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Determination of the aroma properties of Mustard seeds by SPME - GC-MS

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ABSTRACT

The objective of this study was to investigate the volatile aroma constituents of mustard seeds grown in Ethiopia by using SPME-GC-MS. In the analysis several sulfur contained compounds were identified. The most relevant peaks were identified by comparison of their retention indices with literature data and based on their total mass spectra, by comparison with mass spectra databases (NIST mass spectral library). Within the sulfur contained compounds, the ally isothiocyanate was the most dominant. It was revealed that mustard seed powder contained a major aromatic component (peak) at the retention time of 17.69 min for allyl isothiocyanate (71.13%) and 9.57min for 1-Cyano-2,3-Epithiopropane (9.54%). This ally isothiocyanate has beneficial effects on antimicrobial profiles of mustard (brassica nigra) and it is the responsible compound for the pungent aroma of mustard seeds.

INTRODUCTION

The family *Brassicaceae* consists of 350 genera and about 3,500 species, and includes several genera like *Camelina, Crambe, Sinapis, Thlaspi* and *Brassica* (Cartea *et al*, 2010). The most important genus within the Brassiceae is Brassica, which comprises some crops and species having tremendous worldwide economic importance such as Brassica oleracea L., Brassica rapa L and Brassica napus L. The genus is grouped into forage, oilseed, condiment, and vegetable crops by using their leaves, buds, roots, stems and seeds . The mustard group which is made of three species, Brassica Carinata (Ethiopian/Abyssinian mustard), Brassica nigra (Black mustard) and Brassica juncea, is used mainly as a condiment though leaves of B. juncea are also consumed as a vegetable (Cartea et al, 2010).

Mustard seeds are small round seeds of various mustard plants. The seeds are typically about 1 or 2 mm in diameter (Hemmingway et al., 1993). Mustard seeds may be coloured from yellowish white to black.

They normally take about three to ten days to sprout if the proper conditions are present, which includes a relatively moist soil and cold atmosphere. Canada (90%), Hungary, Great Britain, India, Pakistan and the United States are major producers of mustard seeds. Black and brown mustard seeds produce greater yields than their yellow counterparts (Paunović et al. 2012).

Mustard is a condiment prepared from whole, crushed or powdered dried seeds of the mustard plants: Brassica juncea (brown mustard), Brassica nigra (black mustard) or Sinapis alba (white mustard). The seeds are typically mixed with vinegar, wine, water and several spices to create a paste varying in color from bright yellow to dark brown. Mustard plants keep attracting a lot of attention since they have been recognized as a functional food for health maintenance and disease prevention (Hemmingway et al., 1993).

Mustard's spiciness is triggered by a collection of compounds called isothiocyanates. An enzyme called myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) hydrolyses glucosinolates to release isothiocyanates when mustard seeds are crushed and exposed to liquids. The release of isothiocyanates causes structural changes that influences the differences in mustard flavourof. For example, brown and oriental mustard release ally isothiocyanate (AIT), a volatile compound that produces a pungent aroma and sharp taste sensation similar to horseradish. Yellow mustard releases 4-hydroxybenzyl isothiocyanate (PHBIT), a non-volatile compound that causes a hot mouth sensation in condiments.

Mustard products comprise a wide range of such active constituents including isothiocyanates, dithiolthiones, phenolics and dietary fibre. For instance, isothiocyanates of essential mustard oil possess pharmacological and toxic properties by their antibacterial, goitrogenic, antiprotozoal and antifungal activities. The mucilage from yellow mustard both stabilizes prepared mustard products, as well as reduce the glycemic index in both diabetic patients and normal people.

Mustard seeds are made up of protein (23-30%), carbohydrate (12-18%), fixed oil (29-36%), together with minor components including minerals (4%), phytin (2-3%), essential oil (glucosinolates, 0.8-2.3%), as well as phenolic compounds and dithiolthiones (Hemmingway et al., 1993). Concerning the fatty acid content, it was reported that mustard seeds contain a substantial amount of linoleic acid, which is essential for the human body. In the study of Kisbenedek A. (2013) a smaller amount of saturated fatty acids was found during his analysis, such as palmitic acid and stearic acid. Erucic acid content of the mustard seed in his study was 32.78g/100g.

Antioxidant property of mustard seeds

A search for natural antioxidants in foods has resulted from concern over the toxicity of synthetic antioxidants (Barlow, S.M., 1990). The latter include phenolic compounds, phospholipids, tocopherols, and amino acids. Oilseeds generally, are rich in phenolic compounds that retard oxidation of the oil. The presence of phenolic acids in mustard and rapeseed was reported by Kozlowska et al., (1983), in which sinapic acid isomers were predominantly present forms. Saleemi et al., (1993), in their research, compared the antioxidant properties of low pungency ground mustard seed (LPGMS) with synthetic antioxidants butylated hydroxytoluene (BHT) and tertiary-butyl hydroquinone (TBHQ) on the stability of comminuted pork. Additional work by the same researchers revealed that an 85% methanolic extract from low pungency ground mustard seed (LPGMS) displayed the strongest antioxidant activity as a result of the presence of much greater levels of phenolics compared to either water or 10% methanolic extracts. In addition to its antioxidant activity, low pungency ground mustard seed (LPGMS) also improved cook yield without having a detrimental effect on the colour quality of the treated meat samples (Saleemi et al., (1993).

Antimicrobial Properties of mustard seeds

The antibacterial activity of isothiocyanates (the main compound present in mustard seed) was found to be more effective against Staphylococcus aureus. Delaquis and Mazza (1995), in their study showed the influence of vaporized ally isothiocyanate (AIT) on food borne pathogens was dose dependent and the growth of microorganisms was inhibited at concentrations above 500ng/ml. Some isothiocyanates, such as benzyl isothiocyanates, exhibit antibiotic activity in vitro, and are used as pharmaceuticals for the treatment of infections of the urinary and respiratory tracts (Bergmann, M., 1996).

Over half a century ago, Mustard oil was reported to exhibit antifungal activity. The antifungal efficiency of AIT was revealed by Tsunoda (1994). Up-to-date evidence points to ally isothiocyanate and other volatile isothiocyanates being specifically effective against the germination and growth of several fruit pathogens (Mari, M., et al., 1993). In the vapour phase, ally isothiocyanate (AIT) from brown mustard proved to be an effective antifungal agent when added in modified atmosphere packaging of different food samples (Isshiki et al., 1992). Ally isothiocyanate was mostly effective against mycotoxin producing molds such as Aspergillus flavus, Fusarium graminearum and Penicillium citrinum (Delaquis, P.J. and Mazza, G., 1995).

MATERIALS AND METHODS

Sample preparation technique was solid-phase micro extraction (SPME) or purge-and-trap. Briefly, one gram of mustard seed (*brassica nigra*) powder and 0.5g of sodium chloride were mixed with 3ml of distilled water in vial. This vial was placed in automatic Gerster multipurpose sampler, which injects it automatically in GC-MS (Agilent Technologies, 6890N, Network GC System coupled with Agilent 5973 Network Mass Selective Detector).

The aroma properties of the mustard seed (*brassica nigra*) powder was determined by head space solid phase micro extraction method combined with gas chromatographic-mass spectrometric (GC-MS) techniques in laboratory of aroma research for food industry in KU Leuven, technology campus Ghent, Belgium. The most relevant peaks were identified by comparison of their retention indices with literature data and on the basis of their total mass spectra, by comparison with mass spectra databases (NIST mass spectral library).

The percentage of each aromatic chemical compound was calculated using the following formula:

$$\textbf{\% Individual aromatic chemical compound} = \frac{\textbf{Individual chemical compound peak area}}{\textbf{Total chemical compound peak area}} \times 100$$

RESULTS AND DISCUSSION

The chromatograph of the mustard seed powder was presented in Figure 1 and the identified aroma properties or chemical compounds of mustard seed (*brassica nigra*) powder, retention time and their percentage were presented in Table 1.

In fact, dry seeds of *brassica nigra* do not have the pungent flavor. The pungent flavor is only developed when the seeds are ground and macerated in water under specific conditions (Jimmy *et al*., 2001). This is because there are glucosinolate in the seeds of *brassica nigra*, which are the precursors of the essential oil of *brassica nigra*. Glucosinolates are the main secondary metabolites found in mustard seed (*brassica nigra*). Their presence is made known to us whenever we eat mustard as they degrade immediately upon tissue damage to release isothiocyanates or other sulfur-containing compounds, of which isothiocyanates are the most well-known (Mithen R., 2006).

In this experiment, as expected most of the identified chemical compounds were the major sources of sulphur-containing plant compounds, those derived from the glucosinolate–myrosinase (substrate–enzyme) system found in *cruciferous* crops (Figure 2) such as *brassica nigra* and *brassica oleracea* (Mithen R., 2006). The differences in mustard flavor or aroma are due to the structural changes of the released isothiocyanates (Mithen R. 2006; Miyazawa, M. and Jyunichi, K. 2006). For example, brown and oriental mustard (brassica nigra) release a volatile compound, AIT that produces a pungent aroma and sharp taste sensation (Jimmy et al., 2001). Yellow mustard releases 4-hydroxybenzyl isothiocyanate, a non-volatile compound, which elicits a hot mouth feel in condiments. Diversity of product formation in the glucosinolate-myrosinase system is mainly due to structural variation of the glucosinolate substrate as shown in Figure 2.

The identified compoundes which have relatively high contents are isothiocyanate derivatives and 1-Cyano-2,3-Epithiopropane. The analysis (Figure 1) revealed that mustard seed powder contained a major aromatic component (peak) at the retention time of 17.69 min for allyl isothiocyanate (71.13%) and 9.57min for 1-Cyano-2,3-Epithiopropane (9.54%) and this was similar with a result reported by Jimmy *et al*., (2001). Vaughn F. & Boydston R. (1997) also reported the isolation of benzyl isothiocyanate volatile compound from mustard seeds by means of solid phase micro-extraction (SPME).

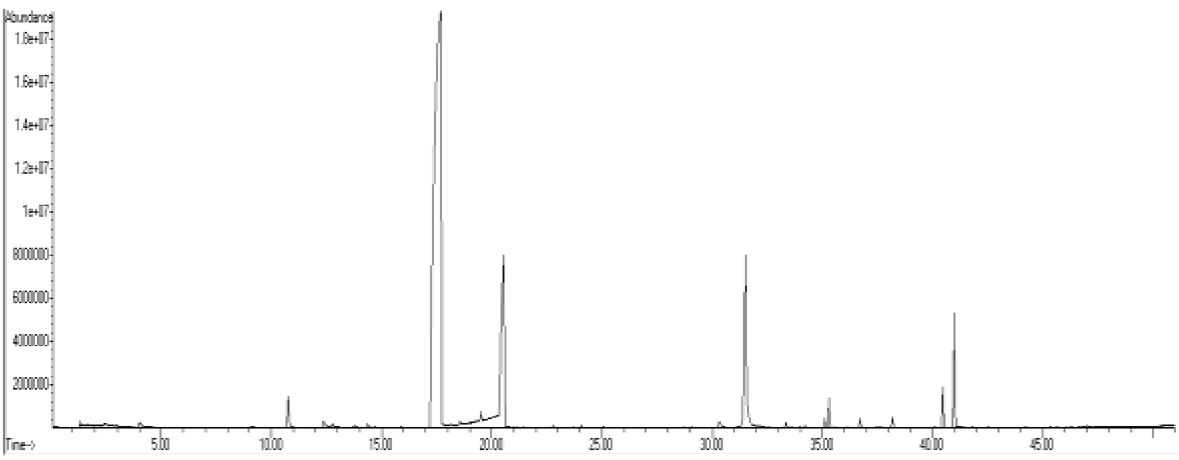

Figure 1 GC chromatograph for the aroma of mustard seeds powder

Table 1 The identified chemical compounds in mustard seed (*brassica nigra*) powder

No	Compounds	Retention time	Percentage
1	Urea	1.336	0.04
2	Carbondisulfide	1.658	0.01
3	Cyclotrisiloxane, Hexamethyl	2.368	0.01
4	N-Ethil-1,3-Dithioisindoline	2.425	0.02
5	Diazoethane	3.995	0.07
6	2-Butenenitril	10.757	0.94
7	Isothiazol, 3-Methyl	12.361	0.24
8	Gamma-Terpinen	12.771	0.08
9	Para Cymene	13.813	0.05
10	Allyliso Thio Cyante	14.347	0.07
11	Isobutyl Isothiocyanate	15.871	0.03
12	Allyl Isothiocyanate	17.693	71.13
13	2-Methyl-3,4-Dihydro-2h-Thiopyran	19.509	0.19
14	Allyl Isothiocyanate	20.533	12.48
15	1h-Indene,Octahydro-2,2,4,4,7,7-Hexamethyl-Trans	22.802	0.05
16	1h-Purin-6-Amine,[(2-Fluorophenyl)Methyl]	24.06	0.03
17	Methyl Nonyl Ketone	25.052	0.02
18	1-Cyano-2,3-Epithiopropane	31.511	9.54
19	1h-Purin-6-Amine,[(2-Fluorophenyl)Methyl]	33.35	0.10
20	Benzeneacetonitrile	33.966	Trace
21	Benzenepropanenitrile	36.713	0.22
22	Octanoic Acid	37.565	0.01
23	Benzyl Isothiocyanate	38.162	0.29
24	Phnol,5-Methyl-2-(1-Methylethyl)	40.449	1.01
25	Phenethylisothiocyanate	40.973	3.31
26	Decanoic Acid	42.5	Trace

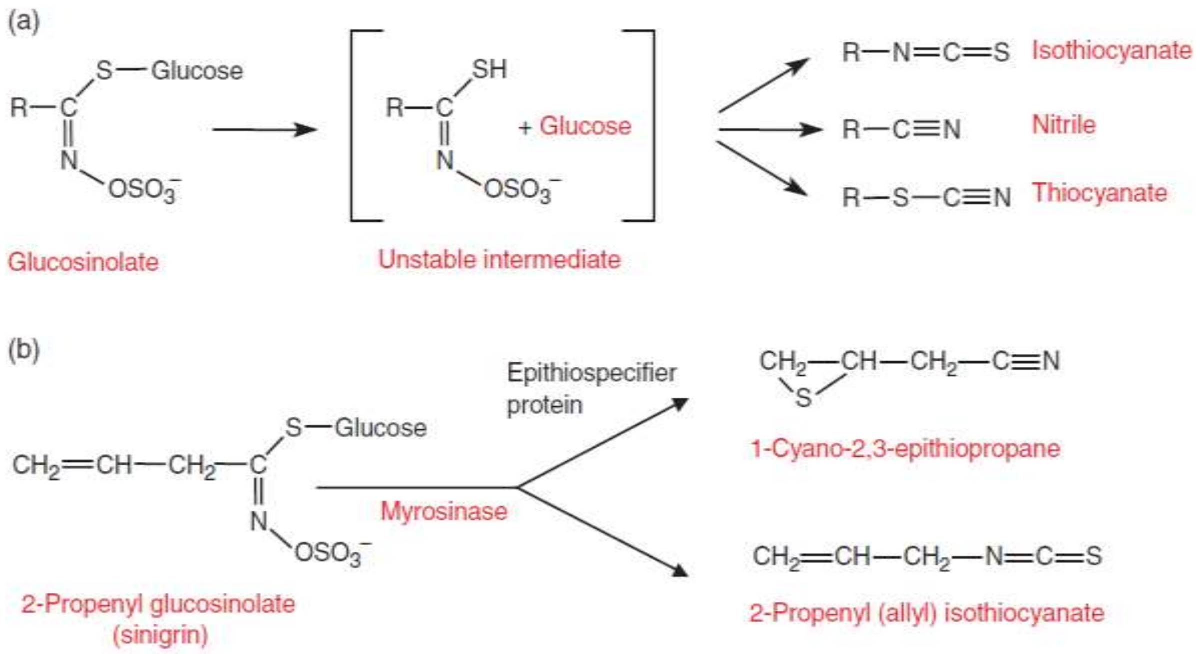

Figure 2 The glucosinolate–myrosinase (substrate–enzyme) system found in *cruciferous* crops
a) Structure of glucosinolates and its enzymic degradation to an unstable intermediate and subsequent conversion to isothiocyanate, nitrile and, more rarely, a thionitrile. (b) Glucosinolates with alkenyl side chains may degrade to either isothiocyanates or, in the presence of the ESP protein, epithionitriles.
Source: Mithen R., (2006)

CONCLUSION

In mustard seeds several sulfur contained compounds had been identified by SPME-GC-MS. Where the ally isothiocyanate were the most dominant. These compounds play a beneficial role on antimicrobial profiles of mustard (*brassica nigra*). The Ethiopian spices have a good antioxidant activity and phenolic compound content and are abundantly available in Ethiopia, hence, can be used as a source of antioxidant for production of shelf stable food products and also as a source of healthy compounds for the development of dietary supplements via advance research and development activities.

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