

Application of Gas Chromatography in the Analysis of Flavour Compounds in Field Peas

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1. Introduction

Flavour compounds influence the taste and quality of foods both of which are very important criteria in food selection and consumer acceptance. Pulse legumes such as field peas are increasingly used in foods such as soup mixes, purees, bakery and other processed products (Heng et al., 2004). In some parts of the world, particularly in Western countries, the presence of off-flavours in peas can be an obstacle to their consumption.

Different chemical compounds such as alcohols, aldehydes, ketones and various heterocyclic compounds play a major role in the flavour of peas. As flavour compounds have different characteristics, changes in their concentrations and profiles can affect the taste and flavour of the finished food product.

Flavour can be analyzed either using sensory methods or with analytical instruments such as gas-chromatography (GC). Separating and analyzing a mixture of volatile compounds in foods without decomposition is an important feature of this latter technique. As most flavour compounds in foods are volatile, simplified GC methods may offer an appropriate technique for the separation and characterisation of volatiles in different food matrices.

In GC, the mobile phase or carrier phase is an inert gas such as helium and the stationary phase is a very thin layer of liquid or polymer on an inert solid support inside a column. The volatile analytes interact with the walls of the column, and are eluted based on the temperature of the column at specific retention times (Grob & Barry, 2004). The eluted compounds are identified with detectors. Flame ionization and mass spectrometry are the most commonly used detectors for flavour analysis (Vas & Vékey, 2004).

Flavour compounds in foods may, however, be at concentrations too low to be accurately detected by GC; concentration of volatiles may, therefore, be required prior to GC operation (Werkhoff et al., 1998; Deibler et al., 1999; Prosen & Zupančič-Kralj, 1999; Zambonin, 2003). Different methods such as purge and trap, static headspace, liquid-liquid, solid phase

extraction, and solid phase microextraction are used for extraction and concentration of volatile compounds. Among various separation and concentration techniques, head space solid phase microextraction (HS-SPME) using a fused-silica fibre combined with gas chromatography-mass spectrometry (GC-MS) has gained increasing attention for the extraction and analysis of volatile, semi-volatile, polar and non-polar compounds in foods such as vegetables, legumes, beverages and dairy products. In comparison with conventional extraction techniques, HS-SPME is a solvent-free, less expensive, fast, and simple technique and involves the adsorption of volatile compounds onto an adsorbent fibre. In fibre-SPME, adsorption is based on the equilibrium partitioning of the analytes between the solid-phase of the SPME fibre, liquid or solid sample matrix. Upon heating, adsorbed analytes are desorbed onto a GC column and analyzed by gas chromatography (Pawliszyn, 1995; Penüalver et al., 1999; King et al., 2003; Vas & Vékely, 2004; Anli et al., 2007).

The flavour profile of legumes, such as peas, is anticipated to become an important quality trait for both traditional and novel food applications. More specifically, knowledge of the flavour profile of peas and the impact of different parameters will be important in selecting the right cultivar as well as storage, handling and processing conditions for different food applications. Unfortunately, data on the impact of different parameters on the flavour profile of peas has been lacking. The main objective of this research, therefore, was to use an optimised HS-SPME-GC-MS technique (Azarnia et al., 2010) to evaluate differences in the flavour profiles of 11 pea cultivars grown in Saskatchewan which is the largest field pea producing province in Canada (AAFC, 2006). Previous work done in our laboratory focused on differences in the flavour properties of different raw pea flours. As pea is cooked before consumption, this work was, therefore, conducted on whole cooked peas.

2. Materials and methods

2.1 Materials

Chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada). Selection of pure volatile standards was carried out as previously reported by Azarnia et al., 2010. Carboxen-polydimethylsiloxane, SPME-fibre (CAR/PDMS, 85 µm, Supelco, Oakville, ON, Canada) was used for the GC analysis. Yellow- (CDC Golden, Eclipse, Cutlass, CDC Centennial), green- (Cooper, CDC Striker, CDC 1434-20), marrowfat- (Rambo, MFR042) and dun- (CDC Dundurn, Kasper) type were evaluated in this study. These field pea cultivars were grown under uniform conditions using recommended agronomic practices for field pea on land managed by the Crop Development Centre, University of Saskatchewan, Canada. These cultivars were selected based on our preliminary results which showed higher differences in the total area of volatile compounds compared to other cultivars. Furthermore, CDC Golden, Eclipse, Cutlass, Cooper and CDC Striker are widely grown in Western Canada. These cultivars were grown in two different locations (i.e. Meath Park, MPK and Wilkie, WIL, near Saskatoon, Saskatchewan, Canada) in crop years of 2008 and 2009.

2.2 Methods

2.2.1 Standard preparation

The preparation of standard solutions as well as the evaluation of the reproducibility of the method during each GC run was carried out as described in Azarnia et al., 2010.

2.2.2 Solid phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS) analyses

Volatile compounds in pea cultivars were determined using HS-SPME-GC-MS as described by Azarnia et al., 2010. Briefly, 3 g of each sample were extracted at 50 °C for 30 min using CAR/PDMS fibre. A MPS2 multipurpose sampler (Gerstel Inc., Baltimore, MD) was used for HS-SPME. Analyses were carried out with a Varian CP-3800 gas chromatograph (Palo Alto, CA). Adsorbed volatile compounds were desorbed at 300 °C for 3 min into a split/splitless injector (Glass insert SPME, 0.8 ID; Varian, Mississauga, ON, Canada). Pure helium gas (1 mL/min) was used for the elution of compounds on a VF-5MS capillary column (30 m x 0.25 mm x 0.25 µm, Varian Inc., Mississauga, ON, Canada). The initial temperature of the GC oven was 35 °C which was held for 3 min, and then increased to 80 °C at a rate of 6 °C per min, and finally to 280 °C at a rate of 20 °C per min, and held for 2 min. The total time of analysis was 22.5 min. A Saturn 2000 MS detector (Varian Inc., Palo Alto, CA) was used for detection of compounds, and the mass range was 30–400 m/z. The total ion current was obtained using an electron impact ionization source at 70 eV at a scan rate of 1 s/scan. Calibration and tuning of the equipment were carried out as recommended by the manufacturer. Identification of volatile compounds were carried out either using National Institute of Standards and Technology (NIST) database (V. 05) through mass spectra library search or by comparing mass spectra and retention times of the compounds with those of the pure commercial volatile standards. After determination of the area count of each volatile compound from the average of two replicate assessments, a semi-quantitative comparison was carried out by calculation of the relative peak area, RPA, of each volatile compound. Results were expressed as percentage of total volatile compounds.

2.2.3 Preparation of cooked-whole seeds

Seeds were soaked in water (ratio of 1:2, seeds:water) and kept at room temperature (~22°C) for 24 h. After draining, the seeds were cooked in boiling water (ratio of 1:2; seeds:water) for 20 min. 3 g of the cooked-whole seeds were weighed into 10 mL headspace amber vials (Supelco, Oakville, ON, Canada) and then mashed twice inside the vial by using a spatula.

2.2.4 Statistical analysis

Each experiment was carried out in two replicates. Peak area count of each volatile compound was obtained for each replicate. Analysis of variance (ANOVA) using a general linear model (GLM) procedure of the Statistical Analysis System (SAS, 2004, Cary, USA) was performed to evaluate differences between parameters. The parameters evaluated were type, cultivar, location, crop year, and interactions between them. Means comparison between parameters was carried out by Duncan's multiple range test using SAS software.

3. Results and discussion

3.1 Effect of type, cultivar, and location on Total Volatile Compounds (TVC) and chemical families

The impact of type, cultivar, and location on TVC and different chemical families (i.e. alcohols, aldehydes, ketones, esters, sulfur compounds, hydrocarbons) in field pea cultivars

was evaluated and results are, respectively, presented in Figures 1-7. The data were subjected to ANOVA and Duncan's multiple range test and were separately reported for each crop year (Tables 1-4). Furthermore, the effect of crop year on the flavour profile of pea cultivars was studied and statistical results are presented in Table 5.

3.1.1 Effect of type, cultivar and location on TVC

Changes in the value of TVC in different field pea cultivars grown in the year of 2008 and 2009 are shown in Fig. 1. ANOVA results showed that TVC in peas grown in different crop years was significantly ($P < 0.01$) affected by the pea type and cultivar (Tables 1 & 3). Based on Duncan's test (Table 1), in the year of 2008, peas grown in MPK had higher TVC compared to those grown in WIL. Rambo from marrowfat type had the highest mean value of TVC, whereas CDC Striker from green-type had the lowest value of TVC. The highest mean value of TVC was observed in the field peas from marrowfat-type, whereas peas from green-type had the lowest value of TVC (Table 1). In the year of 2009, no significant ($P > 0.05$) differences were found between the cultivars grown in different locations (Table 3). Rambo and Kaspia, respectively, had the highest and the lowest mean value of TVC. Amongst the different pea types, marrowfat-type had the highest value of TVC, whereas dun-type had the lowest value of TVC (Table 3).

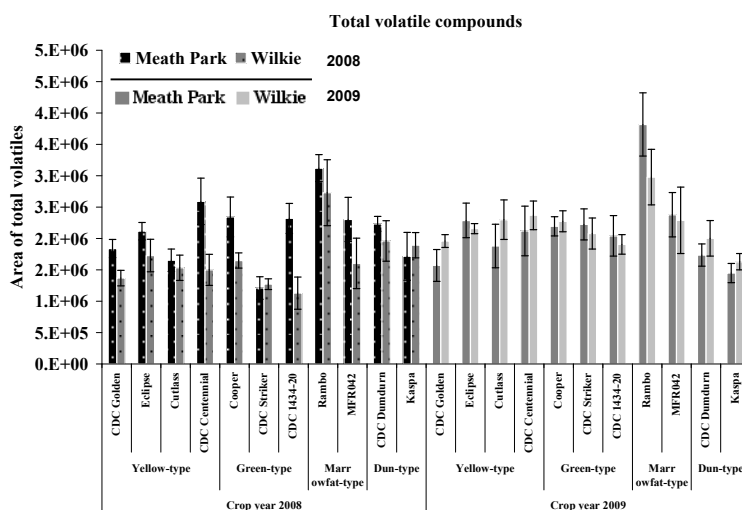


Fig. 1. Changes in total volatile compounds content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean \pm standard deviation.

3.1.2 Effect of type, cultivar and location on different chemical families

3.1.2.1 Alcohols

Changes in the alcoholic compounds in pea cultivars are shown in Fig. 2. In the year of 2008, the mean value of alcohols were significantly ($P < 0.01$) affected by the type, cultivar and

location (Table 2). Pea cultivars grown in WIL location had higher mean value of alcohols than those grown in MPK location. 3-Methyl-1-butanol and 1-hexanol had, respectively, the highest and the lowest mean values (Table 2). In the year of 2009, the mean value of alcohols was significantly affected by the type and cultivar, whereas no significant differences were found between the cultivars grown in different locations (Table 3). 1-Propanol and 2-ethyl-1-hexanol had the highest mean values and 1-hexanol had the lowest mean value (Table 4).

Alcohols in peas are mostly formed from enzymatic oxidation of lipids. Physical damage, storage and processing of seeds could lead to the formation of alcohols (Eriksson, 1967; de Lumen et al., 1978; Oomah & Liang, 2007). Volatile alcoholic compounds have distinct characteristics and they could therefore affect the taste and flavour of peas. For example, 1-propanol has an alcoholic odour and a fruity flavour; 2-methyl-1-propanol has a wine odour, 3-methyl-1-butanol has a fruity, banana, sweet odour with a bittersweet taste; 1-hexanol has an herbaceous, mild, sweet, green fruity odour and an aromatic flavour; 1-heptanol has an aromatic and fatty odour and a spicy taste, whereas 1-octanol has a fresh, orange-rose odour and an oily, sweet taste (Burdock, 2002).

| ¹ ANOVA | | | | | | | | | | | |
|--------------------|----------------|----------------|----------------|------------------|-----------------|------------------|-----------------|-----------------|--------|---------|---------|
| Main effects | | | Interactions | | | | | | | | |
| ² cv | ³ l | ⁴ t | ⁵ r | cv* ¹ | l* ^t | cv* ^r | t* ^r | l* ^r | | | |
| (+++) | (+++) | (+++) | (++) | (+++) | (NS) | (NS) | (NS) | (NS) | | | |
| Duncan grouping | | | | | | | | | | | |
| Cultivar | Rambo | CDC | CDC | Cooper | MFR042 | Eclipse | Kaspa | CDC | CDC | Cutlass | CDC |
| | (a) | Dundurn | Centen- | (bcd) | (bcd) | (bcd) | (cde) | 1434- | Golden | (e) | Striker |
| | | (b) | nial (bc) | | | | | 20 | (e) | (f) | |
| | | | | | | | | (de) | | | |
| Location | Meath | Wilki (b) | | | | | | | | | |
| | Park (a) | | | | | | | | | | |
| Type | Marro | Dun (b) | Yellow | Green (c) | | | | | | | |
| | wfat (a) | | (bc) | | | | | | | | |

¹ANOVA performed using general linear model. +++= $P < 0.01$, NS= Not significant ($P > 0.05$).

²cv=Cultivar, ³l=Location, ⁴t=Type, ⁵r=Replicate. Items with different letters within a row are significantly different at $P < 0.05$ (a>b>c>d>e>f).

Table 1. ANOVA results and Duncan's multiple range test for total volatile compounds in field pea cultivars grown in 2008

3.1.2.2 Aldehydes

Relative peak area of aldehydes in pea cultivars grown in different locations and crop years is presented in Fig. 3. The mean value of aldehydes was significantly ($P < 0.01$) affected by the type of cultivar. However, no significant ($P > 0.05$) differences in aldehydes were observed between cultivars grown in different locations (Tables 2 & 4). 3-Methyl butanal was the most abundant aldehyde in all the pea cultivars studied (Tables 2 & 4).

Enzymatic or autoxidative decomposition of unsaturated fatty acids, mainly linoleic and linolenic acids could lead to the formation of aldehydes in peas (Hornostaj & Robinson, 2000; Barra et al., 2007). Differences observed in the concentration of these carbonyl compounds could be due to differences in linoleate compositions in pea cultivars (Oomah &

| Chemical family | ¹ ANOVA | | | | | | | | |
|------------------|--------------------|----------------|----------------|----------------|--------------|-----|------|-----|-----|
| | Main effects | | | | Interactions | | | | |
| | ² cv | ³ l | ⁴ t | ⁵ r | cv*l | l*t | cv*r | t*r | l*r |
| Alcohols | +++ | +++ | +++ | NS | +++ | NS | ++ | NS | NS |
| Aldehydes | +++ | NS | +++ | NS | +++ | NS | NS | NS | NS |
| Ketones | +++ | +++ | ++ | NS | +++ | NS | ++ | NS | NS |
| Esters | +++ | NS | +++ | NS | +++ | +++ | +++ | NS | NS |
| Sulfur compounds | +++ | ++ | +++ | NS | +++ | NS | ++ | NS | NS |
| Hydro-carbons | +++ | +++ | +++ | NS | +++ | +++ | NS | NS | NS |
| Pyrazines | +++ | +++ | +++ | NS | +++ | NS | NS | NS | NS |

Duncan grouping for each chemical family in peas belonging to different pea- types and grown in different location

| | Pea-type | | | | Location | |
|------------------|-----------|------------|-------------|---------------|----------------|----------------|
| | Marrowfat | Dun | Yellow | Green | Wilkie | Meath Park |
| Alcohols | (a) | (b) | (bc) | (c) | (a) | (b) |
| Aldehydes | Green (a) | Dun (b) | Yellow (b) | Marrowfat (b) | Meath Park (a) | Wilkie (a) |
| Ketones | Dun (a) | Green (ab) | Yellow (ab) | Marrowfat (b) | Meath Park (a) | Wilkie (b) |
| Esters | Green (a) | Yellow (b) | Dun (b) | Marrowfat (c) | Meath Park (a) | Wilkie (a) |
| Sulfur compounds | Dun (a) | Yellow (b) | Green (b) | Marrowfat (c) | Wilkie (a) | Meath Park (b) |
| Hydro-carbons | Green (a) | Dun (b) | Yellow (b) | Marrowfat (b) | Meath Park (a) | Wilkie (b) |
| Pyrazines | Dun (a) | Yellow (b) | Green (b) | Marrowfat (c) | Wilkie (a) | Meath Park (b) |

Duncan grouping for individual flavor compounds in peas belonging to each chemical family

| | | | | | | | |
|-----------|---|-----------------------|-------------------------|----------------|----------------|----------------|---------------|
| Alcohols | 3-Methyl-1-butanol (a) | 2-Ethyl-1-hexanol (b) | 2-Methyl-1-propanol (c) | 1-Propanol (c) | 1-Octanol (dc) | 1-Heptanol (e) | 1-Hexanol (d) |
| Aldehydes | 3-Methyl-butanal, (a) | Hexanal (b) | 2-Methyl-butanal, (c) | | | | |
| Ketones | 2-Butanone (a) | 2-Pentanone (b) | | | | | |
| Esters | Ethyl acetate Hexanoic acid, methyl ester (b) | | | | | | |
| | (a) | | | | | | |

Table 2. (Continued)

| | | | | |
|------------------|-------------------------------|-------------------|------------------------|----------------------|
| Sulfur compounds | Dimethyl sulfide (a) | Methanethiol (b) | Dimethyl disulfide (c) | 2-Acetylthiazole (d) |
| Hydrocarbons | Trichloromethane (a) | Furan,2-ethyl (b) | Toluene (c) | |
| Pyrazines | 2,3-Diethyl-5-methyl pyrazine | | | |

¹ANOVA performed using general linear model. +++=P<0.01, ++=P<0.05, NS= Not significant (P>0.05).

²cv=Cultivar, ³l=Location, ⁴t=Type, ⁵r=Replicate. Items with different letters within a row are significantly different at P<0.05 (a>b>c>d>e).

Table 2. ANOVA results and Duncan's multiple range test for chemical families in cooked pea cultivars grown in the year of 2008

Liang, 2007). Hexanal and pentanal are commonly identified in fruits and vegetables (Oomah & Liang, 2007). Propanal and hexanal, have been reported to be responsible for off-flavour in stored unblanched frozen peas (Barra et al., 2007). Timely harvesting of peas may prevent the formation of undesirable flavours derived from enzymatic reactions (Hornostaj & Robinson, 2000). Aldehyde compounds are known to contribute to the flavour and aroma of various plants and plant foods (Hornostaj & Robinson, 2000). Hexanal, as an example has a fatty, green, grassy, fruity odour and taste; 3-methyl butanal has a choking, acrid, fruity, fatty, almond odour; 2-methyl butanal has a choking odour and a coffee or chocolate flavour and taste, whereas benzaldehyde has a bitter almond taste (Burdock, 2002).

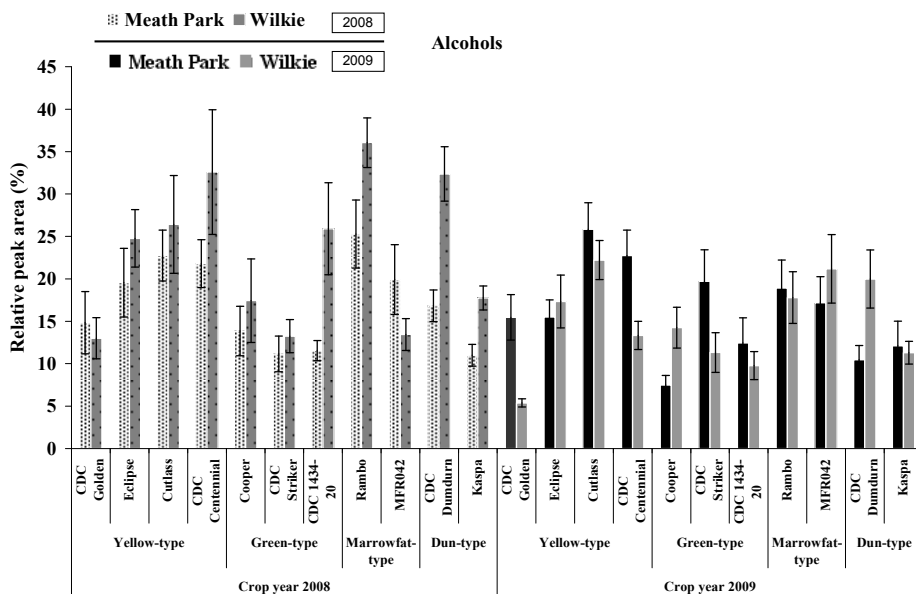


Fig. 2. Changes in total alcohol content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean \pm standard deviation. Relative peak area (%) = Peak area of total alcohols/ Total peak area of volatile compounds \times 100.

¹ANOVA

| Main effects | | Interactions | | | | | | |
|-----------------|----------------|----------------|----------------|-------|-------|-------|------|----------|
| ² cv | ³ l | ⁴ t | ⁵ r | cv*l | l*t | cv*r | t*r | l*r |
| (+++) | (NS) | (+++) | (++) | (+++) | (+++) | (+++) | (NS) | l*r (++) |

| Duncan grouping | | | | | | | | | | | |
|-----------------|-------|--------|-----------------|--------|---------|---------------|---------|---------------|--------------|-------------|-------|
| Cultivar | Rambo | MFR042 | CDC | Cooper | Eclipse | CDC | Cutlass | CDC | CDC | CDC | Kaspa |
| | (a) | (b) | Centennial (bc) | (bc) | (bc) | Striker (bcd) | (bcd) | 1434-20 (cde) | Dundurn (de) | Golden (ef) | (f) |

| | | |
|-----------------|------------|-------|
| Location | Meath Park | Wilki |
| | (a) | (a) |

| | | | | |
|-------------|-----------|-------|--------|-----|
| Type | Marrowfat | Green | Yellow | Dun |
| | (a) | (b) | (b) | (c) |

¹ANOVA performed using general linear model. . +++=P<0.01, NS= Not significant (P>0.05).
²cv=Cultivar, ³l=Location, ⁴t=Type, ⁵r=Replicate. Items with different letters within a row are significantly different at P<0.05 (a>b>c>d>e>f).

Table 3. ANOVA results and Duncan’s multiple range test for total volatile compounds in field pea cultivars grown in the year of 2009

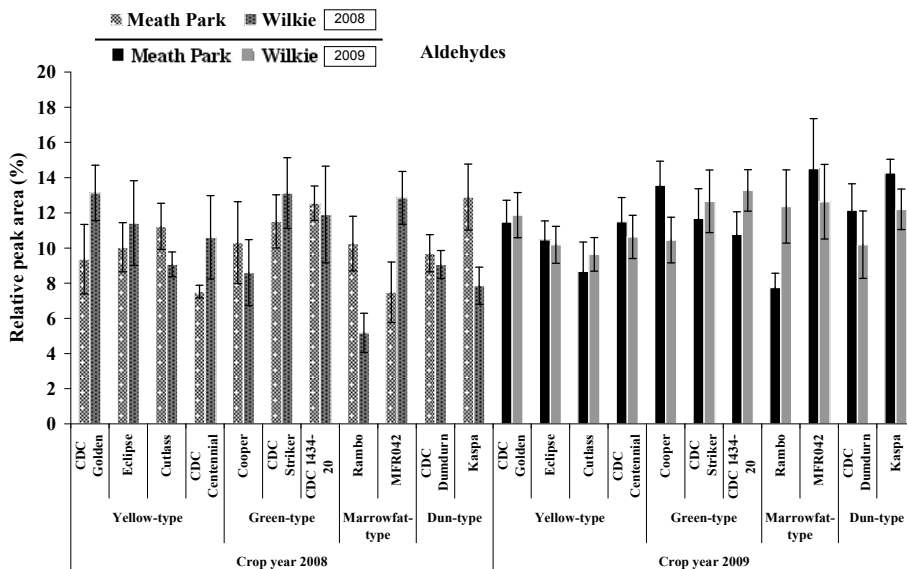


Fig. 3. Changes in total aldehyde content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean ± standard deviation. Relative peak area (%) = Peak area of total aldehydes/ Total peak area of volatile compounds x 100.

3.1.2.3 Ketones

Fig. 4 shows relative peak areas of ketones in the different pea cultivars studied. A significant difference ($P < 0.01$) in the mean value of ketones was observed between pea cultivars from different locations (Tables 2 & 4). Pea cultivar grown in MPK had higher mean value of ketones compared to those from WIL (Table 2). In the 2009 crop year, pea cultivar grown in WIL had higher mean value of ketones than those from MPK (Table 4). 2-Butanone had higher mean value compared to 2-pentanone in all the pea cultivars studied (Tables 2 & 4).

Ketones are products derived from lipid oxidation. They have different characteristics which could affect the flavour of peas. 2-Pentanone, and 2-butanone have been described as having a wine or acetone odour, and a sweet apricot odour, respectively (Burdock, 2002).

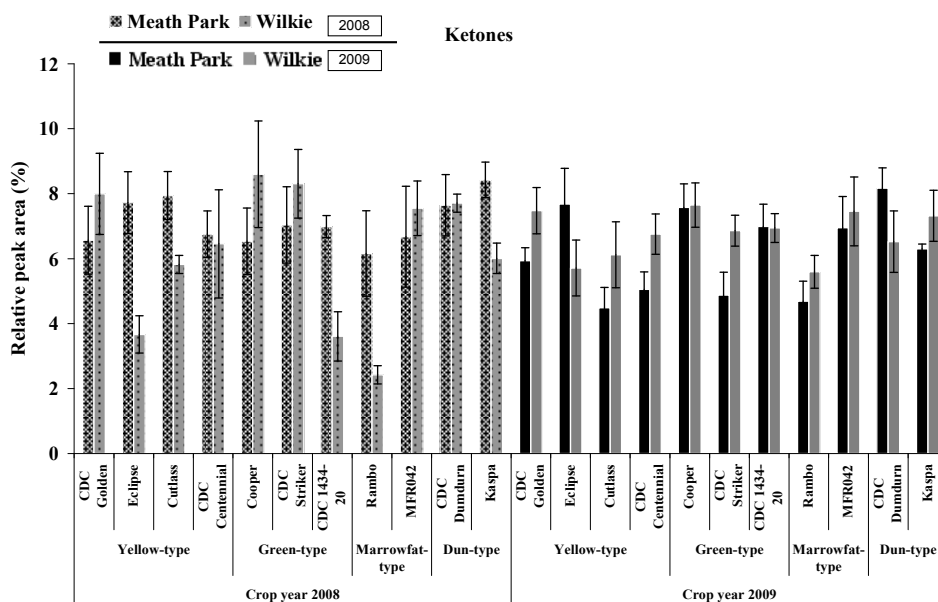


Fig. 4. Changes in total ketone content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean \pm standard deviation. Relative peak area (%) = Peak area of total ketones/ Total peak area of volatile compounds \times 100.

3.1.2.4 Esters

The relative peak area of esters found in the pea cultivars is shown in Fig. 5. No differences ($P > 0.05$) were found between the cultivars grown in different locations (Tables 2 & 4). Ethyl acetate was the most abundant ester in all the pea cultivars studied (Tables 2 & 4). This compound has an ether and brandy odour and a fruity, sweet taste and has also been reported in soybeans and beans (Burdock, 2002; del Rosario et al., 1984). Hexanoic acid, methyl ester also identified in the peas reportedly has an ether and pineapple odour (Burdock, 2002).

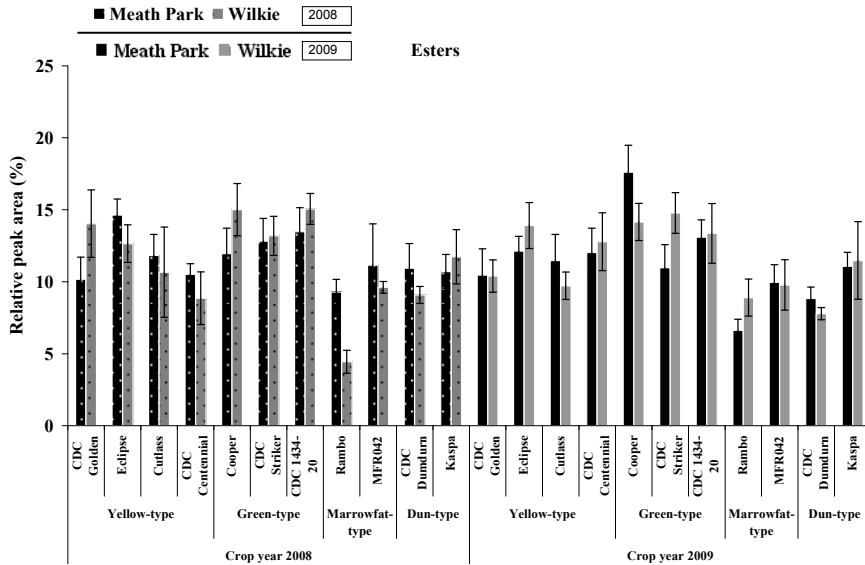


Fig. 5. Changes in total ester content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean ± standard deviation. Relative peak area (%) = Peak area of total esters/ Total peak area of volatile compounds × 100.

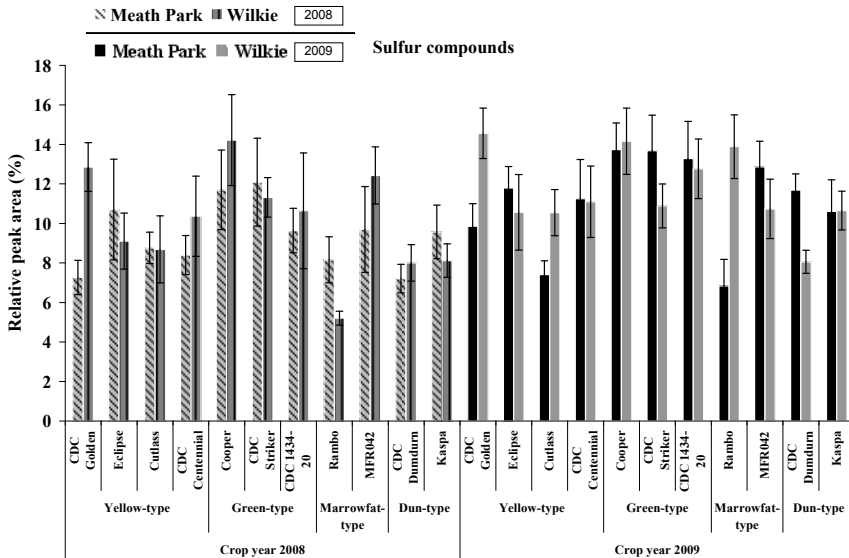


Fig. 6. Changes in total sulfur compounds content in cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean ± standard deviation. Relative peak area (%) = Peak area of total sulfur compounds/ Total peak area of volatile compounds × 100.

3.1.2.5 Sulfur compounds

Differences in sulphur compounds found in the pea cultivars are presented in Fig. 6. Significant differences ($P < 0.01$) were found between the pea cultivars. In both years, pea cultivars grown in WIL had higher mean value of sulfur containing volatile compounds than those grown in MPK (Tables 2 & 4). Dimethyl sulfide was the most abundant sulfur compound in the peas studied (Tables 2 & 4).

Volatile sulphur compounds are natural compounds in foods and could be formed during heat processing and storage (Maga et al., 1973). Formation of these compounds has been reported in blanched peas (Jakobsen et al., 1998). Sulphur compounds contribute to the overall flavour and aroma of foods (Jakobsen et al., 1998). For example, dimethyl disulfide, one of the major sulphur containing compounds identified, has a diffuse, intense onion odour. Dimethyl sulfide, on the other hand, has an intense, cabbage odour (Burdock, 2002).

3.1.2.6 Hydrocarbons

The relative peak area of hydrocarbons found in the pea cultivars is presented in Fig. 7. In the 2008 and 2009 crops, significant ($P < 0.01$) differences in the mean value of hydrocarbons were observed between the peas grown in different locations. In both years, peas grown in MPK had higher hydrocarbons compared to the ones from WIL (Tables 2 & 4). The most abundant hydrocarbon was trichloromethane, followed by furan,2-methyl and toluene (Tables 2 & 4).

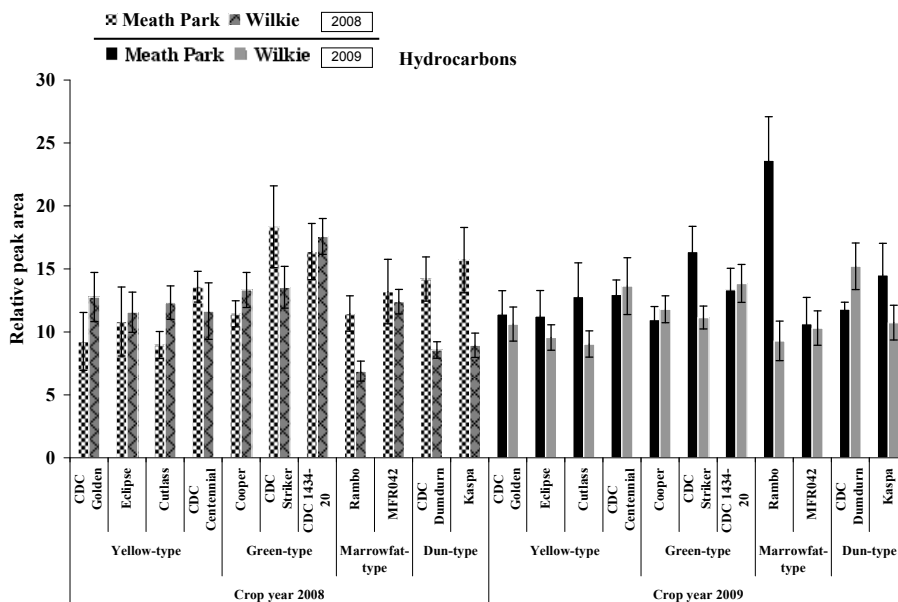


Fig. 7. Changes in total hydrocarbons content in different cooked field peas as affected by type, cultivar, location and crop year. Results are from a two replicate analysis and expressed as mean \pm standard deviation. Relative peak area (%) = Peak area of total hydrocarbons/ Total peak area of volatile compounds \times 100.

| Chemical family | ¹ ANOVA | | | | | | | | |
|---|----------------------|-----------------------|------------------|------------------------|----------------|----------------|------|-----|-----|
| | Main effects | | | | Interactions | | | | |
| | ² cv | ³ l | ⁴ t | ⁵ r | cv*l | l*t | cv*r | t*r | l*r |
| Alcohols | +++ | NS | +++ | NS | +++ | NS | NS | NS | NS |
| Aldehydes | +++ | NS | +++ | NS | +++ | ++ | NS | NS | NS |
| Ketones | +++ | ++ | +++ | NS | +++ | NS | NS | NS | NS |
| Esters | +++ | NS | +++ | NS | +++ | NS | NS | NS | NS |
| Sulfur compounds | +++ | +++ | +++ | ++ | +++ | ++ | +++ | NS | NS |
| Hydrocarbons | +++ | +++ | ++ | +++ | +++ | NS | NS | NS | NS |
| Pyrazines | +++ | +++ | +++ | ++ | +++ | NS | ++ | NS | NS |
| Duncan grouping for each chemical family in peas belonging to different pea- types and grown in different location | | | | | | | | | |
| | Pea-type | | | | Location | | | | |
| Alcohols | Marrowfat (a) | Yellow (ab) | Dun (bc) | Green (c) | Wilkie (a) | Meath Park (a) | | | |
| Aldehydes | Dun (a) | Green (a) | Marrowfat (b) | Yellow (c) | Wilkie (a) | Meath Park (a) | | | |
| Ketones | Dun (a) | Green (ab) | Yellow (bc) | Marrowfat (c) | Wilkie (a) | Meath Park (b) | | | |
| Esters | Green (a) | Yellow (b) | Dun (c) | Marrowfat (c) | Wilkie (a) | Meath Park (a) | | | |
| Sulfur compounds | Green (a) | Marrowfat (b) | Dun (b) | Yellow (b) | Wilkie (a) | Meath Park (b) | | | |
| Hydrocarbons | Green (a) | Dun (ab) | Marrowfat (ab) | Yellow (b) | Meath Park (a) | Wilkie (b) | | | |
| Pyrazines | Dun (a) | Yellow (ab) | Green (b) | Marrowfat (c) | Meath Park (a) | Wilkie (b) | | | |
| Duncan grouping for individual flavor compounds in peas belonging to each chemical family | | | | | | | | | |
| Alcohols | 1-Propanol (a) | 2-Ethyl-1-hexanol (a) | 1-Octanol (b) | 3-Methyl-1-butanol (c) | 1-Hexanol (d) | | | | |
| Aldehydes | 3-Methyl-butanal (a) | Hexanal (b) | Benzaldehyde (c) | 2-Methyl-butanal (d) | | | | | |

Table 4. (Continued)

| | | | | | |
|------------------|-------------------------------|---------------------------------|------------------------|-------------------------|------------------------|
| Ketones | 2-Butanone (a) | 2-Pentanone (b) | | | |
| Esters | Ethyl acetate (a) | 3-Methyl-1-butanol- acetate (b) | | | |
| Sulfur compounds | Dimethyl sulfide (a) | Methan-ethiol (b) | 2-Acethyl-thiazole (c) | Dimethyl trisulfide (d) | Dimethyl disulfide (e) |
| Hydro-carbons | Trichloro-methane (a) | Furan,2-ethyl (b) | Toluene (c) | Undecane (c) | |
| Pyrazines | 2,3-Diethyl-5-methyl pyrazine | | | | |

¹ANOVA performed using general linear model. +++= $P < 0.01$, ++= $P < 0.05$, NS= Not significant ($P > 0.05$).

²cv=Cultivar, ³l=Location, ⁴t=Type, ⁵r=Replicate. Items with different letters within a row are significantly different at $P < 0.05$ (a>b>c>d>e).

Table 4. ANOVA results and Duncan's multiple range test for chemical families in cooked pea cultivars grown in the year of 2009

In general, hydrocarbons are derived from oxidation of unsaturated fatty acids in foods (Märk et al., 2006; Oomah & Liang, 2007). Trichloromethane (chloroform), produced on exposure to chlorinated organic compounds, is a natural compound in plants (Lovegren et al., 1979). Volatile alkanes reportedly contribute to the desirable odour or flavour characteristics of green beans and peas (Perkins, 1988).

3.1.2.7 Pyrazines

2,3-Diethyl-5-methyl pyrazine was the only pyrazine identified in the pea cultivars studied. Significant ($P < 0.01$) differences were observed between pea cultivars grown in different locations (Tables 2 & 4). CDC Golden and Rambo had, respectively, the highest and the lowest mean value of this compound (Tables 2 & 4). In 2008, peas grown in WIL had higher values of this compound compared to those grown in MPK (Table 2). In the 2009 crop, peas from MPK had higher values of this compound than those from WIL (Table 4).

Pyrazines have low vapour pressure and an intense smell and contribute to desirable flavours and aroma of fresh vegetables (Müller & Rappert, 2010). 2,3-Diethyl-5-methyl pyrazine has a nutty, meaty, roasted hazelnut odour (Burdock, 2002).

3.2 Effect of the crop year on the flavour profile of field pea cultivars

ANOVA analysis was carried out on the data pooled from the two crop years to evaluate the impact of this parameter on the flavour profile of pea. Results showed that TVC in pea was significantly ($P < 0.01$) affected by crop year (Table 5). Cultivars grown in the year 2009 had higher TVC than those from the 2008 year (Table 5). There were significant differences in alcohols, aldehydes, sulfur compounds and pyrazine between the cultivars grown in different years. No significant differences in ketones, hydrocarbons and esters were found between the crops grown in different years (Table 5). In general, higher values of alcohols, sulfur compounds and pyrazine were observed in the peas from 2008, whereas crops from 2009 had higher values of aldehydes (Table 5).

¹ANOVA

| Main effects | Interactions | | | | | | | | | |
|------------------|-----------------|----------------|----------------|-----------------|----------------|------|-------|------|-----|------|
| | ² cv | ³ l | ⁴ t | ⁵ cy | ⁶ r | cv*l | cv*cy | cy*l | t*l | t*cy |
| Total volatiles | +++ | +++ | +++ | +++ | NS | +++ | +++ | +++ | +++ | +++ |
| Alcohols | +++ | +++ | +++ | +++ | NS | +++ | +++ | +++ | ++ | NS |
| Aldehydes | +++ | NS | +++ | +++ | NS | +++ | +++ | NS | +++ | NS |
| Ketones | +++ | +++ | +++ | NS | NS | +++ | ++ | +++ | NS | NS |
| Esters | +++ | NS | +++ | NS | NS | NS | NS | NS | NS | NS |
| Sulfur compounds | +++ | NS | +++ | ++ | NS | +++ | +++ | +++ | NS | NS |
| Hydrocarbons | +++ | +++ | +++ | NS | NS | +++ | +++ | NS | +++ | +++ |
| Pyrazines | +++ | NS | +++ | ++ | NS | +++ | +++ | +++ | NS | NS |

Duncan grouping

| Compound | Crop year | |
|------------------|-----------|----------|
| Alcohols | 2008 (a) | 2009 (b) |
| Aldehydes | 2009 (a) | 2008 (b) |
| Ketones | 2008 (a) | 2009 (a) |
| Esters | 2009 (a) | 2008 (a) |
| Sulfur compounds | 2008 (a) | 2009 (b) |
| Hydrocarbons | 2008 (a) | 2009 (a) |
| Pyrazines | 2008 (a) | 2009 (b) |
| Total volatiles | 2009 (a) | 2008 (b) |

¹ANOVA performed using general linear model. +++= $P<0.01$, ++= $P<0.05$, NS= Not significant ($P>0.05$).

²cv=Cultivar, ³l=Location, ⁴t=Type, ⁵cy=Crop year, ⁶r=Replicate. Compounds belonging to each chemical family with different letters within a row are significantly different at $P<0.05$ (a>b).

Table 5. ANOVA and Duncan's multiple range test results for total volatile compounds and chemical families in peas grown in two different crop years

4. Conclusion

Our results showed that the flavour profile of peas was affected by market class, cultivar location, and crop year. The highest total volatile compound (TVC) was observed in cultivars from marrowfat-market class. Crops grown in Meath Park location had the highest TVC. Furthermore, different volatile compounds were identified in pea cultivars. In both crop years, cultivars from the green-market class had the highest mean values of esters and hydrocarbons, whereas the highest value of alcohols was observed for the marrowfat-market class, and the dun-market class had the highest mean values of ketones and pyrazine. 3-Methyl-butanol, 1-propanol, 2-ethyl-hexanol, 3-methyl-butanol, trichloromethane, 2-butanone, dimethyl sulfide, ethyl acetate and 2,3-diethyl-5-methyl pyrazine were the most abundant volatile compounds observed in the pea cultivars.

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The aim of this book is to describe the fundamental aspects and details of certain gas chromatography applications in Plant Science, Wine technology, Toxicology and the other specific disciplines that are currently being researched. The very best gas chromatography experts have been chosen as authors in each area. The individual chapter has been written to be self-contained so that readers may peruse particular topics but can pursue the other chapters in the each section to gain more insight about different gas chromatography applications in the same research field. This book will surely be useful to gas chromatography users who are desirous of perfecting themselves in one of the important branch of analytical chemistry.

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