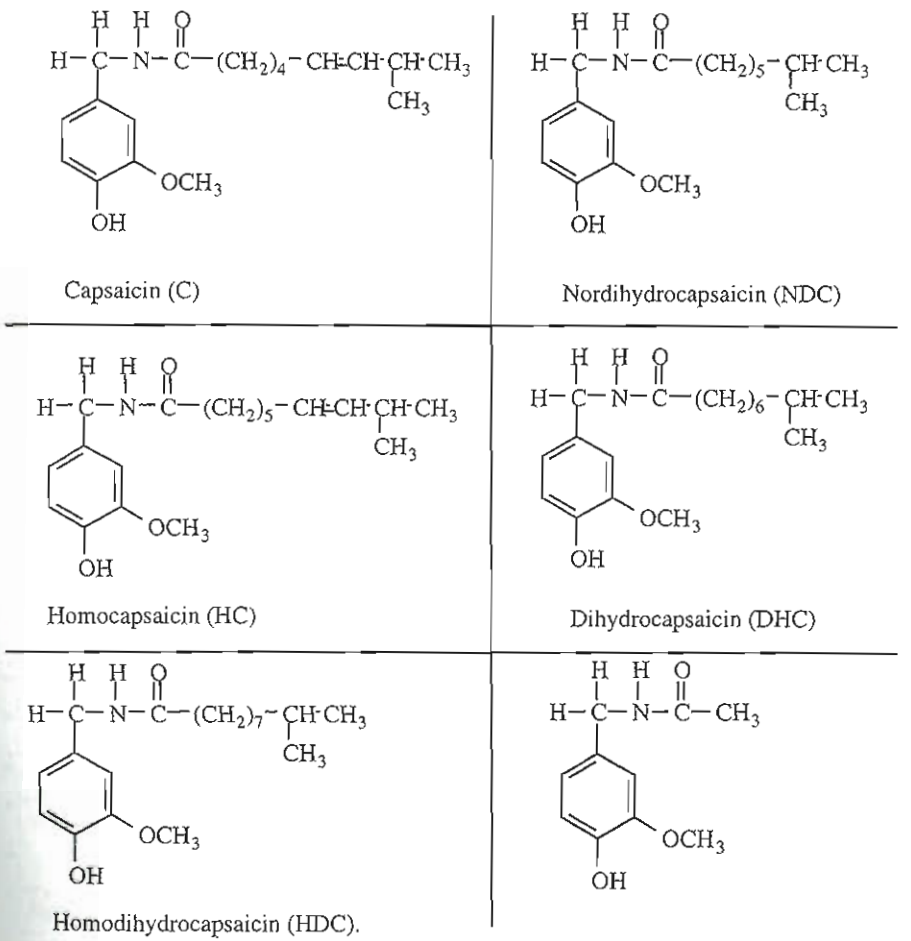

Figure 1 *The structural formula of different closely related amides of capsicum oleoresin and zingerone*

2 EXPERIMENTAL

2.1 Apparatus

The mass spectrometer used was an LCQ Ion Trap Mass Analyzer of Finnigan MAT (ThermoQuest, San Jose, CA, USA) equiped with an exchangable ESI (ElectroSpray Ionization) and APCI (Atmospheric Pressure Chemical Ionization) interface. The HPLC system consisted of a quaternary gradient pump Model P4000, a vacuum degasser and a AS3000 autosampler (TSP, San Jose, USA) and a Symmetry C18 column (5µm, 150x2.1 mm, Waters, Milford, USA). The mobile phase consisted of a mixture of 60:40 methanol-1% acetic acid. This mixture was isocratically pumped at a flow rate of 0.3 ml min^{-1}.

3 RESULTS AND DISCUSSION

3.1 Infusion-ESI-MS

For capsicum oleoresin a working solution in methanol of 100 ng/µl was directly infused into the mass spectrometer equiped with an electrospray interface. In figure 2 the infusion-ESI-MS spectrum is given.

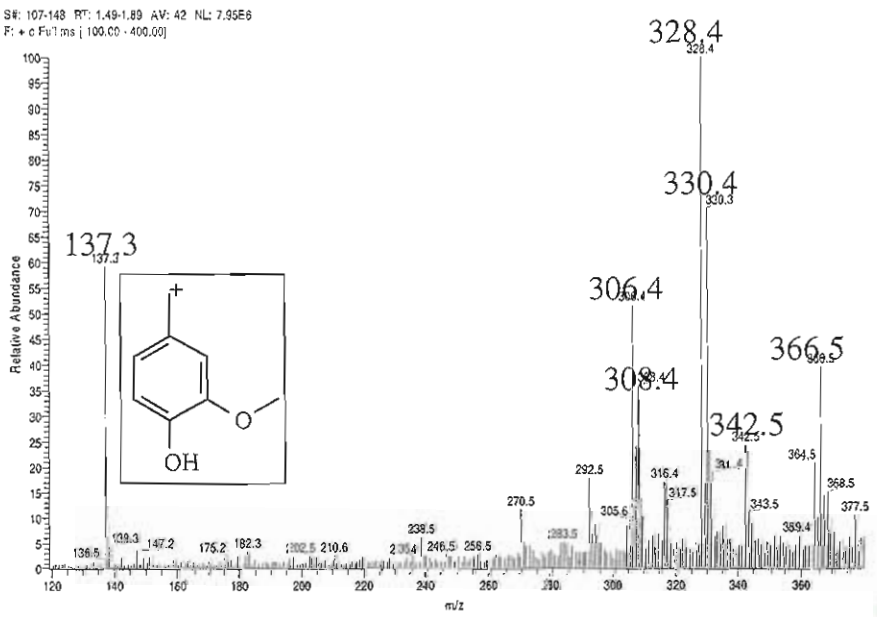

Figure 2 *Infusion-ESI-MS of Capsicum Oleoresin with structural formula of fragment*

An intense pseudo-molecular ion and sodium adduct (MH^+ and MNa^+) of capsaicin and dihydrocapsaicin were observed, 306.4 and 308.4. No MH^+ ions were observed of HC, HDC and NDC. For C and DHC the sodiumadducts were the most abundant ions, 328.4 and 330.4. Knowing this, the ion 342.5 could be a sodiumadduct of HC. Also an intense ion with m/z 137.3 was present in the MS spectrum. This ion is a common fragment ion of all capsaicinoids and also zingerone.

3.2 LC-MS

The two ionization methods, ESI and APCI, were compared. In a first stage electrospray ionization was used. Tenfold dilutions were injected on column. C, DHC and NDC eluted at different retention times. No chromatographic peaks were found for HDC and HC for 1 ug on column. The same amount was injected on column but the analytes were ionized using Atmospheric Pressure Chemical Ionization. Using APCI the five amides were detected. APCI was prefered to ESI. In figure 3 the different mass traces of the capsaicinoids are compared.

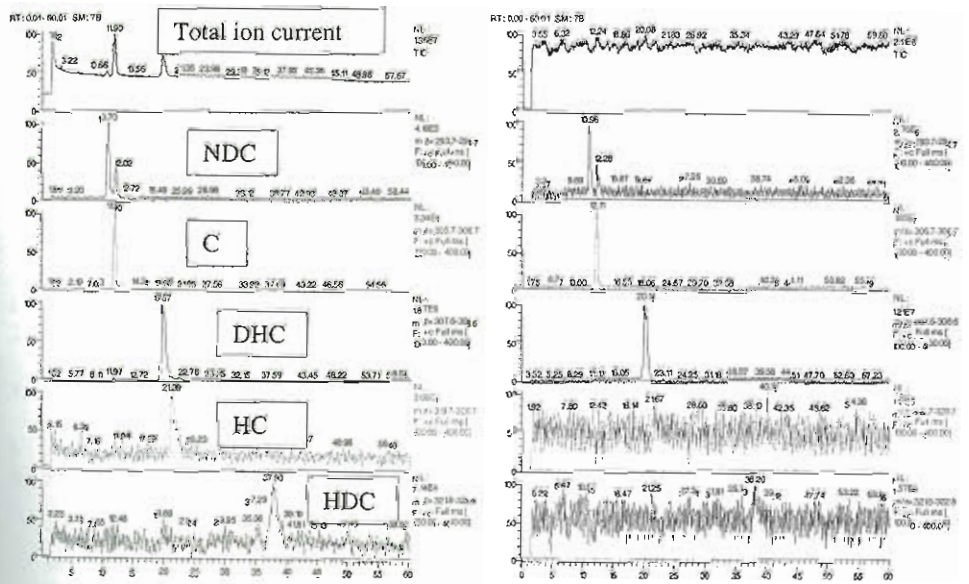

Figure 3 *Comparison of the different mass traces of the capsaicinoids in APCI (left) and ESI (right)*

Using APCI, a chromatographic peak was obtained for HC and HDC. To improve the sensitivity and specificity, the pseudo-molecular ions were isolated in the ion trap and fragmented to obtain a product ion spectrum. The identity of the fragment ions is illustrated in figure 4.

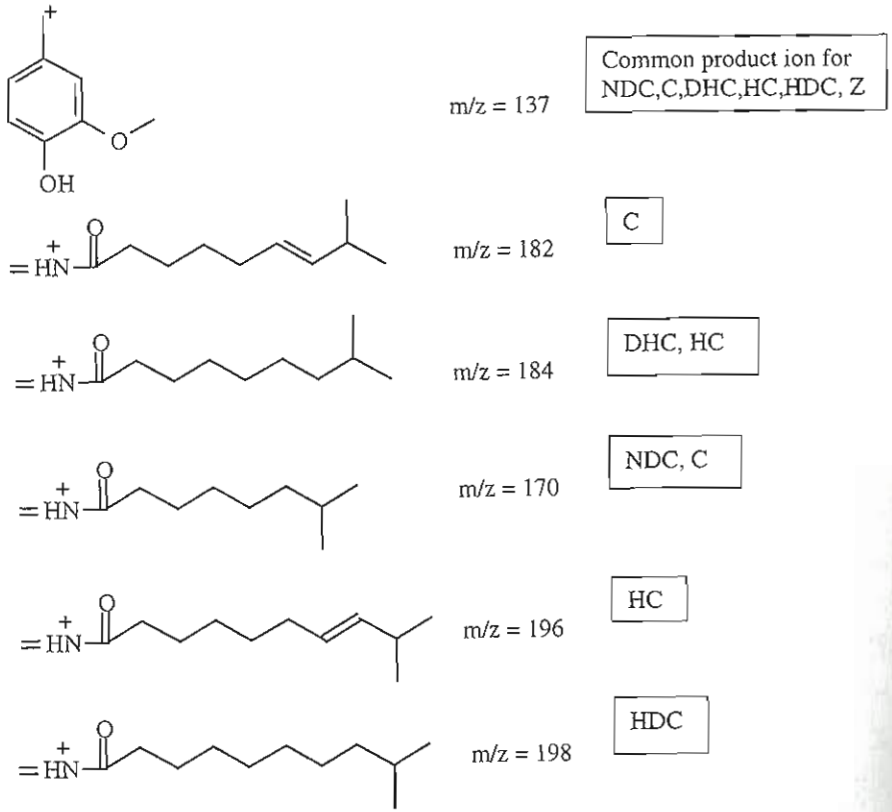

Figure 4 *Identity of the product ions*

An important remark is that by using APCI fragmentation occurs already in MS. This implies that by isolating the parent ion, not all of the injected amount is detected in MSMS. Absolute peak areas are smaller using APCI but the number of capsaicinoids detected is larger.

For zingerone a distinct spectrum was obtained which indicates the same product ion 137 as previously analysed capsicum oleoresin. This is not surprising since the structures (given in figure 1) of both compounds are very similar. They both contain an aromatic ring with two ortho oxygen atoms in an ether-phenol combination. The carbonylgroup is located in the same position for capsaicin and zingerone, although the latter is a ketone, whereas capsaicin is an amide. The fragment ion with m/z 137 is derived from the structure they have in common and is given in figure 2.

3.3 Lowest detectable concentration

The lowest detectable amount injected on column was determined for the individual amides. For capsaicin and dihydrocapsaicin the lowest detectable amount on column is 100 pg. Nordihydrocapsaicin can be detected up to 1 ng. Homocapsaicin and homodihydrocapsaicin can be detected up to 100 nanogram on column.

4 CONCLUSION

In this work a preliminary study is performed of the detection of the different capsaicinoids with LC-APCI-MSMS and the structural relation with zingerone. Specific spectra are obtained for each individual compound. Since capsaicin and dihydrocapsaicin are the main components of the preparation, the overall detection limit is also determined by these two compounds. To detect residues of C and DHC 100 pg has to be injected on column.

If the limit of detection is the most important factor in determining the presence of capsicum oleoresin then LC-ESI-MSMS should be used and capsaicin is the target analyte. If all capsaisinoids have to be determined LC-APCI-MSMS will be the method of choice.

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