SENSORY ANALYSIS OF REFINED AND WHOLE WHEAT BREADS MADE FROM RED AND WHITE WHEAT USING ELECTRONIC NOSE AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

BY

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfillment of the Degree of

MASTER OF SCIENCE

Department of Food Science

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ACKNOWLEDGEMENTS

I offer my sincere gratitude to my advisor, Dr. H. D. Sapirstein for his knowledge, support and patience throughout this project. I also thank him for allowing me the room to work in my own way and consequently grow. I could have certainly not attained the knowledge of sensory science without help of Dr. Michel Aliani. I am grateful for his time, generosity and willingness to help in this project as my co-advisor. I also thank Dr. Nancy Edwards for participating in my thesis committee.

Special thanks are offered to the Canadian International Grains Institute (CIGI) and to Tony Tweed of CIGI for his time, effort and expertise in producing the bread used in my research, and to Ashok Sarkar also of CIGI who expertly milled the wheat and produced the flour and bran used in the research. Also, I thank Donna Ryland, Haifeng Yang and Randy Roller for their technical guidance. I thank the Richardson Centre for Functional Foods and Nutraceuticals for providing me with their E-nose and GC-MS facilities. I appreciate all the efforts of the staff at the Department of Food Science for their contributions.

I offer my thanks to all those who supported me in any respect during the completion of this project. The financial assistance provided by the Manitoba Graduate Scholarship, St. John's College bursary, William B. Malchy Scholarship, Alfred Rea Tucker Memorial Scholarship, Food Science Department Entrance Scholarship and the Canadian Wheat Board, are greatly acknowledged.

I cannot thank enough my best friends, Ari and Romeo, for their unconditional love and support, and also Arshala and Daiva, for always being there for me.

A big thank you to Mummy and Papa for their encouragement and unwavering support. Thanks also to my little brother Pravesh for always being there for me and cheering me on and always bringing a smile on my face.

Abstract

Aroma is one of the most important quality attributes of bread or any food. It will determine whether the product will be tasted and eaten in the first instance and is a major factor in establishing acceptability and preference. The dominant preference by consumers of bread made from refined flour in contrast to whole wheat flour is at least in part due to the strong and different aroma of whole wheat bread. White wheats may have an advantage over red wheat in this regard according to some industry reports, but the science is extremely limited. The goal of this research was to add more science-based knowledge to this topic via the use of machine olfaction technology, specifically electronic (E) nose and gas chromatography combined with mass spectrometry (GC-MS).

A state-of-the-art E-nose system (AlphaMOS FOX 3000) with metal oxide sensors (MOS) was used to capture aroma volatiles from crumb, crust and whole slices of breads made from sound Canadian Western Red Spring (CWRS) wheat as well as representative samples of two hard white wheats, viz. Snowbird, a cultivar belonging to the Canada Western Hard White Spring (CWHWS) class of wheat, and Platte, a U.S. Hard White Winter (HWW) wheat. The same CWRS wheat provided the base flour for all the breads. A commercial formula and size format was used to produce breads from four flours for the study, i.e. refined CWRS wheat, and three whole wheat flours comprising blends of 85% CWRS flour and 15% bran from CWRS wheat, Snowbird and Platte.

As there was no established protocol in the literature to evaluate bread aroma by E-nose, one was developed. Five temperatures (35, 40, 45, 50 and 60°C) were tested along with two incubation times (5 and 10 min) and four sample sizes (0.05, 0.1, 0.25 and

0.50 g) of ground bread crumbs. Through optimization using E-nose software including principal component analysis, a procedure was adopted using 40 °C, 5 min incubation time and 0.05 g of sample to acquire MOS data from 12 sensors for crust, crumb and whole slices of refined and whole wheat breads. Multivariate analysis methods were used to evaluate the capabilities of the E-nose system to discriminate and correctly classify samples according to bread type. Data for analysis comprised approximately 24 samples each of crust, crumb and whole slices randomly selected from three loaves each of refined and the three whole wheat breads.

Results varied according to the nature of the sample, i.e. crust, crumb or whole slices. For crusts, the greatest distinction in aroma was found between refined and whole wheat breads. Refined bread crust was correctly classified 67% of the time. When refined bread crust was misclassified, samples were confused with whole white wheat crust predominantly from Platte bread. For whole wheat bread crusts, the pattern of classification depended mainly on bran colour. Whole wheat bread crust samples had correct classification scores in the range 54-58%. When misclassified, whole wheat CWRS crust was equally confused with the aroma of crust of the white whole wheat breads, Platte and Snowbird. Whole wheat Platte crust tended to be misclassified with the counterpart white whole wheat Snowbird or refined bread crust. In contrast, Snowbird whole wheat crust tended to be misclassified as either its counterpart HW wheat Platte or whole CWRS wheat. Accordingly, Platte bread crust appeared to possess an aroma more in line with refined wheat bread as opposed to whole wheat bread.

For bread crumb, the pattern of E-nose differentiation of samples was different. In this case, CWRS whole wheat bread aroma was clearly and perfectly distinguished from

the crumb aroma of all the other breads, either whole white wheat or refined CWRS. The latter tended to cluster on its own, as might be expected, and had a correct classification score of 75%, with the balance of samples largely misclassified as Snowbird crumb. Whole wheat Platte and Snowbird bread crumb had identical correct classification scores of 42%, and were similarly confused with the other's aroma (average 3 4% classified) or the aroma of refined wheat bread (average 21% classified). E-nose results for crumb indicated a clear distinction in aroma between the hard red and white wheats in this study.

E-nose analysis of bread samples representing whole slices produced results that provided unsatisfactory discrimination among bread types likely due to the blending of the different aromas of constituent crust and crumb. For whole slices, discrimination between refined and whole wheat breads was substantially lower than that for either crust and crumb samples. Based on this result, analysis of samples that combine both crust and crumb is not recommended for sensory analysis of bread, whether by instruments or human sensory panel.

Further understanding of the differences between different types of bread made from refined wheat flour and whole wheat, and how the inclusion of bran from red and white-grained wheats modifies the composition and content of volatile and non-volatile compounds in crust and crumb was determined by gas chromatography-mass spectrometry (GC-MS). In total, 50 compounds were found, the greater majority of which have been previously reported in bread. Major Maillard reaction compounds like furfural, 2-furanmethanol, pyranone, maltol and 5-hydroxymethyl- 2-furancarboxaldehyde were present in highest concentration in whole CWRS bread. Significantly fewer compounds were found in the crust and crumb of CWRS refined wheat bread compared to the other

whole wheat breads. In contrast, whole CWRS bread crumb and crust had the highest number of compounds, and in considerably higher total concentration compared to the other two whole white wheat breads, Snowbird and Platte. The higher concentration and number of compounds in whole CWRS bread was attributed to the wheat bran fraction. White whole wheat breads, Snowbird and Platte, had a total number of compounds in crust and crumb approximately intermediate between refined and whole CWRS bread, although Platte whole wheat bread crust was closer to refined bread crust in compound numbers. In terms of total compound concentrations, crust and crumb samples of the whole white breads were clearly more similar to refined CWRS bread, and in the case of whole wheat Platte bread crust, compound concentrations were much lower. On the whole, these aggregate totals of compound numbers and concentrations by GC-MS mirrored the discrimination and classification results obtained by E-nose, and supported the contention that whole wheat bread made with white wheat bran was milder in aroma compared to bread formulated using red wheat bran.

While the number of samples of red and white wheats were very few in this study, results support the contention that different wheat genotypes and specifically, the bran tissue of these genotypes, contain differences in compound composition and/or concentration which when processed by breadmaking, manifest volatiles characteristic of those genotypes even between genotypes possessing the same colour of bran. E-nose instrumentation appears to be very capable of accommodating these sorts of complex tasks on fresh bread. It would be highly beneficial in future research to carry out similar studies in parallel with a human sensory panel, and ideally with many more genotypes of red and white grained wheat with an aim to firmly establish the relative superiority of

particular genotypes to produce whole wheat bread with aroma profiles more similar to those of white pan bread. The long term goal of such studies would be to foster increased consumption of whole wheat products and constituent bioactive compounds which confer favourable health benefits in the general population.

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List of Abbreviations

AACC	American Association of Cereal Chemists
CIGI	Canadian International Grain Institute
CGC	Canadian Grain Commission
CWB	Canadian Wheat Board
CWHWS	Canada Western Hard White Spring
CWRS	Canada Western Red Spring
EI	Electron Ionization
FIA	Flow Injection Analysis
GC-MS	Gas Chromatography-Mass Spectrometry
HMF	5-Hydroxymethylfurfural
HR	Hard Red
HW	Hard White
LRI	Linear Retention Index
MD	Method of Detection
MOS	Metal Oxide Sensors
MS	Mass Spectrum
NAMA	North American Millers Association
NIST	National Institute of Standards and Technology
PCA	Principal Component Analysis
SHA	Static Headspace Analysis
USDA	United States Department of Agriculture

CHAPTER 1

Introduction

Bread is the most widely consumed processed food. In North America, most bread is made from hard red (HR) wheat. In Canada, bread wheat flour is mainly derived from the Canada Western Red Spring (CWRS) wheat class. Canada Western Hard White Spring (CWHWS) is a relatively new class of wheat in Canada that was first put into commercial production in 2003 with the release of the cultivar Snowbird. In the U.S., hard white (HW) wheat was put into limited commercial production 15 years earlier. In North America, HW wheat production represents a small proportion of total bread wheat acreage, but significant and growing demand exists in domestic and export markets. In contrast, much of the wheat grown in China, South Asia and Australia is HW wheat.

The distinct benefit of HW wheat over its HR counterpart is a flour and product colour advantage at higher extraction rates when flour contains more bran (Ambalamaatil et al., 2006). Apart from colour, there have been comments and reports made in the scientific literature and wheat industry publications of a flavour or aroma advantage of HW wheats, specifically that whole wheat bread made from HW wheat has a milder taste as compared to bread made from HR wheat (Chang and Chambers IV, 1992; Ambalamaatil et al., 2006; Ransom et al., 2006). Marketing of Ultragrain^{TM} whole wheat flour derived from the U.S. HW winter wheat variety, Platte is based on this supposition. However, there are very few scientific studies to support this claim that HW wheat bread is a milder tasting or a sensory-wise different product compared to HR wheat bread.

Limited sensory panel research has noted differences, but not preferences in flavour and volatiles between both refined flour and whole wheat breads made from HR

and HW wheats (Lang and Walker, 1990; Chang and Chamber IV, 1992). The latter study is significant as it appears to be the only peer-reviewed report of evidence of the perception of bitterness in HR wheat derived products and sweetness in HW wheat products. Along with visual appeal, bread aroma and flavour can substantially affect the consumer perception of bread quality. Flavour perception involves aroma, taste and texture. Aroma refers to the volatile flavour components that are sensed by smell and is usually the first sense to assess product quality. Taste comprises sweet, sour, salty and bitter components that are sensed by the tongue and the mouth.

There are various types of breads present in market today which attract a broad range of consumers. Some consumers prefer whole wheat bread with its higher fiber content, while some prefer refined breads because of its milder sensory qualities. Market research has shown that the majority of consumers predominantly prefer refined bread over whole wheat bread (Bakke and Vickers, 2007) in spite of latter's superior nutrient profile. As with human sensory work, there is very limited scientific research attempting to explain the difference in flavour or volatile components of refined and whole wheat bread. Many differences using gas chromatography mass spectrometry (GC-MS) technology were found between white pan and whole wheat bread, and many fewer differences were found between whole wheat breads made from HR and HW wheat (Chang et al, 1995). Clearly, machine olfaction, if proved to be accurate and precise, would be an excellent complement to sensory analysis due to its objective nature. Instrument olfaction includes conventional instruments like GC-MS which has been extensively used since the early 1970s to analyze the composition of volatiles from food and beverages to give a better understanding of products. A more recent technology is

the electronic nose or E-nose. E-noses are rapidly gaining popularity in the food industry. Sensory analysis also offers the possibility to compare E-nose evaluation with those of expert human sensory panels which would help in establishing the effectiveness of E-nose instrumentation. In contrast to many reports of GC-MS application to bread volatiles (but very few on whole wheat products), there have been essentially no publications on the application of E-nose to bread aroma. This thesis research has focused on several of these knowledge gaps using both E-nose and GC-MS technologies applied to HR and HW wheat breads made from both refined and whole wheat flour.

The objectives of this study were as follows:

- To develop a protocol for analyzing bread using state-of-the-art E-nose technology.
- To use the E-nose to establish its capabilities to differentiate between crust, crumb and whole slices of refined and whole wheat bread and between whole wheat breads from red and white wheats.
- To develop a better understanding of the differences between different types of bread made from refined wheat flour and whole wheat, i.e. how the inclusion of bran from red and white-grained wheats modifies the composition and content of volatiles and non-volatile compounds determined by GC-MS.
- To determine to what extent results obtained by the GC-MS approach were comparable to the discrimination and classification results obtained by E-nose where aroma compounds are not specifically identified.

CHAPTER 2

Literature Review

2.1. Wheat and wheat composition

Wheat is grown throughout the world and is the most widely consumed cereal grain. The uses of wheat are very varied and extensive. Wheat is the major ingredient in most bread, rolls, chapattis, pancakes, cookies, cakes, doughnuts, muffins, waffles, noodles, pizza, puddings, breakfast cereals, and many other foods. Although durum wheat (*Triticum durum*) production is significant, the majority of the wheat grown in the world is *Triticum aestivum*, or so called common wheat. It is also the largest crop produced in Canada.

Wheat grain has three main parts viz. endosperm, bran and germ consisting of typically, 82%, 15% and 3% of grain weight respectively. When wheat is milled to provide refined flour, the objective is to separate endosperm from the germ and bran tissues including the aleurone layer (botanically the outermost layer of the endosperm). Figure 2.1 show the structure of wheat kernel.

2.1.1. Wheat Composition

Wheat grain varies widely in chemical composition and these differences have wide-ranging effects on processing, use and nutritive value. (Reitz, 1976). Proteins are the most important components of wheat flour for breadmaking purposes. Wheat proteins, based on their solubility in different solvents, can be divided into four types: albumins, globulins, gliadins and glutenins. Wheat can vary widely in protein content,

from e.g. 7 – 20%, due to genetic and environmental factors. Typically, wheat bran can contain higher protein content than whole grain. Table 2.1 presents a typical composition of whole wheat and major fractions. The minerals in wheat, as represented by ash content, are mainly concentrated in the bran. This is mainly a result of the presence of aleurone tissue in bran (Fig. 2.1) which adheres very tightly to the seed coat when wheat is milled (Peyron et al., 2003). The minerals present in bran include potassium, phosphorus and magnesium (about 85% of total, USDA Nutrient Database 2009) with the balance comprising mainly selenium, calcium, manganese and zinc. Flour lipids in wheat are also concentrated in germ and bran (Table 2.1). Lipids are believed to have a role in breadmaking by interacting with proteins to modify gluten structure (Chung et al., 1978). Vitamins are another class of wheat nutrients that are concentrated in bran (Table 2.1). Bran vitamins are mainly represented by the soluble B vitamins. However, wheat has the highest concentration of tocopherols, i.e. vitamin E, than any other cereal grain (Morrison, 1978), and it is mostly present in the wheat germ. Arguably the most important nutrient component of whole wheat is fibre which is composed mainly of non-starch polysaccharides that are highly concentrated in bran (Table 2.1).

Table 2.1. Proximate composition of different layers of whole wheat grain (%).

Nutrients	**Whole wheat**	**Refined flour (endosperm)**	**Wheat bran**	**Wheat germ**
Moisture	10.3	11.9	9.9	11.1
Protein	13.7	10.3	15.6	23.2
Total lipid	1.9	0.98	4.3	9.7
Ash	1.6	0.47	5.8	4.2
Carbohydrates	72.6	76.3	64.5	51.8
Total dietary fibre	12.2	2.7	47.8	13.2
Sugars	0.41	0.27	0.41	0
Vitamins	0.11	0.01	0.09	0.01

Source: USDA Nutrient Database (2009).

Wheat bran is derived from various stages of the milling process and may differ in purity i.e. contamination by endosperm in chemical composition (Posner, 2000) depending on milling technology, type of wheat used (hard or soft) and environmental (mainly weather) conditions. When considering the human nutritional value of whole wheat products, clearly, the major beneficial components of the wheat kernel are the bran and germ. The majority of bioactive components in whole wheat grain are concentrated in the bran. Apart from important vitamins and minerals (Laubin et al., 2008), bran contains a broad spectrum of phytochemicals, most notably phenolic compounds (Smith and Hartley, 1983; Kim et al., 2006), but also other bioactive constituents like betain and choline (Graham, 2009). On a quantitative basis, the predominant bioactive components of wheat bran are the non-starch polysaccharides which comprise soluble and insoluble dietary fibre (Cui et al., 2000). The aleurone layer contains high levels of protein, lipid, vitamins, minerals and phytochemicals. As noted above, the aleurone layer is separated from endosperm during milling and constitutes inner most layer of bran (Peyron et al., 2003). It is rich in phenolic compounds such as ferulic acid and ρ-coumaric acid (Smith et al., 1983). In addition, aleurone cells are rich in phytic acid, lysine and many vitamins (B1, B2, B3, B6, B9 and E), minerals (P, K, Mg, Mn, Fe) (Laubin et al., 2008), betain and choline (Graham et al., 2009).

2.2. Types of wheat

The major factors that differentiate wheats are texture of the grain (hard or soft), bran colour (red, white or other), growing habit (winter or spring types) and protein content (Cracknell and Williams, 2004; Mahesh et al., 2008). The seven basic classes of

wheat grown in western Canada are: Canada Western Red Spring (CWRS), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Red Winter (CWRW), Canada Western Amber Durum (CWAD), Canada Western Soft White Spring (CWSWS) and Canada Western Hard White Spring (CWHWS) (CGC, 2006).

CWRS and CWAD are the major classes based on production and value to the industry in terms of end products. The relative hardness and softness of wheat are milling characteristics related to the resistance encountered during milling. The harder the texture, the greater the force required to mill the wheat. Hardness is believed to be related to the adhesion between endosperm starch granules and protein (Greenwell and Schofield, 1989; Gooding, 2009). Flour from hard wheat is free-flowing, easily sifted and relatively coarse, while soft wheat yields very fine flour which sifts with difficulty and consists of irregular shaped fragments of endosperm cells (Kent and Evers, 1994a). Hard wheat is favoured for breadmaking due to its higher gluten protein content and typically higher level of starch damage and water absorption and soft wheat is desired for products like cakes and cookies.

The intensity of pigmentation in red wheat is higher than that in white wheat because of the presence of one or more dominant red alleles in red varieties (Flintham, 2000). Wheat grain colour is controlled by the *R* genes on wheat chromosomes 3A, 3B and 3D (McIntosh et al., 1998). HR wheat is the predominant class produced in North America because of its wide adaptation and excellent bread making attributes. It also has superb milling properties and produces high-volume pan breads (CWB, 2006a). HW wheat, on the other hand, is a newer class of wheat produced in North America.

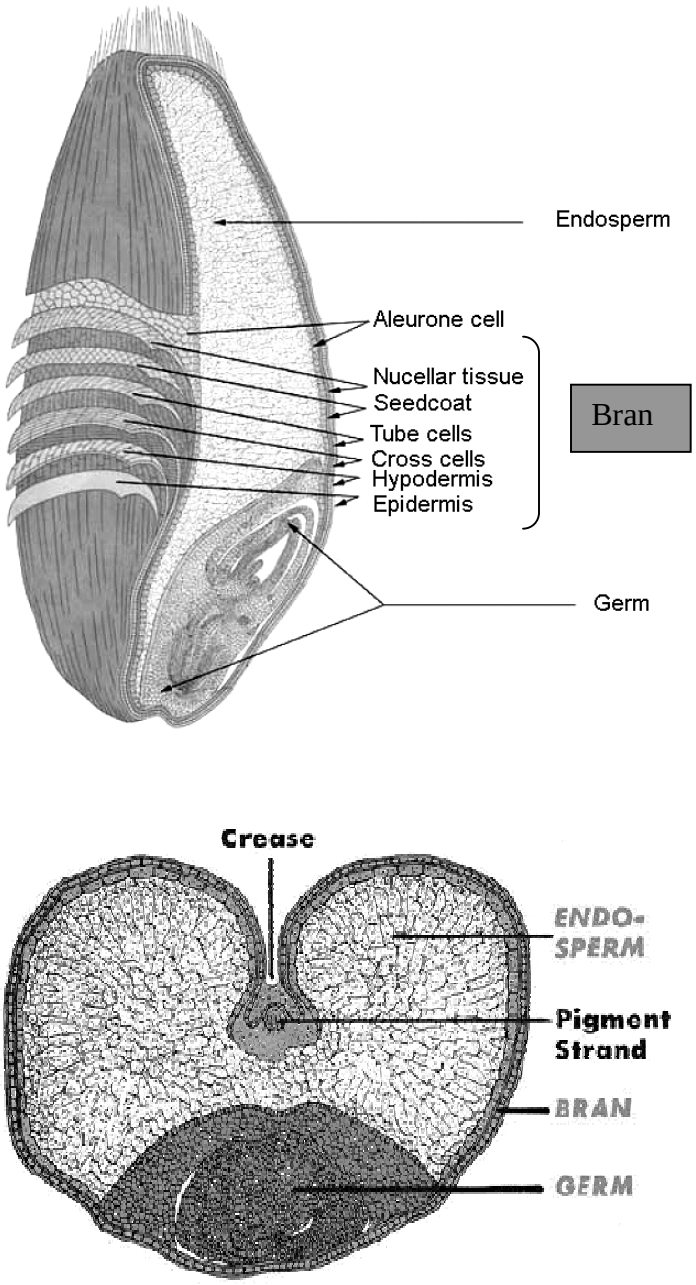


Figure 2.1. Longitudinal (above) and cross-sectional (below) view of wheat kernel (North American Millers Association, 2006 a and b).

However, it is the major type produced in other parts of the world such as China, South Asia and Australia. CWHWS is a new class of Canadian wheat that possesses many qualities like CWRS but also has an additional benefit of improved flour colour and reduced level of speckiness due to bran residue when wheat is milled to higher extraction rates. It also has excellent milling quality similar to that of CWRS (CWB, 2006b). Marquart et al. (2006) suggested use of white whole wheat instead of red whole wheat to reduce the typical brown appearance of whole wheat bread, as one of the ways to introduce more whole grains into diets of consumers who might prefer the whiter appearance of refined wheat products.

A few studies have shown that HW wheat may be preferred over HR wheat for many reasons including lighter coloured end products (hence better appeal), higher flour extraction rate, higher protein content, reduced bitterness, less strong aftertaste and preference from export markets (Miller, 1979; Lang and Walker, 1990; Matus-Cadiz et al., 2008). Chemically, the sensory preference for white wheat could be related to fewer or less concentrated phenolic compounds owing to less pigmentation. Phenolic compounds impart bitterness and astringency to food products like tea, wine, several fruits, nuts and chocolate (Lesschaeve and Noble, 2005).

Red pigment in the seed coat tissue is a derivative of catechins (Fig. 2.2), probably, phlobaphene or proanthocyanidin (Miyamoto and Everson, 1958; Himi and Noda, 2005). These polyphenol compounds including anthocyanin are plant pigments that are synthesized through the flavonoid biosynthesis pathway (Winkel-Shirley, 2001; Lesschaeve and Noble, 2005). Flavonoids are composed of seven major groups, viz. aurones, chalcones, flavones, flavonols, flavandiols, anthocyanins and proanthocyanidins

which are synthesized through the flavonoid biosynthesis pathway (Winkel-Shirley, 2001). White wheat has lesser amounts of proanthocyanidins in their seed coats than red wheat (Matus-Cadiz et al., 2008). Chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H) and dihydroflavanol reductase (DFR) are enzymes that catalyse the flavonoid synthesis pathway and have a reduced role in white wheat as compared to red wheat (Winkel-Shirley, 2001; Himi and Noda, 2005; Matus-Cadiz et al., 2008).

2.3. Whole grains and wheat bran

Historically, the baking and milling industry has strived to meet consumers' preferences for flour and its products. A rapid change has occurred in the past few decades with much more demand for cereal products with high fibre content or whole grain. The nutritional and health benefits of fiber have led to an increase in the production of whole wheat products. American Association of Cereal Chemists (AACC) International defines whole grain as follows, "Whole grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components - the starchy endosperm, germ and bran - are present in the same relative proportions as they exist in the intact caryopsis " (AACC, 1999). Numerous favourable health effects of wheat bran and whole wheat have been reported in the last few decades related to reduced risk of various cancers and cardiovascular disease (Cohen et al., 1996; Earnest et al., 1999; Baublis et al., 2002; Yu et al. 2002; Anderson, 2003; Yu et al., 2003), type II diabetes (Bornet et al., 1987; Plaami et al., 1997; Montonen et al., 2003) and obesity (Liu et al., 2003).

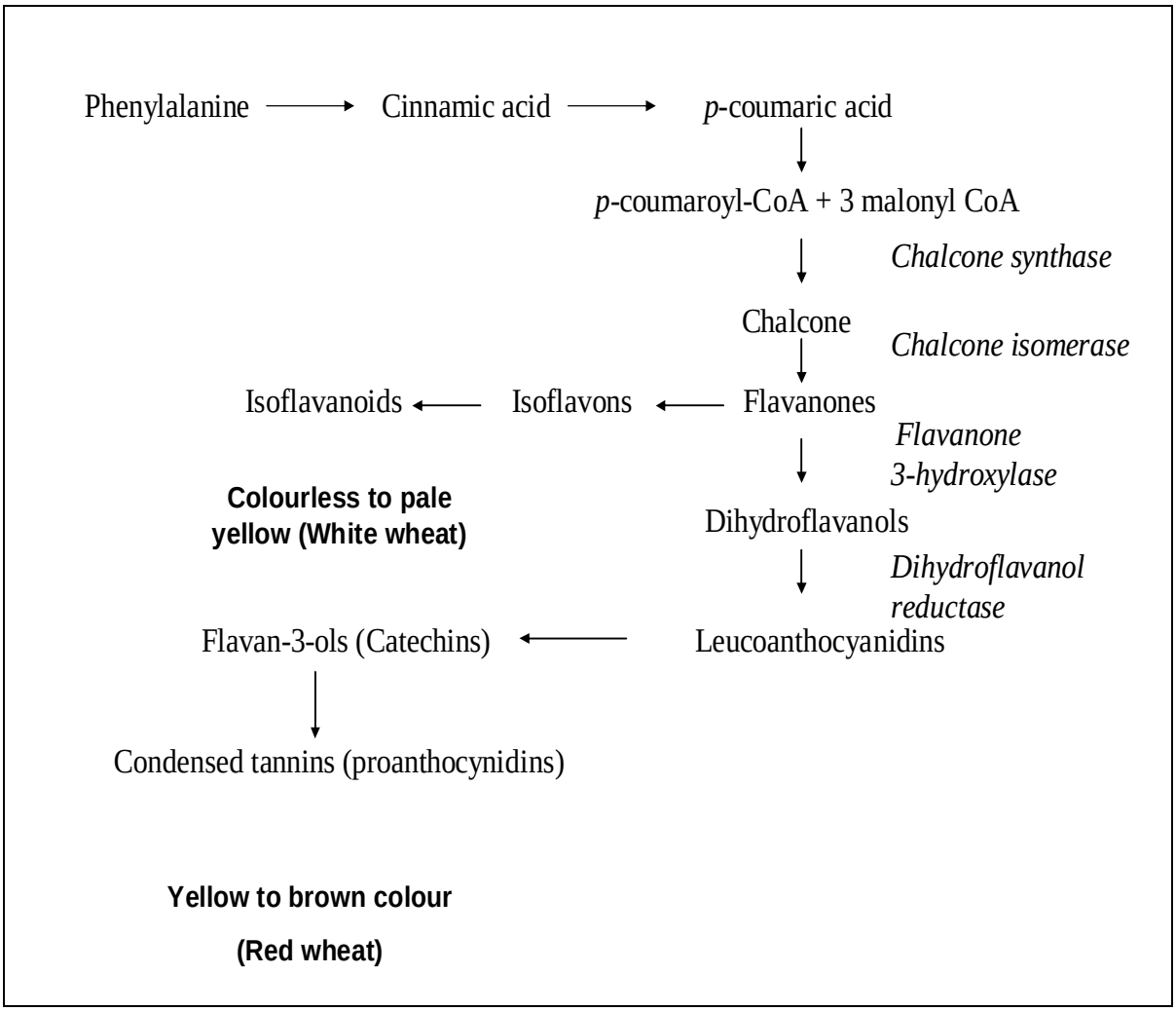


Figure 2.2. Major pathways of flavonoid biosynthesis leading to formation of key pigments of red and white wheat (Matus-Cadiz et al., 2008).

In addition to dietary fibre, wheat contains a wide range of nutrients and bioactive compounds which are believed to have many health benefits. These phytochemicals such as lignans, phenolic acids, carotenoids, flavonoids, tocopherols, tocotrienols, phytosterols, phytostanols, and vitamins are deficient in endosperm, but are concentrated in the germ and in particular, the bran fraction of the grain. Ferulic acid is the primary phenolic acid in wheat and most cereals, accounting for up to 90% of total phenolic acids (Adom et al., 2003). Ferulic acid and many other phenolic compounds are strong

antioxidants and this antioxidant activity appears to be an important factor in reducing risk of diseases mentioned above. Antioxidants function essentially to combat oxidative stress in the human body by maintaining a balance between oxidants and antioxidants (Temple, 2000). The health benefits of cereal grains have significant implications for the improvement of food quality, particularly through applications in functional foods and nutraceuticals (Truswell, 2003).

2.4. Sensory Properties of Bread

Sensory properties of foods significantly influence food habits and consumption patterns (Beilder, 1966; Maga, 1978). Aroma and flavour are very important factors governing the food choices consumers make. Sensory evaluation as a measure of food quality has been in practice ever since food was first produced (Kramer, 1966).

2.4.1. Bread aroma and flavour

Although there are many sensory parameters affecting the quality of bread and its acceptance by the consumers like colour, flavour, texture and appearance, the characteristic aroma of bread is one of the most important factors (Quilez et al., 2006). Palatability of bread is mainly due to its flavour. Bread flavour is a result of many factors, such as the type of wheat, flour, ingredients and the process used in bread formulation. Though much of the bread flavour arises from milling the wheat itself (McWilliams and Mackey, 1969), differences in flour composition, genotype and environmental effects, bread formula and baking techniques make it possible to offer a range of breads having varied aroma and flavour (Schieberle and Grosch, 1989; Kihlberg et al., 2006).

Two conditions essential in producing bread aroma and flavour are fermentation reaction products and formation of crust during baking (Baker and Mize, 1939; Schieberle and Grosch, 1985; Schieberle and Grosch, 1991; Schieberle and Grosch, 1994). Yeast fermentation plays an important role in bread flavour. Difference in fermentation time and temperature results in formation of different flavour compounds (Schieberle and Grosch, 1987; Zehentbauer and Grosch, 1998b; Hansen and Schieberle, 2005). Schieberle and Grosch (1991) reported an increase in malty, sweet and honey like odour in bread in long fermented dough. Development of the Maillard browning products during crust formation delivers different flavour profiles to different types of bread with varied crust to crumb ratios (Cauvain, 2007). According to Chang et al. (1995), longer baking times impart stronger flavour to bread because of formation of more products from browning reactions.

The chemistry of bread flavour involves both volatile and non-volatile components of dough and bread. More than 540 compounds have been identified in bread so far (Schieberle, 1996) which include organic acids, alcohols, ketones, aldehydes, furans, pyrazines, lactones, and sulphur compounds (Schiberle, 1996; Quilez et al., 2006). The bread flavour that originates during the baking process can broadly be divided into three categories: alcohol odours, bread odours and burnt odours (Yoshimo et al., 2007).

Compounds accountable for the aroma profile of crust have a significant influence on consumers' liking and disliking of bread (Schieberle and Grosch, 1985; Belitz et al., 2004). The major compounds responsible for wheat bread crust odour are 2-acetyl-1-pyrroline (roasty smell), 2-acetyltetrahydropyridine (roasty smell), 3-methylbutanal (malty smell), methylpropanal (malty), 4-hydroxy-2, 5-dimethyl-3 (2H)-furanone

(caramel-like smell), acetic acid (sour, pungent), methional (potato-like) and (E)-2-nonenal (green, tallowy) (Schieberle, 1990; Rychlik and Grosch, 1996; Grosch and Schieberle 1997; Zehentbauer and Grosch 1998a; Belitz et al., 2004).

The compound believed to be responsible for the major difference between wheat crust and crumb is 2-acetyl-1-pyrroline. It has been described as a "character impact compound" which has a characteristic roasty aroma (Schieberle and Grosch, 1987 and 1994; Grosch and Schieberle, 1991; Grosch and Schieberle, 1997). Its aroma also has been described as popcorn like (Buttery et al., 1983) and cracker like (Schierberle and Grosch, 1985). It has also been reported to be responsible for difference between wheat bread and rye bread crust (Schieberle and Grosch, 1987 and 1994; Grosch and Schieberle, 1991; Grosch and Schieberle, 1996). The compounds that contribute to caramel like flavour of the crust are 2-methyl-3-hydroxy-4H-pyran-4-one (maltol) and 2-acetyl-3-hydroxyfuran (isomaltol) (Schieberle and Grosch, 1985). The concentration of 2-acetyl-1-pyrroline and 2-acetyltetrehydropyridine has been reported to be 30 times lower in the crumb of wheat bread and thus results in complete absence of a roasty aroma in the crumb (Schieberle and Grosch, 1991).

The aroma of wheat bread crumb is contributed by many important compounds such as, diacetyl (buttery), 3-methylbutanol (malty), 2-phenylethanol (floral), butanoic acid (rancid), 2- and 3- methylbutanoic acid (rancid, sweaty), hexanal (green), (Z)-4-heptanal (biscuit like), (E)-2-nonenal (green, tallowy), ethyl octaonate (sweet), 1-octen-3-one (mushroom like), (Z)-2-nonenal (green), 2, 4-decadienal (fatty, green), (E, E)- 2, 4-decadienal (fatty, waxy) and phenylacetaldehyde (honey like, sweet) (Schieberle and Grosch, 1991; Schieberle, 1991; Schieberle and Grosch, 1994; Gassenmeier and

Schieberle, 1995). Furan derivatives are considered essential to the aroma of bread with their sweet, fruity and caramel like odour (Fors, 1983). In bread crust and crumb presumably, furans result from thermal degradation of sugars (Martinez- Anaya, 1996). Peroxidation of linoleic acid is responsible for formation of "fatty" aroma compounds such as (E)-2-nonenal, (Z)-2-nonenal and 1-octen-3-one in the bread crumb (Schieberle, 1991; Schieberle and Grosch, 1991). Studies show decrease in concentration of (E)-2-nonenal by 46% when omitting fat in the bread making formula. (E)-2-nonenal, give fatty impression and is formed by peroxidation of linoleic acid. Linoleic acid is one of the major fatty acids in the wheat flour. (E)-2-nonenal also naturally occurs in wheat flour (Schieberle and Grosch, 1991). However, upon baking, it was reported to increase five fold in the crumb and more than six fold in the crust. Reducing fat in the dough formula can also reduce the concentration of (E)-2-nonenal in the crumb (Schieberle and Grosch, 1991).

The compounds contributing to bread aroma are present in trace amounts. Not all the compounds present in bread contribute to the aroma. The compounds that are present in bread with concentrations higher than their odour threshold are classified as aroma compounds. Pyrazines are important constituents of cereal grain flavour (Zhou et al., 1999). They possess comparatively high flavour thresholds and thus need to be in high concentrations to contribute to aroma (Bredie et al., 1998).

2.4.1.1. Effect of yeast fermentation on bread aroma and flavour

Fermentation is a necessary prerequisite in aroma production (Wiseblatt and Zoumut, 1963). The characteristic crumb aroma is predominantly due to the compounds

formed during fermentation (Schieberle and Grosch 1984; Schieberle and Grosch 1991; Schieberle and Grosch 1994). Yeast plays an important part in bread flavour. Longer dough fermentation results in increase of 2-phenylethanol which has a flowery, yeasty aroma, and 3-methylbutanol which imparts a malty aroma (Hansen and Hansen, 1996). Studies show that reaction of yeast with L-phenylalanine and L-leucine is mainly responsible for formation of 2-phenylethanol and 3-methylbutanol, respectively (Grosch and Schieberle, 1997). Yeast fermentation also influences the concentration of 3-hydroxy-2-butanone (Gassenmeier and Schieberle, 1995), a major bread aroma compound. Fermentation also produces higher amounts of acetic acid and lactic acid in bread contributing to better flavour (El-Dash and Johnson, 1970; Cayot, 2007).

The major compound in wheat crust, 2-acetyl-1-pyrroline, is formed from baker's yeast. Interestingly, the crumb has ornithine and 2-oxopropanal, the precursors of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine, responsible for roasty aroma, but only the temperature in crust area is high enough to result in their occurrence (Grosch and Schieberle, 1997). Another key crust compound, 6-acetyltetrahydropyridine, is formed by the reaction of proline and 2-oxopropanal, which is a product of thermal degradation of sugars (Grosch and Schieberle, 1997). The amount of iso organic acids like 2-methyl propanoic and 3-methyl-butanoic acid formed in the dough is also proportional to the amount of baker's yeast added in the formula (Pozo-bayon et al., 2006).

2.4.1.2. Amino acids as precursors of bread aroma

Yeast plays an important role in formation of bread aroma. It helps increase the level of substances that, during the baking process are thermally degraded to release

odorants. Amino acids are key precursors in that process. While free amino acids represent a small proportion of protein amino acids, it is notable that wheat bran has more than six times the level than that in sifted wheat flour (~ 0.30% vs. 0.04%, Mustafa et al., 2007). Addition of yeast to dough substantially increased the level of free amino acids in dough by approximately 400%, particularly lysine, alanine, proline, cysteine and dicarboxylic acids (El-Dash and Johnson, 1970).

Free amino acids are metabolized by yeast to produce different aroma compounds (Pozo-bayon et al., 2006). The increase in concentration of free amino acids by yeast has a major effect on bread flavour (El-Dash and Johnson, 1970). Amino acid content is reduced in the crust during the course of baking and thus its importance in non-enzymatic browning is revealed (El-Dash and Johnson, 1970). This could be in part due to deactivation of yeast due to high temperatures in the crust (El-Dash and Johnson, 1970). Reduction of free amino acids is also expected due to the Maillard reaction, in which they are reactants. It was also found that amount of carbonyl compounds like furfural, hydroxymethyl furfural, 2-propanone, 2-methylpropanal, butanal and 2-methylpentanal increases in the crust due to fermentation (El-Dash and Johnson, 1970). Addition of amino acids to the bread formula is believed to increase carbonyl compounds in crust but has no effect on levels in crumb (Salem et al., 1967).

Increase in fermentation time can increase proteolysis and formation of greater amounts of free amino acids which act as precursors of so called “Stecker aldehydes” which were reported to be responsible for malty aroma of baguettes (Zehentbauer and Grosch, 1998a). Amino acids are also important source of 2-acetyl-1-pyrroline and 2-acetyltetrapyridine in wheat bread crust and thus, its roasty smell (Schieberle 1990;

Zehentbauer and Grosch 1998a). The free amino acid ornithine in yeast is known to be the most important precursor of 2-acetyl-1-pyrroline (Schieberle, 1990). In wheat bread crumb, long fermentation increases concentration of 2-phenylethanol, methylpropanal, and 3-methylbutanol which impart floral, malty and honey like odours respectively (Gassenmeier and Schieberle, 1995; Grosch and Schieberle, 1991). Table 2.2 provides a list of amino acids and their associated aromas upon heating.

Table 2.2. Aroma produced on boiling aqueous amino acids and dihydroxyacetone (carbohydrate) mixtures.

Amino acids	**Aroma**
Proline	Cracker, crusty, toasty
Lysine	Dark corn syrup like
Valine	Yeasty
Alanine	Caramel
Glutamic acid	Chicken broth
Aspartic acid	Very weak
Leucine	Cheesy, baked potato
Isoleucine	Crusty
Arginine	Very weak
Cysteine	Hydrogen sulphide
Hydroxyproline	Weak, vaguely like proline
Phenylalanine	Hyacinth
Serine	Vaguely breadlike
Threonine	Very weak
Methionine	Baked potato
Glycine	Baked potato
Histidine	Very weak

Source: Wiseblatt and Zoumut (1963).

2.4.1.3. Contribution of the Maillard reaction to bread aroma

Among the many reactions occurring in food processing, the Maillard reaction plays a very important role in formation of aromatic or flavour compounds and colour (Van Boekel, 2006). It is a major reaction that occurs during baking. This complex

reaction involves the interaction between a free amino group in peptides or proteins and a carbonyl group in reducing sugars. During the Maillard reaction, a myriad of products are formed, which have direct impact on nutritional and sensory qualities of foods. Elimination of water during heating produces a Schiff's base (functional group that contains a carbon-nitrogen double bond) which cyclizes to give corresponding N-substituted aldosylamine which converts into 1-amino-1-deoxy-2-ketose, an Amadori product. Dehydration produces furfural from pentose sugars or 5-hydroxymethylfurfural (HMF) from hexose sugars (Pozo-Bayon et al., 2006), or reductones and dehydroreductones. Dehydroreductones also can generate aldehydes through the Strecker reaction, containing one less carbon atom than the original amino acid. Disintegration of Amadori products generates compounds like 2, 3-butnediol (diacetyl), 2-oxopropanal, etc. which are α-dicarbonyl compounds (Pozo-Bayon et al., 2006) and 1-hydroxy-2-propanone, 2-hydroxy-ethanal, etc. which are hydroxycarbonyl compounds (Pozo-Bayon et al., 2006). Further interaction between compounds formed from the Maillard reaction and Strecker degradation results in formation of compounds containing nitrogen and sulphur containing pyrazines, oxazoles, and thiopenes. Interestingly, many compounds formed as a result of the Maillard reaction are considered toxic or carcinogenic (Lee and Shibamoto, 2002), but the majority of the compounds possess antioxidant properties (Lee and Shibamoto, 2002; Nursten, 2005; Chawla et al., 2009). The antioxidant property of the Maillard reaction products has been shown to elongate the shelf life of heat-treated food products (Ciesarová et al., 2009). Figure 2.3 describes the major reactions involved in the Maillard reaction. Table 2.3 provides a summary of those reactions.

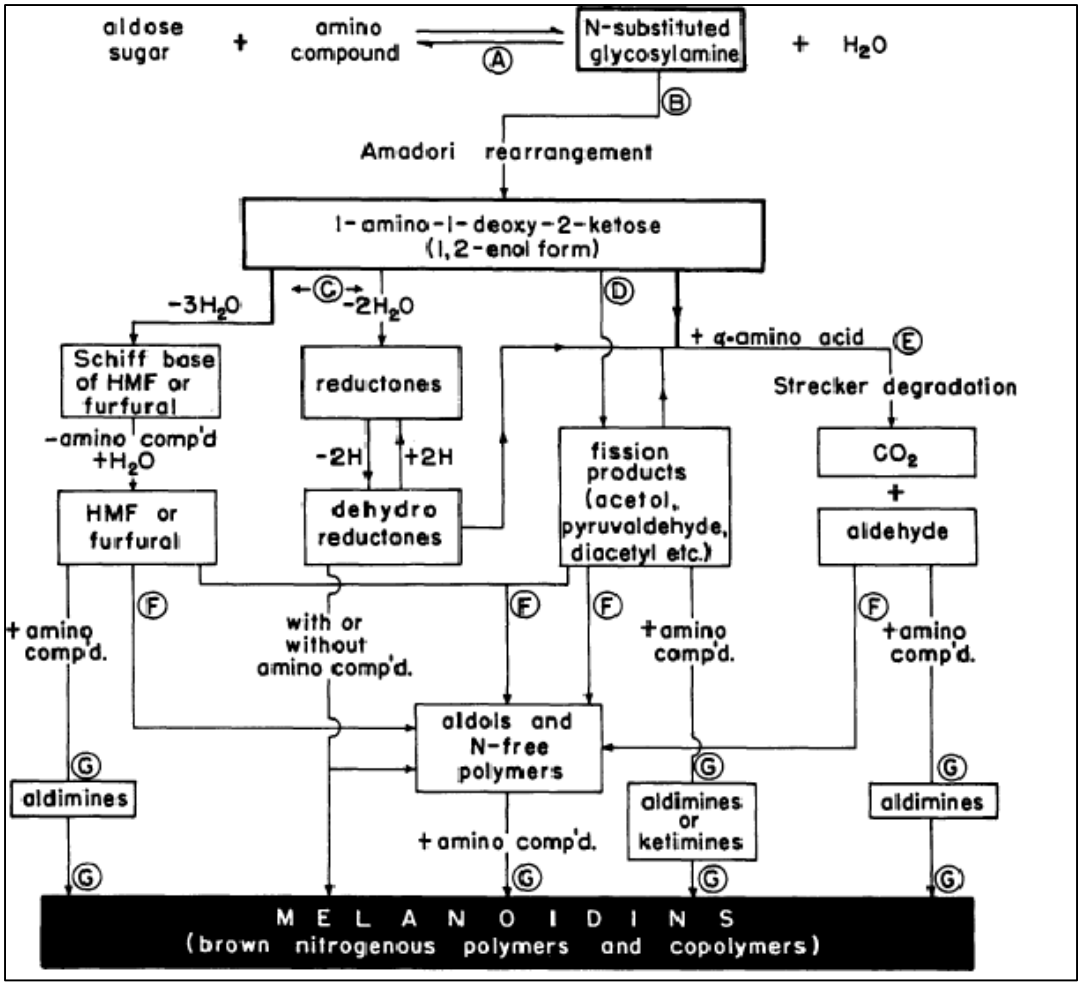


Figure 2.3. Outline of the Maillard reaction (Hodge, 1953).

Table 2.3. Summary of the Maillard reaction based on Hodge's outline.

Initial stage (colourless products)	**Intermediate stage (colourless or yellow products)**	**Final stage (brown products called melanoidins)**
Sugar–amine condensation	Sugar dehydration	Aldol condensation
Amadori rearrangement (acid or base catalyzed isomerization)	Sugar fragmentation	Aldehyde–amine condensation and formation of heterocyclic nitrogen compounds
	Strecker degradation (Amino acid degradation)	

Source: Nursten (2005).

2.4.1.4. Breakdown of phenolic compounds

It is clear from the discussion above that many chemical compounds influence the perceived flavour of food including, non-volatile compounds like sugars, free amino acids and fatty acids (Belitz and Grosch, 1999; Decker et al., 2002). Phenolic compounds also contribute flavour as has been shown in rye and oat breads (Heinio, 2003; Heinio et al., 2008). The outermost bran fraction and some endospermic parts of rye have been reported to be bitter tasting and this bitterness has been associated with significant amounts of alkylresorcinols, lignans and phenolic acids concentrated in those parts of the grain (Liukkonen et al., 2003; Lesschaeve and Noble, 2005). The same can be said in case of wheat bread. The bitterness and astringency of phenolic compounds (Huang and Zayas, 1991; Drenowski and Gomez-Carneroz, 2000) is perhaps a major reason for customer preference of refined or white pan bread over whole wheat bread (Bakke and Vickers, 2007). Although many studies have been done to assess and quantify phenolic acids in wheat and wheat bran, there are no studies reported describing their specific contribution to bread flavor.

2.4.2. Whole wheat bread flavour

Whole wheat bread is far superior to refined flour bread in terms of nutritional composition such as fibre and mineral content. Nevertheless, white bread is the most commonly consumed bread (Bakke and Vickers, 2007). The likely reason is better perceived sensory properties of white bread mainly in texture, colour and flavour. Whole wheat bread has very different sensory properties than white bread. Addition of wheat bran and germ has a major effect on the resultant properties of bread. It lowers the final

loaf volume, impairs crumb texture, increases crumb firmness, darkens the crumb and crust and changes taste and mouth feel (Pomeranz, 1977; Pomeranz et al., 1977; Galliard 1986; Lai et al., 1989; Gan et al., 1992; Heinio, 2006). Despite these unfavorable properties, many consumers prefer eating whole wheat bread for its high bran and fibre content. Many more prefer refined breads because of better sensory qualities (Bakke and Vickers, 2007). Many phenolic compounds with important health benefits are characterized by bitterness and astringency which, sensory-wise, are often perceived as aversive (Drewnowski and Gomez-Carneros, 2000; Lesschaeve and Noble, 2005).

There is ample literature on counteracting the detrimental effects of wheat bran or other non-endosperm components on bread to improve the quality of whole wheat bread to make the product more appealing to consumers. Rogers and Hoseney (1982) concluded that an unknown factor in whole wheat interferes with the rheological changes expected from oxidants and fermentation. The chemical effect of bran on breadmaking has been studied (Galliard, 1986). Gan et al. (1992) reported that bran in wheat flour doughs appears to disrupt the starch gluten matrix adversely affecting the final loaf volume.

It is clear that adding wheat bran to flour imparts a different or off-flavour to the bread. The flavour components of bread have been previously studied but there is limited science concerning the sensory aspects of refined versus whole wheat bread and/or bread made from bran of red and white wheats. Moder et al. (1984) reported that bread produced from white wheat bran was more acceptable to consumers, compared to bread made from red wheat bran. Lang and Walker (1990) compared hamburger buns made from hard white and red winter wheat. While taste panelists easily differentiated red

wheat from white wheat buns, there was no preference for one type over the other. Chang and Chambers IV (1992) compared hard red and white refined and whole wheat pan breads and found more differences in bread crust than crumb. Refined red wheat crust was found to be more sour, bitter and astringent compared to refined white wheat crust. The authors stated that aroma and flavour attributes were "more balanced and blended" in the white bread crust. Whole wheat bread results were less clear. The author suggested that white wheat bread had more after taste than red wheat bread for crust and crumb. Panelists in a study by Zhang and Moore (1999) compared the baking quality and sensory properties of breads prepared with several different brans including soft white and HR wheat bran. Panelists preferred the flavour, mouthfeel and general acceptability of the former over the latter.

Chang et al. (1995) conducted dynamic headspace analysis (using purge and trap) of volatile flavour components of white pan breads and whole wheat breads made from HR winter and HW winter wheat. A total of 63 compounds were identified across all samples using GC-Fourier transform infrared spectroscopy-MS. A range of differences was found depending on the type of bread and colour of bran. In contrast to the authors' conclusion that wheat colour was associated with few differences compared to differences between crumb and crust, careful analysis of the data indicate a more complicated outcome. Compound concentration differences between white and red wheat crumb of the same type of bread (i.e. within pan or within whole wheat bread) were fewer (2-3 compounds) than for crust (6-10), with whole wheat bread producing more differences (6-10) compared to pan bread (2-3). For the pan bread vs. whole wheat bread contrast, the number of compound differences increased in the following order: white

crumb (n=8) < white crust (n=9) < red crumb (n=11) < red crust (n=17). For the crumb vs. crust contrast, the number of compound differences increased in the following order: white whole (n=3) < red pan (n=4) < white pan (n=12) < red whole (n=14).

2.5. Aroma and flavour analysis

The main sensory system used by humans to sense flavour is olfaction. It is plausible that when humans smell any food, bread for example, we perceive its smell as an easily distinguishable and unmistakable aroma. The anatomy of smell is complex and subtle. There are several methods used in the aroma and flavour industry to characterize flavour compounds. Flavour analysis of various food products aims at addressing all aspects of food flavour, including the perception of consumers and their contentment on eating.

2.5.1. Gas Chromatography and Mass Spectrometry (GC-MS) to analyse bread flavour

Aromatic characteristics of wheat bread have been studied in detail. Bread crumb and crust odorants have been previously screened using different methods. Charm analysis (Acree et al., 1984) and aroma extraction dilution analysis (Ullrich and Grosch, 1987; Zehentbauer and Grosch, 1998a) are two such methods. Solvent extraction, headspace analysis and purge and trap are among the popular methods used. More recently, solid phase microextraction (SPME) has been used for headspace analysis of bread volatiles (Ruiz et al., 2003; Poinot et al., 2008). To identify and separate bread compounds, high resolution GC-Olfactometry (Schieberle and Grosch, 1991;

Zehentbauer and Grosch, 1998a), paper chromatography, (Salem et al., 1967), thin layer chromatography (Salem et al., 1967), gas –liquid chromatography (Salem et al., 1967) have been used.

When GC-MS methods were combined together for identification of compounds in white bread (Mulders and Dhont, 1972), 42 new volatiles in white bread were discovered that were never previously reported. Since then, GC-MS has been the most popular technique. Solvent extraction methods for bread component analysis have utilized various solvents e.g. dichloromethane (Schieberle and Grosch, 1985; Schieberle and Grosch, 1987; Schieberle and Grosch, 1991; Gassenmeier and Schieberle, 1995; Zehentbauer and Grosch, 1998a), pentane (Mulders et al., 1973), diethyl ether (Mulders et al. 1973; Folkes and Gramshaw 1977; Zehentbauer and Grosch, 1998a), ethanol (Schieberle and Grosch, 1987), methanol (Wiseblatt and Zoumut, 1963), hexane (Mulders and Dhont, 1972), chloroform (Salem et al., 1967) and water (Wiseblatt and Zoumut, 1963).

2.5.2. Machine olfaction

The perception of flavour in cereal products is most conveniently analysed by descriptive sensory methods (Heinio et al., 2003). In addition to sensory profiling, the main methods for analysing the volatile compounds that influence the aroma and flavour perception are instrumental approaches such as the headspace GC-MS technique and GC/ olfactometry, which involve simultaneously analysing and combining the sensory perception of aroma with the instrumental analysis of odourous volatile compounds (Zhou et al., 1999; Vanderhaegen et al., 2003).

Table 2.4. Review of studies on bread flavour.

No.	Author	Method	Product	Solvent/ Headspace	Results
1.	Wiseblatt, 1960	Vapour-phase chromatography	Wheat bread and dough	Acetone, ether	Major volatile acids were identified
2.	Mulders and Dhont, 1972	This layer chromatography, GC	White bread	Hexane, pentane-ether, ether	24 carbonyl compounds and 13 acids identified, 9 new compounds
3.	Mulders et al., 1973	GC-MS	White bread crust	Pentane-ether	52 compounds identified, 42 new compounds
4.	Folkes and Gramshaw, 1977	Gas liquid chromatography- MS, Flame ionization detector	White bread crust	Diethyl ether	190 compounds identified, 97 new compounds
5.	Schieberle and Grosch, 1985	High resolution gas chromatography (HRGC)	Wheat and rye bread crust volatiles	Dichloromethane	Total 26 compounds identified in both
6.	Schieberle and Grosch, 1987	HRGC	Wheat and rye bread crust	Dichloromethane	Less odorants in wheat crust and more in rye (total 43 compounds identified)

No.	Author	Method	Product	Solvent/ Headspace	Results
7.	Luning et al., 1991	Aroma Isolation Apparatus, GC-MS	White bread, with and without enzyme active soya flour	Headspace analysis	61 compounds identified, 9 new in white bread, 2 additional in bread with soy flour
8.	Schieberle and Grosch, 1991	HRGC-MS	White bread crumb	Dichloromethane	Effect of longer fermentation on odorants
9.	Frasse et al., 1992	GC-O	French bread dough	Dichloromethane	54 known and 19 unknown compounds identified
10.	Schieberle and Grosch, 1992	Aroma extraction dilution analysis and GC-MS	White bread crust	Headspace volatiles, diethyl ether	Change in concentration of crust odorants during storage
11.	Schieberle and Grosch, 1994	Column chromatography, HRGC-olfactometry (O), HRGC-MS	Rye bread crust and crumb	Dichloromethane	Differences between rye crust and crumb and comparison with wheat bread crust (29 neutral/basic and 12 acidic odorants)

No.	Author	Method	Product	Solvent/ Headspace	Results
12.	Chang et al., 1995	GC-Fourier transform infrared spectroscopy-MS	White and whole wheat bread from hard red and hard white winter wheat	Headspace analysis	Many differences between white and whole wheat bread and few between red and white whole wheat bread
13.	Gassenmeier and Schieberle, 1995	HRGC-MS	French type wheat bread crumb	Dichloromethane	Determined most important contributors to crumb aroma
14.	Hansen and Hansen, 1996	GC-MS	Sourdough wheat bread crumb	Headspace analysis	19 compounds identified, flavour intensity higher in sourdough breads
15.	Rychlik and Grosch, 1996	Column chromatography, HPLC, HRGC-O, HRGC-MS	Toasted wheat bread	Dichloromethane, headspace volatiles	51 compounds identified, major compounds responsible for toasted odour determined
16.	Zehentbauer and Grosch, 1997	Purge and trap HRGC	Baguette crust	Headspace analysis	Change in concentration of odorants during storage

No.	Author	Method	Product	Solvent/ Headspace	Results
17.	Zehentbauer and Grosch, 1998a	Column chromatography, HRGC-O, HRGC-MS, GC-O	Baguette crust	Dichloromethane, diethyl ether, headspace analysis	48 odorants identified
18.	Zehentbauer and Grosch, 1998b	Column chromatography, HRGC-O, HRGC-MS, GC-O	Baguette crust	Dichloromethane, diethyl ether, headspace analysis	Change in concentration of odorants by different baking processes
19.	Kirchhoff and Schieberle, 2001	Stable isotope dilution assay, HRGC-O	Sourdough rye bread crumb	Dichloromethane	35 compounds identified
20.	Ruiz et al., 2003	GC-MS	Wheat bread crumb	Solid phase microextraction (SPME) headspace	18 major and 24 minor crumb volatiles identified
21.	Poinot et al., 2008	GC-MS	Wheat bread crust and crumb, fully and partially frozen	SPME headspace analysis	46 compounds identified in total
22.	Maeda et al, 2009	GC-MS	Sourdough and wheat bread	Headspace sorptive extraction method	Total 90 compounds identified in both

Although sensory evaluation by a well trained sensory panel is still the best and most comprehensive method of sensory analysis, it is challenged by a number of factors. A few disadvantages of the human sensory approach include limited availability of panelists, fatigue, high costs and time requirements. Separation-based instrumental techniques are also usually expensive and time-consuming, requiring trained personnel to execute the measurements. As the need for more and better knowledge of food composition increases, the need for techniques or instruments that are simpler and faster also increases (Marsili, 2001; Poprawski et al., 2006). The electronic nose (E-nose) is one such equipment. The term E-nose was first used by Gardner in 1988 in reference to an instrument composed of sensors combined with a pattern recognition system, enabling recognition and discrimination of simple and complex odours (Gibson et al., 2000). Sensorial analysis is one of the branches of food analysis which should benefit most from introduction of the E-nose as an analytical tool (Di Natale et al., 1998).

Gardner and Bartlett (1994) defined the E-nose as "an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognizing system, capable of recognizing simple or complex odours". The three major parts of an E-nose system, odour sensory array, data pre-processor and pattern recognition have been inspired by and designed to operate as a human nose-olfactory receptor cell, olfactory bulb and the brain of humans, respectively (Deisingh et al., 2004). Figure 2.4 graphically illustrates the relationship between a human nose and an E-nose.

There are various types of E-noses that differ in terms of the sensors they use. The first commercial E-nose devices were launched in the early 1990s. Among the first were

systems from Alpha MOS in 1993, Neotronics and Aromascan in 1994, and Bloodhound Sensors and HKR Sensorsyteme in 1995 (Gibson et al., 2000). A list of commercially available E-nose instruments is presented in Table 2.5.

Table 2.5. Commercially available E-noses.

Company name	**Product name**
Airsense Analysentechnik, Germany	PEN-3, i-PEN, PEN-EDU
Agilent Technologies, US	Chem Sensor 4440
Alpha MOS, France	Fox 2000, 3000, 4000, 5000 alphaKronos, alphaPrometheus, alphaCentauri, alphaGemini
Bloodhound Sensors, UK	Bloodhound BH114, OEM-modules under development
Chemsensing Inc.	Chemsensing
Cyrano Sciences, US	Cyranose 320
Daimler Chyrsler Aerospace	SAM system
Element, Iceland	FreshSense
Environics Industry, Finland	MGD-1
Estcal, US	zNose
E2V (Marconi Applied Technologies), UK	e-nose 5000
Forschungszentrum Karlsruhe	Sagas and Gasys
HKR SensorSystem, Germany	QMB6/HS40XL, HS40/MS, MS-Sensor SensiTOF
Hewlett Packard, US	HP4440A
Lennartz Electronic, Germany	MOSES II
Microsensor Systems	ProSat
MoTech Sensoric, Germany	VOCmeter, VOCcheck, OEM-modules
Nordic Sensor Technologies	NST 3210, NST 3220, NST 3220A
Applied Sensor, Sweden	VOCseries, VOCcheck
Osmetech, UK	OMA, MultiSampler-SP, CP sensors
Quartz technology	QTS-1
RST Rostock, Germany	Sam
Smart Nose, Switzerland	SMart Nose-300

Sources: Strike et al., 1999; Gibson et al., 2000; Vanneste and Geise, 2003; Zhang, 2003 and Needham, 2004

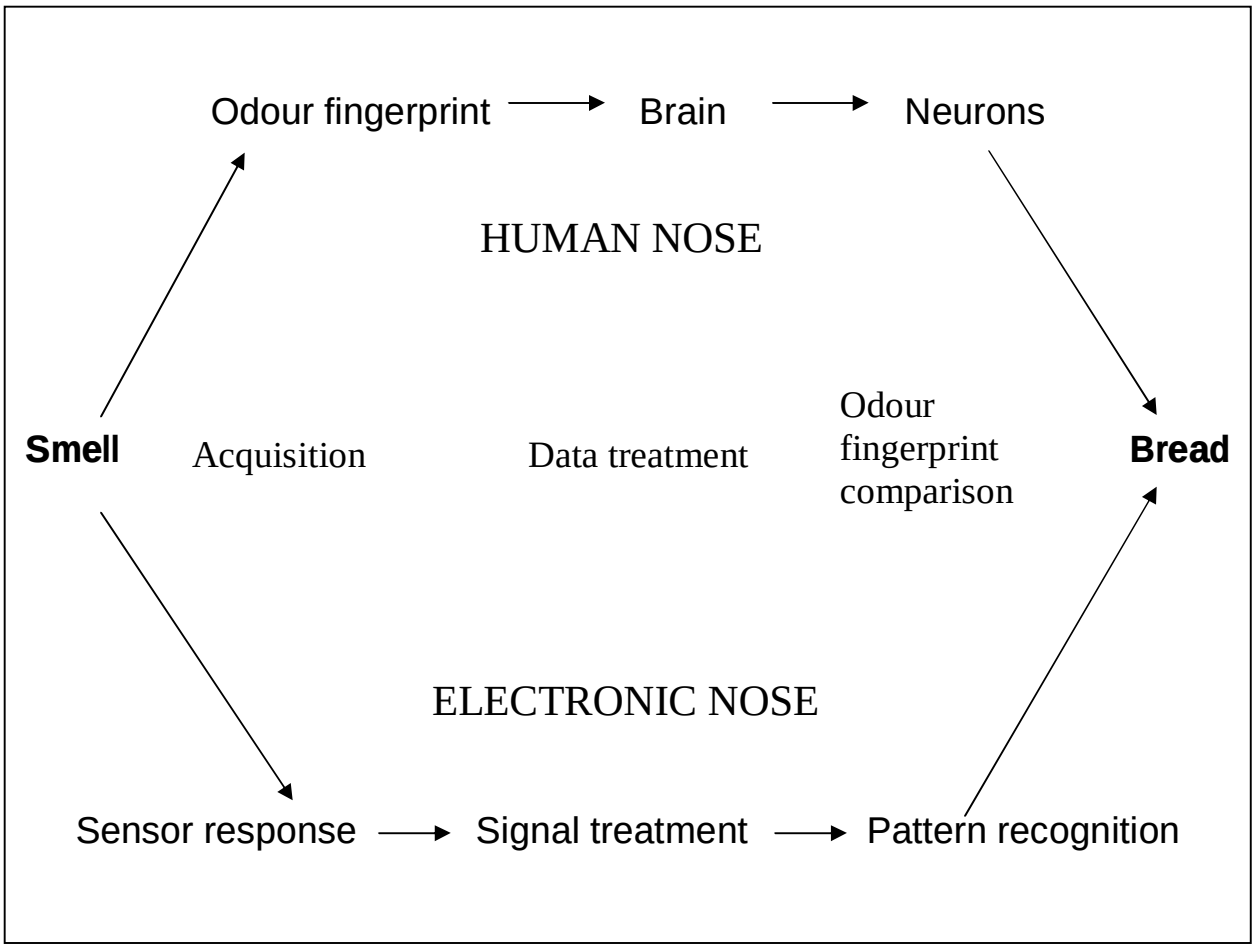


Figure 2.4. Relationship between human nose and E-nose (adopted from Clanchin et al., 2003).

The E-nose used in this thesis research (Fig. 2.5) was an AlphaMOS FOX 3000 (AlphaMOS, Toullouse, France), which uses metal oxide sensors (MOS) which is one of the most common types of sensor coatings. Sensor output to an input volatile stimulus corresponds to a change in electrical resistance of the metal oxide coating. There are twelve sensors in FOX 3000 comprising six so called L-types sensors, two T-type sensors and four P-type sensors. Each sensor in the E-nose is characterized by its own degree of selectivity and this is the basis of the principle on which it works (Sinesio et al., 2000).

The E-nose sensors are sensitive to both odourous and odourless volatile compounds (Haugen, 2001).

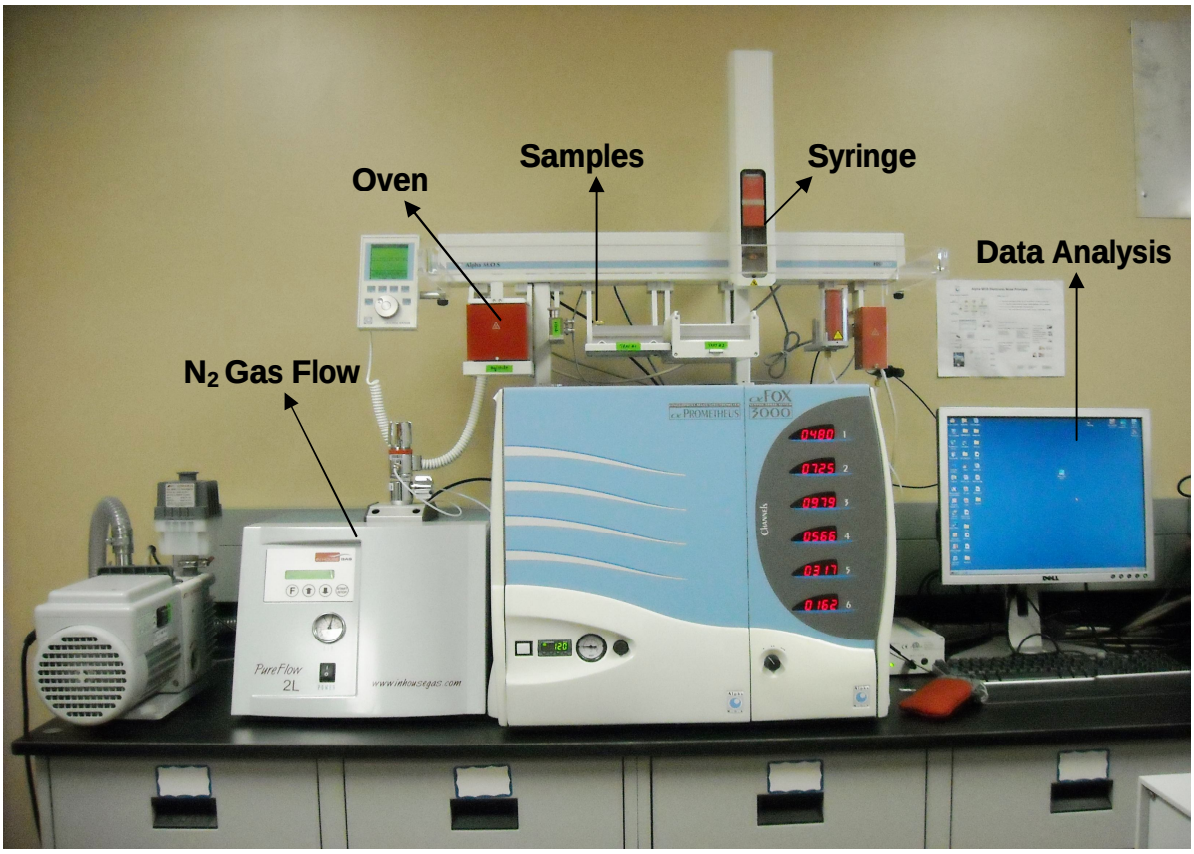


Figure 2.5. AlphaMOS FOX 3000 E-nose system

Ideally, E-nose sensors should be non-specific in their response to volatile organics and should possess good stability and sensitivity coupled with a fast response, and low sensitivity towards humidity and temperature (Bartlett et al., 1997). The most popular sensors (Needham, 2004) used to develop E-noses suitable for uses in the food industry are discussed below.

Semiconductor metal oxide sensors: The oxide materials in the sensor contain chemisorbed oxygen species with which interaction of odour molecules alters the conductivity of the oxide (Bartlett et al., 1997). Sensor selectivity can be altered to different compounds by modification of the films by incorporating different amounts of noble metals or by changing operating temperatures (Börjesson et al., 1996; Dickinson et al., 1998). They are quite sensitive to combustible materials such as alcohols but are less efficient at detecting sulphur- or nitrogen-based odours (Bartlett et al., 1997). They are sensitive and relatively resistant to humidity and ageing. Metal oxides can also be used in a field-effect-transistor configuration, which are termed metal oxide semiconductor field effect transistor sensors (MOSFETs). MOSFET sensors are sensitive to a number of organic compounds.

Quartz-resonator sensors: Quartz resonator sensors consist of a piezoelectric quartz crystal oscillator coated with a sensing membrane such as acetyl cellulose or lecithin (Bartlett et al., 1997). The selectivity of these sensors is dictated by different coatings that are applied to the crystals surface (Dickinson et al., 1998). Adsorption of odour molecules onto the membrane leads to changes in the resonant frequency which is related to the mass of the compound. The magnitude of the change determines differences between odours.

Conducting polymers: There are two main classes of conducting polymers, which are poly(pyrrole)s and poly(aniline)s (Bartlett et al., 1997). The materials are easy to process, so preparation of reproducible gas sensors is possible (Bartlett et al., 1997). The

reversible adsorption of molecules to the films induces a temporary change in the electrical conductance of the film by altering the population of active charge carriers in the polymer structure (Dickinson et al., 1998).

E-noses have found wide and diverse uses. Some of these applications include assessing changes in sensory quality of pork during storage (Vestergaard et al., 2007), classification of spoiled and unspoiled fresh beef in terms of microbiological spoilage (Panigrahi et al., 2005), differentiation of cheese aroma (Drake et al., 2003), differentiation of aging beer (Chmielewski et al., 2007), monitoring oil oxidation (Mildner-Szkudlarz et al., 2008), qualitative and quantitative analysis of perfumed cleaner products (Poprawski et al., 2006), environmental and industrial analysis, water contamination (Ameer and Adeloju, 2005; Canhoto and Magan, 2005), medical sciences to diagnose illnesses (Gardner et al., 2000; Pavlou et al., 2004) and many more. In general, E-noses are mainly used for flavour and aroma identification (Deisingh et al., 2004).

In cereal grain science and industry, the E-nose technique has also had many diverse applications. It has been used for detection of spoilage of grain and mycotoxins, ergosterol and odour volatiles in durum wheat (Jonsson et al., 1997; Magan and Evans, 2000), mite infestation in wheat (Ridgeway et al., 1999), fungal contamination of soft white seeds with *Penicillium* and *Fusarium* (Paolesse et al., 2006), microbial spoilage of bakery products (Needham et al., 2005) and testing shelf life of cereal grains (Zhang et al., 2007). Borjesson et al. (1996) classified samples of wheat, barley and oats according to mouldy/musty odour and concluded that E-nose was equally effective in distinguishing

between samples compared to humans. In their experiment, the E-nose proved to be 90% accurate in classifying samples.

The first and only baking application so far reported for the E-nose (Ponzoni et al. 2008) was to monitor key aromas of baking processes as contributed by acetaldehyde, diacetyl, acetylpyridine, acetylpyrazine and 2-ethyl, 3-methylpyrazine. The authors reported that the E-nose was capable to distinguish these key aromas of bread at different stages of the baking process primarily based on their chemical nature.

The E-nose is considered a dynamic headspace analysis technique (Rouseff and Cadwallader, 2001). Though the characteristics of E-nose are designed to mimic the human nose in certain ways, it has large differences in sensitivity and selectivity from the human nose (Haugen, 2001). The data recorded on the computer is a result of resistance experienced by the E-nose sensors on exposure to volatiles (Zhang et al., 2007). The E-nose has shown correlation with data from human sensory panels for several applications, and is often more sensitive than the human nose (Harper, 2001). E-nose technology has many inherent advantages for machine olfaction. Instruments have fast analysis times, can run continuously for extended periods of time without operator supervision, can run many samples via an autosampler and can detect complex odours. They do not require extensive sample preparation as needed in analytical techniques such as GC-MS. While not a replacement for GC-MS which can quantify and identify compounds, nor a replacement for human sensory panels, the E-nose nonetheless offers an excellent complementary methodology to monitor and discriminate odours (Van Deventer, 2001). The main disadvantage of the moderns E-nose system is its high cost.

Despite many applications of the E-nose, as cited above, none has dealt with differences between refined and whole wheat breads or breads that differ according to the colour of the seed coat. A major focus of this thesis research was on these applications.

CHAPTER 3

Determining a Suitable E-Nose Protocol for Fresh Bread and Discrimination Between Bread Types

3.1. Abstract

A suitable protocol was developed for use of an electronic (E) nose system with metal oxide sensors to analyze aroma differences in refined and whole wheat bread. Bread was prepared using a commercial formula based on two flours, Canada Western Red Spring (CWRS) wheat straight grade flour (refined bread) and a blend of 85% CWRS flour and 15% bran milled from the same CWRS wheat. Five incubation temperatures (35, 40, 45, 50 and 60°C) were tested along with two incubation times (5 and 10 min) and four sample sizes (0.05, 0.1, 0.25 and 0.50 g) of ground bread crumb. Through optimization using E-nose software including principal component analysis, a procedure was adopted using 40°C, 5 min incubation time and 0.05 g of sample. Possible changes in volatiles of crumb samples stored at room temperature over a period of 24 h were also examined. There were no apparent changes in sample volatiles and sensor response to bread aroma analyzed within this time interval. As the E-nose instrument possessed an autosampler capable of handling up to 64 samples, the result supported a protocol whereby many samples of bread could be prepared and analyzed in a given time frame thus improving the overall efficiency of use of the instrument.

3.2. Introduction

There are numerous types of breads present in market today which attract a broad range of consumers. Some consumers prefer eating whole wheat bread with its higher fiber content, while some prefer refined breads because of its milder sensory qualities. Market research has shown that the majority of consumers predominantly prefer refined bread over whole wheat bread (Bakke and Vickers, 2007) in spite of latter's superior nutrient profile. Consumers are familiar with differences between refined and whole wheat breads in terms of appearance, aroma, taste and texture, and it is widely accepted that adding wheat bran to flour imparts a different or off-flavour to the bread. The flavour components of bread have been previously studied, and more than 540 compounds have been identified in bread so far (Schieberle, 1996) which include organic acids, alcohols, ketones, aldehydes, furans, pyrazines, lactones, and sulphur compounds (Schiberle, 1996; Quilez et al., 2006). Gas chromatography combined with mass spectrometry (GC-MS) is the preferred method for the separation and identification of bread compounds that contribute to aroma.

Despite the large body of knowledge on the quantification of aroma compounds in bread by GC-MS and related methods (Buttery et al., 1983; Schieberle and Grosch, 1985, 1987; Schieberle, 1990; Grosch and Schieberle, 1991; Rychlik and Grosch, 1996; Grosch and Schieberle 1996, 1997; Zehentbauer and Grosch 1998a; Belitz et al., 2004), there is very limited science on the sensory aspects of refined versus whole wheat bread and/or breads made from different types of wheat, e.g. red versus white wheats (Moder et al., 1984; Lang and Walker, 1990; Chang and Chambers IV, 1992; Zhang and Moore, 1999). These studies documented various degrees of difference between the bread types.

Although sensory evaluation by a well trained panel is still the best and comprehensive method of sensory analysis, it is challenged by a number of factors. A few disadvantages of the human sensory approach include limited availability of panelists, fatigue, and time requirements encompassing training and evaluation phases. As the need for more and better knowledge of food composition increases, the need for techniques or instruments that are simpler and faster also increases (Marsili, 2001; Poprawski et al., 2006). The electronic nose (E-nose) is one such instrument. The term E-nose was first used by Gardner and Bartlett (1988) in reference to an instrument composed of sensors combined with a pattern recognition system, enabling recognition and discrimination of simple and complex odours (Gibson et al., 2000). Di Natale et al. (1998) acknowledged that sensory analysis is one of the branches of food analysis which should benefit most from introduction of the E-nose as an analytical tool. It has also shown high sensitivity and correlation with data from human sensory panels for several applications (Harper, 2001).

E-nose technology has many inherent advantages for machine olfaction. Instruments have fast analysis times, can run continuously for extended periods of time without operator supervision, can run many samples via an autosampler and can detect complex odours. They do not require extensive sample preparation as needed in analytical techniques such as GC-MS. While not a replacement for GC-MS which can quantify and identify compounds, nor a replacement for human sensory panels, the E-nose nonetheless offers an excellent complementary methodology to monitor and discriminate odours (Van Deventer, 2001).

E-noses have found wide and diverse uses in science and industry for many different products and/or problems such as pork quality due to storage (Vestergaard et al., 2007), microbiological spoilage of beef (Panigrahi et al., 2005), cheese aroma (Drake et al., 2003), beer (Chmielewski et al., 2007), oil oxidation (Mildner-Szkudlarz et al., 2008), perfumed cleaner products (Poprawski et al., 2006), water contamination (Ameer and Adeloju, 2005; Canhoto and Magan, 2005), damaged grain (Jonsson et al., 1997; Ridgeway et al., 1999; Magan and Evans, 2000; Paolesse et al., 2006; Zhang et al., 2007).

Only four baking-related applications have so far been reported for the E-nose. Needham et al. (2005) studied the detection of odour differences in modeled bread inoculated with various spoilage microorganisms. Botre and Gharpure, (2006) evaluated bread aroma changes due to staling to predict the state of bread freshness. Ponzoni et al. (2008) applied an experimental E-nose system to distinguish pure aroma compounds known to be present in baked bread. Piazza et al. (2008) examined the differentiation of five commercial toasted breads by e-nose as part of a larger study to correlate texture with the release of volatile compounds. Among these studies, only the latter provided E-nose method details for the analysis of samples including sample size (0.8 g), incubation temperature and time (40 ^{o}C, 10 min).

The aim of this study was to develop a suitable E-nose method appropriate to the analysis of fresh bread samples for the purpose of assessing the capabilities of the E-nose to differentiate different forms of bread (crust and crumb), different types of bread (refined or white bread versus whole wheat bread), and bread from different wheat (red and white wheats). This study also examined the stability of the E-nose system, i.e. sensor response, over a sufficient period of type (24 h) commensurate with using the

instrument most efficiently via an autosampler wherein samples could wait for analysis for periods up to 16 h or more.

3.3. Materials and Methods

3.3.1 Milling and baking

Bread made from Canada Western Red Spring (CWRS) wheat straight grade flour (refined bread) and a 85-15% blend of CWRS flour with coarse wheat bran (referred to as whole wheat throughout this study) were used to develop the E-nose protocol. The flour was milled from sound wheat on the Buhler pilot mill (Buhler AG, Uzwil, Switzerland) of the Canadian International Grains Institute (CIGI), Winnipeg, Canada. The typical amount of wheat milled for each sample was 1 tonne. Flour was generated at an extraction rate of 75%. Coarse bran was obtained from unsifted millstream of the final break roll. Bran was subsequently ground on a Jacobson model 120B hammer mill (Jacobson inc., Minneapolis, MN) to pass through a 1.6 mm sieve.

All the breads were baked at CIGI in their pilot baking facility. Table 3.1 summarizes the breadmaking formula. Flour was mixed with a baking absorption determined at panning, using an Erika S-35 Spiral Revolving Bowl Mixer (Erica Records, Clifton, NJ, U.S.A). The baking absorption of refined CWRS, whole CWRS, whole Platte and whole Snowbird bread were 63%, 65%, 66% and 64% respectively. Dough was removed from the mixer at 28 ^{o}C and had a floor time in bulk of 10 min. Dough was divided into 640 g portions and subjected to an intermediate proof at room temperature for 15 min in covered boxes. Dough was subsequently moulded by straight curling on a B and B Moulder (Bloemhof Industries, Edmonton, AB), panned and subjected to a final

proof for 60 min at 30 ^{o}C and 95% relative humidity. Bake time was 27 min at 210 ^{o}C. After cooling, bread weights and volumes were determined. Average specific volumes of the four types of bread were as follows: refined CWRS, 6.70; whole CWRS, 5.45, whole Platte, 5.81 and whole Snowbird, 5.65. Subsequently, bread loaves were sliced on a commercial machine to 12 mm thickness, separated into three portions with each wrapped completely in aluminum foil and wrapped again using two layers of food grade plastic wrap. Bread was subsequently stored in plastic containers at -35°C until analysis. Prior to analysis, the bread samples were thawed overnight in ziplock bags. Bread samples when thawed showed no evidence of freezer burn or moisture loss.

Table 3.1. Breadmaking formula.

Ingredients	**Amount (g)**
CWRS wheat flour (corrected to 14% moisture) or flour substituted with 15% bran	5000
Sugar	200
Salt	100
Shortening	200
Yeast	200
Non fat dry milk solids	100
Conditioner (Puratos “No Soy Alpaga”); custom improver without soy flour to avoid bleaching effect	50
Water	Baking absorption determined according to dough handling properties at panning stage

3.3.2. Bread sample preparation for E-nose

Loaves of refined and whole wheat bread were used. Bread crumb was processed using a Cuisinart Mini-Prep Processor (model DLC-2RC). Crumb samples were taken as a rectangular portion with corners approximately 3.3 cm from the edge of the bread slice. The amount of sample ground in the processor was approximately 6 g. Preliminary experiments showed no effect of grinding time on results. Accordingly, crumb was ground for 30 s.

3.3.3. Electronic nose

There are many variables that can be manipulated when undertaking E-nose experiments including temperature of the sample prior to injection, time to accumulate headspace volatiles in a sealed vial, injection volume and temperature, oven temperature where sensors reside, carrier gas parameters and recovery time between samples.

3.3.3.1. E-nose sampling

An AlphaMOS FOX 3000 E-nose system was used comprising 64 place headspace autosampler, E-nose unit with oven and 12 metal oxide semiconductor sensors, and control and multivariate analysis software (AlphaSoft version 8.01). There are two main odour sampling methods used in E-nose analysis, static headspace analysis (SHA), and flow injection analysis (FIA). SHA is the more popular and lower cost method. The sample to be analyzed is placed in a vial and left in the Alpha MOS auto sampler so that the headspace becomes saturated with the odour (Craven et al., 1996). The vial is then agitated while being heated for a period of time (incubation temperature

and time) and the headspace is then transferred into the chamber containing the sensor array. The initial magnitude of response of the sensor array to the odour is large because the gas reaching the sensor array is saturated with the vapour, after a time (usually brief in the order of a few min) the headspace containing the volatiles is removed from the chamber and replaced by clean air and the sensor responses return to baseline values (Craven et al., 1996; Needham, 2004). SHA has been used in all the experiments conducted for this thesis research.

3.3.3.2. Temperature

The temperature that samples could be incubated in the E-nose system to generate headspace volatiles ranged from 35° to 150 °C. In this experiment, five different temperatures were tested (35, 40, 45, 50 and 60 °C). The syringe temperature suggested by the manufacturer is 5 or 10°C above that of the sample incubation temperature. In the method used, the syringe temperature was set at 10°C above the incubation temperature that was determined via experimentation.

3.3.3.3. Vials and injection volume

Glass vials with a total capacity of 10 ml were used (London Scientific, ON). The type of syringe used in FOX 3000 had a capacity of 5 ml. So the maximum amount of headspace volatiles that could be injected was also 5 ml. The filling speed of the syringe could be selected between 10 µL/sec to 2500 µL/ second. In this experiment it was set at 500 µL/ second. Magnetic caps with silicon septa (London Scientific, ON) were used to seal the vials. A hand crimper was used for sealing the caps onto the vials.

3.3.3.4. Acquisition time, period and gas flow

Acquisition time is the time that sensors are exposed to sample volatiles for which sensor data from a sample is acquired. Acquisition time, data acquisition period and gas flow as recommended by the manufacturer were used, i.e. 120 seconds, 0.5 seconds and 150 ml/min respectively.

3.3.3.5. Delay between samples

Delay time is the time between the two consecutive sample runs. It can range from very low values, e.g. 10 s to several hr. It is important to get the sensors back to baseline prior to injection of the next sample. This implies that the sensors are freed from any volatiles or contaminants from the previous sample. This was achieved by supplying the sensory array with clean air. Usually 10 - 20 min of delay time is required, according to the manufacturer, to return sensors to a baseline state. An 18 min delay time was adopted for this study.

3.3.3.6. Carrier gas

Sample volatiles are transferred to the metal oxide sensors by a constant flow of dry carrier gas (air) at the rate of 150 ml/min. The dry (H_2O < 5ppm) carrier gas composition was 19.8% to 20.2% O_2 with the balance being N_2. Other organic impurities were specified as < 5ppm.

3.3.3.7. Calibration of sensors

Chemical calibration of sensors with standard solutions (propanol and acetone) was performed weekly, according to manufacturer's specifications, during the course of all studies related to this thesis research. This calibration was done to maintain a proper functioning E-nose and reduce the effect of external elements like humidity and temperature. This ensured low sensor drift over time, hence repeatability and reproducibility of sensor output which was reflected in the reproducibility of discrimination and grouping results.

3.3.4. Design of experiments and statistical analysis

Samples used to establish E-nose conditions for all subsequent experiments as carried out in Chapter 4 were crumb from refined and whole wheat CWRS bread. As refined and whole wheat bread are very different based on their characteristic aromas, considerable differentiation would be expected by the E-nose. For establishing protocol E-nose analysis conditions, two loaves of bread of each of refined and whole CWRS wheat were used. One loaf of each type of bread was used to obtain crumb samples from different slices to establish the headspace generation/incubation time (5 or 10 min) at 40 and 50 ^{o}C as described below. A different loaf of bread was used to obtain crumb samples in experiments determining sample size and incubation temperature conditions. Samples were prepared as needed for different temperature conditions that were assessed on different days. For each condition of incubation time, temperature and sample size, crumb samples were prepared in triplicate from the same bread slice.

For the experiment aimed at assessing the effect of sample wait time in the autosampler, the ideal crumb sample set should be as similar as possible. Accordingly crumb samples for this experiment were obtained from one slice each of refined and whole wheat bread.

Pre-processing the E-nose data is an important step before classification can be performed (Dickinson et al., 1998). Signals from the sensor array are generally pre-processed in some way in order to improve the quality of the information available and to optimize sensor output before data is passed onto the pattern recognition system (Bartlett et al., 1997). Sensors are allowed to reach equilibrium and, after a predetermined time, the signal intensities from each sensor are measured (Dickinson et al., 1998). The resulting responses are time independent and represent the absolute change in sensor signal with a measured odour. A variety of different pattern recognition techniques can be applied including simple linear statistical methods, pattern recognition algorithms, principal component analysis, discriminant function analysis, and artificial neural networking approaches (Bartlett et al., 1997; Magan and Evans, 2000).

In this experiment, principal component analysis (PCA) was used to evaluate the E-nose system sensor data to differentiate among the samples. This basic multivariate statistical analysis method was a part of the E-nose system software. The sensor response provided by the Alpha MOS software is the sensitivity DR /R_0 value, where R_0 is baseline resistance (resistance at 0 seconds) and DR is $R_0 - R$, where R is the resistance at a selected time (60 s). For maximum sensor intensity, default values for the sensors were based on highest level response of each sensor. The accepted values according to the manufacturer were between 0.05 and 0.9.

The discrimination index (DI) was an essential parameter used to determine optimal conditions for analysis of bread samples. DI values could theoretically range from -100 to + 100. The DI computation was derived from PCA results and provided a numerical value corresponding to the degree of separation of volatiles between sample groups. A positive value indicated that samples were distinct in volatile composition or aroma, while a negative value indicated that groups overlapped. A value between +80 and 100 indicated the most useful separation of the samples. Data analysis was based on DR /R_0 values and DI.

3.4. Results and Discussion

3.4.1. Finding the appropriate headspace generation time

There is no information published on the sorts of conditions appropriate for E-nose analysis of fresh bread samples. Sample incubation temperature is arguably one of the most important variables that needs to be established. Several different incubation temperatures ranging from room temperature (Zhang et al., 2007) to 30°C (Paolesse et al., 2006) and 50°C (Jonsson et al., 1997) have been used for detection of grain quality. Piazza et al (2008) used 40 ^{o}C for E-nose of toasted dry bread samples. Finding an appropriate temperature depends on the type of sample and the content of volatile compounds. The basic rule according to the manufacturer's documentation (Alpha MOS, 2002) is based on the level of volatiles in a category of samples. If the content of volatiles is high, the suggested incubation temperature is in the range 35°C to 50°C with 3 to 5 min incubation time and sample size ranging from 50 mg to 500 mg. For samples with

lower levels of volatiles, higher incubation temperatures are recommended (usually above 80°C) with longer incubation times and greater sample size.

Taking into consideration that bread has a high volatile content, the initial temperatures chosen for protocol development were 40°C and 50°C. Initial experiments with these two temperatures and a sample size of 0.5 g were conducted to find an appropriate headspace generation time, i.e. sample incubation time in the sealed vials prior to injection of the headspace sample. The E-nose oven agitates during heating to maximize volatile production. An injection volume of 500 µL was used according to the manufacturer's guidelines for samples with high volatile content. Table 3.3 summarizes the analytical conditions and results.

As seen in the sensor response curves and PCA (Appendix A and B), although there were differences when samples were heated at 40°C and 50°C, there was no major differences in sensor output between the 5 and 10 min headspace generation times. Also, the DI corresponding to 5 min was much higher than that for 10 min (Table 3.3). Hence, 5 min incubation time was used in all further analyses. This value was also consistent with the manufacturer's specification (Anonymous, 2002a) that samples with high volatile content should be heated for 3 to 5 min.

3.4.2. Finding the appropriate sample size and incubation temperature

The DI values at both 40°C (DI=32) and 50°C (DI=45) with a sample size of 0.5g was much lower than needed for acceptable discrimination of samples. This could be because the sample size was too large resulting in volatile concentrations too high for proper sensor array performance. Accordingly, three smaller sample sizes were

evaluated: 0.25 g, 0.1 g and 0.05 g. These quantities were in the range suggested by the manufacturer (Anonymous, 2002a) for material with high content of volatile compounds These three different sample sizes were tested with five different incubation temperatures of 35°C, 40°C, 45°C, 50°C and 60°C. To maximize E-nose performance to produce reproducible results, a so-called "warm-up" sample was used at the beginning of every sequence of sample analyses. The warm-up sample was meant to stabilize or condition the sensors before test samples are run. Warm-up samples were either refined or whole wheat bread crumb.

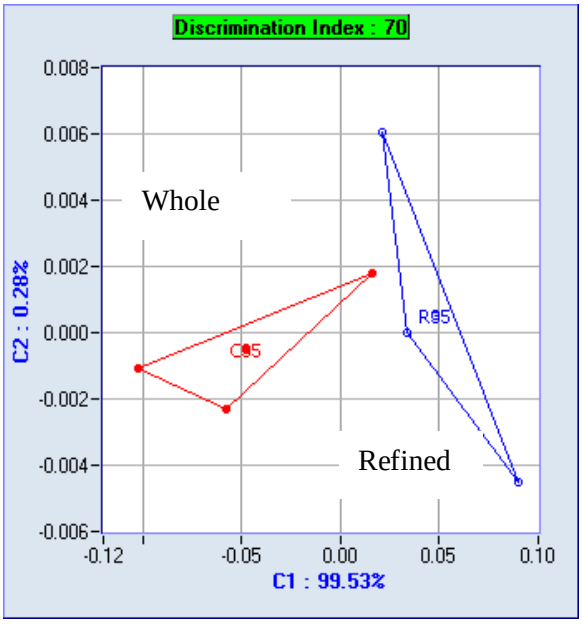
Table 3.3 summarizes the condition variables and DI results for these experiments. Each DI value is based on triplicate analysis for the specified condition. Sensor output, i.e. DR/R_0 values were within acceptable ranges for all conditions. The objective therefore was to find a set of conditions that maximized DI values. Figure 3.1 shows representative principal component analysis (PCA) results along with DI values for the refined and whole wheat bread crumb contrasts at all temperatures and sample sizes tested. Based on results in Table 3.3 and Fig. 3.1, the best discrimination between refined and whole wheat bread crumb was obtained using a sample size of 0.05 g incubated for 5 min at temperatures 40 °C (DI=96) , 45 °C (DI=97) and 50 °C (DI=90). The lowest temperature, 40 °C, was chosen as there was essentially no difference in DI value between that and 45 ^{o}C.

Table 3.2: Discrimination Index (DI) results for indicated E-nose conditions for comparing refined and whole CWRS bread crumb.

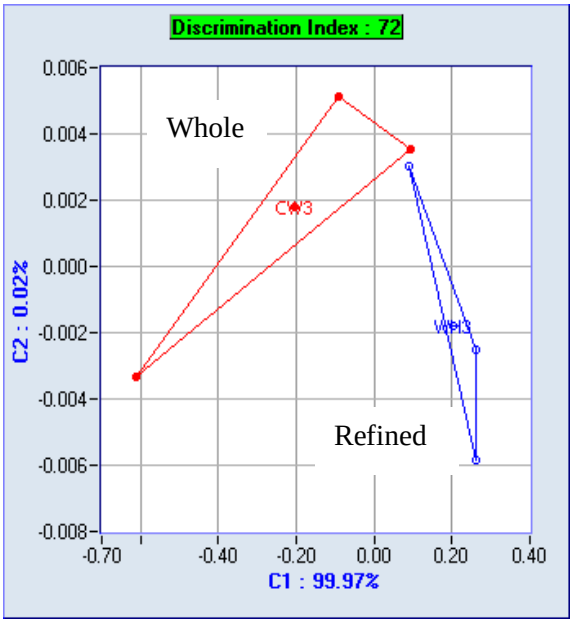
Temperature (°C)	Time (min)	Sample size (g)	DI
35	5	0.25	70
35	5	0.10	-1
35	5	0.05	72
40	5	0.50	32
40	10	0.50	-10
40	5	0.25	72
40	5	0.10	78
40	5	0.05	96
45	5	0.25	46
45	5	0.10	73
45	5	0.05	97
50	5	0.50	45
50	10	0.50	0
50	5	0.25	57
50	5	0.10	76
50	5	0.05	90
60	5	0.25	66
60	5	0.10	54
60	5	0.05	82

Figure 3.1. Figure Principal component analysis results and discrimination indices (DI) for all crumb sample sizes (S = 0.25, 0.10, and 0.05 g) tested against all temperatures (T = 35°C, 40°C, 45°C, 50°C and 60°C).

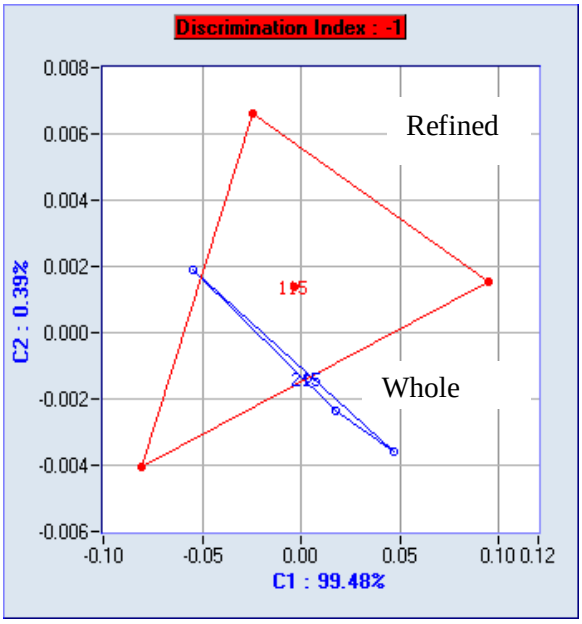
T =35°C; S=0.25 g



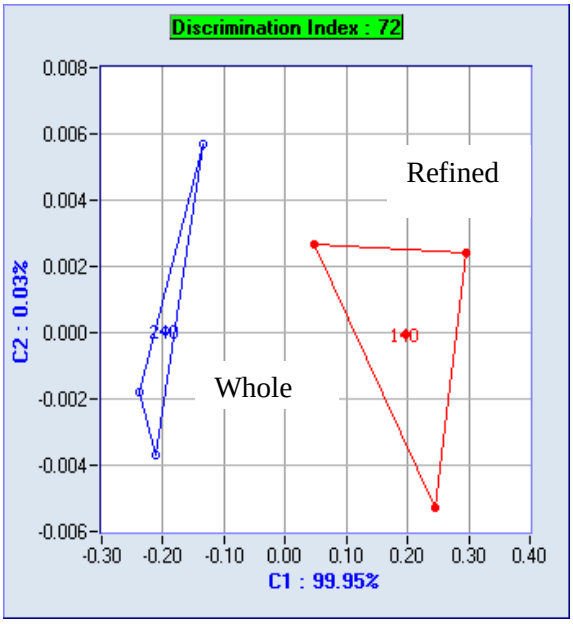
T =35°C; S=0.10 g



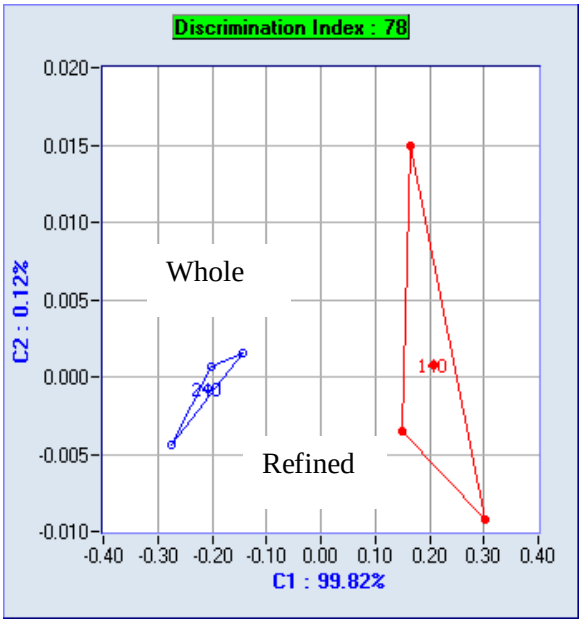
T =35°C; S=0.10 g



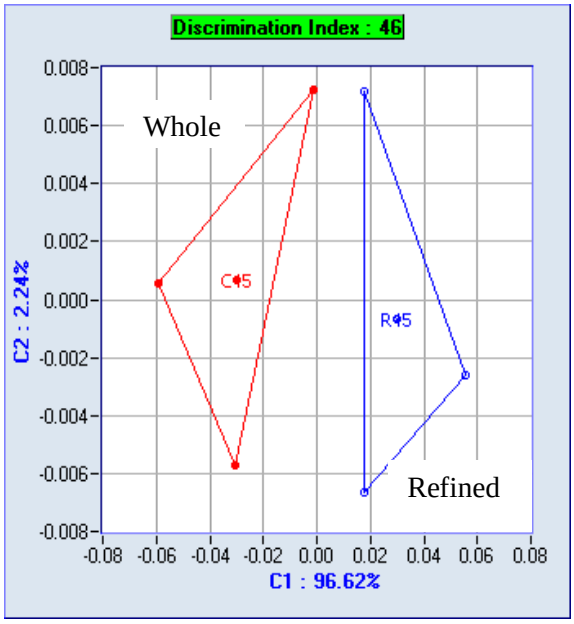
T=40°C S=0.25 g



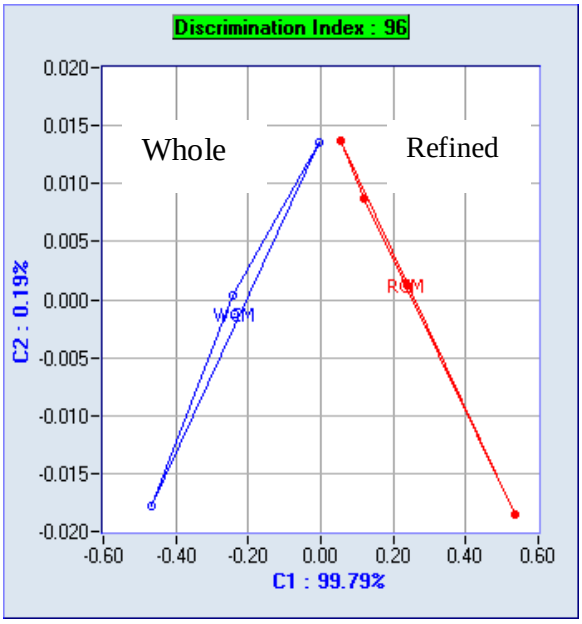
T=40°C S=0.10 g



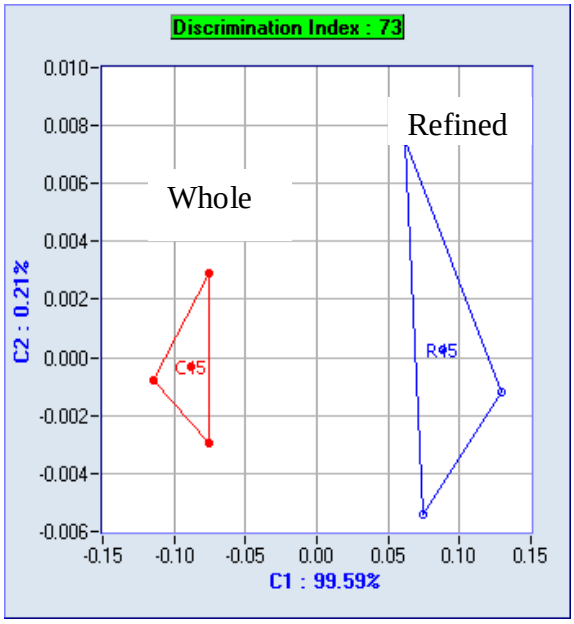
T=45°C S=0.25 g



T=40°C S=0.05 g



T=45°C S=0.10 g



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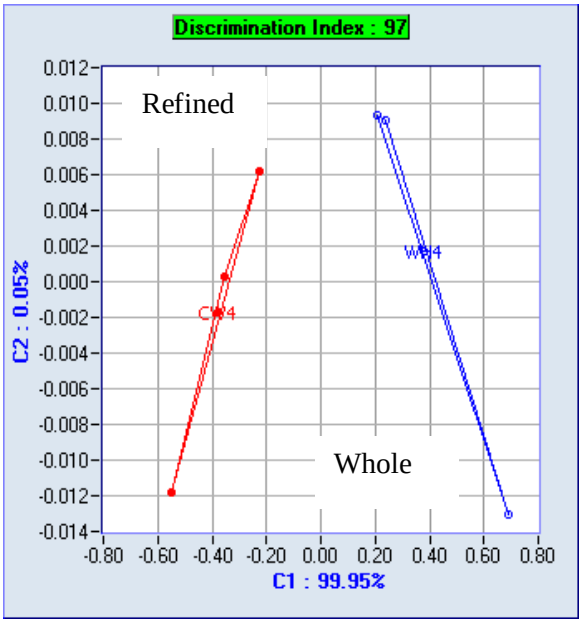
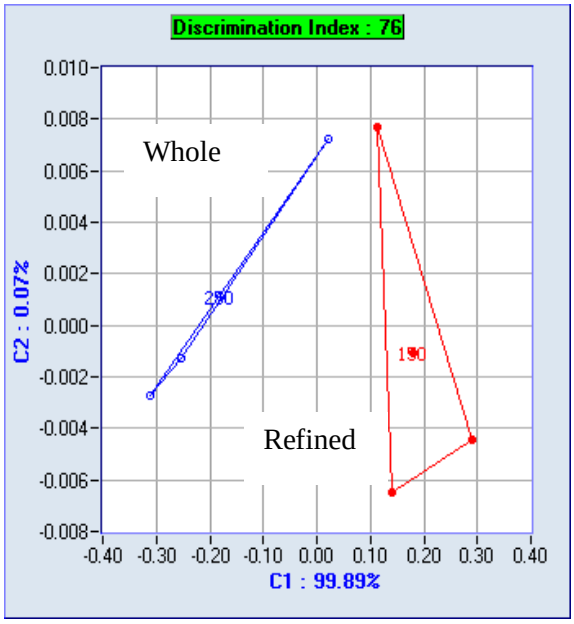
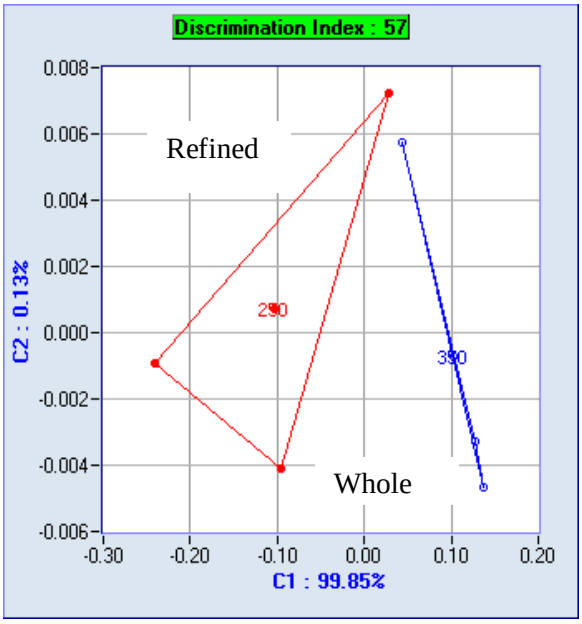


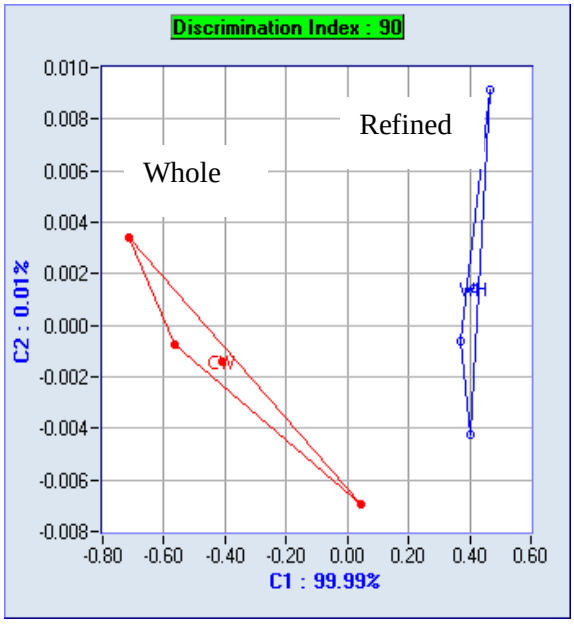
T=50°C S=0.10 g



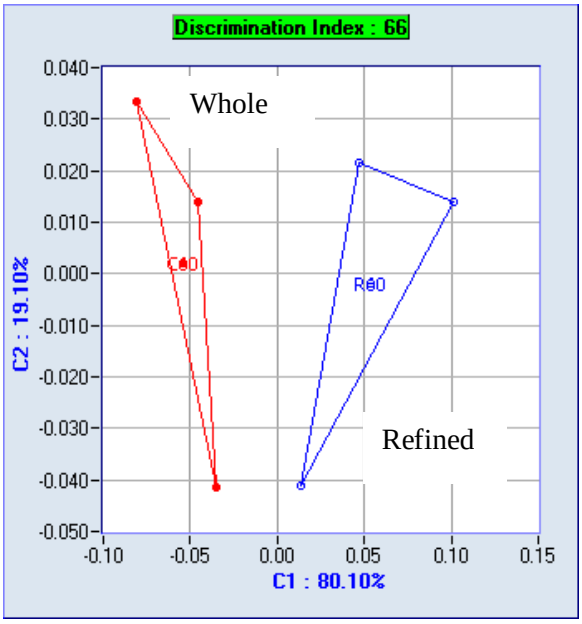
T=50°C S=0.25 g



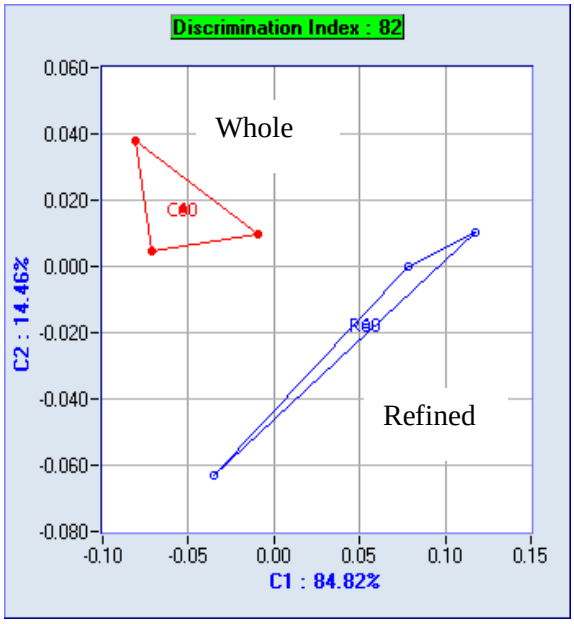
T=50°C S=0.05 g



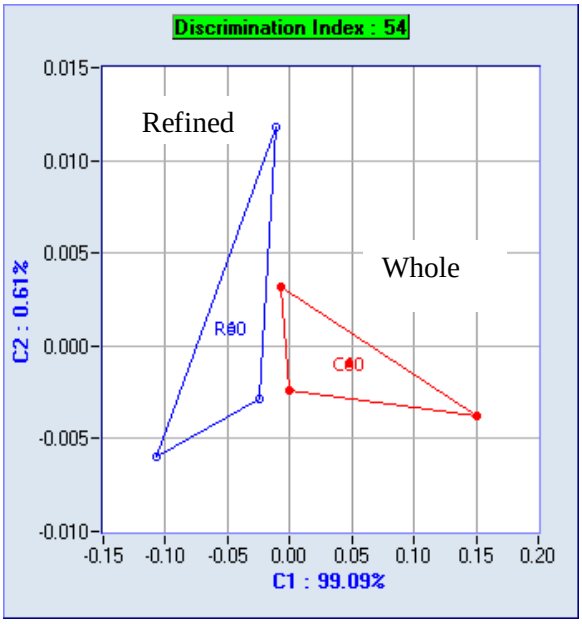
T=60°C S= 0.25 g



T= 60°C S= 0.05 g



T= 60°C S= 0.10 g



3.4.3. Effect of sample wait time in a sequence on E-nose performance

One of the major advantages of modern E-nose systems with autosamplers is that samples can be run in a long sequence with minimal or no supervision. However, it was not known whether the time a bread sample in a sealed vial waits to be analyzed was a significant factor in the quality of results. There is no heating or cooling of samples in the autosampler of the FOX 3000 system; samples sit at room temperature until the analysis run begins. Accordingly, an experiment was conducted to assess this potential issue, i.e whether sample volatiles change over the time frame of a practical analysis of a sequence of samples and/or whether sensors drift over this sequence time.

Total number of samples that can be analyzed in one sequence run in the FOX 3000 autosampler is 64. This corresponds to a total sequence run time of about 26 hr considering a total cycle time of 25 min comprising an individual sample incubation time of 5 min, sample run time over the sensors of 2 min, and 18 min delay for sensors to return to baseline until the start of the next sample run.

An experiment was designed to verify if there was any significant change in E-nose sensor output affecting the grouping of samples. Refined and whole CWRS bread crumb was analyzed. Sealed vials containing refined and whole wheat bread crumb were spread out among the 64 places in the autosampler tray which was additionally populated with “dummy” bread samples in order to increase the total analysis time. Samples were analyzed using the protocol conditions established above. In principle, if sample wait time in the autosampler had no effect on volatile composition and concentration, there should be no obvious differentiation of samples within each of the two types of bread.

Additionally, there should be satisfactory discrimination of samples between refined and whole wheat bread.

Results were consistent with these expectations. Figures 3.2 and 3.3 show principal component analysis results for the sequenced crumb samples from refined and whole wheat, respectively. In both cases there was considerable overlap of samples in the PCA plots with negative DI values. When samples were classified prior to PCA analysis according to bread type, refined and whole wheat samples were very well separated from each other in the PCA plot (Fig. 3.4), although the DI value was only 0. The results taken together indicate that there were no major changes in sensor response and in bread samples sealed in vials waiting over many hours to be analyzed in the E-nose.

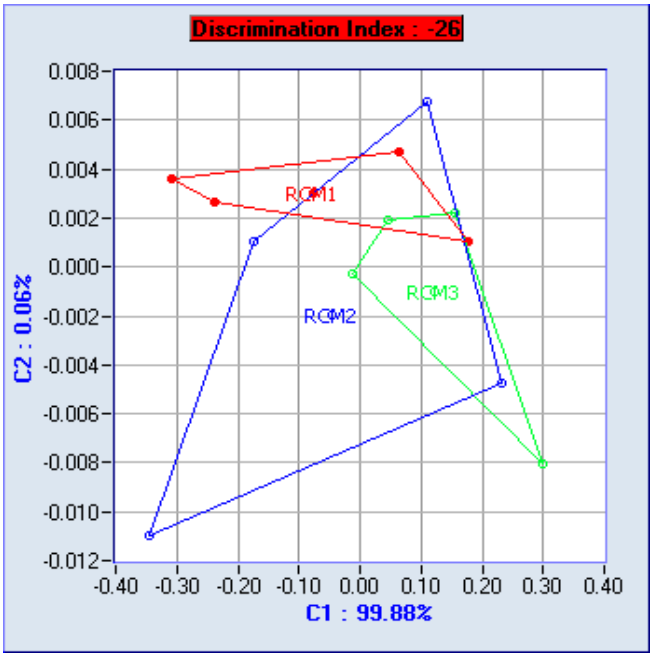


Figure 3.2. Principal component analysis result for a sequence of 12 samples of crumb from refined CWRS bread. The sequence was run over a period of 14.6 h.

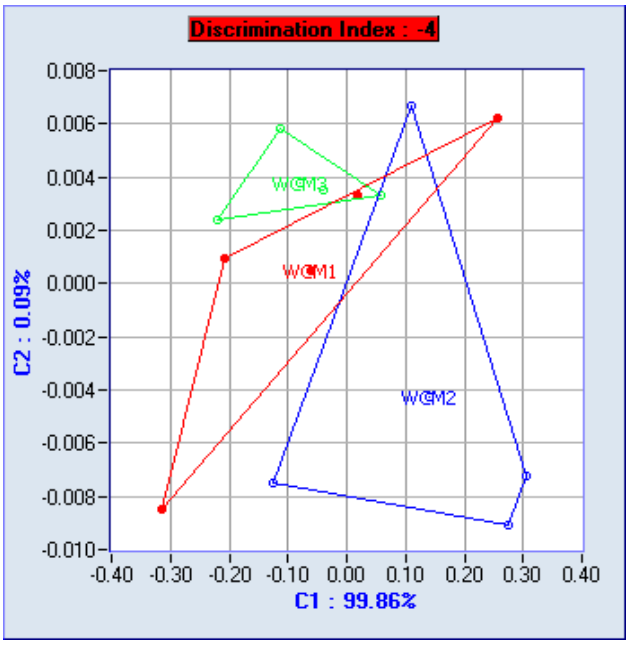


Figure 3.3. Principal component analysis result for a sequence of 12 samples of crumb from whole CWRS bread. The sequence was run over a period of 14.6 h.

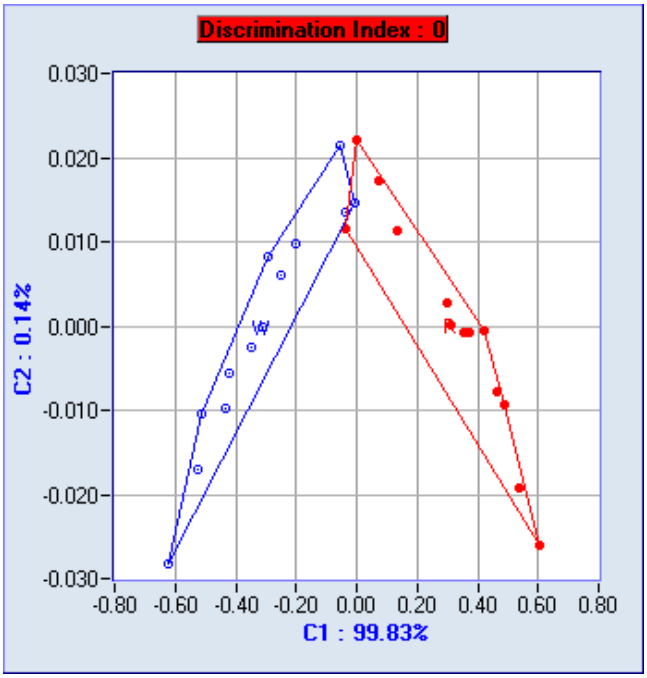


Figure 3.4. Principal component analysis result for the sequence of 12 samples each of crumb from refined and whole CWRS bread, classified according to bread type. The sequence was run over a period of approximately 24 h.

3.5. Conclusions

This study illustrated the potential for use of an E-nose system to differentiate refined and whole wheat bread. It represents the first reported use of an E-nose instrument to analyze fresh bread samples. A suitable protocol for analyzing refined and whole wheat bread aroma was developed based on experiments that evaluated a range of sample incubation temperatures and times, and sample sizes. Through optimization using E-nose software including principal component analysis, a procedure was adopted using 40 °C, 5 min incubation time, 0.05 g of sample with an injection volume of 500 μL, 2 min sensor exposure to volatiles, and an 18 min delay between samples. Possible changes in volatiles of crumb samples stored at room temperature in sealed vials over a period of 24 h were also examined. There were no apparent changes in bread sample volatiles within this time interval that affected the quality of results. As the E-nose instrument possessed an autosampler capable of handling up to 64 samples, the result supported a protocol whereby many samples of bread of different types could be prepared and analyzed in a given time frame thus improving the overall efficiency of use of the instrument.

Machine olfaction using the E-nose appears to be a very good, robust and highly sensitive instrumental technique to complement or even substitute human sensory panel analysis when dealing with complex bread aroma compounds in the context of establishing whether differences in samples exist or not. A well maintained E-nose can be a remarkable technique for qualitative sensory analysis and could prove to be very promising when used in conjunction with human sensory analysis and GC-MS. Proof of the value of the E- nose for fresh bread analysis needs to be established with more

challenging sample sets. That is the subject of the next chapter of this thesis research where the efficacy of the E-nose to discriminate crumb, crust and whole slice samples of refined of whole wheat bread from red and white wheats was evaluated.

CHAPTER 4

Electronic Nose Analysis of Crumb, Crust and Whole Slice of Refined and Whole Wheat Breads Made From Red and White Wheat

4.1. Abstract

A state-of-the-art E-nose system (AlphaMOS FOX 3000) with metal oxide sensors (MOS) was used to capture aroma volatiles from crumb, crust and whole slices of breads made from sound Canadian Western Red Spring (CWRS) wheat as well as representative samples of two hard white wheats, viz. Snowbird, a cultivar belonging to the Canada Western Hard White Spring (CWHWS) class of wheat, and Platte, a U.S. Hard White Winter (HWW) wheat. The same CWRS wheat provided the base flour for all the breads. A commercial formula was used to produce pan breads from four flours for the study, i.e. CWRS straight grade flour (refined), and three whole wheat flours comprising blends of 85% CWRS flour and 15% bran from CWRS wheat, Snowbird and Platte.

Volatiles of ground bread were acquired using a protocol established in previous experiments (Chapter 3); 0.05 g at 40 °C, and 5 min incubation time. Multivariate discriminant analysis was applied to 12 sensor output data comprising 24 samples each of crust, crumb and whole slices randomly selected from three loaves each of refined and the three whole wheat breads. Results varied according to the nature of the sample, i.e. crust, crumb or whole slices. For crust, the greatest distinction in aroma was found between refined and whole wheat breads. Refined bread crust was correctly classified 67% of the time. When refined bread crust was misclassified, samples were confused with whole white wheat crust predominantly from Platte bread. For whole wheat bread

crusts, the pattern of classification depended mainly on bran colour. Whole wheat bread crust samples had correct classification scores in the range 54-58%. When misclassified, whole wheat CWRS crust was equally confused with the aroma of crust of the white whole wheat breads, Platte and Snowbird. Whole wheat Platte crust tended to be misclassified with the counterpart white whole wheat Snowbird or refined bread crust. In contrast, Snowbird whole wheat crust tended to be misclassified as either its counterpart HW wheat Platte or whole CWRS wheat. Accordingly, Platte bread crust appeared to possess an aroma more in line with refined wheat bread as opposed to whole wheat bread.

For bread crumb, the pattern of E-nose differentiation of samples was different. CWRS whole wheat bread aroma was clearly and perfectly distinguished from the crumb aroma of all the other breads, either whole white wheat or refined CWRS. The latter tended to cluster on its own, as might be expected, and had a correct classification score of 75%, with the balance of samples largely misclassified as Snowbird crumb. Whole wheat Platte and Snowbird bread crumb had identical correct classification scores of 42%, and were similarly confused with the other's aroma (average 34% classified) or the aroma of refined wheat bread (average 21% classified). Results for crumb indicated a clear distinction in aroma between the hard red and white wheats used in this study. E-nose analysis of bread samples representing whole slices produced results that provided generally unsatisfactory discrimination among bread types likely due to the blending of the different aromas of constituent crust and crumb. Based on this result, analysis of samples that combine both crust and crumb is not recommended for sensory analysis of bread, whether by instruments or human sensory panels.

Results support the contention that different wheat genotypes and specifically, the bran tissue of these genotypes, contain differences in compound composition and/or concentration which when processed by breadmaking, manifest volatiles characteristic of those genotypes, even between genotypes possessing the same colour of bran. E-nose instrumentation appears to be very capable of accommodating these sorts of complex assessments on fresh bread.

4.2. Introduction

Aroma is one of the most important quality attributes of bread or any food. It will determine whether the product will be tasted and eaten in the first instance and is a major factor in establishing acceptability and preference. The dominant preference by consumers of bread made from refined flour in contrast to whole wheat flour (Bakke and Vickers, 2007) is at least in part due to the strong and different aroma of whole wheat bread. White wheats may have an advantage over red wheat in this regard according to some reports reviewed below, but the science is limited. In North America, most bread, whether it is refined flour pan bread or whole wheat bread, is made from hard red (HR) wheat. Since the early 1990s in the U.S. and over a decade later in Canada, hard white (HW) wheat was introduced as an alternative to HR for manufacturing of bread and noodle products. While HW wheat production represents a small proportion of total bread wheat acreage in North America, much of the wheat grown elsewhere in the world such as in China, South Asia and Australia is HW wheat.

The distinct benefit of HW wheat over its HR counterpart is a flour and product colour advantage at higher extraction rates when the flour contains more bran

(Ambalamaatil et al., 2006). Marquart et al. (2006) suggested the use of white whole wheat instead of red whole wheat to reduce the typical brown appearance of whole wheat bread, as one of the ways to introduce more whole grains into diets of consumers who might prefer the whiter appearance of refined wheat products.

Apart from colour, there have been comments and reports made in the scientific literature and in wheat industry publications of a flavour or aroma sensory advantage of HW wheats, specifically that whole wheat bread made from HW wheat has a milder taste as compared to bread made from HR wheat (Chang and Chambers IV, 1992; Ambalamaatil et al., 2006; Ransom et al., 2006). It is clear that adding wheat bran to flour imparts a different or off-flavour to the bread. The flavour components of bread have been previously studied but there is limited science concerning the sensory aspects of refined versus whole wheat bread and/or bread made from bran of red and white wheats. Moder et al. (1984) reported that bread produced from white wheat bran was more acceptable to consumers, compared to bread made from red wheat bran. Lang and Walker (1990) compared hamburger buns made from hard white and red winter wheat. While taste panelists easily differentiated red wheat from white wheat buns, there was no preference for one type over the other. Chang and Chambers IV (1992) compared hard red and white refined and whole wheat pan breads and found more differences in bread crust than crumb. Refined red wheat crust was found to be more sour, bitter and astringent compared to refined white wheat crust. The authors stated that aroma and flavour attributes were “more balanced and blended” in the white wheat bread crust. Whole wheat bread results were less clear. The authors suggested that white wheat bread had more aftertaste than red wheat bread for crust and crumb. Panelists in a study by

Zhang and Moore (1999) compared the baking quality and sensory properties of breads prepared with several different brans including soft white and HR wheat bran. Panelists preferred the flavour, mouthfeel and general acceptability of the former over the latter. Chang et al. (1995) conducted dynamic headspace analysis (using purge and trap) of volatile flavour components of white pan breads and whole wheat breads made from HR winter and HW winter wheat. A total of 63 compounds were identified across all samples using GC-Fourier transform infrared spectroscopy-MS. A range of differences was found depending on the type of bread and colour of bran. The authors concluded that many differences were noted between white pan and whole wheat bread, however many fewer differences were found between bread made from HR and HW wheat.

Chemically, the sensory preference for white wheat could be related to fewer or less concentrated phenolic compounds owing to less pigmentation that derive from polyphenol content in the seed coat. Red pigment in the seed coat tissue is a derivative of the polyphenol catechins, probably, phlobaphene or proanthocyanidin (Miyamoto and Everson, 1958; Himi and Noda, 2005). It has been reported that white wheats have lesser amounts of proanthocyanidins in their seed coats than red wheat (Matus-Cadiz et al., 2008). It is known in general that phenolic compounds impart bitterness and astringency to food products like tea, wine, several fruits, nuts and chocolate (Lesschaeve and Noble, 2005).

Clearly, more science using instrumental methods is needed to quantify differences in aroma and flavour of bread made from refined and whole wheat flour and different types of wheat. Information on these specific and practical contrasts are not provided in the very large body of knowledge that exists on the quantification of aroma

compounds in bread by GC-MS and related methods (Buttery et al., 1983; Schieberle and Grosch, 1985, 1987; Schieberle, 1990; Grosch and Schieberle, 1991; Rychlik and Grosch, 1996; Grosch and Schieberle 1996, 1997; Zehentbauer and Grosch 1998a; Belitz et al., 2004).

The electronic (E) nose may the ideal technology to address these sorts of questions. E-nose has generated much interest in last fifteen years for its potential to classify and differentiate odours in areas of research and development, quality control, and marketing for applications related to food and beverage, pharmaceuticals, plastics and packaging and environment. A modern E-nose system is comprised of sampling hardware, a heated compartment containing an array of semiconductor-based sensors with different selectivities to detect different volatile organic compounds, and software to both control the apparatus and compute and interpret, by pattern recognition, sensor output data in the form of conductivity or resistance values induced by the adsorption of volatile molecules. E-nose sensors are typically sensitive to both odourous and odourless volatile compounds (Haugen, 2001), have high sensitivity, often more than the human nose and produce results that have been shown to correlate with data from human sensory panels (Harper, 2001).

E-nose technology has many inherent advantages for machine olfaction. Instruments require minimal sample preparation compared to GC-MS, have fast analysis times, can run continuously and analyze many samples for extended periods of time without operator supervision, and can be applied to detect toxic odours. Limitations of sensory panels encourage the use of E-nose in sensory analysis of food products. It offers advantages like less time consumption, lower labour costs, longer runs (multiple samples)

with minimal supervision, higher sensitivity and ability to test toxic samples. While not a replacement for GC-MS which can quantify and identify compounds, nor a replacement for human sensory panels, the E-nose nonetheless offers an excellent complementary methodology to monitor and discriminate odours (Van Deventer, 2001).

Only four baking-related applications have so far been reported for the E-nose. Needham et al. (2005) studied the detection of odour differences in modeled bread inoculated with various spoilage microorganisms. Botre and Gharpure, (2006) evaluated bread aroma changes due to staling to predict the state of bread freshness. Ponzoni et al. (2008) applied an experimental E-nose system to distinguish pure aroma compounds known to be present in baked bread. Piazza et al. (2008) examined the differentiation of five commercial toasted breads by e-nose as part of a larger study to correlate texture with the release of volatile compounds.

The aim of this study was to evaluate the capabilities of a state-of-the-art E-nose system to differentiate between bread made from refined wheat flour and whole wheat flour where bran was derived from red and white-grained wheat. A separate objective was to determine how the nature of the sample, whether crust, crumb or whole slice, affected the quality of the pattern recognition results.

4.3 Materials and Methods

4.3.1. Milling and bread preparation

Breads for this study were made from sound Canadian Western Red Spring (CWRS) wheat as well as representative samples of two hard white wheats, viz.

Snowbird, a cultivar belonging to the Canada Western Hard White Spring (CWHWS) class of wheat, and Platte, a U.S. Hard White (HW) Winter wheat.

Flour was milled from approximately 1 tonne of sound wheat on the Buhler pilot mill (Buhler AG, Uzwil, Switzerland) of the Canadian International Grains Institute (CIGI), Winnipeg, Canada. Each wheat sample represented a composite of wheat sourced from many producers. Accordingly, environmental influences on results of this study were effectively minimized. Flour was generated at an extraction rate of 75%. Coarse bran was obtained from unsifted millsteam of the final break roll. Bran was subsequently ground on a Jacobson model 120B hammer mill (Jacobson inc., Minneapolis, MN) to pass through a 1.6 mm sieve.

Four types of bread were produced. One was bread made from Canada Western Red Spring (CWRS) wheat straight grade flour and is referred to as refined bread. The same CWRS wheat provided the base flour to produce three whole wheat flours comprising blends of 85% CWRS flour and 15% bran from CWRS wheat, Snowbird and Platte. All the breads were baked at CIGI in the pilot baking facility. The baking formula and procedure, and how breads were sliced and prepared for storage prior to sample preparation for E-nose analysis are described in Chapter 3.

Four slices were taken from each of three loaves of the four types of bread. The different types of samples were prepared for analysis, crust, crumb, and whole slices. For crust, the top portion of bread slices was used. Crumb samples were taken as a rectangular portion with corners approximately 3.3 cm from the edge of the bread slice. For whole slice samples, one-half of a slice was taken cut vertically. Samples were processed using a Cuisinart Mini-Prep Processor (model DLC-2RC). The amount of

sample ground in the processor was approximately 6 and 2 g for crumb and crust, respectively. Preliminary experiments showed no effect of grinding time on results. Accordingly, crumb was ground for 30 s, while crust and whole slices were processed for 1 min in order to achieve ground material with similar appearance and particle size. All bread samples were carefully handled using latex gloves to prevent possible transfer of extrinsic organic compounds.

4.3.2. E-nose

Samples were analyzed on FOX 3000 e-nose (Alpha MOS, Toulouse, France) incorporating an array of 12 metal oxide semiconductor sensors of type P, T and LY types (Table 4.1) as recommended by the manufacturer for food volatile analysis. The sensor array comprised six L (LY2/LG, LY2/G, LY2/AA, LY2/Gh, LY2/gCTl, LY2/gCT), two T (T30/1, T70/2), and four P-type (P10/1, P10/2, P40/1, PA2) sensors.

Table 4.1. Sensors in FOX 3000 E-nose.

Type of sensors	**Detection type**
P: Plate type using thick film technology	Non polar volatiles
T : conventional sensor using alumina tube gold printed electrodes	Organic solvents
LY : Non tin-based oxides	Organic compounds and gases including aldehydes, alcohols, ammonia, amines

Source: Anonymous (2002b)

The analysis was conducted over three days. Each day, four random slices from one loaf were taken for analysis. The slices were ground individually and two samples were taken from slice. Total samples analyzed were 24 from three different loaves.

4.3.3. Headspace analysis

Sample (50 mg) was placed into a vial of 10 ml capacity (London Scientific, Toronto, ON). Vials were sealed with polytetrafluoroethylene lined silicon rubber caps (London Scientific, Toronto, Canada) and mechanical crimper. Samples were then placed in the auto sampler (HS 100, AlphaMOS) tray in a random order with a warm-up sample leading other samples for headspace analysis. The warm-up sample serves as a conditioning sample for subsequent analyses. Each type of sample had eight replicates. The samples were incubated for 5 min at 40°C for headspace volatile generation. Agitation speed of the incubator was set at 500 rpm. Using a 5 ml syringe, 500 µl of headspace gas was injected into the E-nose injection port. The temperature of syringe was set at 50°C. Volatiles were transferred to the metal oxide sensors (MOS) over 2 min by a constant dry air carrier gas flowing at a rate of 150 ml/min. The delay time between samples was 18 min. Delay time is the time during which the sensors re-establish baseline response. The analytical conditions used are summarized in Table 4.2.

When interacting with the volatiles, there is a change in sensor conductivity which is measured as maximum change in resistance for each sensor.

Table 4.2. Summary of E-nose analysis parameters.

Parameter	Value
Size of vial	10 ml
Sample size	50 mg
Incubation temperature	40 °C
Incubation (and agitation) time	5 min
Agitation speed	500 rpm
Injection volume	500 µL
Elution speed	500 µL/sec
Data acquisition time	2 min
Delay time between samples	18 min
Rate of air flow	150 ml/min

4.3.4. Data analysis

To evaluate the pattern recognition aspects of the sensor data, several multivariate statistical methods were used using the SAS program (version 9.1, SAS Institute, Cary, NC). These included procedures STEPDISC (stepwise discriminant analysis), CANDISC (canonical discriminant analysis) and DISCRIM (discriminant analysis and classification). This analysis was carried out on a personal computer. Results from the stepwise discriminant analysis, which reveal the relative power of individual sensors to discriminate the different bread types, are shown in Appendix D.

4.4. Results and Discussion

Figure 4.1 shows a typical sensor response for refined and whole wheat crumb generated by the E-nose system. The sensor response provided by the Alpha MOS software is a sensitivity measure, DR /R_0, where R_0 is baseline resistance (resistance at 0 s) and DR = R_0 – R, where R is the resistance at a selected time (i.e. 60 s). For maximum sensor intensity, default values for the sensors are based on the maximum response from

each sensor. The accepted values of DR /R_0 according to the manufacturer are between 0.05 and 0.9. Fig. 4.1 shows that sensors, depending on type, have positive and negative responses. Each individual sensor is a separate line. There are two groups of six metal oxide sensors. Each group is doped differently. One group exhibits negative signals and the other group exhibits positive signals. The x axis is time in seconds and theY axis is the normalized change in sensor resistivity. It can be seen that whole wheat bread crumb generates sensor responses of larger magnitude that those for refined bread wheat crumb.

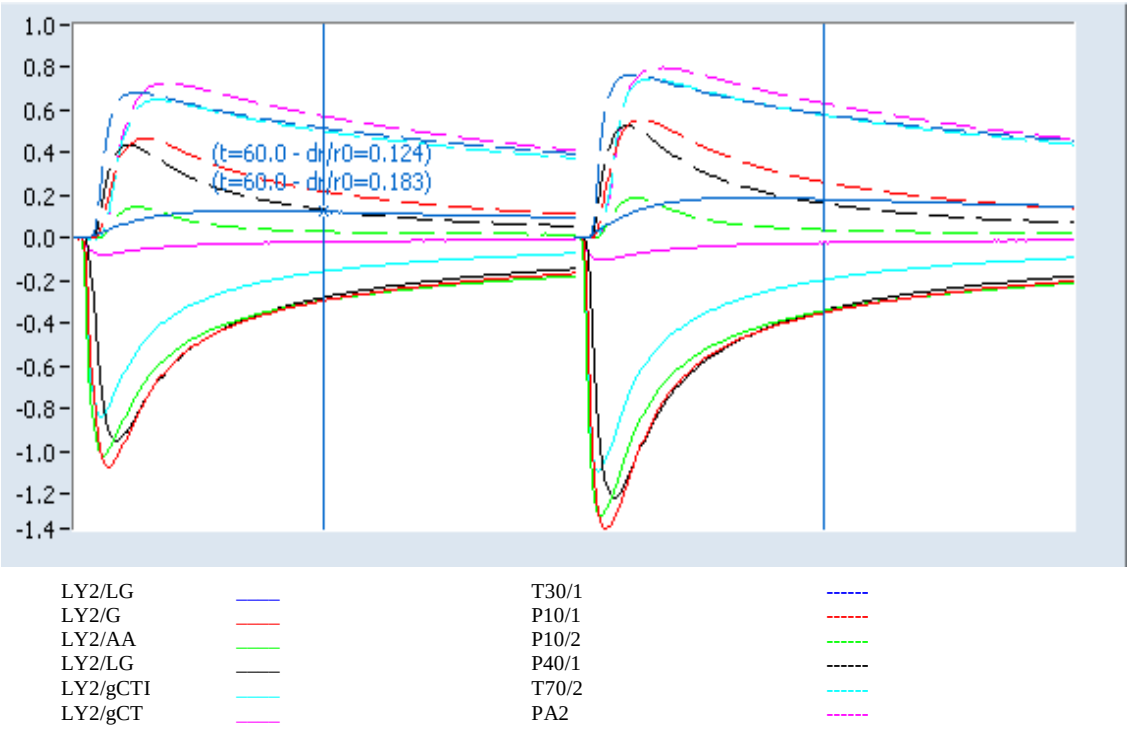


Figure 4.1. A typical sensor response generated by AlphaMOS Fox3000 for crumb from refined CWRS bread (left) and whole CWRS bread (right).

4.4.1. Crust

A graphical result of a canonical discriminant analysis of E-nose data is shown in Fig. 4.2. This type of analysis was applied in order to graphically evaluate, in three-dimensional space, the level of discrimination that exists in the 12-sensor E-nose data. In this type of analysis, the greatest degree of discrimination is revealed across the first "canonical" axis (Can1). The second canonical axis (Can2) accounts for a lower degree of discrimination or separation of samples, and so on. The result (Fig. 4.2) shows that crust from both refined wheat bread and CWRS whole wheat bread are distinct from each other, whereas the crust of both HW whole wheat breads (Snowbird and Platte) appear to be relatively similar to each other and relatively distinct from both CWRS refined and whole wheat bread crust. The result clearly demonstrates the capability of the E-nose system to discriminate among crust types.

The corresponding discriminant analysis classification (Table 4.3) confirmed the graphical result (Fig. 4.2). Refined bread crust was correctly classified 67% of the time. Interestingly, when refined bread wheat crust was misclassified, samples were confused with white whole wheat bread crust, and predominantly from Platte bread.

For whole wheat bread, the pattern of classification depended on the type of bread, specifically the colour of the wheat. All whole wheat crust samples were correctly classified in the range 54-58%. When misclassified, whole wheat CWRS aroma was about equally confused with the aroma of crust of the other whole wheat breads, Platte and Snowbird. Whole wheat Platte crust tended to be misclassified with the counterpart white whole wheat Snowbird or refined bread crust. In contrast, Snowbird whole wheat crust tended to be misclassified as either its counterpart HW white wheat Platte or whole CWRS

wheat. Accordingly, Platte bread crust appeared to possess an aroma more in line with refined wheat bread as opposed to whole wheat bread.

According to Schieberle and Grosch (1985), it is the aroma profile of crust that affects the consumer preference for the bread. Refined CWRS bread crust and whole CWRS bread crust were nearly distinct in their aroma profile by E-nose. The aroma of wheat bread crust depends on formation of roasty smelling compounds arising from Maillard reaction during the baking process and also their stability during storage (Schieberle and Grosch, 1985). The substantially higher concentration of phenolic compounds in wheat bran is likely to generate more compounds which would make its crust aroma stronger and distinct than that from refined wheat bread which contains much less bran.

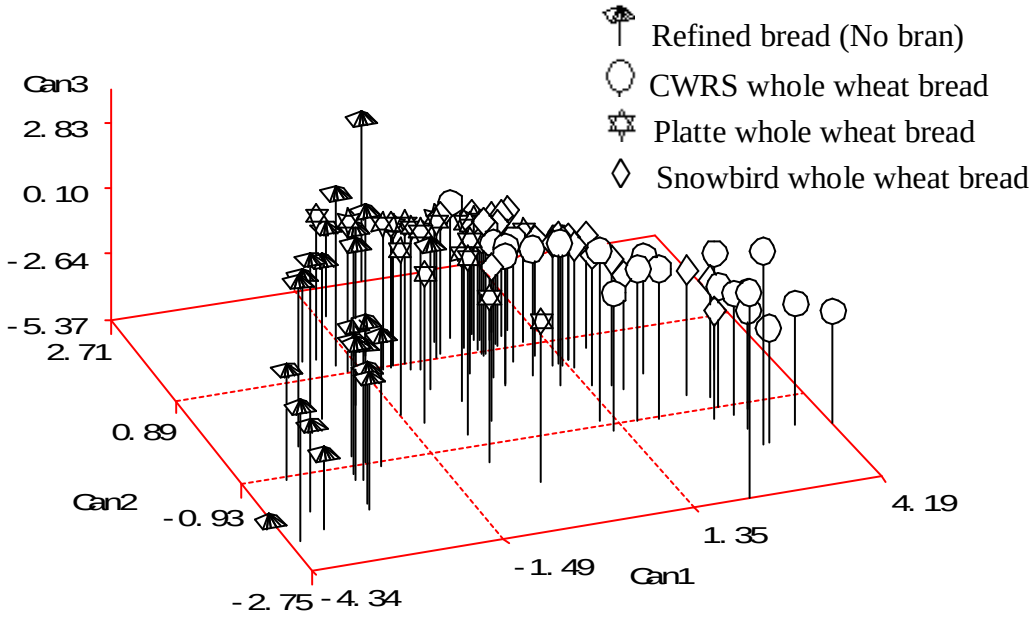


Figure 4.2. Clustering of refined and whole wheat bread crusts by canonical discriminant analysis of E-nose sensor data.

Table 4.3. E-nose classification of refined and whole wheat bread crust*.

	Refined	**Whole CWRS**	**Whole Platte**	**Whole Snowbird**
Refined	67	0	29	4
Whole CWRS	4	58	17	21
Whole Platte	17	4	54	25
Whole Snowbird	0	25	17	58

* Percent of samples correctly classified

4.4.2. Crumb

For bread crumb, the pattern of E-nose differentiation of samples was different. CWRS whole wheat bread aroma was clearly and completely distinguished from the crumb aroma of all the other breads (Fig. 4.3, Table 4.4), either whole white wheat or refined CWRS. The latter tended to cluster on its own (Fig. 4.3), as might be expected, and had a correct classification score of 75% (Table 4.4) with the balance of samples largely misclassified as Snowbird crumb. Whole wheat Platte and Snowbird bread crumb had identical correct classification scores of 42%, and were similarly confused with the other's aroma (average 34% classified) or the aroma of refined wheat bread (average 21% classified). E-nose results for crumb indicated a clear distinction in aroma between the hard red and white wheats used in this study, and support the notion that whole wheat bread from white wheats have sensory properties closer to refined wheat bread than that which exists for whole wheat bread from red wheat.

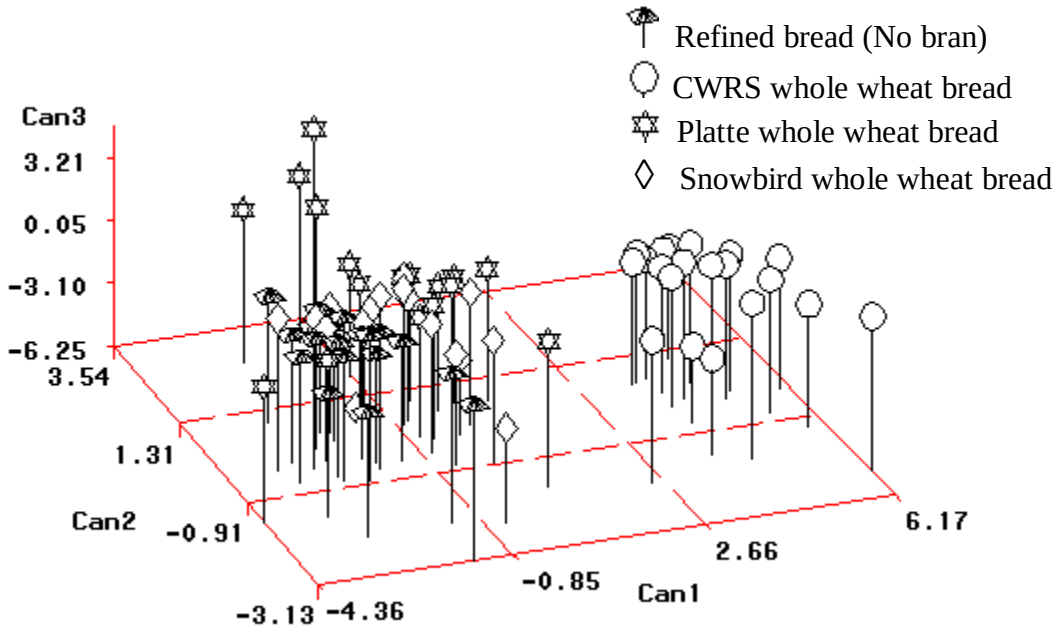


Figure 4.3. Clustering of refined and whole wheat bread crumb by canonical discriminant analysis of E-nose sensor data.

Table 4.4. E-nose classification of refined and whole wheat bread crumb*.

	Refined	**Whole CWRS**	**Whole Platte**	**Whole Snowbird**
Refined	75	0	4	21
Whole CWRS	0	100	0	0
Whole Platte	17	4	42	37
Whole Snowbird	25	0	33	42

* Percent of samples correctly classified

4.4.3. Whole slice

The clustering and classification of whole slices of the four bread types are shown in Fig. 4.4 and Table 4.5, respectively. Results were clearly distinct from those of crust or crumb samples in the degree of discrimination which was noticeably poorer for whole slices compared to crust or crumb samples. The correct classification score for refined wheat bread based on whole slices (34%, Table 4.5) was considerably degraded compared to crust (67%, Table 4.3) and crumb samples (75%, Table 4.4). Similarly, correct classification of whole wheat CWRS (54%, Table 4.5) was much lower than the corresponding results for crumb samples (100%, Table 4.4). For white whole wheat bread Platte, which was very well differentiated from red whole wheat CWRS bread using crust or crumb material (4% misclassification, Tables 4.2 and 4.3), misclassification outcomes based on whole slices (33% misclassification, Table 4.5) were significantly higher.

Overall, E-nose analysis of bread samples representing whole slices produced results that provided unsatisfactory discrimination among bread types likely due to the blending of the different aroma volatiles of constituent crust and crumb. It can be concluded that analysis of bread samples by E-nose that combine both crust and crumb is not recommended for sensory analysis of bread, whether by instruments or human sensory panels.

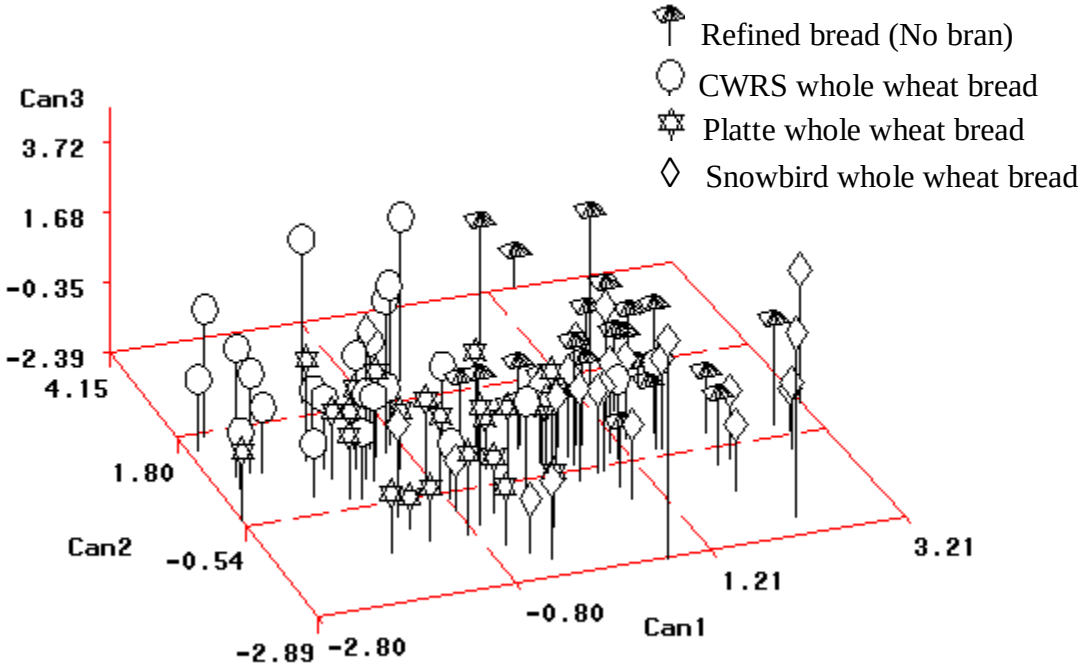


Figure 4.4. Clustering of refined and whole wheat bread whole slices by Canonical discriminant analysis of E-nose sensor data.

Table 4.5. E-nose classification of refined and whole wheat bread whole slices*.

	Refined	**Whole CWRS**	**Whole Platte**	**Whole Snowbird**
Refined	34	8	8	50
Whole CWRS	0	54	33	13
Whole Platte	0	33	46	21
Whole Snowbird	33	4	21	42

* Percent of samples correctly classified

4.4.4. Relative concentration of crust and crumb volatiles

E-nose sensor output is in effect electrical resistance caused by the combustion of organic volatiles on the sensor surface, and typical sensor response has been presented (Fig. 4.1). Accordingly, apart from capabilities related to discrimination of samples as shown in Figs. 4.2-4.4 and related sample classifications (Tables 4.3-4.5), the E-nose can also be used to evaluate the overall concentration of volatiles based on the same data used to produce the discriminant analysis outcomes. In that regard, an interesting question that can be asked concerns the relative concentration of volatiles from crust and crumb samples. Figures 4.5 and 4.6 address that question and show clearly that bread crumb contributed considerably more volatiles than bread crust for both refined (Fig. 4.5) and whole wheat bread (Fig. 4.6). Appendix E provides the complete set of E-nose sensor output data plus summary charts corresponding to the classification results documented in this chapter. In brief, those results show that refined CWRS wheat bread crust and crumb clearly had the lowest concentrations of volatiles compared to whole wheat breads. Among the whole wheat breads, Platte crust had the lowest concentration of volatiles among the crust samples, whereas CWRS and Snowbird crust samples had comparably high concentrations of volatiles. For whole wheat bread crumb samples, CWRS bread crumb clearly had the highest concentration of volatiles, whereas Platte and Snowbird had lower volatile concentrations similar to those of refined CWRS bread crumb.

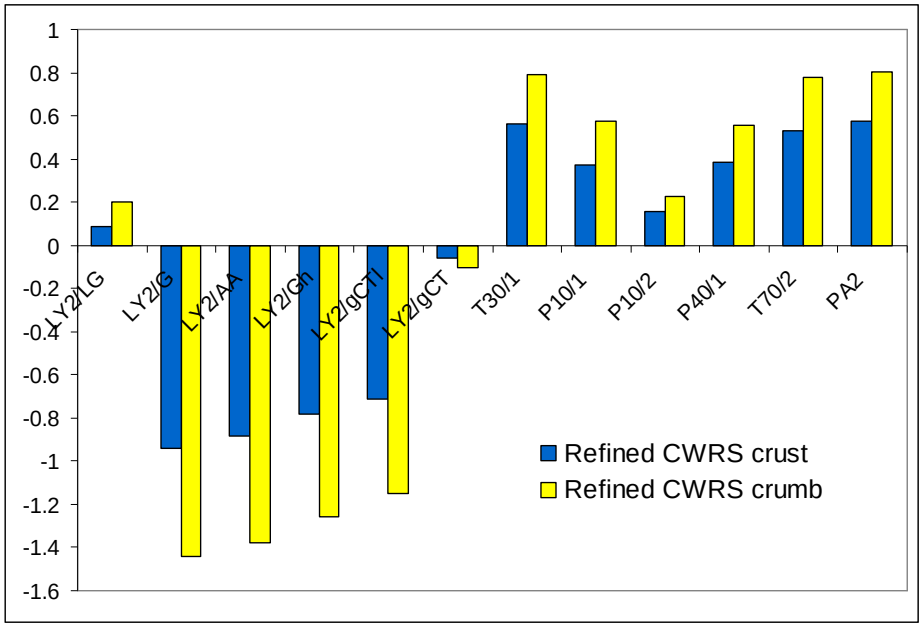


Figure 4.5. Average E-nose sensor output for crust and crumb samples of refined CWRS wheat bread. Result indicates that crumb samples had approximately 59% higher concentration of volatiles compared to crust.

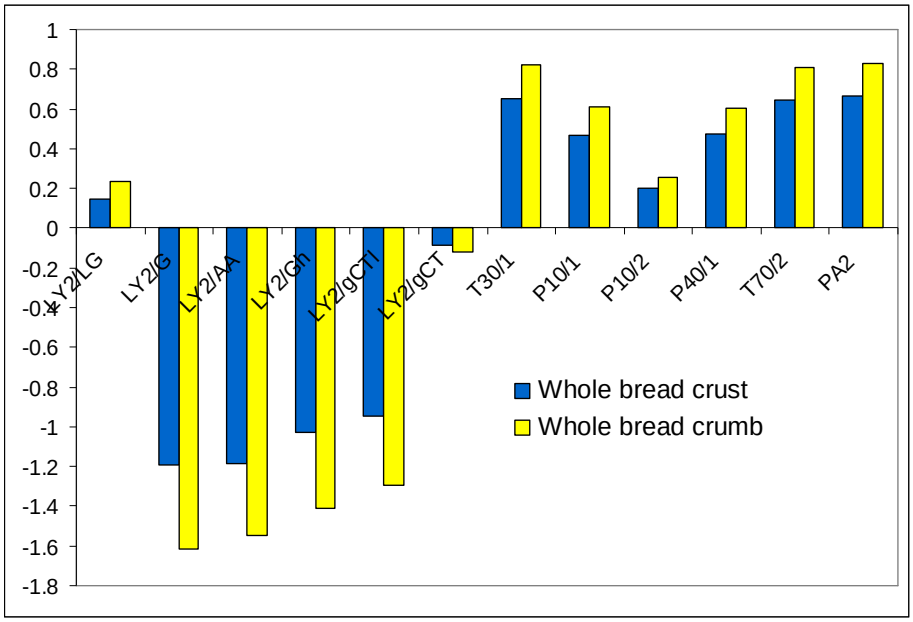


Figure 4.6.Average E-nose sensor output for crust and crumb samples of whole wheat breads CWRS, Snowbird and Platte. Result indicates that crumb samples had approximately 34% higher concentration of volatiles compared to crust.

4.6. Conclusions

E-nose was able to establish 100% differentiation between crust and crumb of all bread types (Appendix C) as well as between refined and whole red wheat CWRS bread using crust and crumb samples. Whole wheat bread produced using white wheat brans of cultivars Platte and Snowbird tended to be misclassified with the counterpart whole wheat bread; combined correct classification of whole wheat white bread whether using crust or crumb samples was in the range 75-79%. Interestingly for whole wheat Platte bread (both crust and crumb), the balance of misclassifications was predominantly with refined wheat bread and not with the counterpart whole wheat bread made with red wheat bran. While the number of samples of red and white wheats were very few in this study, results support the contention that different wheat genotypes and specifically, the bran tissue of these genotypes, contain differences in compound composition and/or concentration which when processed by breadmaking, manifest volatiles characteristic of those genotypes even between genotypes possessing the same colour of bran. E-nose instrumentation appears to be very capable of accommodating these sorts of complex tasks on fresh bread. It would be highly beneficial in future research to carry out similar studies in parallel with a human sensory panel, and ideally with many more genotypes of red and white grained wheat with an aim to firmly establish the relative superiority of particular genotypes to produce whole wheat bread with aroma profiles more similar to those of white pan bread. The long term goal of such studies would be to foster increased consumption of whole wheat products and constituent bioactive compounds which confer favourable health benefits in the general population.

CHAPTER 5

Quantitative Analysis of Aroma and Flavour Compounds in Solvent Extraction of Bread Using Gas Chromatography - Mass Spectrometry

5.1. Abstract

Gas chromatography in combination with mass spectrometry (GC-MS) was used to analyze the volatile and non volatile content of crumb and crust of refined Canada Western Red Spring (CWRS) wheat and whole wheat breads made from refined CWRS wheat flour to which bran was added from one red (CWRS) and two white grained wheats (cultivars Snowbird and Platte). Bread samples were extracted using methanol. More than 50 compounds were found in total, the greater majority of which have been previously reported in bread. Major Maillard reaction compounds like furfural, 2-furanmethanol, pyranone, maltol and 5-hydroxymethyl- 2-furancarboxaldehyde were present in highest concentration in whole CWRS bread. Significantly fewer compounds were found in the crust and crumb of CWRS refined wheat bread compared to the other whole wheat breads. In contrast, whole CWRS bread crumb and crust had the highest number of compounds, and in considerably higher total concentration compared to the other two whole white wheat breads, Snowbird and Platte. The higher concentration and number of compounds in whole CWRS bread was attributed to the wheat bran fraction. White whole wheat breads, Snowbird and Platte, had a total number of compounds in crust and crumb approximately intermediate between refined and whole CWRS bread, although Platte whole wheat bread crust was closer to refined bread crust in compound numbers. In terms of total compound concentrations, crust and crumb samples of the whole white breads were clearly more similar to refined CWRS bread, and in the case of

whole wheat Platte bread crust, compound concentrations were much lower. On the whole, these aggregate totals of compound numbers and concentrations by GC-MS mirrored the discrimination and classification results obtained by E-nose, and supported the contention that whole wheat bread made with white wheat bran was milder in aroma compared to bread formulated using red wheat bran.

5.2. Introduction

Flavor is generally thought to consist of the volatile components sensed in the nose and nonvolatile compounds sensed on the tongue. Aroma is considered more important than taste in determining flavor. Large molecules like proteins, carbohydrates and fats which are present in bread do not have much flavour but can break down into smaller molecules like amino acids, sugars and free fatty acids that generate the characteristic bread flavour. Where volatile substances contribute greatly to the odour of bread, non volatile substances influence bread flavour directly or indirectly as products of fermentation or baking (Johnson and El-Dash, 1969).

It has been suggested that volatile compounds of commercial wheat baguettes can influence consumer preferences (Quilez et al., 2006). Although flour furnishes a small amount of volatile compounds and aroma precursors, the contribution of these compounds to bread aroma is considered very small (Martinez-Anaya, 1996). It is the breadmaking process that involves participation of flour components and other ingredients, which at the right temperature, moisture and pH, produce the characteristic flavour for which bread is known. The two most important contributors to bread flavour are crust formation and browning during baking (Baker et al., 1953). There are two main

types of reactions that can occur during baking; caramelization and the non-enzymatic Maillard reaction although the Maillard reaction, involving free amino acids and reducing sugars, is believed to have the more significant role in developing bread flavour during baking (Johnson et al., 1966). It accounts for formation of many important aldehydes and furans. Sugars in bread dough can come from flour, or from oligosaccharides or polysaccharides produced by enzyme action during fermentation, and from added dough ingredients (Johnson and El-Dash, 1969). The type of amino acid is believed to affect the kind of carbonyl compounds formed and sugar type influences the amount of compounds formed (Salem et al., 1967). Sugar influences the rate of the Maillard reaction but odour is believed to be mainly controlled by amino acids (Johnson and El-Dash, 1969). An increase in free amino acids can influence both the crust colour and bread aroma (Salem et al., 1967).

Fermentation is an important process in breadmaking that contributes to flavour. Yeast is the driving force behind fermentation. Baker's yeast, *Saccharomyces cerevisiae*, is the primary agent of fermentation in bread. Yeast utilizes amino acids as a nutrient source of nitrogen which is oxidized to imino acids. The latter react with water to form carboxylic acids. Carboxylic acids upon cleaving yield aldehydes (Johnson et al., 1966). Reduction and oxidation of aldehydes result in formation of alcohols and acids. Bacterial degradation of amino acids also forms organic acids, alcohols and aldehydes. Longer fermentation time increases the content of precursors from which other major flavour compounds form (Schieberle and Grosch, 1987). Bakers' yeast is also believed to be an important source of Maillard-type bread-flavour compounds (Schieberle, 1990).

More than 540 volatile compounds have been previously reported in bread (Schieberle, 1996) and several techniques has been used to separate and identify bread compounds: aroma extraction dilution analysis (Schieberle and Grosch, 1987), headspace analysis (Luning et al., 1991; Ruiz et al., 2003; Poinot et al., 2008), organic solvent extraction (Frasse et al., 1993), distillation (Gasenmeier and Schieberle, 1995) and purge and trap (Seitz et al., 1998). When GC-MS methods were combined together for the first time for identification of compounds in white bread (Mulders and Dhont, 1972), 42 new volatiles were discovered that had not been previously reported. Since then, GC-MS has become the most popular technique for bread volatile analysis and identification.

Compounds accountable for the aroma profile of crust have a significant influence on consumers' liking and disliking of bread (Schieberle and Grosch, 1985; Belitz et al., 2004). Volatile compounds of wheat and rye bread crusts (Schieberle and Grosch, 1987) were analysed using aroma extract dilution analysis and high resolution GC. The results indicated that the "characteristic crust aroma" of wheat bread was due to 2-acetyl-1-pyrroline which has a roasted and popcorn-like aroma. This has been by far the most important aroma compound in bread. Rye bread crust has more compounds than wheat bread crust that are responsible for aroma. Another major compound that has been reported to contribute to the aroma of wheat bread crust aroma is 2-acetyl-1,4,5,6-tetrahydropyridine (Schieberle and Grosch, 1987; Grosch and Schieberle, 1991; Schieberle and Grosch, 1994; Grosch and Schieberle, 1996). The concentration of 2-acetyl-1-pyrroline and 2- acetyl-1,4,5,6-tetrahydropyridine has been reported to be 30 times lower in the crumb of wheat bread compared to crust which substantiates the complete absence of a roasty aroma in the crumb (Schieberle and Grosch, 1991). The

compounds that contribute to caramel like flavour of the crust are 2-methyl-3-hydroxy-4H-pyran-4-one (maltol) and 2-acetyl-3-hydroxyfuran (isomaltol) (Schieberle and Grosch, 1985). Other major compounds important to crust aroma are 3-methylbutanal (malty smell), methylpropanal (malty), 4-hydroxy-2, 5-dimethyl-3 (2H)-furanone (caramel-like smell), acetic acid (sour, pungent), methional (potato-like) and (E)-2-nonenal (green, tallowy) (Schieberle, 1990; Rychlik and Grosch, 1996; Grosch and Schieberle 1997; Zehentbauer and Grosch 1998a; Belitz et al., 2004). Crust aroma of baguettes was studied by Zehentbauer and Grosch (1998a and 1998b) using aroma extraction dilution analysis and GC-MS and gas chromatography/olfactometry. The authors identified several important compounds and concluded that the amount of yeast, fermentation time and fermentation temperature cause difference in flavours for two types of baguettes. Schieberle and Grosch (1991), while analyzing odorants from wheat bread crumb, reported that prolongation of dough fermentation resulted in changes in crumb flavour, mainly due to increased levels of 2-phenylethanol and 2-methybutanal.

The aroma of wheat bread crumb is contributed by many important compounds such as, diacetyl (buttery), 3-methylbutanol (malty), 2-phenylethanol (floral), butanoic acid (rancid), 2- and 3- methylbutanoic acid (rancid, sweaty), hexanal (green), (Z)-4-heptanal (biscuit like), (E)-2-nonenal (green, tallowy), ethyl octaonate (sweet), 1-octen-3-one (mushroom like), (Z)-2-nonenal (green), 2, 4-decadienal (fatty, green), (E, E)- 2, 4-decadienal (fatty, waxy) and phenylacetaldehyde (honey like, sweet) (Schieberle and Grosch, 1991; Schieberle, 1991; Schieberle and Grosch, 1994; Gassenmeier and Schieberle, 1995). Furan derivatives are considered essential to the aroma of bread and

contribute sweet, fruity and caramel like odour (Fors, 1983). In bread crust and crumb, furans presumably arise from thermal degradation of sugars (Martinez- Anaya, 1996).

All the GC-MS research on bread aroma, as cited above, has involved refined wheat bread only. There are very few studies so far conducted on whole wheat bread. Chang et al. (1995) performed dynamic headspace analysis (using purge and trap) of volatile flavour components of white pan breads and whole wheat breads made from HR winter and HW winter wheat, and identified volatiles using GC-Fourier transform infrared-MS (GC-FTIR-MS). They reported more compound differences between whole wheat breads and white pan breads made with refined wheat flour compared to differences between whole wheat breads made from hard red (HR) winter and hard white (HW) winter wheat. Fifteen volatile compounds were reported to have significantly higher concentrations in whole wheat breads than white pan breads both made from either HR and HW winter wheats (ethanol, ethyl acetate, 2-methylbutanal, 1-propanaol, hexanal, isoamyl acetate, 1-butanol, heptanal, 1-pentanol, 2-octanone, 1-hexanol, ethyl octanoate, 1-octen-3-ol, 1-heptanol and 2-furfural). The authors also reported that the higher quantities of ethyl acetate, ethanol, 2-ethyl-3-methylpyrazine and ethyl octanoate in HR bread and 2-butoxy-ethanol and 2-furfural in HW bread could partly be responsible for flavour differences.

The aim of this study was to develop a better understanding of the differences between bread made from refined wheat flour and whole wheat and how the inclusion of bran from red and white-grained wheats modifies the composition and content of volatiles and non volatile compounds determined by GC-MS. A separate objective was to determine to what extent results obtained by this approach were comparable to the

discrimination results previously obtained by E-nose (Chapter 4) where aroma compounds are not specifically identified.

5.3. Materials and Methods

5.3.1. Breads

Wheat was milled and breads were prepared along with crumb and crust samples as described in Chapter 4.

5.3.2 Solvent extraction of bread samples and GC-MS conditions

After thawing an entire loaf of bread overnight, bread crust and crumb samples were carefully separated using scalpel and tweezers to prevent contamination. The samples were then ground using a Cuisinart Mini-Prep Processor DLC-2RC (The Bay, Canada) as previously described (Chapter 4). Bread crumb (50 g) and 25 g of crust were suspended in 400 ml and 100 ml of 100% methanol (HPLC standard, grade, Fisher Scientific, Ottawa, ON), respectively and shaken at room temperature (200 rpm) for 24 h (Max^{Q} 4000-Barnstead/Lab-line, Artisan Scientific, Champaign, IL, U.S.A). Methanol extraction is considered a suitable method for analyzing volatile organic compounds (VOC). The mixture was then filtered (Whatman # 54 hardened circle, 125mm) and the filtrate was concentrated to approximately 5 ml using a rotary evaporator (Buchi Rotavor R-205, 30°C). Triplicate analysis of the extracts was performed by manual injection with a 10 µl syringe (Hamilton Co., Reno, NV, and U.S.A). The remaining extract was stored in dark glass bottles at -20°C. The GC and quadrupole MS (EI) instruments used were an

Agilent Technologies 6890N and 5975, respectively. The amount of sample injected in the GC injection port was 0.8 µl plus 0.4 µl of internal standard solution (see below) and 0.8 µl alkane (C_8 to C_{20}) solution, to yield a total injection volume of 2 µl. An Agilent DB-VRX column (Agilent, Canada) with dimensions 0.18mm X 20m X 1.0µm was used to separate compounds. This column is moderately polar and separates VOC well. The GC oven temperature was set at 40 °C where it was held for 2 min. Temperature was then increased at a rate of 3°C/min to a final temperature of 230 °C where it was held for 5 min, for a total run time of 70.33 min. The gas flow was maintained at 5 ml/min with helium as the carrier gas. The GC injector port was set at 250 °C in splitless mode and a delay time of 8 min was used for MS, which was recorded by electronic impact (EI) at 70 eV. The ion source temperature was 230 °C and the mass quadrupole temperature was 150 °C. The liner used for the splitless injections was Splitless Injection Sleeve Single Taper, packed with glass wool (Supelco, Bellefonte, PA, USA). Split injection is preferred when working with samples with high analyte concentrations (> 0.1%), whereas splitless injection is best suited for trace analysis with low content of analytes. (< 0.01%).

A blank sample was run after every bread sample extract to avoid compound carryover and other contamination. Table 5.1 summarizes the GC-MS parameters.

5.3.3. GC-MS internal and external standards

Adding known quantities of an internal standard in GC helps correct matrix effects and provides a reference for calculating concentrations. 2-ethylbutyric acid was chosen as internal standard. The standard mixture of n-alkane was used as an external

standard reference to calculate linear retention index (LRI) for the compounds detected from methanol extraction of bread samples. The LRI obtained were matched with the LRI database of The University of Reading (www.odour.org.uk). The equation used to calculate LRI of each compound is as follows (Madruga and Mottram, 1998):

$$LRI = \frac{[RTx - RTn + n]}{[RTn+1-RTn]} \times 100$$

Where, *RTx* = retention time of compound

RTn = retention time of *n*-alkane before peak

RTn+1 = retention time of *n*-alkane after peak

n = carbon number of *n*-alkane before peak

Table 5.1. Summary of GC-MS parameters.

Initial temperature	40°C (2 min hold)
Final temperature	230 °C (5 min hold) with increase of 3 °C/ min
Column	Agilent DB-VRX (0.18mm X 20m X 1.0µm)
Mode	Splitless
Front inlet temperature	250°C
Pressure	25 psi
Gas flow (helium)	1.3ml/min

5.3.4. Statistical analysis

The NIST 98 library built into the GC-MS computer system and LRI database of The University of Reading (www.odour.org.uk) were used to identify possible compounds. Data presented are means of triplicate analyses.

5.4. Results and Discussion

5.4.1. Differences between crust and crumb of bread types by GC-MS

Figure 5.1 presents the total number and concentration of crust and crumb compounds quantified by GC-MS of the four bread types. More than 50 different compounds were identified in total. Significantly fewer compounds were found in the crust and crumb of CWRS refined wheat bread compared to the other whole wheat breads (Fig. 5.1). In contrast, whole red wheat (CWRS) crumb and crust had the highest number of compounds, and in considerably higher total concentration compared to the other two whole white wheat breads, Snowbird and Platte. These white whole wheat breads, had a total number of compounds in crust and crumb approximately intermediate between refined and whole CWRS bread, although Platte whole wheat bread crust was closer to

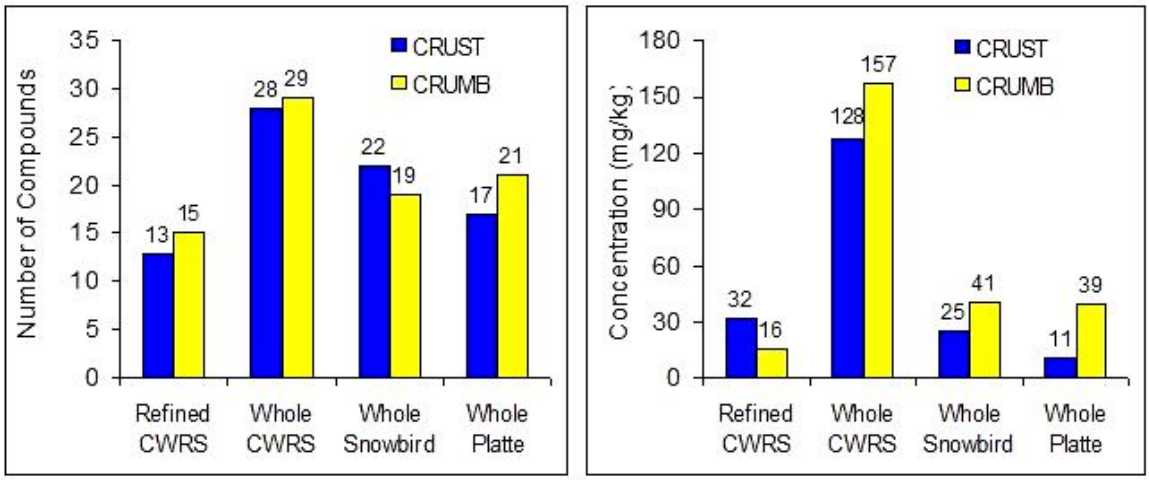


Figure 5.1. Total number (left) and concentration (right) of compounds in crust and crumb samples of bread types by GC-MS.

refined bread crust in compound numbers. In terms of total compound concentrations, crust and crumb samples of the whole white breads were clearly more similar to refined CWRS bread, and in the case of whole wheat Platte bread crust, compound

concentrations were much lower. On the whole, these aggregate totals of compound numbers and concentrations by GC-MS appear to support the discrimination and classification results obtained by E-nose as presented in Chapter 4.

Table 5.2 summarizes all the compounds generated from the crust and crumb of all breads. Major compounds responsible for crust aroma, 2-acetyl-1-pyrroline, and 6-acetaltetrahydropyridine (Schieberle and Grosch, 1991; Grosch and Schieberle, 1997) were not detected. This could be because they were present below the limit of detection of the instrument or they were lost because of bread storage, or it could be present in very low concentration. 2-acetyl, 1-pyrroline was also not detected during headspace analysis of volatile flavour components of white pan breads and whole wheat breads from HR and HW winter wheats (Chang et al., 1995). As previously reported (Schieberle and Grosch, 1992), there is rapid decline in the concentration of 2-acetyl-1-pyrroline within hours and days of baking. Moreover, industrial methods use a greater quantity of yeast compared with the traditional method which produces larger quantities of 2-acetyl-1-pyrroline (Zehentbauer and Grosch, 1998a). Ornithine from yeast is a precursor of this compound (Schieberle, 1990). One of the foremost compounds responsible for formation of 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine is 1-hydroxy, 2-propanone (Hofmann and Schieberle, 1998; Adams et al., 2004). This compound, however, was present in all the bread crust and crumb samples, and remarkably in high concentration in whole CWRS.

Acetic acid was present in all the samples but was difficult to detect due to contaminants (siloxanes). The levels were higher in crumb than crust except for Snowbird. Snowbird crumb and whole CWRS crust had the highest concentration of 2-

hexanol, which was present in all samples but refined CWRS. 3-hydroxy-2-butanone, an important ketone in bread with smell of butter was present only in whole Platte crumb and in all crust samples except refined CWRS.

One of the major compounds contributing significantly to bread crumb aroma has been identified as 2-methylbutanal (Schieberle and Grosch, 1991). 2-methylbutanal was found only in whole CWRS crumb. It is formed due to Strecker degradation of amino acids isoleucine (Johnson et al., 1966) and smells malty. In old bread, concentration of malty-smelling 3-methylbutanal, which is another very important crust and crumb odorant, is reduced significantly (Schieberle, 1996). Freezing bread for long period as was done in this study could alter changes in compound composition. 3-methylbutanal was not detected in any of the samples. Zehentbauer and Grosch (1998a) noted that an increase in intensity and duration of dough kneading reduces the amount of 2-methylbutanal, 3-methylbutanal and 2, 3-butanedione. 2, 3-butanedione, an important crumb aroma compound was not identified in any of the bread types. 2, 3-butanediol, a fermentation product, was found in all crumb samples, but it was present in higher concentration in whole CWRS and whole Snowbird crumb. It was also present in all crust samples but refined CWRS.

Important Maillard reaction compounds like furfural, 2-furanmethanol, 2(5H)-furanone, 2-furancarboxylic acid, maltol and 5-hydroxymethyl furancarboxaldehyde were present in all crumb samples, however, whole CWRS crumb had significantly higher amounts. This could explain the more bitter flavour of red CWRS whole wheat bread. Though furfural was missing only in refined CWRS crust, 2-furanmethanol, 2(5H)-furanone, maltol and 5-hydroxymethyl furancarboxaldehyde were present in all crust

samples with, yet again, highest concentrations in whole CWRS bread. Furfural was found to be present in significantly higher amounts in whole wheat bread as compared to refined bread (Chang et al., 1995) as was found in this study as well. 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one (pyranone) was present in all crumb and crust samples. 2-furanmethanol and pyranone are among the few Maillard reaction compounds that have been reported to possess antioxidant properties (Osada and Shibamoto, 2006).

2-Furancarboxylic acid was not detected in refined CWRS and Platte crusts., Other Maillard reaction products like Isomaltol and 2(3H)-furanone were found only in whole CWRS crumb and crust. Isomaltol was also detected in refined CWRS. Isomaltol and maltol have caramel like aroma and flavour but it has not been established yet that they both are important contributors to bread flavour. Maillard reaction doesn't exclusively contribute towards flavour but it is the interaction between Maillard reaction products and products of lipid degradation that is important in releasing desirable flavour compounds (Huang et al., 1987). Melanoidins, formed during browning of bread, in general, are not perceived to be as important to bread flavor as the intermediates of browning (Johnson et al., 1966). 4-hydroxy-2, 5-dimethyl-3(2H)-furanone (strawberry furanone) was absent only in refined CWRS and evidently was highest in Snowbird. In crust, it was present only in Snowbird. 5-methyl -2- furancarboxaldehyde was only present in crumbs of refined and whole CWRS and only whole CWRS crust. From the data, it can be said that influence of bran in production of various maillard reaction is possible.

Isopropyl alcohol and benzeneacetaldehyde (phenylacetaldehyde) were found only in whole CWRS crumb. Isopropyl alcohol was also present in whole CWRS crust at

very low concentration. Benzeneacetaldehyde is formed by amino acids phenylalanine via Strecker degradation. Phenylethyl alcohol (2-phenylethanol) has been reported in wheat bread crumb in higher amounts than crust (Grosch and Schieberle, 1997). It was only detected in whole CWRS crust. It is possible to find aroma compounds from crust in crumb, as upon cooling and during aging, most of the volatile compounds, originating only in the crust migrate to the crumb (Baker et al., 1953; Wiseblatt and Kohn, 1960; Schieberle and Grosch, 1991). This could be the reason that most of the compounds found in crust according to literature are present in our crumb samples as well. 1-octanol was assumed to be present in all samples, though had a clear peak only in Snowbird. Larger amounts of formic acid in all the samples dominated the 1-octanol peak. Although formic acid has been found in bread previously, methanol as an extraction solvent could possibly trigger the production of larger amount of formic acid.

Major fatty acid identified in all the samples was saturated fatty acid n-hexadecanoic acid (palmitic acid). Higher amounts of it were in crusts than crumbs of refined CWRS and whole CWRS while Snowbird and Platte crumbs had higher amounts of it than their respective crusts. Only refined CWRS and whole CWRS crumb demonstrated presence of polyunsaturated (Z, Z)-9, 12- octadecadienoic acid (linoleic acid), which was present in all crust samples. Only Platte crumb and refined CWRS crust had (E)-9- octadecenoic acid (oleic acid). Dodecanaoic acid (lauric acid) and n-decanoic acid (capric acid) occurred only in whole CWRS crust. Docecanoic acid has been known to have antimicrobial properties (Ouattara et al., 2000). Important aromatic acids like hexanoic acid and benzoic acid were found only in crust samples. Benzoic acid, which is known to have antifungal properties, was found only in whole CWRS crust, and only

refined CWRS and Snowbird crusts had hexanoic acid. Shortening and conditioner in the breadmaking process could also have contributed to these fatty acids.

The compounds common and unique to crust and crumb samples of each bread type are listed in Tables 5.3 to 5.6.

The interactions between the products of the Maillard reaction and those of the Strecker degradation lead to the formation of many important classes of flavor compounds. These include heterocyclic compounds such as pyrazines, oxazoles, thiophenes, and heterocyclic compounds with more than one sulfur atom (Pozo-Bayon, 2006; Mottram, 2007). Though certain pyrazines were present in the whole CWRS bread, sulphur containing compounds were not detected. Flavonoids have inhibitory effects on the formation of melanoidins, which is one of the final products of the Maillard reaction (Zhang, 2009). This could explain the reason why many high molecular weight melanoidins were not detected in whole CWRS bread, assuming that red wheat bran contains higher levels of flavonoids than white wheat bran. The reason why these compounds could not be detected in other bread types including refined CWRS remains unclear. It could also be possible that these compounds were poorly extracted by methanol as used in this study or they were very volatile and escaped during extraction or were below limits of detection. A very large peak corresponding to glycerin was present in all the samples. Glycerin can be derived from saponification of fats and oils; it could be removed by defatting bread samples before methanol extraction (Wiseblatt and Zoumut, 1963). This step was not included in this experiment.

Table 5.2. Possible identified compounds in crumb and crust of breads.

#	Possible compound	Crumb (Concentration in µg/kg of bread[A]±%)				Crust (Concentration in µg/kg of bread[A]±%)				MD[B]	LRI[C]	Odour[D]
		Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte			
1.	Acetic acid	-	840±	738±1	-	-	-	-	-	MS	621 [2]	Sour, pungent[4, 6, 10]
2.	1-hydroxy- 2 - propanone	681±1	1730±3	277±1	567±1	447±0	407±1	416±1	144±0	MS	625 [2]	-
3.	1,3-butanediol	-	173±1	142±1	397±6	-	1410±2	377±1	-	MS	-	-
4.	2-hexanol	148±	213±1	622±1	126±1	-	592±1	230±1	201±1	MS	777 [2]	
5.	2-methylbutanal	-	984±1	-	-	-	-	-	-	MS	652 [2]	Malty [5, 6, 7, 9]
6.	Butanoic acid, methyl ester	-	772±1	-	-	-	223±0	-	-	MS	796 [2]	Sweaty[6, 7, 9]
7.	2,3-butanediol	379±1	1554±4	1198±4	552±2	-	2619±5	502±1	239±0	MS	-	Buttery, cheesy[3]
8.	3-furanmethanol	-	579±1	-	133±1	-	-	43.6±0	34±1	MS	825 [1]	Burnt[3]
9.	Furfural	639±6	6532±6	1194±5	601±3	-	4158±6	601.4±1	44±0	MS, LRI	840 [1,2]	Sweet, toasted[3]
10.	2-furanmethanol	1270±6	10149±13	1451±5	2122±5	1418±1	5052±6	1157±7	391±1	MS, LRI	856 [1,2]	Burnt[3]
11.	Isopropyl alcohol	-	5642±7	-	-	-	18±0	-	-	MS	610 [2]	
12.	2-cyclopentene-1, 4-dione	88±2	1912±3	-	150±1	-	1024±1	110±0	-	MS	889 [1]	Caramel[8]
13.	2(5H)-furanone	295±1	2544±6	967±2	606±1	285±0	1195±1	202±0	91±1	MS	920 [1]	-

#	Possible compound	Crumb (Concentration in µg/kg of bread[A]±%)				Crust (Concentration in µg/kg of bread[A]±%)				MD[B]	LRI[C]	Odour[D]
		Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte			
14.	1, 3-cyclopentanedione	-	1743±3	623±1	923±1	-	-	-	-	MS	927 [1]	Caramel[8]
15.	5-methyl, 2-furancarboxaldehyde	482±2	2166±6	-	-	-	662±4	-	-	MS, LRI	972 [1,2]	Sweet, toasted, bitter [10]
16.	Isomaltol	-	3706±2	-	-	825±0	1339±8	-	-	MS, LRI	988 [1,2]	Caramel, burnt[8]
17.	Benzeneacetaldehyde	-	293±2	-	-	-	-	-	-	MS, LRI	1054 [1,2]	Honey-like[6, 11]
18.	4-hydroxy-2,5-dimethyl-3(2H)-furanone	-	242±1	551±1	112±1	-	-	187.5±1	-	MS	1059 [1]	Strawberry like[14], Caramel[4, 6, 9, 10]
19.	Formic acid	7398±12	14117±14	12332±17	14210±12	6855±11	19462±60	9524±9	6766±10	MS, LRI	1065 [1,2]	
20.	5-methyl-2-pyrazinylmethanol	-	2276±4	-	-	-	-	-	-	MS	1082[1]	-
21.	2-furancarboxylic acid	468±3	3802±6	648±1	209±5	-	1010±5	322±1	-	MS	1090 [1]	
22.	Maltol	181±2	3165±2	324±2	2908±1	308±1	3975±7	502±2	107±1	MS, LRI	1120 [1,2]	Caramel[8, 9, 10]
23.	N-methyl-N-nitroso, 2-Propamine	-	1262±4	-	-	-	-	-	-	MS	1148[1]	-

#	Possible compound	Crumb (Concentration in µg/kg of bread[A]±%) Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	Crust (Concentration in µg/kg of bread[A]±%) Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	MD[B]	LRI[C]	Odour[D]
24.	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (pyranone)	916±8	17450±9	4834±8	4001±7	2126±1	10435±10	3446±4	771±3	MS	1150 [1]	Fruity, caramel[14]
25.	2(3H) Furanone	-	5614±5	-	-	-	-	-	-	MS	1167 [1]	-
26.	5-hydroxymethyl-2-furancarboxaldehyde	1031±5	57413±24	11345±5	7396±25	45753±3	50116±29	5414±9	544±1	MS, LRI	1228 [1,2]	Warm, caramel, hay like[6]
27.	2-methoxy-4-vinylphenol	-	9223±10	190.2±1	-	-	521±2	72.7±0	-	MS, LRI	1330 [1,2]	Spicy[4, 6, 10]
28.	(Z,Z)-9,12-octadecadienoic acid	898±3	588±0	-	-	476±1	5670±11	272±2	130±1	MS	1822[1]	-
29.	n-hexadecanoic acid	423±2	1028±4	802±3	1769±7	9554±7	13506±20	604±1	360±1	MS	1720[1]	-
30.	n-decanoic acid	-	-	-	-	-	82±1	-	-	MS	1359[1]	Soapy [6, 9]
31.	Dodecanoic acid	-	-	-	-	-	95±0	-	-	MS	1490[1]	-
32.	(E)-9-octadecenoic acid	-	-	-	488±1	2294±2	-	-	-	MS	1828[1]	-
33.	Octadecanoic acid					-	-	218±0	292±1	MS	1814[1]	-
34.	2-methylpropyl ester,2-propanoic acid	-	-	-	754±1	-	-	-	-	MS	874 [1]	-
35.	1,4-pentadiene	-	-	-	130±0	-	-	-	-	MS	947 [1]	-

#	Possible compound	Crumb (Concentration in µg/kg of bread[A]±%)				Crust (Concentration in µg/kg of bread[A]±%)				MD[B]	LRI[C]	Odour[D]
		Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte			
36.	Trans-tetrahydro-5-methyl-2-furanmethanol	-	-	-	1719±3	1206±2	-	-	134±0	MS	1066 [1]	-
37.	1,2,3-propanetriol, monoacetate	-	-	-	1459±4	-	-	-	-	MS	1247[1]	
38.	2-methyltetrahydropyrazine	-	-	-	270±1	-	-	-	-	MS	1323 [1]	-
39.	2,3-dimethylheptane	-	-	164±0	-	-	-	-	-	MS	806 [1]	-
40.	1-octanol	-	-	2475±4	-	-	-	-	-	MS, LRI	1061 [1,2]	Musty, hay like[3]
41.	2-propenoic acid	-	-	-	-	-	60±0	-	-	MS	-	
42.	3-hydroxy, 2-butanone	-	-	-	186±1	-	520±1	159±1	79±0	MS	690 [2]	-
43.	4-methyl, 2-pentanol	-	-	-	-	128±0	-	-	-	MS	900 [1]	-
44.	3-methyl, 2 -pentanol	-	-	-	-	-	-	-	86±1	MS	-	-
45.	3-furaldehyde	-	-	-	-	-	482±1	-	-	MS	825[1]	-
46.	3-methyl-2-butanol	-	-	-	-	-	1634±2	444±1	-	MS	884[1]	-
47.	2-hydroxy, 2-cyclopenten-1-one	-	-	-	-	1366±2	571±1	-	-	MS	928[1]	-
48.	Phenylethyl alcohol (2-phenylethanol)	-	-	-	-	-	213±0	-	-	MS, LRI	1117 [1,2]	Sweet, honey like[9, 10]
49.	Benzoic acid	-	-	-	-	-	1143±1	-	-	MS	1172[1]	-

#	Possible compound	Crumb (Concentration in µg/kg of bread[A]±%) Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	Crust (Concentration in µg/kg of bread[A]±%) Refined CWRS	Whole CWRS	Whole Snowbird	Whole Platte	MD[B]	LRI[C]	Odour[D]
50.[8],	Hexanoic acid	-	-	-	-	354±2	-	414±1	-	MS	1483[1]	Sweaty, goat like[6, 7, 9]

[A]Each value is expressed as mean of triplicate analysis
[B]MD- Method of detection of volatiles, based on NIST 98 library ; MS= Mass spectrum (data provided in appendix F)
[C] LRI- Linear Retention Index
[1] LRI (± 10) calculated; [2] LRI (± 50) indicated in literature (www.odour.org.uk)
[3]Chang et al., 1995
[4]Gassenmeier and Schieberle, 1995
[5]Mottram, 2007
[6]Pazo-Bayon et al., 2006
[7]Rychlik and Grosch, 1996
[8] Hodge et al., 1972
[9] Zehentbauer and Grosch, 1998a
[10] Schieberle and Grosch, 1994
[11] Schieberle and Grosch, 1987

Table 5.3. Compounds common and unique to crust and crumb of Refined CWRS bread*.

Common compounds in crust and crumb	**Compounds unique to crumb**	**Compounds unique to crust**
1-hydroxy- 2 -propanone	2-hexanol	Trans-tetrahydro-5-methyl-2-furanmethanol
2-furanmethanol	2,3-butanediol	(E)-9-octadecenoic acid
2(5H)-furanone	Furfural	4-methyl, 2-pentanol
Formic acid	2-cyclopentene-1, 4-dione	Hexanoic acid
Maltol	5-methyl, 2-furancarboxaldehyde	
Pyranone	2-furancarboxylic acid	
5-hydroxymethyl- 2-furancarboxaldehyde		
(Z,Z)-9,12-octadecadienoic acid		
n-hexadecanoic acid		
Total compounds = 9	Total compounds = 6	Total compounds = 4

Table 5.4. Compounds common and unique to crust and crumb of whole CWRS bread*.

Common compounds in crust and crumb	**Compounds unique to crumb**	**Compounds unique to crust**
1-hydroxy- 2 -propanone	**Acetic acid**	**Dodecanoic acid**
2-furanmethanol	**2-methylbutanal**	**n-decanoic acid**
2, 3-butanediol	**3-furanmethanol**	**3-hydroxy, 2-butanone**
Formic acid	**Strawberry furanone**	**2-propenoic acid**
Maltol	**5-methyl-2-pyrazinylmethanol**	**3-furaldehyde**
Isomaltol	**2 (3H)-furanone**	**3-methyl-2-butanol**
2-furancarboxylic acid	**Benzeneacetaldehyde**	**2-hydroxy, 2-cyclopenten-1-one**
Pyranone	**1, 3-cyclopentanedione**	**Benzoic acid**
5-hydroxymethyl- 2-furancarboxaldehyde		**Phenylethyl alcohol**
5-methyl, 2-furancarboxaldehyde		
Furfural		
2(5H)-furanone		
n-hexadecanoic acid		
1, 3-butanediol		
2-hexanol		
Butanoic acid		
Isopropyl alcohol		
2-cyclopentene-1, 4-dione		
2-methoxy-4-vinylphenol		
(Z,Z)-9,12-octadecadienoic acid		
Total compounds = 20	Total compounds = 8	Total compounds = 9

*Compounds in bold were not present in refined bread.

Table 5.5. Compounds common and unique to crust and crumb of whole Snowbird bread*.

Common compounds in crust and crumb	**Compounds limited to crumb**	**Compounds limited to crust**
1-hydroxy- 2 -propanone	**Acetic acid**	**3-furanmethanol**
1,3-butanediol	**1-octanol**	2-cyclopentene-1, 4-dione
2-hexanol	**2,3-dimethylheptane**	(Z,Z)-9,12-octadecadienoic acid
2,3-butanediol	**1, 3-cyclopentanedione**	**Octadecanoic acid**
2(5H)-furanone		**3-hydroxy, 2-butanone**
2-furanmethanol		**3-methyl-2-butanol**
Furfural		Hexanoic acid
Pyranone		
Formic acid		
2-furancarboxylic acid		
Maltol		
5-hydroxymethyl- 2-furancarboxaldehyde		
2-methoxy-4-vinylphenol		
n-hexadecanoic acid		
Total compounds = 14	Total compounds = 4	Total compounds = 7

*Compounds in bold were not present in refined bread.

Differences in the amount of yeast and fermentation conditions can change the roasty and malty notes (Zehentbauer and Grosch, 1998b). Higher yeast activity decreases lipoxygenase activity. Lipoxygenase enzyme uses oxygen to convert fatty acids into aldehydes, like hexanal, and alcohols, like 1-propanol, 1-pentanol, 1-hexanol, and 1-octen-3-ol (Frasse et al., 1992). GC-MS results of this study indicate samples had low lipoxygenase activity. None of the above mentioned compounds were detected. Enzymes like lipoxygenase, polyphenol oxidase and peroxidase are highly concentrated in bran fractions and these enzymes might accelerate lipid oxidation (Maeda et al., 2009). The main enzymes which account for bread flavor during bread making are α-amylase, β-amylase, proteases and lipoxygenases (Martinez-Anaya, 1996) due to the products of these reactions. If freezing of dough or bread is part of the process, there is reduction in

compounds from lipid oxidation like- E-2-nonenal, 1-octen-3-one, pentanol and hexanal (Ullrich and Grosch, 1987). None of these compounds were found in any bread samples. The general increase of aldehydes and ketones during processing is usually associated with a decrease of alcohol and ester compounds (Kermasha et al., 1988).

Table 5.6. Compounds common and unique to crust and crumb of whole Platte bread*.

Common compounds in crust and crumb	**Compounds limited to crumb**	**Compounds limited to crust**
1-hydroxy- 2 -propanone	**1,3-butanediol**	**3-methyl, 2 -pentanol**
2-hexanol	**2-methyltetrahydropyrazine**	(Z,Z)-9,12-octadecadienoic acid
2,3-butanediol	**1,2,3-propanetriol monoacetate**	**Octadecanoic acid**
3-hydroxy, 2-butanone	**1,4-pentadiene**	
Trans-tetrahydro-5-methyl-2-furanmethanol	**2-methylpropyl ester, 2-propanoic acid**	
n-hexadecanoic acid	(E)-9-octadecenoic acid	
5-hydroxymethyl- 2-furancarboxaldehyde	2-furancarboxylic acid	
Maltol	**1, 3-cyclopentanedione**	
Furfural		
Formic acid		
2(5H)-furanone		
2-furanmethanol		
3-furanmethanol		
Total compounds = 13	Total compounds = 8	Total compounds = 3

*Compounds in bold were not present in refined bread.

Table 5.7 summarizes the total number of compounds possibly identified in the crust and crumb samples of bread types. According to the results (Table 5.7), it can be seen that the greatest total number of compounds were identified in whole CWRS bread crumb and crust. Refined CWRS had the least number of compounds detected, a result likely due to the absence of bran in the flour used to make the refined CWRS bread. Refined and

whole CWRS wheat bread also had the greatest difference in number of compounds for both crust and crumb (Table 5.7). In contrast, the total number of compounds in whole white wheat breads (Snowbird and Platte) was similar. Also Platte crust tended to have a lower number of compounds somewhat similar to the result for refined CWRS wheat bread. These results generally support the E-nose results presented in Chapter 4, where Snowbird and Platte whole wheat breads were more similar to each other compared to whole CWRS bread for both crust and crumb samples, Platte whole wheat bread crust was partially similar to refined CWRS wheat bread crust, and a large separation existed between refined CWRS and whole CWRS bread aroma.

Table 5.7. Summary of number of common and unique compounds.

	Crumb	**Crust**	**Total**[1]
Refined CWRS	15 (6)[2]	13 (4)	19
Whole CWRS	29 (8)	28 (9)	37
Whole Snowbird	19 (4)	22 (7)	25
Whole Platte	21 (8)	17 (3)	24

[1] Total refers to total number of different compounds in crumb and crust
[2] Numbers in parentheses are unique compounds in crumb or crust

5.4.2. Refined bread vs. whole wheat bread

Absence of wheat bran in refined wheat flour likely explains the lower concentration of many Maillard reaction products, making the bread from refined wheat flour smell and taste less strong. This is likely the main reason other than colour, why refined bread is preferred by consumers over whole wheat bread (Bakke and Vickers, 2007). As wheat bran contains several times the concentration of unsaturated fatty acids compared to endosperm, refined bread has the potential to produce fewer or less

concentrated lipid oxidation products which are responsible for the fatty, grassy and tallowy smell of bran that can cause undesirable flavours commonly associated with rancidity. Refined bread imparts a less caramel flavour and it certainly differs from breads made with whole wheat bran in many organoleptic aspects. Dehulling oats reduces the concentration of volatiles by 80%, (Klensporf and Jelen, 2008) which could also provide an explanation for the fewer volatile compounds in refined bread (Table 5.7).

Tables 5.8 -5.10 compare the crust and crumb compounds unique to refined bread, and red and white whole wheat breads. It can be concluded that the compounds unique to red and white whole wheat breads are due to bran. Conversely, refined bread lacks the strong aroma present in whole wheat bread due to the absence of breakdown products of bran phenolic compounds. Comparing refined to whole CWRS crust, 17 compounds were unique to the latter. Of those 17 compounds, seven were present in concentrations above 1 mg/kg of bread, viz. furfural (toasted, burnt aroma), 2, 3-butanediol (fruity), 1, 3-butanediol, 2-furancarboxylic acid (tobacco), 3-methyl, 2-butanol, 2-cyclopentene-1, 4-dione and benzoic acid (pleasant aroma) (Table 5.8). Similarly, whole CWRS crumb had seven major compounds (out of a total of 14) with concentrations above 1 mg/kg, viz. 2-methoxy, 4-vinylphenol (spicy), isomaltol (caramel, burnt), isopropyl alcohol (alcoholic), 1, 3-cyclopentanedione (caramel), 3(2H)-furanone, 5-methyl, 2- pyrazinylmethanol and N-methyl-N-nitroso, 2-propamine (Table 5.8). The high concentration of these compounds however, may not in fact be relevant to bread aroma as even trace compounds may contribute to bread aroma if their concentrations are higher than their odour thresholds.

Whole white breads (Snowbird and Platte) had 12 and 13 compounds in crust and crumb, respectively that were not present in refined CWRS, however, none of the compounds were present in concentration above 1 mg/kg in the crust, and 1-octanol (musty), 1, 2, 3-propanetriol monoacetate (bitter taste, fatty odour) and 1, 3-cyclopentanedione (sweet, toasted aroma) was present in concentration above 1 mg/kg in the crumb (Tables 5.9 and 5.10).

5.4.3. Whole red wheat bread vs. whole white wheat bread

Ferulic acid, a major phenolic acid, is a source of 2-methyl-4-vinylphenol, which is described as having a burnt or tar-like flavour (Hansen, 1995). 2-methoxy-4-vinylphenol was present only in Snowbird whole wheat bread and whole CWRS suggesting that Platte may contain lower amounts of phenolic acids than Snowbird. More research is needed to support this suggestion. Whole CWRS bread contained significantly higher amounts of volatile and non-volatile compounds responsible for stronger, more caramel, malty, pungent, sweeter and toastier notes than its refined wheat counterpart. These differences can be attributed to phenolic compounds in red wheat bran (Huang and Zayas, 1991). Phenolic compounds in general are believed to influence odour, taste and colour of food (Weston, 2005). Fewer phenolic compounds and tannins in the bran of white wheat likely make it less bitter in taste.

Table 5.8. Compounds unique to refined CWRS and whole CWRS crust and crumb.

	Refined CWRS	Whole CWRS
Crust	(E)-9-octadecenoic acid	Furfural*
	Trans-tetrahydro-5-methyl-2-furanmethanol	2,3-butanediol*
	Hexanoic acid	3-methyl-2-butanol*
	4-methyl, 2-pentanol	1,3-butanediol*
		Benzoic acid*
		2-cyclopentene-1,4-dione*
		2-furancarboxylic acid*
		5-methyl, 2-furancarboxaldehyde
		2-hexanol
		2-methoxy-4-vinylphenol
		3-hydroxy, 2-butanone
		3-furaldehyde
		2-phenylethanol
		Butanoic acid
		dodecanoic acid
		n-decanoic acid
		2-propenoic acid
		Isopropyl alcohol
Crumb		2-methoxy, 4-vinylphenol*
		3(2H)-furanone*
		Isopropyl alcohol*
		Isomaltol*
		5-methyl-2-pyrazinylmethanol*
		1,3-cyclopentanedione*
		N-methyl-N-nitroso, 2-propamine*
		2-methylbutanal
		Acetic acid
		Butanoic acid
		3-furanmethanol
		Benzeneacetaldehyde
		4-hydroxy-2,5-dimethyl-3(2H)-furanone
		1,3-butanediol

* Compounds present in concentration > 1 mg/kg.

Table 5.9. Compounds unique to refined CWRS and whole Snowbird crust and crumb.

	Refined CWRS	**Whole Snowbird**
Crust	Isomaltol	2-hexanol
	(E)-9-octadecenoic acid*	2,3-butanediol
	Trans-tetrahydro-5-methyl-2-furanmethanol*	1,3-butanediol
	4-methyl, 2-pentanol	3-furanmethanol
	2-hydroxy, 2-cyclopenten-1-one*	Furfural
		2-cyclopentene-1,4-dione
		4-hydroxy-2,5-dimethyl-3(2H)-furanone
		2-furancarboxylic acid
		2-methoxy, 4-vinylphenol
		Octadecanoic acid
		3-hydroxy, 2-butanone
		3-methyl, 2-butanol
Crumb	2-cyclopentene-1,4-dione	Acetic acid
	5-methyl, 2-furancarboxaldehyde	1,3-butanediol
	(Z, Z)-9, 12-octadecadienoic acid	1, 3-cyclopentanedione
		2-methoxy, 4-vinylphenol
		4-hydroxy-2,5-dimethyl-3(2H)-furanone
		2,3-demethylheptane
		1-octanol*

* Compounds present in concentration > 1 mg/kg.

Between the two white whole wheat breads, Snowbird contained higher amounts of major flavour compounds compared to Platte. Only Platte whole wheat bread and refined CWRS bread contained provisionally identified trans-tetrahydro-5-methyl-2-furanmethanol which has never been reported in literature. More analysis is needed to confirm the identity of this compound. Among the whole wheat breads, the least number of compounds were identified in Platte product. Platte whole wheat bread had somewhat similar compound characteristics to refined CWRS bread.

Table 5.10. Compounds unique to refined CWRS and whole Platte crust and crumb.

	Refined CWRS	**Whole Platte**
Crust	Isomaltol (E)-9-octadecenoic acid* 4-methyl, 2-pentanol Hexanoic acid 2-hydroxy, 2-cyclopenten-1-one*	2-3, butanediol 3-furanmethanol Furfural Octadecanoic acid 3-hydroxy, 2-butanone 3-methyl, 2-pentanol
Crumb	5-methyl, 2-furancarboxaldehyde (Z, Z)-9, 12-octadecadienoic acid	1,3-butanediol 3-furanmethanol 4-hydroxy-2,5-dimethyl-3(2H)-furanone (E)-9-octadecenoic acid 2-methyltetrahydropyrazine 1,2,3-proanetriol, monoacetate * 1,4-pentadiene 2-methylpropyl ester, 2-propanoic acid Trans-tetrahydro-5-methyl-2-furanmethanol* 1,3-cyclopentanedione

* Compounds present in concentration > 1 mg/kg.

Tables 5.11, 5.12 and 5.13 compare the compounds unique to crust and crumb fractions of whole red and whole white breads. Whole red and whole white wheat breads had eight and six unique crust compounds, respectively. Benzoic acid (faint aroma) and isomaltol (caramel) in whole red, however, were the only two compounds with a concentration higher than 1 mg/ kg. For crumb, whole CWRS wheat bread and whole white bread (Snowbird and Platte) each had 10 compounds that were not present in the other bread. Six of these compounds in whole red crumb had concentrations higher than 1 mg/ kg, viz. 5-methyl, 2-furancarboxaldehyde, isopropyl alcohol (alcoholic), isomaltol (caramel), N-methyl, N-nitroso, 2-propamine, 2(3H)-furanone and 5-methyl, 2-

pyrazinylmethanol. Whole white bread crumb had three compounds with concentrations greater than 1 mg/kg, viz. 1-octanol (musty), 1, 2, 3-propanetriol monoacetate (bitter taste, fatty odour) and trans-tetrahydro, 5-methyl, 2-furanmethanol.

2-methoxy-4-vinylphenol was found only in crumb and crust of whole CWRS and Snowbird breads, with lower concentrations in crust. 2-methoxy-4-vinylphenol is derived from thermal degradation of ferulic acid (Bredie et al., 2006). Whole CWRS bread compared to Snowbird bread had 50 times more 2-methoxy-4-vinylphenol in the crumb and seven times higher concentration in the crust. Presence of 2-methoxy, 4-vinylphenol in whole Snowbird bread suggested higher ferulic acid content in Snowbird bran compared to that of Platte.

Whole Snowbird bread crust had seven compounds not present in whole Platte bread crust, while Platte bread crust had two compounds that were unique, but none were present in concentration higher than 1 mg/ kg. Whole Platte bread crumb had eight unique compounds not present in Snowbird crumb, while Snowbird crumb had four unique compounds not present in Platte. The unique compounds with concentrations greater than 1 mg/kg were 1, 2, 3-proanetriol monoacetate and trans-tetrahydro, 5-methyl, 2-furanmethanol in whole Platte, and 1-octanol in whole Snowbird (Table 5.13).

Table 5.14 lists provisionally identified compounds found in the various breads including with compound type or class, possible nature of formation and chemical structures.

Table 5.11. Compounds unique to whole Snowbird and whole CWRS crust and crumb.

	Whole Snowbird	Whole CWRS
Crust	3-furanmethanol	Butanoic acid
	4-hydroxy-2,5-dimethyl-3(2H)-furanone	Isopropyl alcohol
	Octadecanoic acid	5-methyl, 2-furancarboxaldehyde
		n-decanoic acid
		Dodecanoic acid
		2-propenoic acid
		3-furaldehyde
		Benzoic acid*
		2-phenylethanol
		2-hydroxy, 2-cyclopenten-1-one
		Isomaltol*
Crumb	1-octanol*	2-methylbutanal
	2,3-dimethylheptane	Butanoic acid
		3-furanmethanol
		Isopropyl alcohol*
		2-cyclopentene-1,4-dione*
		5-methyl, 2-furancarboxaldehyde*
		Isomaltol*
		Benzeneacetaldehyde
		5-methyl-2-pyrazinylmethanol*
		N-methyl-N-nitroso, 2-propamine*
		2(3H) furanone*
		(Z, Z)-9, 12-octadecadienoic acid

* Compounds present in concentration > 1 mg/kg.

Table 5.12. Compounds unique to whole Platte and whole CWRS crust and crumb.

	Whole Platte	**Whole CWRS**
Crust	3-furanmethanol Octadecanoic acid 3-methyl, 2-pentanol Trans-tetrahydro-5-methyl-2-furanmethanol	1, 3-butanediol* Butanoic acid Isopropyl alcohol 5-methyl, 2-furancarboxaldehyde Isomaltol* 2-propenoic acid Hexanoic acid 2-hydroxy, 2-cyclopenten-1-one 2-furancarboxylic acid* n-decanoic acid Dodecanoic acid 2-phenylethanol Benzoic acid* 3-methyl-2-butanol 2-methoxy-4-vinylphenol 3-furaldehyde
Crumb	3-hydroxy, 2-butanone (E)-9- octadecenoic acid 2-methyltetrahydropyrazine 1,2,3-proanetriol, monoacetate* 1,4-pentadiene (E)-9-octadecenoic acid 2-methylpropyl ester, 2-propanoic acid Trans-tetrahydro-5-methyl-2-furanmethanol*	(Z, Z)-9, 12-octadecadienoic acid N-methyl-N-nitroso, 2-propamine* Benzeneacetaldehyde 5-methyl-2-pyrazinylmethanol* Isomaltol* 5-methyl, 2-furancarboxaldehyde* Isopropyl alcohol* Acetic acid Butanoic acid

* Compounds present in concentration > 1 mg/kg.

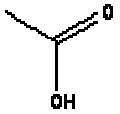
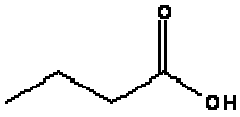
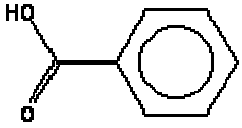
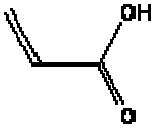
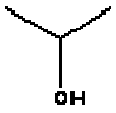
Table 5.13. Compounds unique to whole Snowbird and whole Platte crust and crumb.

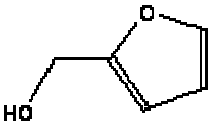
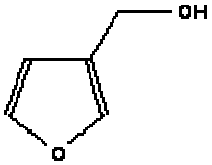
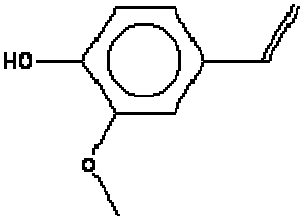
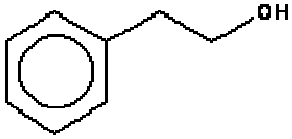
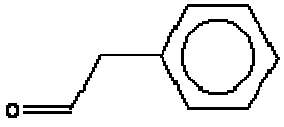
	Whole Snowbird	**Whole Platte**
Crust	1,3-butanediol 4-hydroxy-2,5-dimethyl-3(2H)-furanone 2-furancarboxylic acid 2-methoxy-4-vinylphenol 3-methyl-2-butanol Hexanoic acid 2-cyclopentene-1,4-dione	Trans-tetrahydro-5-methyl-2-furanmethanol 3-methyl, 2-pentanol
Crumb	1-octanol* 2,3-dimethylheptane 2-methoxy-4-vinylphenol Acetic acid	3-hydroxy, 2-butanone 3-furanmethanol 2-methyltetrahydropyrazine 1,2,3-proanetriol, monoacetate* Trans-tetrahydro-5-methyl-2-furanmethanol* 1,4-pentadiene (E)-9-octadecenoic acid 2-methylpropyl ester, 2-propanoic acid

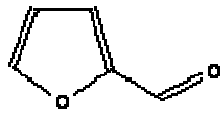
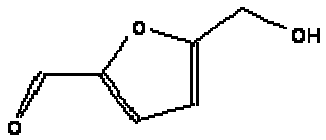
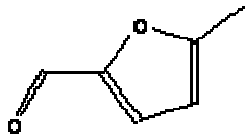
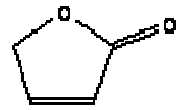
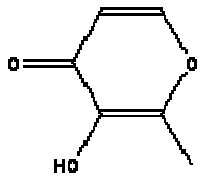
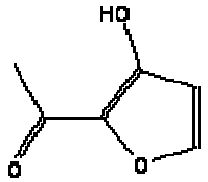
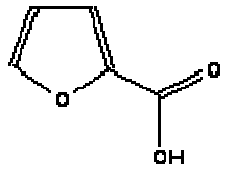
* Compounds present in concentration > 1 mg/kg.

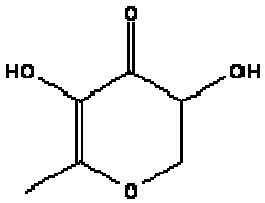
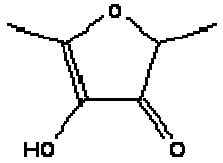
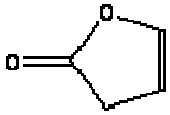
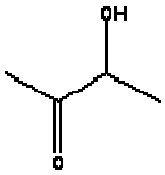
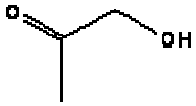
Table 5.14 lists possible identified compounds with their classes, possible nature of formation and chemical structures.

Table 5.14. Some of the possible identified compounds with their classes, possible nature of formation and chemical structures.

Class	Possible identified compounds	Possible nature of formation	Chemical structure [1]
Acids	Acetic acid	Fermentation[2]	
	Formic acid (methanoic acid)	-	
	Butyric acid (butanoic acid)	Fermentation[3]	
	Benzoic acid	-	
	Hexanoic acid	Lipid oxidation[3]	
	2-propenoic acid	-	
	Decanoic acid	-	
Alcohols	Isopropyl alcohol	-	

Class	Possible identified compounds	Possible nature of formation	Chemical structure [1]
Alcohols	2-hexanol	-	
	2-furanmethanol	Maillard reaction[3]	
	3-furanmethanol	-	
	1-octanol	-	
	2-methoxy-4-vinylphenol	Maillard reaction and cysteine degradation	
	Phenylethyl alcohol (2-phenylethanol)	Fermentation[4, 5]	
Aldehydes	2-methylbutanal	Maillard reaction[3, 7]	
	Phenylacetaldehyde	Fermentation and lipid oxidation	

Class	Possible identified compounds	Possible nature of formation	Chemical structure [1]
Furans and Pyrans	Furfural	Maillard reaction, fermentation[3, 6]	
	5-(hydroxymethyl)-2-furancarboxaldehyde	Maillard reaction[3, 6]	
	5-methyl-2-furancarboxaldehyde	Maillard reaction[3, 6]	
	2(5H)-furanone	Maillard reaction[6]	
	Maltol (3-hydroxy-2-methyl-4H-pyran-4-one)	Maillard reaction[3, 6]	
	Isomaltol	Maillard reaction[3, 6]	
	Furancarboxylic acid	-	

Class	Possible identified compounds	Possible nature of formation	Chemical structure [1]
Furans and Pyrans	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Maillard reaction[3, 6]	
	2,5-dimethyl-4-hydroxy-3(2H) furanone (Strawberry furanone)	Maillard reaction[3, 6]	
	2(3H)- furanone	Maillard reaction[6]	
Ketones	3- hydroxy, 2-butanone (acetoin)	Fermentation[2]	
	1-hydroxy-2-propanone (acetol)	-	
Ketones	2-cyclopentene, 1, 4-dione	-	
	1,3-cyclopentanedione	-	
Alkenes	1,4-pentadiene	-	

[1]NIST Chemistry WebBOOK (2008)
[2] Hansen and Schieberle, 2005; [3] Pazo-Bayon et al., 2006 ; [4] Frasse et al., 1992 ; [5] Grosch and Schieberle, 1997; [6] Hodge et al., 1972; [7] Rychlik and Grosch, 1996

5.5. Conclusions

GC-MS was used to study differences between different types of bread made from refined wheat flour and whole wheat, and how the inclusion of bran from red and white-grained wheats modified the composition and content of volatile and non-volatile compounds in crust and crumb. In total, 50 compounds were found, the greater majority of which have been previously reported in bread. Major Maillard reaction compounds like furfural, 2-furanmethanol, pyranone, maltol and 5-hydroxymethyl- 2-furancarboxaldehyde were present in highest concentration in whole CWRS bread. Significantly fewer compounds were found in the crust and crumb of CWRS refined wheat bread compared to the other whole wheat breads. In contrast, whole CWRS bread crumb and crust had the highest number of compounds, and in considerably higher total concentration compared to the other two whole white wheat breads, Snowbird and Platte. The higher concentration and number of compounds in whole CWRS bread was attributed to the wheat bran fraction. White whole wheat breads, Snowbird and Platte, had a total number of compounds in crust and crumb approximately intermediate between refined and whole CWRS bread, although Platte whole wheat bread crust was closer to refined bread crust in compound numbers. In terms of total compound concentrations, crust and crumb samples of the whole white breads were clearly more similar to refined CWRS bread, and in the case of whole wheat Platte bread crust, compound concentrations were much lower. On the whole, these aggregate totals of compound numbers and concentrations by GC-MS mirrored the discrimination and classification results obtained by E-nose, and supported the contention that whole wheat bread made

with white wheat bran was milder in aroma compared to bread formulated using red wheat bran.

CHAPTER 6

General Discussion and Conclusions

6.1. General discussion and conclusions

Aroma and flavour are undoubtedly two of the most important quality attributes of bread or any food. Aroma in particular, as it will be sensed first, will determine whether the product will be tasted and eaten, and is a major factor in establishing acceptability and preference. In that regard, the dominant preference by consumers of bread made from refined flour in contrast to whole wheat flour (Bakke and Vickers, 2007) is at least in part due to the strong and different aroma of whole wheat bread. White wheats may have an advantage over red wheat in this regard according to some reports (reviewed in Chapter 4) but the science is limited. In North America, most bread, whether it is refined flour pan bread or whole wheat bread, is made from hard red (HR) wheat. Sensory panel research has noted differences, but not preferences in flavour and volatiles between both refined flour and whole wheat breads made from HR and hard white (HW) wheats (Lang and Walker, 1990; Chang and Chamber IV, 1992). As with human sensory work, there is very limited instrument-based research that has been published with the aim to explain differences in volatile or flavour components of refined and whole wheat bread or how the colour of bran can influence volatile outcomes in whole wheat bread. This despite the fact that GC-MS methods have been used to identify several hundred compounds in bread that contribute to aroma and flavour (Schieberle, 1996). This thesis research has focused on several of these knowledge gaps using both E-nose and GC-MS "instrument olfaction" technologies applied to HR and HW wheat breads made from both refined and whole wheat flour.

The first sets of experiments (Chapter 3) were tasked to determine a protocol for E-nose analysis of bread as none has been previously reported. The strategy employed in these experiments was to identify a set of conditions that would maximize the discrimination of refined and whole wheat bread crumb. Sample size proved to be one of the most important experimental parameters for the E-nose, and a sample size of 50 mg in contrast to higher amounts (100 – 500 mg) of ground bread crumb, gave the best results. This indicated that the E-nose system had very high sensitivity, likely higher than that for the human nose (Harper, 2001).

This E-nose protocol was applied in Chapter 4 to establish the capabilities of a state-of-the art E-nose system to differentiate between crust, crumb and whole slices of refined and whole wheat bread and between whole wheat breads from red and white wheats. Results varied according to the nature of the sample, i.e. crust, crumb or whole slices. For crust, the greatest distinction in aroma was found between refined and whole wheat breads. When refined bread crust was misclassified, samples were confused with whole white wheat crust predominantly from Platte bread. Interestingly, average E-nose sensor outputs for crust samples of Platte and refined wheat breads were the lowest among the four bread types that were studied (Appendix E, Fig. 1) suggesting a milder aroma profile; not surprisingly refined wheat bread crust (and crumb) had the lowest concentration of volatiles according to E-nose sensor data. Conversely, whole wheat Platte bread crust, when misclassified, appeared to possess an aroma more in line with crust of its counterpart whole white wheat Snowbird or that of refined CWRS bread. For crumb samples, the aroma of the whole red wheat bread was found to be completely distinct from other bread types, including the two whole white breads. This result was

explained by noticeably higher concentration of volatiles as reflected in average E-nose sensor output (Appendix E, Fig. 2). These results support the notion that white wheat bran is certainly different if not milder in aroma than red wheat bran (Miller 1979; Lang and Walker, 1990; Matus-Cadiz et al., 2008).

With regard to the type of sample that is best used for E-nose analysis of bread products, results recommend both crust and crumb. In contrast, whole slice samples that combine both crust and crumb in their appropriate proportions, are not recommended as the overall discrimination among different bread types was poorer compared to that of crust and crumb. Blending of volatiles from crust and crumb is the likely reason. This recommendation would seem to be justified not only for E-nose analysis of bread, but also for sensory analysis of any kind, whether by instruments (e.g. GC-MS) or human sensory panels.

It can be concluded from the study reported in Chapter 4 that E-nose instrumentation appears to be capable of accommodating sophisticated aroma assessments on fresh bread, and results indicated that it was the LY-type of MOS sensor (Appendix E, Figs. 1 and 2) that provided the greatest discrimination of different samples. However, while the results obtained in this thesis research were compelling and plausible, the need remains to validate those results with experiments using more genotypes of red and white-branned wheats as well as with traditional sensory panel studies, particularly in view of the high sensitivity provided by the E-nose instrumentation. “Would humans respond the same way?” is a key practical question that needs to be answered with more research.

The final phase of this thesis research (Chapter 5) involved a more traditional analysis of bread samples by GC-MS of methanol extracts. It is worth pointing out that numerous GC-MS studies have evaluated bread aroma and flavour compounds (see Table 2.4), however, very few have analyzed whole wheat bread. The aim of this study was to develop a better understanding of the differences between different types of bread made from refined wheat flour and whole wheat, especially how the inclusion of bran from red and white-grained wheats modified the composition and content of volatile and non-volatile compounds in crust and crumb.

Fifty compounds in total were detected by GC-MS (Table 5.2). It is noteworthy that with very few exceptions, all compounds have been previously reported. Major compounds responsible for crust aroma such as 2-acetyl-1-pyrroline, and 6-acetyl-1,4,5,6-tetrahydropyridine (Schieberle and Grosch, 1991; Grosch and Schieberle, 1997) were not detected in this study. In other work, these compounds were present in very low concentration, but had a low odour threshold which explains their significance for bread aroma. These compounds could either be below the limit of detection of the instrument or were lost during storage of bread. As previously reported (Schieberle and Grosch, 1992), there is a rapid decline in the concentration of 2-acetyl-1-pyrroline soon after baking. It could also be possible that methanol was not effective to extract these compounds. Storage conditions in general can contribute to altering the aroma profile of bread (Pohjanheimo et al., 2006), particularly if bread stales. However, as noted in Chapter 4, bread samples were carefully and thoroughly sealed soon after baking, first with aluminum foil which provided a neutral barrier for volatiles, and second with two layers of polyethylene film to further restrict migration of moisture and volatiles in the samples.

The latter were then stored until analysis at -35 ^{o}C which is a temperature below which staling can occur (Kent and Evers, 1994; Pateras, 2007). Upon thawing, bread samples appeared to retain their natural aroma. Accordingly, storage conditions were likely not a significant factor on the aroma of the breads as determined by E-nose or GC-MS.

Detection of 2-acetyl, 1-pyrroline was not reported in headspace analysis of volatile components of white pan breads and whole wheat breads from HR and HW winter wheats (Chang et al., 1995). Also, not many pyrazines were detected in this thesis research. Pyrazines, which are important constituents of cereal grain flavour (Zhou et al., 1999) possess comparatively high flavour thresholds and thus need to be in high concentrations to contribute to aroma (Bredie et al., 1998). Pyrazines provisionally identified included 2-methyltetrahydropyrazine in whole Platte crumb, and 5-methyl, 2-pyrazinylmethanol and N-methyl, N-nitroso, 2-propamine in whole CWRS crumb.

Aggregate GC-MS results of the total number of compounds and compound concentrations by bread and sample type supported outcomes obtained using the E-nose. For example, bread crust and crumb made using refined and whole CWRS wheat flour produced the greatest contrast in number and concentration of compounds. The E-nose produced parallel results (Figs. 4.2 and 4.3) showing that refined and whole CWRS wheat breads were the two types with the highest degree of discrimination. Also, E-nose results indicated that white whole wheat breads (crust and especially crumb material) tended to cluster in aroma profile with refined wheat bread as opposed to bread made with the red-grained CWRS whole wheat flour. GC-MS results likewise revealed that white whole wheat breads were much more similar in total compound concentration to that of refined wheat bread, and that whole CWRS bread was very distinct, having at least four-fold

higher concentration of volatile compounds, again supporting the contention that whole wheat bread made with white wheat bran is milder in aroma than that for red wheat bran.

As discussed above, the E-nose produced a corresponding result in that regard, i.e. the qualitative and quantitative distinctiveness of whole CWRS crumb samples as evidenced by Fig. 4.3 and Fig. 2, Appendix E, respectively. Accordingly there was generally very good agreement between E-nose and GC-MS results even though the former analyzed pure headspace volatiles of bread with no specific identification of aroma compounds, while the latter was based on quantification of GC separated volatiles of methanol soluble bread extracts.

Major Maillard reaction compounds like furfural, 2-furanmethanol, pyranone, maltol and 5-hydroxymethyl- 2-furancarboxaldehyde were present in highest concentration in whole CWRS bread. Also, there were several compounds in crumb and crust samples that were unique only to the white whole wheat breads made from Snowbird and Platte. Again, this suggests a chemical composition difference between the two hard white cultivars and between those cultivars and red wheat bran as represented by the CWRS class of wheat that was milled for this study. Results were more than likely genotype based as each of the three wheat samples milled to produce the bran for whole wheat breadmaking were large-scale commercial composites of wheat each derived from many different producers over a broad geographical region in western Canada (for CWRS and Snowbird) and Colorado for Platte wheat.

The main difference between white and red (or other pigmented) wheat bran has been ascribed to phenolic flavonoid compounds in seed coat tissue (Matus-Cadiz et al, 2008). Older research has shown a positive relationship between catechin tannin content

and seed coat color in immature kernels, with red, light red, and white varieties showing the highest to lowest levels, respectively, of these precursors of brown pigment that were believed to be either phlobaphene or proanthocyanidin (Miyamoto and Everson, 1958). Feng and McDonald (1989) reported that red-branned wheats contained higher levels of total flavonoids compared to white common wheat and durum wheat that contains no pigment in the seed coat. Accordingly, total flavonoid content was not correlated with wheat bran color. More recent work (Himi et al, 2005) found that the expression of early genes of the flavonoid biosynthesis pathway was almost completely suppressed in the developing grains of white-grained wheats, compared with that in red-grained wheats. The authors concluded that grain pigment of wheat was primarily proanthocyanidin rather than phlobaphene. Regardless of the specific compound, evidently the bran tissue of white and red wheats contain differences in specific flavonoid composition, which when processed by breadmaking, manifest volatiles characteristic of those genotypes, even between genotypes possessing the same colour of bran. There appears to be no other literature on flavonoid content and composition of red and white wheats. Much more research is needed on this topic to develop a better understanding of the mechanisms by which bread aroma and flavour appear to be differentiated according to bran colour.

In conclusion, while the number of samples of red and white wheats were very few in this study, results support the view that different wheat genotypes and specifically, the bran tissue of these genotypes, contain differences in compound composition and/or concentration which when processed by breadmaking, manifest volatiles characteristic of those genotypes even between genotypes possessing the same colour of bran. GC-MS and E-nose results additionally indicated that whole wheat bread formulated using white

wheat bran had a milder aroma, or at least lower concentration of volatiles, compared to bread made with red wheat bran. It would be highly beneficial in future research to carry out similar studies in parallel with a human sensory panel, and ideally with many more genotypes of red and white grained wheat with an aim to firmly establish the relative superiority of particular genotypes to produce whole wheat bread with aroma profiles more similar to those of white pan bread. The long term goal of such studies would be to foster increased consumption of whole wheat products and constituent bioactive compounds which confer favourable health benefits in the general population.

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Appendix A: Response curves for each of 12 sensors for the two-minute data acquisition period for refined and whole wheat breads at 40°C and 50°C for 5 and 10 minutes

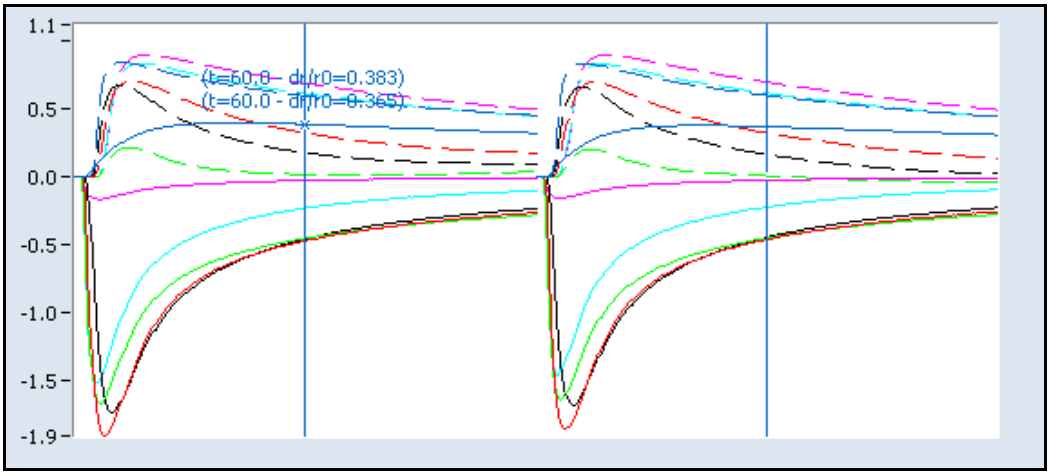


Figure 1. Refined and whole wheat CWRS incubated at 40°C for 5 minutes

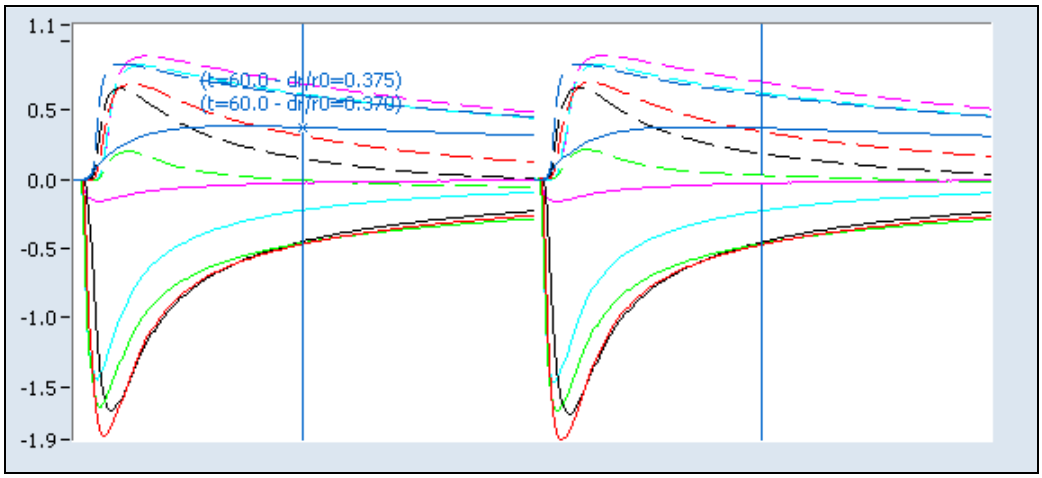


Figure 2. Refined and whole wheat CWRS incubated at 40°C for 10 minutes

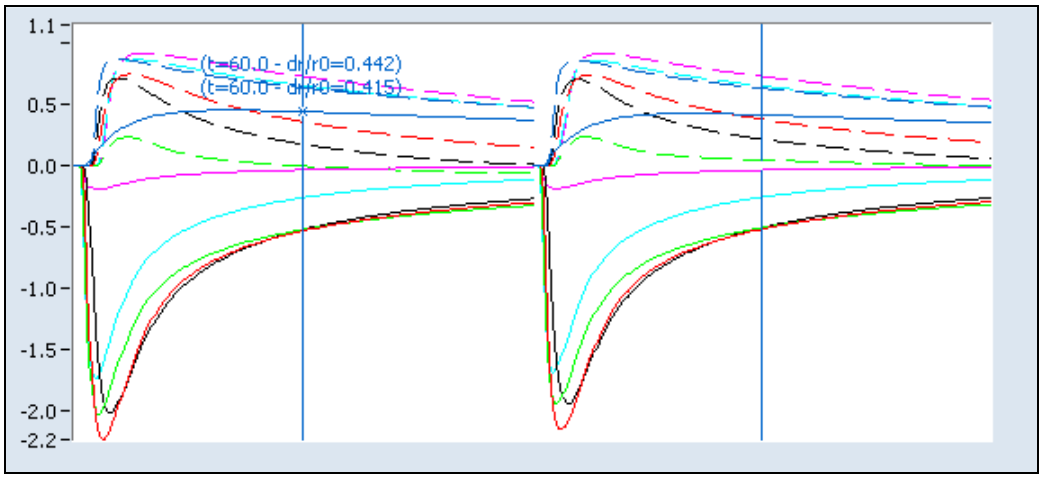


Figure 3. Refined and whole wheat CWRS incubated at 50°C for 5 minutes

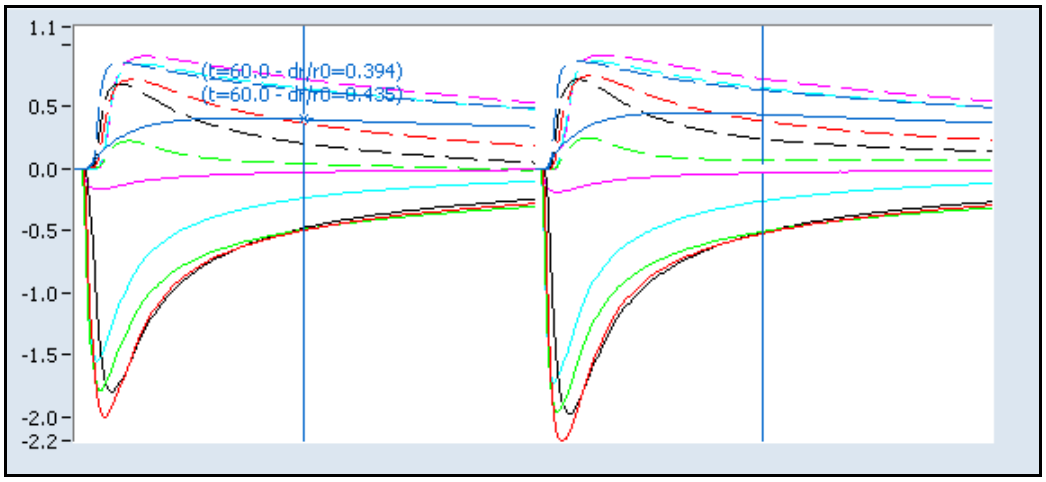


Figure 4. Refined and whole wheat CWRS incubated at 50°C for 10 minutes

Appendix B: PCA plots of data from appendix A

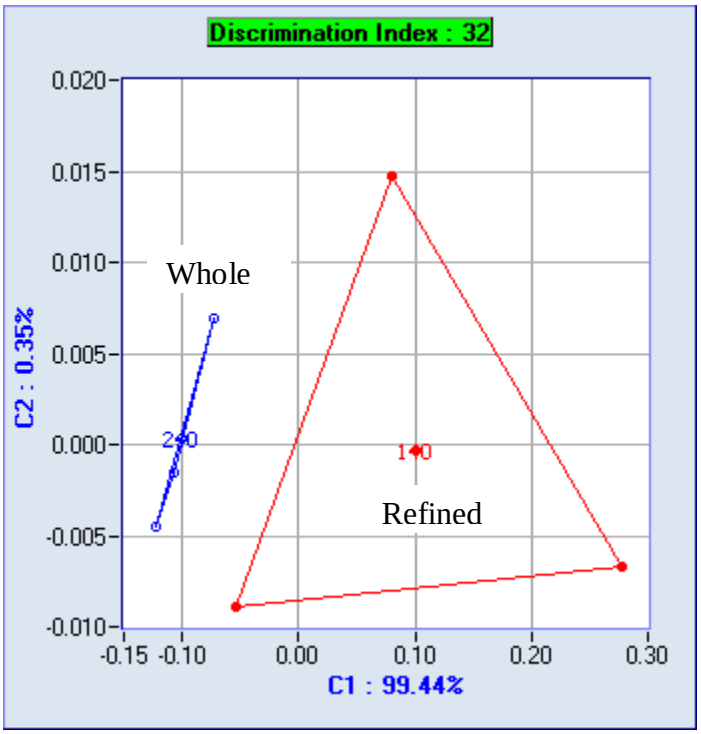


Figure 1. PCA plot of 0.5 gram of refined and whole CWRS bread at 40°C and 5 minutes against the first and second principal components. The three points represent triplicate analysis.

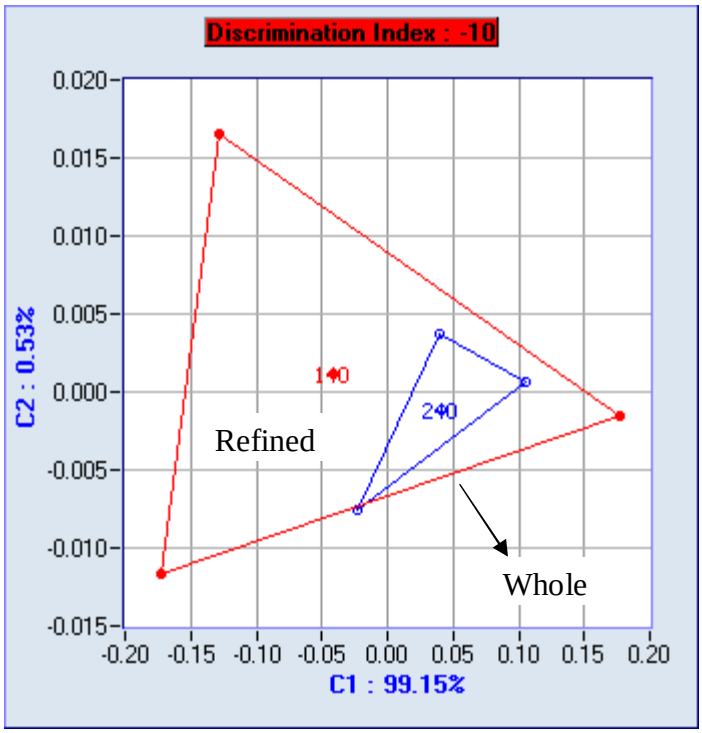


Figure 2. PCA plot of 0.5 gram refined and whole CWRS bread at 40°C and 10 minutes against the first and second principal components. The three points represent triplicate analysis.

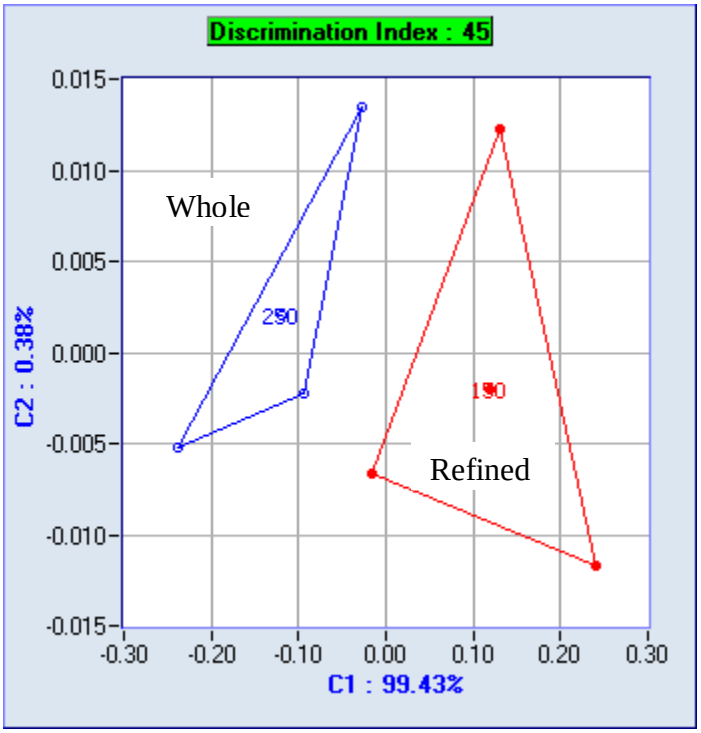


Figure 3. PCA plot of 0.5 gram refined and whole CWRS bread at 50°C and 5 minutes against the first and second principal components. The three points represent triplicate analysis.

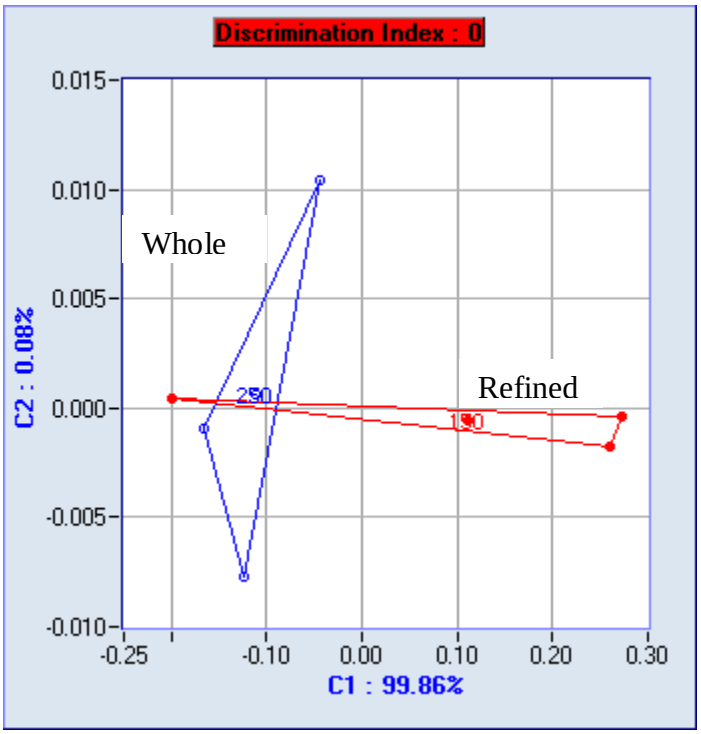


Figure 4. PCA plot of 0.5 gram refined and whole CWRS bread at 50°C and 10 minutes against the first and second principal components. The three points represent triplicate analysis.

Appendix C: Electronic nose analysis of crust and crumb of four different types of bread

Refined CWRS crust and crumb

Table 1. Cross-validation summary for refined CWRS crust and crumb.

	Crust (% classification)	Crumb (% classification)
Refined crust	100	0
Refined crumb	0	100

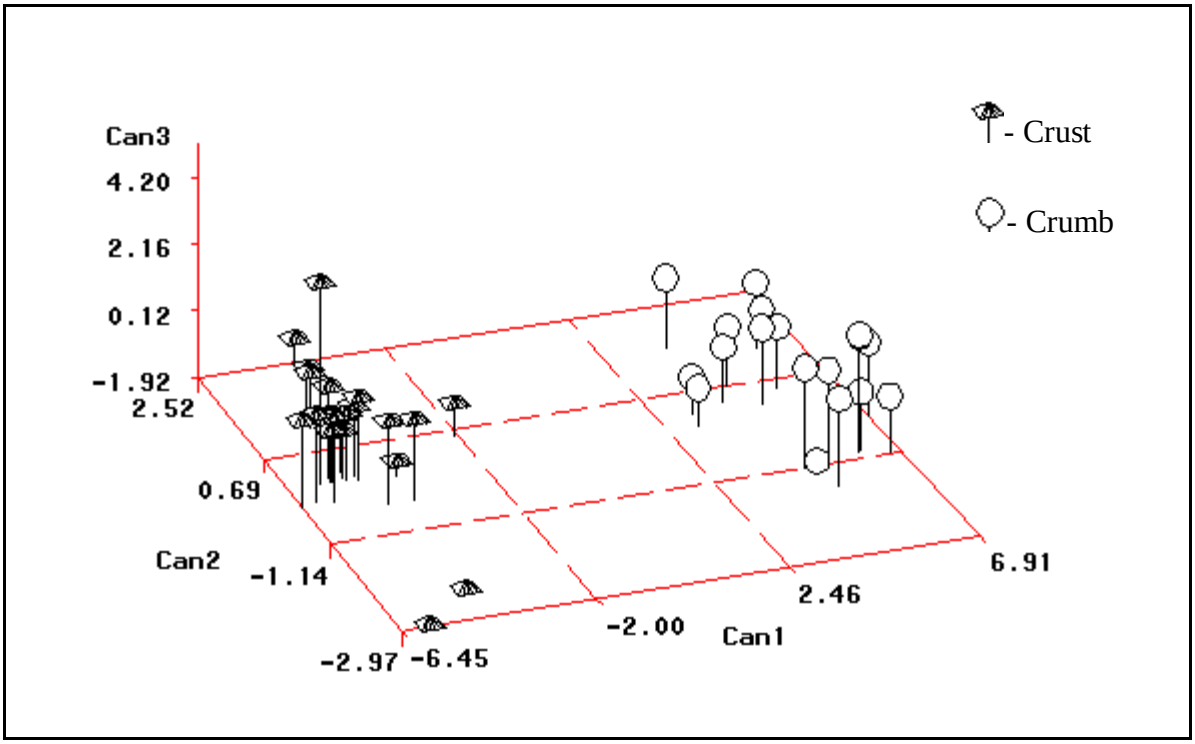


Figure 1. Discriminant analysis for refined CWRS bread crust and crumb

Whole CWRS crust and crumb

Table 2. Cross-validation summary for whole wheat CWRS bread crust and crumb.

	Crust (% classification)	**Crumb (% classification)**
CWRS crust	100	0
CWRS crumb	0	100

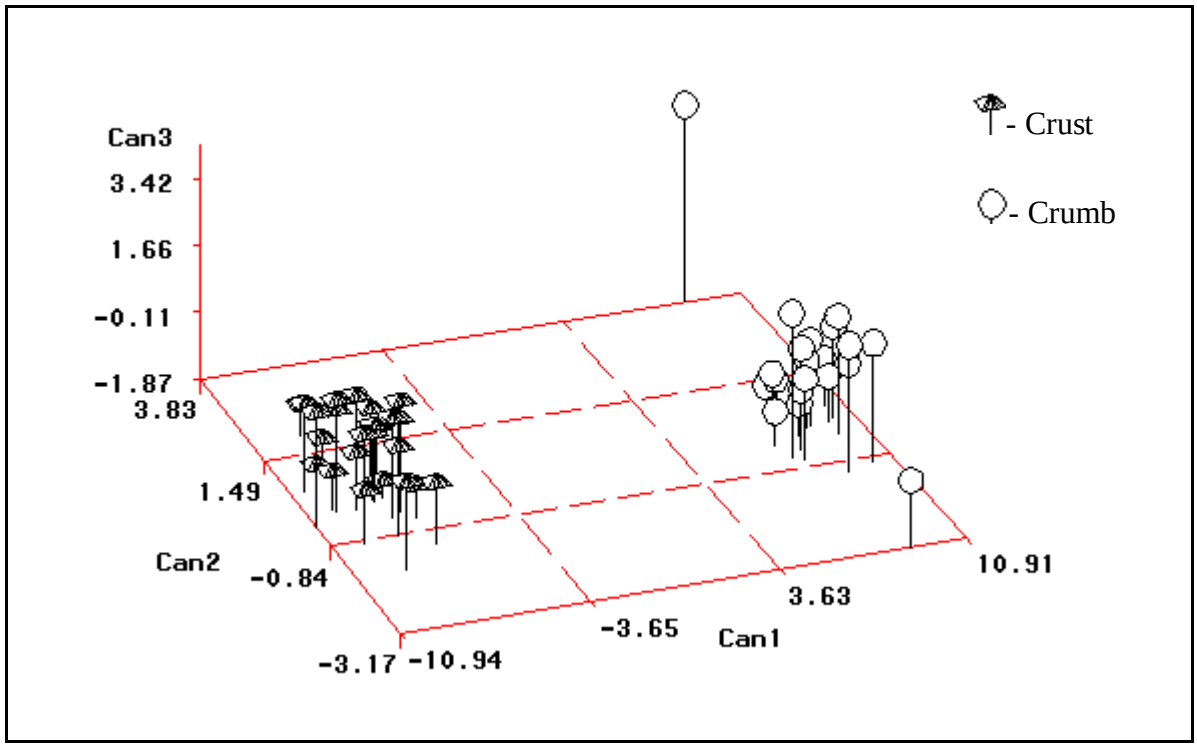


Figure 2. Discriminant analysis for whole wheat CWRS bread crust and crumb.

Whole Snowbird crust and crumb

Table 3. Cross-validation summary for Snowbird whole wheat bread crust and crumb.

	Crust (% classification)	**Crumb** (% classification)
Snowbird crust	100	0
Snowbird crumb	0	100

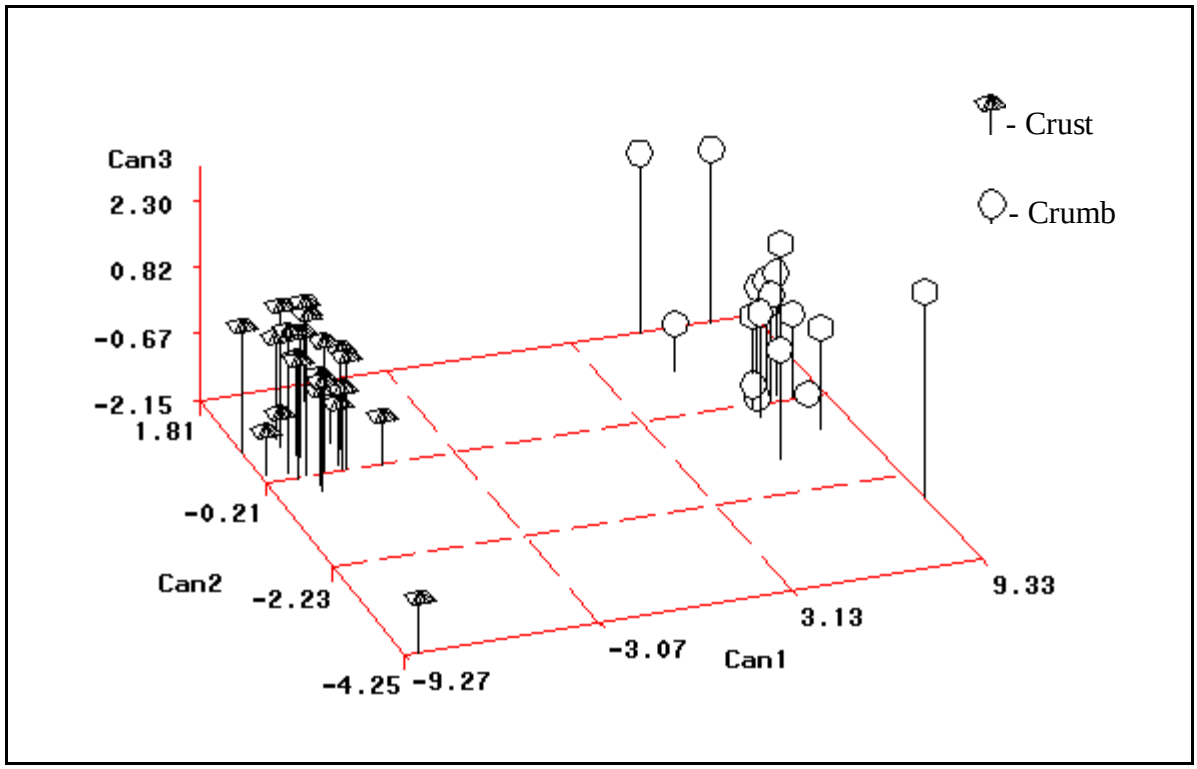


Figure 3. Discriminant analysis for whole wheat Snowbird bread crust and crumb.

Whole Platte crust and crumb

Table 4. Cross-validation summary for whole wheat Platte bread crust and crumb.

	Crust (% classification)	**Crumb (% classification)**
Platte crust	100	0
Platte crumb	0	100

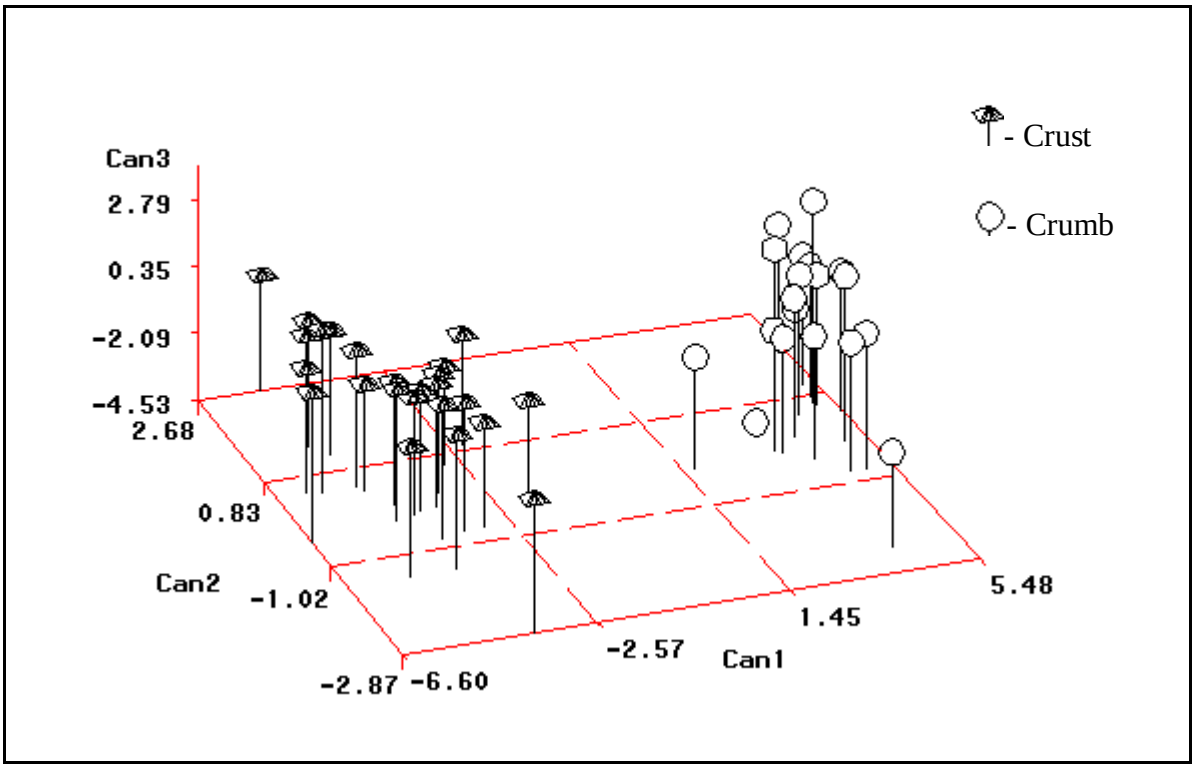


Figure 4. Discriminant analysis for whole wheat Platte bread crust and crumb.

Appendix D: Stepwise Selection Summary of all the bread samples

Table 1. Stepwise selection summary for all crust samples (corresponding to Fig. 4.2)

Step	Number In	Entered	Removed	Label	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Squared Canonical Correlation	Pr > ASCC
1	1	LY2_LG		LY2/LG	0.6403	54.59	<.0001	0.35971229	<.0001	0.21342924	<.0001
2	2	PA2		PA2	0.1794	6.63	0.0004	0.29518048	<.0001	0.23677265	<.0001
3	3	LY2_gCTl		LY2/gCTl	0.0869	2.85	0.0416	0.26953614	<.0001	0.26406583	<.0001
4	4	T70_2		T70/2	0.0515	1.61	0.1929	0.25566802	<.0001	0.26968871	<.0001
5	5	P10_1		P10/1	0.0190	0.57	0.6368	0.25080202	<.0001	0.27390020	<.0001
6	6	LY2_AA		LY2/AA	0.0198	0.59	0.6252	0.24582696	<.0001	0.27966509	<.0001
7	7	LY2_Gh		LY2/Gh	0.0802	2.50	0.0648	0.22610700	<.0001	0.30050573	<.0001
8	8	LY2_G		LY2/G	0.0449	1.33	0.2697	0.21596213	<.0001	0.30945948	<.0001
9	9	P40_1		P40/1	0.0338	0.98	0.4060	0.20865500	<.0001	0.31505180	<.0001
10	10	T30_1		T30/1	0.0668	1.98	0.1230	0.19470758	<.0001	0.32893834	<.0001
11	11	P10_2		P10/2	0.0107	0.30	0.8289	0.19262858	<.0001	0.33079849	<.0001
12	12	LY2_gCT		LY2/gCT	0.0078	0.21	0.8886	0.19113559	<.0001	0.33203586	<.0001

Table 2. Stepwise selection summary for all crumb samples (corresponding to Fig. 4.3)

Step	Number In	Entered	Removed	Label	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Squared Canonical Correlation	Pr > ASCC
1	1	LY2_LG		LY2/LG	0.6935	69.40	<.0001	0.30645121	<.0001	0.23118293	<.0001
2	2	PA2		PA2	0.4308	22.96	<.0001	0.17443740	<.0001	0.27655327	<.0001
3	3	LY2_Gh		LY2/Gh	0.0909	3.00	0.0347	0.15857979	<.0001	0.29734359	<.0001
4	4	T70_2		T70/2	0.0788	2.54	0.0617	0.14608682	<.0001	0.31635336	<.0001
5	5	P10_1		P10/1	0.1053	3.45	0.0199	0.13070637	<.0001	0.32816234	<.0001
6	6	LY2_gCTl		LY2/gCTl	0.1370	4.60	0.0049	0.11279741	<.0001	0.34474351	<.0001
7	7	LY2_AA		LY2/AA	0.0559	1.70	0.1735	0.10649003	<.0001	0.35865270	<.0001
8	8	LY2_gCT		LY2/gCT	0.0535	1.60	0.1950	0.10079255	<.0001	0.36199724	<.0001
9	9	P10_2		P10/2	0.0510	1.50	0.2194	0.09565425	<.0001	0.37421119	<.0001
10	10	LY2_G		LY2/G	0.0666	1.97	0.1242	0.08928312	<.0001	0.38987294	<.0001
11	11	T30_1		T30/1	0.0356	1.01	0.3935	0.08610707	<.0001	0.39631726	<.0001
12	12	P40_1		P40/1	0.0212	0.58	0.6267	0.08428176	<.0001	0.39740246	<.0001

Table 3. Stepwise selection summary for all whole slice samples (corresponding to Fig. 4.4)

Step	Number In	Entered	Removed	Label	Partial R-Square	F Value	Pr > F	Wilks' Lambda	Pr < Lambda	Average Squared Canonical Correlation	Pr > ASCC
1	1	LY2_gCTl		LY2/gCTl	0.3815	18.92	<.0001	0.61850037	<.0001	0.12716654	<.0001
2	2	LY2_G		LY2/G	0.1052	3.57	0.0172	0.55343661	<.0001	0.14957931	<.0001
3	3	LY2_Gh		LY2/Gh	0.0511	1.61	0.1917	0.52518339	<.0001	0.16521319	<.0001
4	4	T30_1		T30/1	0.1577	5.55	0.0015	0.44238552	<.0001	0.21448642	<.0001
5	5	LY2_AA		LY2/AA	0.0367	1.12	0.3462	0.42614334	<.0001	0.22362052	<.0001
6	6	LY2_LG		LY2/LG	0.0943	3.02	0.0340	0.38594025	<.0001	0.24903077	<.0001
7	7	P10_2		P10/2	0.0618	1.89	0.1375	0.36208341	<.0001	0.26371381	<.0001
8	8	P40_1		P40/1	0.0602	1.81	0.1508	0.34030118	<.0001	0.27466236	<.0001
9	9	PA2		PA2	0.0287	0.83	0.4829	0.33054391	<.0001	0.28042685	<.0001
10	10	P10_1		P10/1	0.0787	2.36	0.0772	0.30454207	<.0001	0.29916701	<.0001
11	11	LY2_gCT		LY2/gCT	0.0532	1.54	0.2114	0.28833901	<.0001	0.30896615	<.0001
12	12	T70_2		T70/2	0.0373	1.05	0.3772	0.27759478	<.0001	0.32016252	<.0001

Appendix E: E-nose sensor data for all bread types and their crust, crumb and WS fractions.

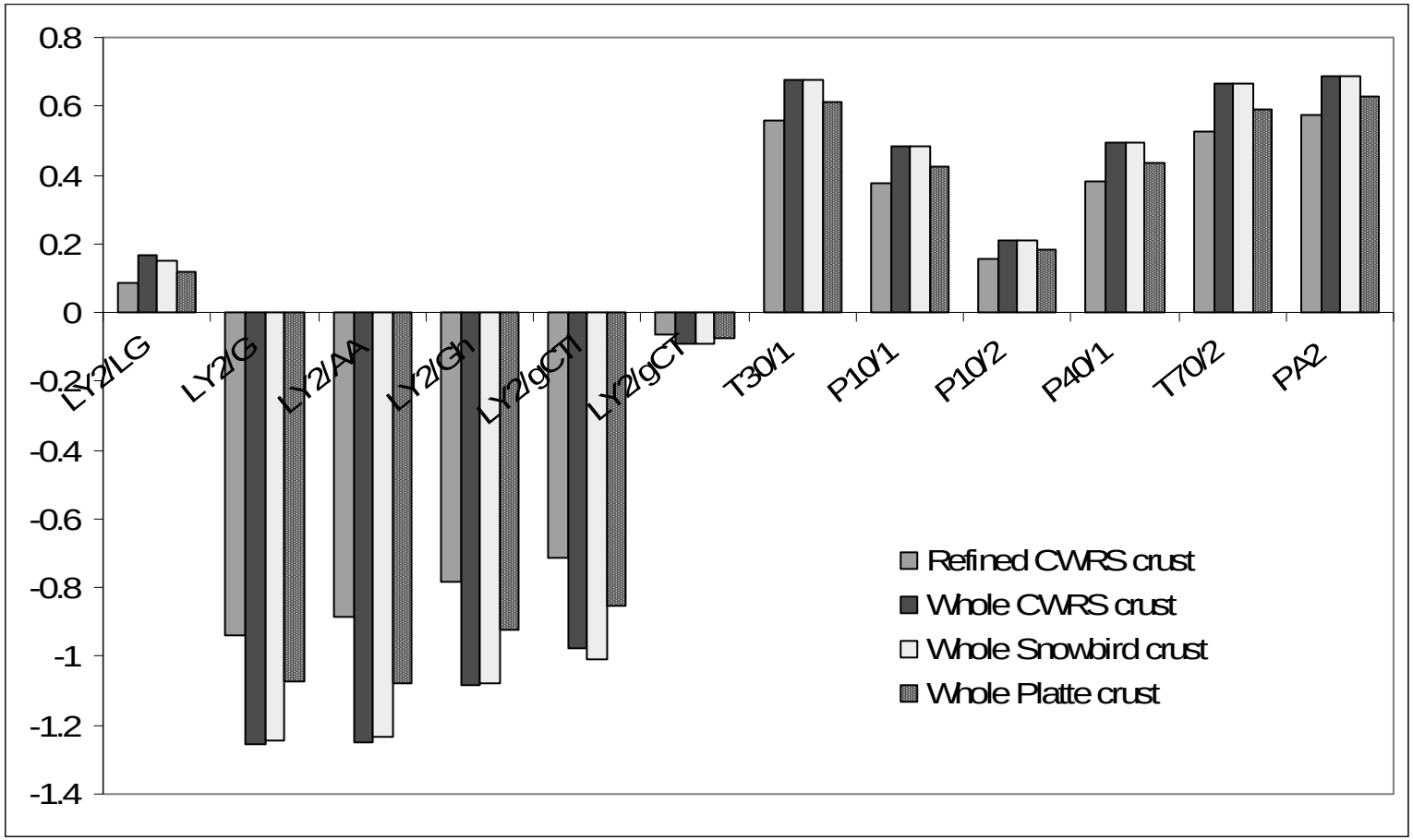


Figure. 1. Average E-nose sensor output for crust samples of indicated breads corresponding to Fig. 4.2. Data follows in Tables 1.1-1.4. Based on sensor output, refined bread crust clearly had the lowest concentration of volatiles; whole Platte bread crust clearly had the next lowest concentration of volatiles; whole CWRS crust and whole Snowbird crust had comparable concentrations of volatiles.

Table 1.1. Sensor data for refined CWRS crust (corresponding to Fig. 4.2).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Refined CWRS crust	0.068427	-0.9594	-0.99767	-0.81785	-0.78136	-0.06269	0.397134	0.319715	0.146977	0.368378	0.403237	0.423235
Refined CWRS crust	0.065232	-0.93059	-0.96817	-0.7944	-0.75506	-0.05962	0.383543	0.309751	0.140873	0.357017	0.388652	0.411749
Refined CWRS crust	0.064976	-0.93264	-0.96821	-0.79419	-0.75558	-0.05962	0.38497	0.309151	0.140995	0.356634	0.388889	0.411301
Refined CWRS crust	0.066847	-0.91471	-0.94934	-0.78127	-0.74269	-0.05891	0.379953	0.309353	0.141046	0.356025	0.383713	0.409124
Refined CWRS crust	0.064019	-0.91928	-0.95261	-0.78303	-0.74165	-0.0582	0.378121	0.302521	0.138456	0.349486	0.382089	0.402998
Refined CWRS crust	0.074719	-1.01664	-1.05464	-0.86857	-0.82621	-0.06576	0.417348	0.333022	0.153245	0.38163	0.424463	0.439815
Refined CWRS crust	0.075366	-0.93887	-0.97046	-0.79976	-0.75596	-0.05976	0.383916	0.306542	0.140856	0.353197	0.387812	0.408207
Refined CWRS crust	0.078056	-0.9529	-0.99291	-0.81393	-0.78032	-0.06033	0.39593	0.318268	0.14562	0.366438	0.401622	0.42108
Refined CWRS crust	0.074948	-0.69714	-0.67087	-0.59607	-0.50431	-0.04529	0.526996	0.324053	0.1294	0.311123	0.470772	0.560763
Refined CWRS crust	0.108675	-0.8415	-0.79126	-0.69329	-0.72533	-0.05068	0.636966	0.408776	0.13426	0.374483	0.507311	0.645079
Refined CWRS crust	0.085624	-0.77302	-0.73645	-0.66008	-0.56726	-0.0513	0.569224	0.356317	0.143807	0.344433	0.517136	0.59513
Refined CWRS crust	0.094093	-0.82844	-0.79103	-0.70933	-0.61406	-0.05442	0.602969	0.377797	0.151859	0.365062	0.553202	0.621925
Refined CWRS crust	0.110084	-1.07766	-0.92946	-0.83173	-0.7309	-0.06431	0.708203	0.471347	0.193509	0.409292	0.677133	0.716744
Refined CWRS crust	0.096842	-1.74	-0.81197	-0.71781	-0.61909	-0.06304	0.612262	0.40109	0.161385	0.383984	0.571037	0.632939
Refined CWRS crust	0.103546	-0.90642	-0.85392	-0.95025	-0.85611	-0.07565	0.71483	0.480338	0.197385	0.466624	0.687541	0.726985
Refined CWRS crust	0.082474	-0.7498	-0.72006	-0.64172	-0.54775	-0.04921	0.561667	0.346362	0.138633	0.332069	0.50661	0.591102
Refined CWRS crust	0.073619	-0.78808	-0.84007	-0.7535	-0.66187	-0.06867	0.6736	0.344196	0.112704	0.326432	0.607992	0.639247
Refined CWRS crust	0.091228	-0.81952	-0.88734	-0.79676	-0.71091	-0.06436	0.644355	0.36811	0.123985	0.374919	0.602538	0.65807
Refined CWRS crust	0.116736	-0.94586	-0.89701	-0.81532	-0.72583	-0.06477	0.66242	0.429718	0.17199	0.416087	0.623313	0.669127
Refined CWRS crust	0.101486	-0.99278	-0.92614	-0.85459	-0.75353	-0.071	0.675536	0.436475	0.191057	0.44743	0.661695	0.671157
Refined CWRS crust	0.10099	-0.94733	-0.89009	-0.81479	-0.72625	-0.06503	0.673853	0.434672	0.187291	0.430902	0.636305	0.663836
Refined CWRS crust	0.0916	-0.93038	-0.766	-0.85964	-0.7784	-0.06943	0.702311	0.446409	0.175516	0.465704	0.640486	0.694139
Refined CWRS crust	0.108213	-1.03324	-0.97597	-0.86821	-0.78559	-0.07387	0.718182	0.450097	0.188579	0.470622	0.656663	0.696527
Refined CWRS crust	0.11212	-0.93101	-0.89378	-0.80479	-0.71824	-0.06452	0.646956	0.422731	0.159013	0.401486	0.606724	0.67482
Mean	0.087913	-0.9403	-0.88481	-0.7842	-0.71518	-0.06168	0.560469	0.375284	0.154518	0.383727	0.528622	0.574379
STDEV	0.017149	0.193619	0.100084	0.080739	0.087197	0.007636	0.131767	0.059417	0.023747	0.045482	0.110981	0.120477
%RSD	19.50727	-20.5912	-11.3114	-10.2957	-12.1924	-12.379	23.51007	15.83251	15.36813	11.85279	20.99443	20.97518

Table 1.2. Sensor data for whole CWRS crust (corresponding to Fig. 4.2).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
CWRS crust	0.134168	-1.39898	-1.53357	-1.1589	-0.25167	-0.08923	0.58525	0.414144	0.204992	0.47348	0.581825	0.557622
CWRS crust	0.130269	-1.26131	-1.33041	-1.08432	-1.06052	-0.08748	0.510477	0.410867	0.19461	0.466978	0.529162	0.531563
CWRS crust	0.147627	-1.30652	-1.37823	-1.12412	-1.09899	-0.09063	0.526122	0.422118	0.201255	0.478395	0.546374	0.542374
CWRS crust	0.123093	-1.34386	-1.41622	-1.15577	-1.12928	-0.09366	0.535924	0.430025	0.205269	0.486706	0.557143	0.552764
CWRS crust	0.130847	-1.39646	-1.46973	-1.20047	-1.17299	-0.09819	0.551275	0.441857	0.211991	0.499512	0.572866	0.56544
CWRS crust	0.137399	-1.22313	-1.28474	-1.05414	-1.03181	-0.08692	0.499582	0.404011	0.190985	0.458596	0.516997	0.519715
CWRS crust	0.157666	-1.29336	-1.38199	-1.09515	-1.04572	-0.08522	0.500745	0.402304	0.189607	0.45637	0.518834	0.52193
CWRS crust	0.140882	-1.25544	-1.32505	-1.08168	-1.06099	-0.08679	0.510891	0.410536	0.195429	0.466558	0.529536	0.52839
CWRS crust	0.204334	-1.35445	-1.30006	-1.17981	-1.07498	-0.09732	0.78297	0.550994	0.230493	0.536433	0.766696	0.795007
CWRS crust	0.201883	-1.34928	-1.29832	-1.17428	-1.06958	-0.09653	0.780804	0.550374	0.228748	0.535416	0.764866	0.793271
CWRS crust	0.214586	-1.40382	-1.35235	-1.22273	-1.11812	-0.10024	0.794761	0.567434	0.240227	0.555003	0.781912	0.80433
CWRS crust	0.208333	-1.3579	-1.30287	-1.18766	-1.08383	-0.09803	0.786475	0.552571	0.235831	0.539468	0.770719	0.797861
CWRS crust	0.198325	-1.34048	-1.28625	-1.16437	-1.05968	-0.09593	0.780497	0.550243	0.231179	0.537187	0.764285	0.789431
CWRS crust	0.206006	-1.37915	-1.32421	-1.19705	-1.09272	-0.09866	0.787487	0.560979	0.236784	0.547899	0.773829	0.797597
CWRS crust	0.226435	-1.46493	-1.41237	-1.27041	-1.16281	-0.10624	0.805435	0.584865	0.251174	0.573819	0.796197	0.814496
CWRS crust	0.203362	-1.37383	-1.32125	-1.18651	-1.09223	-0.09858	0.784096	0.561926	0.237784	0.549519	0.772754	0.797427
CWRS crust	0.138429	-1.08142	-1.03702	-0.93979	-0.85254	-0.07547	0.703744	0.476057	0.181943	0.455692	0.674468	0.727646
CWRS crust	0.165089	-1.20794	-1.15628	-1.05015	-0.9591	-0.08675	0.747489	0.518204	0.207431	0.501731	0.726633	0.759608
CWRS crust	0.180227	-1.25796	-1.2008	-1.09306	-1.00332	-0.09019	0.765801	0.537428	0.225613	0.526303	0.748445	0.767804
CWRS crust	0.155066	-0.97218	-0.93499	-0.84514	-0.75855	-0.06756	0.663238	0.439001	0.164664	0.41597	0.626241	0.694931
CWRS crust	0.13414	-1.04303	-0.98516	-0.90235	-0.81155	-0.07205	0.705345	0.46774	0.194866	0.458146	0.674354	0.702421
CWRS crust	0.133862	-1.03028	-0.96914	-0.89266	-0.79947	-0.07217	0.707585	0.466124	0.201779	0.462365	0.67653	0.696292
CWRS crust	0.140274	-1.05032	-0.98607	-0.90601	-0.81703	-0.07472	0.717334	0.475438	0.211928	0.476416	0.687893	0.700063
CWRS crust	0.130014	-1.00558	-0.94404	-0.8661	-0.77209	-0.06979	0.702205	0.457084	0.204104	0.458079	0.668791	0.684993
Mean	0.164263	-1.25632	-1.24713	-1.08469	-0.97415	-0.08826	0.676481	0.485514	0.211612	0.496502	0.667806	0.685124
STDEV	0.034162	0.147158	0.17788	0.126253	0.20134	0.010992	0.114446	0.063167	0.021575	0.04213	0.099955	0.112118
%RSD	20.7969	-11.7135	-14.2632	-11.6395	-20.6683	-12.4531	16.91786	13.01043	10.19539	8.485427	14.96764	16.36465

Table 1.3. Sensor data for whole Snowbird crust (corresponding to Fig. 4.2).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Snowbird crust	0.123401	-1.34681	-1.42439	-1.16234	-1.14306	-0.09509	0.539572	0.434256	0.208366	0.491997	0.561199	0.557897
Snowbird crust	0.127947	-1.37667	-1.45378	-1.18822	-1.16639	-0.09653	0.548134	0.441197	0.211751	0.498908	0.57045	0.563696
Snowbird crust	0.123927	-1.35235	-1.42497	-1.16296	-1.13971	-0.09607	0.539659	0.43217	0.208171	0.489524	0.561077	0.554037
Snowbird crust	0.122819	-1.34704	-1.42235	-1.15883	-1.13103	-0.09517	0.537143	0.430352	0.205951	0.48768	0.557833	0.553066
Snowbird crust	0.138937	-1.45209	-1.5329	-1.25261	-1.22752	-0.10272	0.567453	0.45624	0.221529	0.515109	0.591853	0.580942
Snowbird crust	0.135424	-1.43928	-1.5227	-1.23697	-1.21542	-0.10189	0.560393	0.452782	0.21875	0.511554	0.584871	0.578365
Snowbird crust	0.129834	-1.37466	-1.45481	-1.1869	-1.1748	-0.09819	0.549881	0.444618	0.214258	0.502198	0.573189	0.565546
Snowbird crust	0.130886	-1.38831	-1.46805	-1.19876	-1.18332	-0.09804	0.553425	0.446668	0.215663	0.504882	0.576694	0.568784
Snowbird crust	0.18057	-1.26804	-1.21596	-1.10534	-1.00331	-0.09098	0.756275	0.527798	0.216848	0.51128	0.737669	0.775194
Snowbird crust	0.163462	-1.19826	-1.14562	-1.03885	-0.93982	-0.08468	0.735572	0.507775	0.206045	0.491024	0.713441	0.7552
Snowbird crust	0.166578	-1.20853	-1.1563	-1.05029	-0.94967	-0.08521	0.743211	0.513936	0.212806	0.499348	0.721606	0.757778
Snowbird crust	0.162753	-1.18947	-1.13585	-1.03123	-0.93122	-0.08324	0.736489	0.507476	0.208412	0.492645	0.714376	0.751484
Snowbird crust	0.164788	-1.20487	-1.14849	-1.04246	-0.94122	-0.08452	0.740729	0.513112	0.213538	0.500368	0.719609	0.754181
Snowbird crust	0.165236	-1.21283	-1.15589	-1.04994	-0.94914	-0.08531	0.74354	0.515536	0.215074	0.503379	0.7229	0.75602
Snowbird crust	0.201339	-1.34936	-1.29376	-1.17764	-1.07565	-0.0976	0.779023	0.550735	0.23294	0.535588	0.764116	0.795669
Snowbird crust	0.174972	-1.26724	-1.21177	-1.09631	-1.00197	-0.09026	0.75571	0.532494	0.220514	0.518158	0.739775	0.772192
Snowbird crust	0.14362	-1.08307	-1.03417	-0.93793	-0.84868	-0.07638	0.703546	0.476425	0.183838	0.456931	0.674875	0.724463
Snowbird crust	0.15981	-1.15744	-1.10421	-1.00385	-0.91491	-0.08215	0.731137	0.50327	0.203022	0.488462	0.708238	0.742186
Snowbird crust	0.160943	-1.15152	-1.08961	-0.99723	-0.90517	-0.08076	0.736223	0.503036	0.209563	0.492629	0.712418	0.736479
Snowbird crust	0.164819	-1.1615	-1.09647	-1.00292	-0.91446	-0.08241	0.742443	0.506539	0.215596	0.499162	0.719941	0.73781
Snowbird crust	0.187346	-1.24283	-1.17775	-1.07788	-0.9887	-0.08968	0.772289	0.536148	0.237624	0.532685	0.754988	0.76088
Snowbird crust	0.178484	-1.20223	-1.13291	-1.03896	-0.95083	-0.08571	0.76308	0.522707	0.232596	0.522349	0.743383	0.748009
Snowbird crust	0.133833	-1.02458	-0.98259	-0.89164	-0.80645	-0.07233	0.68284	0.457323	0.173505	0.436158	0.649622	0.710459
Snowbird crust	0.116618	-0.90779	-0.84762	-0.77942	-0.68924	-0.06097	0.659543	0.419726	0.187432	0.421491	0.618516	0.644751
Mean	0.152431	-1.24611	-1.2347	-1.0779	-1.00799	-0.08816	0.674055	0.48468	0.211408	0.49598	0.66636	0.685212
STDEV	0.023682	0.132283	0.186082	0.113338	0.140081	0.009907	0.093651	0.040372	0.014544	0.026416	0.074846	0.090919
%RSD	15.53598	-10.6157	-15.071	-10.5148	-13.8972	-11.2377	13.89363	8.329583	6.879539	5.32606	11.23212	13.26872

Table 1.4. Sensor data for whole Platte crust (corresponding to Fig. 4.2).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Platte crust	0.138889	-1.43338	-1.51645	-1.23732	-1.22266	-0.10347	0.564692	0.455614	0.221003	0.514658	0.58951	0.578891
Platte crust	0.125606	-1.36449	-1.44212	-1.17774	-1.15527	-0.09653	0.544555	0.439008	0.210422	0.49621	0.566655	0.561178
Platte crust	0.118294	-1.32337	-1.39625	-1.13981	-1.11346	-0.09201	0.530564	0.426725	0.20323	0.483092	0.550928	0.547807
Platte crust	0.123503	-1.36449	-1.43921	-1.17125	-1.14589	-0.09585	0.541025	0.435377	0.207644	0.49206	0.562696	0.557816
Platte crust	0.130643	-1.40522	-1.48435	-1.21061	-1.18501	-0.0997	0.553314	0.445608	0.213921	0.503369	0.576386	0.569751
Platte crust	0.119945	-1.34158	-1.41444	-1.15325	-1.12654	-0.09283	0.53093	0.426841	0.20442	0.483842	0.552091	0.550018
Platte crust	0.13045	-1.38168	-1.45939	-1.19577	-1.17755	-0.09977	0.551082	0.446517	0.214736	0.504294	0.575097	0.567251
Platte crust	0.110727	-1.27215	-1.34009	-1.09482	-1.07552	-0.08824	0.51514	0.415378	0.196747	0.471461	0.534324	0.534502
Platte crust	0.116316	-0.96105	-0.91795	-0.82913	-0.7313	-0.0658	0.657722	0.427615	0.168903	0.410206	0.618732	0.681261
Platte crust	0.12068	-0.98021	-0.93388	-0.84469	-0.74901	-0.06743	0.665715	0.43547	0.172923	0.41906	0.628472	0.6858
Platte crust	0.117692	-0.99383	-0.96859	-0.85208	-0.69007	-0.06196	0.638572	0.412538	0.165088	0.397398	0.596096	0.658948
Platte crust	0.115773	-0.96451	-0.91613	-0.82891	-0.73492	-0.06635	0.661645	0.431765	0.174969	0.417317	0.623359	0.678138
Platte crust	0.117772	-0.97561	-0.92901	-0.84136	-0.74206	-0.06672	0.667195	0.435815	0.176251	0.422076	0.630014	0.682803
Platte crust	0.128	-1.02976	-0.97847	-0.88897	-0.78864	-0.07064	0.688115	0.456506	0.186691	0.443001	0.655243	0.70234
Platte crust	0.101985	-0.89596	-0.85264	-0.76913	-0.67193	-0.05943	0.627104	0.405963	0.160344	0.389597	0.584708	0.651653
Platte crust	0.106423	-0.8352	-0.80058	-0.71966	-0.62359	-0.05591	0.604217	0.381434	0.152024	0.365462	0.555901	0.632711
Platte crust	0.112055	-0.92869	-0.88554	-0.80187	-0.71259	-0.06383	0.64698	0.422164	0.161003	0.403327	0.607561	0.669082
Platte crust	0.120167	-0.96989	-0.92411	-0.83947	-0.74984	-0.06798	0.6713	0.44015	0.174658	0.425561	0.635011	0.68138
Platte crust	0.130214	-1.01594	-0.96226	-0.87762	-0.78699	-0.07132	0.6943	0.458273	0.191064	0.448117	0.661418	0.694268
Platte crust	0.116349	-0.93471	-0.97752	-0.80643	-0.71713	-0.06255	0.666074	0.430358	0.184225	0.425298	0.627703	0.65937
Platte crust	0.101779	-0.84554	-0.7925	-0.72574	-0.63546	-0.05794	0.629799	0.39698	0.17541	0.39743	0.584654	0.61881
Platte crust	0.105797	-0.81036	-0.85397	-0.69317	-0.60279	-0.05401	0.612414	0.38327	0.174375	0.388508	0.564587	0.598171
Platte crust	0.118115	-0.81847	-0.76444	-0.69945	-0.7117	-0.05794	0.618752	0.384913	0.173724	0.388132	0.570998	0.605082
Platte crust	0.10075	-0.86688	-0.93397	-0.75019	-0.66118	-0.06058	0.616395	0.399686	0.149306	0.377858	0.572566	0.651357
Mean	0.11783	-1.07137	-1.07849	-0.92285	-0.85463	-0.07412	0.6124	0.424749	0.183878	0.436139	0.592696	0.625766
STDEV	0.010006	0.218704	0.266086	0.189109	0.219617	0.016617	0.05659	0.0229	0.020848	0.046127	0.03507	0.055458
%RSD	8.491576	-20.4134	-24.672	-20.4918	-25.6973	-22.421	9.240702	5.391415	11.33767	10.57623	5.916964	8.862418

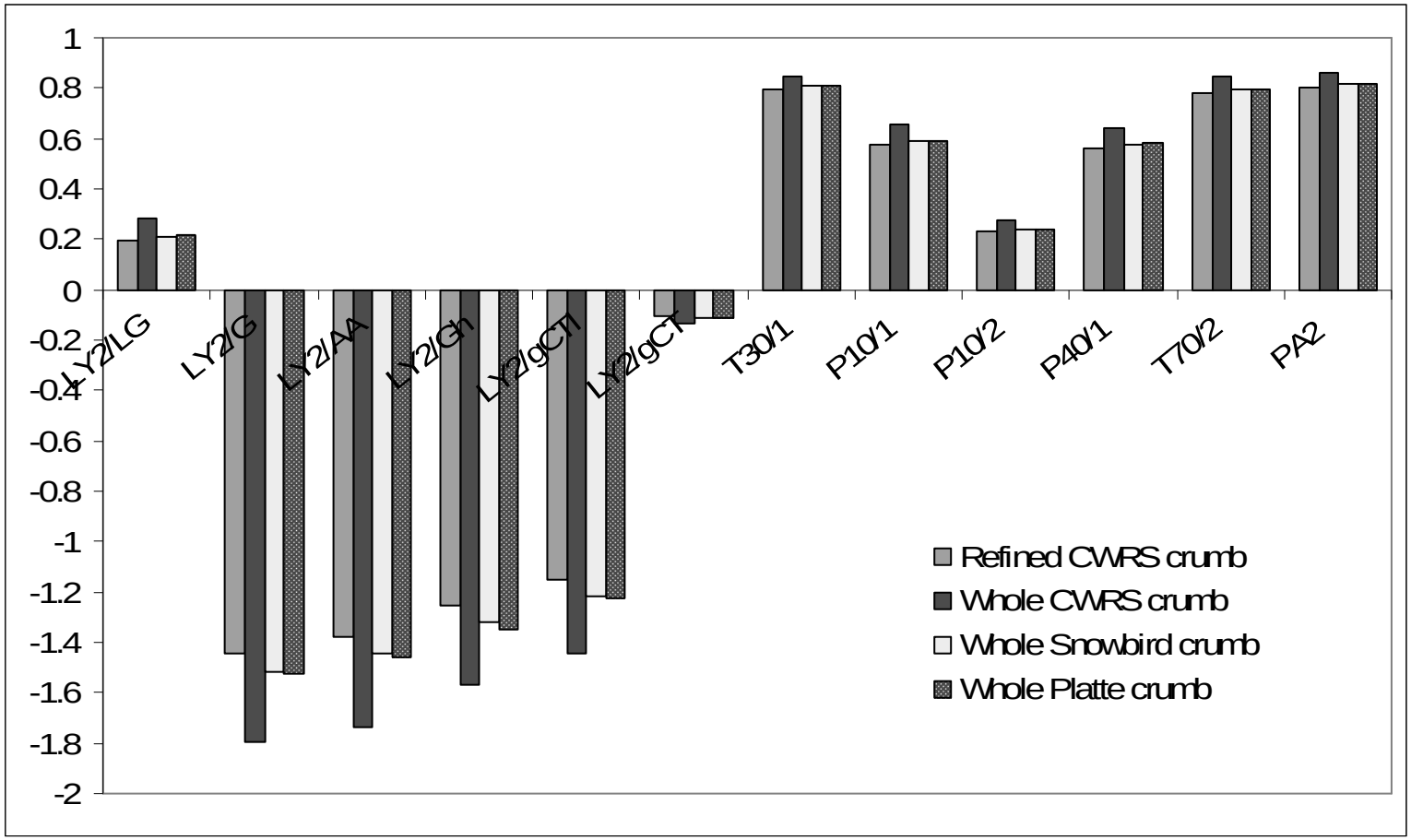


Figure. 2. Average E-nose sensor output for crumb samples of indicated breads corresponding to Fig. 4.3. Data follows in Tables 2.1-2.4. Based on sensor output, whole CWRS bread crumb clearly had the highest concentration of volatiles; whole wheat bread crumb had declining concentrations of volatiles in the following order: whole Platte > whole Snowbird > refined bread.

Table 2.1. Sensor data for refined CWRS crumb (corresponding to Fig. 4.3).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Refined CWRS Crumb	0.217391	-1.57157	-1.51898	-1.3746	-1.2577	-0.11536	0.803116	0.608218	0.238427	0.585618	0.796176	0.836417
Refined CWRS Crumb	0.226989	-1.61804	-1.55708	-1.38676	-1.29052	-0.1252	0.831452	0.62924	0.262295	0.612312	0.825634	0.851642
Refined CWRS Crumb	0.224706	-1.60608	-1.55232	-1.4016	-1.28258	-0.11613	0.80862	0.615975	0.242847	0.595503	0.802917	0.838908
Refined CWRS Crumb	0.210804	-1.54829	-1.48527	-1.3453	-1.22788	-0.1125	0.79888	0.600971	0.23502	0.580655	0.791533	0.827211
Refined CWRS Crumb	0.223723	-1.62081	-1.55847	-1.40509	-1.28389	-0.1171	0.811404	0.617438	0.244695	0.597865	0.805993	0.838521
Refined CWRS Crumb	0.224913	-1.58779	-1.53119	-1.39079	-1.27748	-0.11623	0.809399	0.612804	0.245044	0.592461	0.804097	0.838947
Refined CWRS Crumb	0.232537	-1.60886	-1.53289	-1.40707	-1.29481	-0.12413	0.826542	0.633929	0.221136	0.605886	0.813235	0.85637
Refined CWRS Crumb	0.225393	-1.60063	-1.59734	-1.43828	-1.32863	-0.12297	0.821821	0.63152	0.220685	0.60328	0.808633	0.848825
Refined CWRS Crumb	0.177187	-1.33465	-1.2663	-1.16857	-1.06814	-0.0964	0.774322	0.547548	0.21978	0.532058	0.758269	0.782157
Refined CWRS Crumb	0.183223	-1.3615	-1.29177	-1.19069	-1.08389	-0.09718	0.779413	0.553139	0.222626	0.538412	0.764162	0.785473
Refined CWRS Crumb	0.179287	-1.33731	-1.26825	-1.17728	-1.07543	-0.09781	0.773872	0.550362	0.220248	0.533362	0.757302	0.788176
Refined CWRS Crumb	0.194841	-1.41842	-1.3433	-1.23566	-1.1265	-0.10283	0.790706	0.565884	0.229892	0.552329	0.777103	0.796059
Refined CWRS Crumb	0.180108	-1.33281	-1.25399	-1.16122	-1.05411	-0.09505	0.774795	0.54369	0.222989	0.532161	0.757435	0.773785
Refined CWRS Crumb	0.182672	-1.32132	-1.23435	-1.14939	-1.04086	-0.09291	0.778417	0.542228	0.229561	0.535807	0.760662	0.767571
Refined CWRS Crumb	0.19747	-1.41807	-1.35729	-1.22156	-1.1207	-0.1106	0.797896	0.558	0.208454	0.567921	0.777875	0.826394
Refined CWRS Crumb	0.19281	-1.42101	-1.3211	-1.16174	-1.0857	-0.12291	0.78221	0.548	0.219	0.519804	0.7502	0.76797
Refined CWRS Crumb	0.177187	-1.33465	-1.2663	-1.16857	-1.06814	-0.0964	0.774322	0.547548	0.21978	0.532058	0.758269	0.782157
Refined CWRS Crumb	0.183223	-1.3615	-1.29177	-1.19069	-1.08389	-0.09718	0.779413	0.553139	0.222626	0.538412	0.764162	0.785473
Refined CWRS Crumb	0.179287	-1.33731	-1.26825	-1.17728	-1.07543	-0.09781	0.773872	0.550362	0.220248	0.533362	0.757302	0.788176
Refined CWRS Crumb	0.194841	-1.41842	-1.3433	-1.23566	-1.1265	-0.10283	0.790706	0.565884	0.229892	0.552329	0.777103	0.796059
Refined CWRS Crumb	0.180108	-1.33281	-1.25399	-1.16122	-1.05411	-0.09505	0.774795	0.54369	0.222989	0.532161	0.757435	0.773785
Refined CWRS Crumb	0.194196	-1.39842	-1.36554	-1.23566	-1.1265	-0.10283	0.790706	0.565884	0.229892	0.552329	0.777103	0.796059
Refined CWRS Crumb	0.1933	-1.3615	-1.31177	-1.19069	-1.08389	-0.09718	0.779413	0.553139	0.222626	0.538412	0.764162	0.785473
Refined CWRS Crumb	0.182672	-1.32132	-1.23435	-1.14939	-1.04086	-0.09291	0.778417	0.542228	0.229561	0.535807	0.760662	0.767571
Mean	0.198286	-1.44054	-1.37521	-1.2552	-1.14825	-0.10614	0.791855	0.574201	0.228346	0.558346	0.777809	0.804132
STDEV	0.019357	0.116764	0.126552	0.104102	0.099667	0.011261	0.018201	0.0335	0.011595	0.029807	0.022341	0.030357
%RSD	9.762203	-8.10557	-9.20235	-8.29367	-8.67987	-10.6093	2.298471	5.834194	5.077958	5.338488	2.872236	3.775166

Table 2.2. Sensor data for whole CWRS crumb (corresponding to Fig. 4.3).

Bread type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
CWRS Crumb	0.304917	-1.95426	-1.91893	-1.70497	-1.57688	-0.1491	0.856554	0.684608	0.292008	0.668191	0.858845	0.884914
CWRS Crumb	0.30441	-1.97519	-1.94678	-1.72187	-1.58825	-0.14875	0.85665	0.687309	0.288609	0.669343	0.858997	0.887217
CWRS Crumb	0.297308	-1.93736	-1.89616	-1.68683	-1.55176	-0.14609	0.852904	0.680636	0.285907	0.664327	0.854581	0.880712
CWRS Crumb	0.296624	-1.8626	-1.80721	-1.63543	-1.51257	-0.14063	0.8522	0.670392	0.287483	0.654342	0.852621	0.876383
CWRS Crumb	0.306268	-1.9813	-1.94837	-1.72372	-1.58586	-0.14743	0.858802	0.688792	0.29143	0.672791	0.86125	0.885464
CWRS Crumb	0.303174	-1.97681	-1.93665	-1.71594	-1.5763	-0.14766	0.85781	0.687406	0.291571	0.672411	0.860549	0.883324
CWRS Crumb	0.292149	-1.81008	-1.76234	-1.58161	-1.45312	-0.13591	0.843452	0.660803	0.238342	0.634938	0.833197	0.870398
CWRS Crumb	0.307367	-1.86333	-1.82318	-1.62321	-1.50128	-0.1411	0.85013	0.670817	0.24605	0.645288	0.842039	0.879456
CWRS Crumb	0.276643	-1.79339	-1.73279	-1.56785	-1.45386	-0.13323	0.850132	0.653647	0.282215	0.64276	0.848939	0.860118
CWRS Crumb	0.263032	-1.71004	-1.61653	-1.4752	-1.35854	-0.12294	0.83602	0.630476	0.266467	0.619203	0.832264	0.844364
CWRS Crumb	0.262586	-1.69589	-1.60566	-1.46689	-1.347	-0.12235	0.835426	0.628493	0.26738	0.617557	0.830946	0.841682
CWRS Crumb	0.261123	-1.68585	-1.60914	-1.47085	-1.35037	-0.12441	0.840582	0.631452	0.274366	0.624154	0.836211	0.840514
CWRS Crumb	0.27464	-1.77459	-1.71048	-1.55851	-1.44406	-0.13391	0.848213	0.650101	0.27816	0.63852	0.846356	0.860279
CWRS Crumb	0.279465	-1.72933	-1.64703	-1.50795	-1.38511	-0.12697	0.850932	0.642487	0.28724	0.639582	0.847915	0.845467
CWRS Crumb	0.271392	-1.72729	-1.6789	-1.50726	-1.39131	-0.12789	0.831902	0.648155	0.228947	0.620177	0.820746	0.863425
CWRS Crumb	0.2623	-1.69112	-1.59444	-1.46342	-1.36603	-0.12405	0.85802	0.64509	0.28116	0.64082	0.842311	0.851804
CWRS Crumb	0.276643	-1.79339	-1.73279	-1.56785	-1.45386	-0.13323	0.850132	0.653647	0.282215	0.64276	0.848939	0.860118
CWRS Crumb	0.260303	-1.69004	-1.61653	-1.4752	-1.35854	-0.12294	0.83602	0.630476	0.266467	0.619203	0.832264	0.844364
CWRS Crumb	0.269986	-1.7286	-1.65656	-1.4885	-1.347	-0.12235	0.835426	0.628493	0.26738	0.617557	0.830946	0.841682
CWRS Crumb	0.261123	-1.68585	-1.60914	-1.47085	-1.35037	-0.12441	0.840582	0.631452	0.274366	0.624154	0.836211	0.840514
CWRS Crumb	0.27464	-1.77459	-1.71048	-1.55851	-1.44406	-0.13391	0.848213	0.650101	0.27816	0.63852	0.846356	0.860279
CWRS Crumb	0.26643	-1.73392	-1.71279	-1.553	-1.43908	-0.13289	0.86124	0.6547	0.2795	0.63663	0.840001	0.859442
CWRS Crumb	0.269389	-1.72239	-1.69528	-1.54338	-1.41966	-0.13171	0.85	0.64862	0.273095	0.63373	0.83887	0.849814
CWRS Crumb	0.279465	-1.72933	-1.64703	-1.50795	-1.38511	-0.12697	0.850932	0.642487	0.28724	0.639582	0.847915	0.845467
Mean	0.280057	-1.79277	-1.73396	-1.5657	-1.44333	-0.13337	0.848011	0.654193	0.274823	0.640689	0.84372	0.860717
STDEV	0.016776	0.103282	0.119574	0.089888	0.084268	0.009301	0.008613	0.020369	0.016608	0.01801	0.010906	0.016485
% RSD	5.990177	-5.761	-6.89596	-5.7411	-5.83842	-6.97385	1.015705	3.113618	6.043137	2.810984	1.292631	1.91531

Table 2. 3. Sensor data for whole Snowbird crumb (corresponding to Fig. 4.3).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Snowbird Crumb	0.239577	-1.668	-1.61919	-1.46094	-1.34218	-0.12481	0.819324	0.631272	0.25499	0.610564	0.81651	0.851003
Snowbird Crumb	0.229762	-1.63737	-1.5845	-1.43087	-1.3136	-0.12081	0.812002	0.623191	0.245303	0.601445	0.807694	0.845199
Snowbird Crumb	0.241277	-1.6926	-1.63889	-1.4757	-1.35507	-0.12325	0.821478	0.634463	0.25438	0.614745	0.818635	0.850881
Snowbird Crumb	0.237107	-1.68403	-1.62452	-1.46032	-1.34349	-0.12266	0.820041	0.631823	0.25463	0.613097	0.81705	0.847849
Snowbird Crumb	0.247919	-1.74145	-1.68354	-1.50656	-1.38423	-0.12803	0.828038	0.643194	0.26127	0.625305	0.826584	0.855238
Snowbird Crumb	0.238312	-1.63031	-1.57285	-1.42887	-1.31691	-0.12188	0.818226	0.623235	0.25597	0.604749	0.814246	0.845767
Snowbird Crumb	0.243871	-1.70863	-1.66813	-1.47102	-1.35007	-0.13591	0.842031	0.659033	0.236704	0.632219	0.832084	0.871877
Snowbird Crumb	0.244604	-1.67348	-1.68208	-1.46542	-1.43336	-0.13447	0.838188	0.6587	0.239226	0.632513	0.829129	0.866441
Snowbird Crumb	0.198775	-1.44479	-1.37636	-1.26574	-1.16067	-0.10667	0.796552	0.576682	0.235437	0.562629	0.785513	0.805076
Snowbird Crumb	0.192329	-1.41621	-1.34391	-1.23744	-1.12916	-0.10267	0.789294	0.567531	0.229787	0.552117	0.776401	0.797617
Snowbird Crumb	0.18444	-1.36406	-1.2858	-1.19051	-1.08278	-0.10733	0.780368	0.55251	0.22522	0.539568	0.764593	0.782937
Snowbird Crumb	0.207246	-1.45078	-1.36524	-1.26492	-1.15107	-0.1056	0.801455	0.576827	0.244024	0.569089	0.790163	0.797902
Snowbird Crumb	0.213319	-1.45504	-1.36239	-1.26277	-1.15261	-0.10489	0.806251	0.579582	0.251592	0.575388	0.795609	0.79686
Snowbird Crumb	0.20626	-1.47905	-1.40613	-1.2956	-1.19484	-0.10798	0.802178	0.585212	0.239628	0.571239	0.791933	0.812972
Snowbird Crumb	0.20716	-1.55223	-1.29757	-1.228	-1.19755	-0.12983	0.83384	0.597142	0.228445	0.590098	0.781589	0.801341
Snowbird Crumb	0.1929	-1.4122	-1.35087	-1.2439	-1.19363	-0.10252	0.77684	0.569087	0.21999	0.557132	0.764532	0.7909
Snowbird Crumb	0.198775	-1.44479	-1.37636	-1.26574	-1.16067	-0.10667	0.796552	0.576682	0.235437	0.562629	0.785513	0.805076
Snowbird Crumb	0.192329	-1.41621	-1.34391	-1.23744	-1.12916	-0.10267	0.789294	0.567531	0.229787	0.552117	0.776401	0.797617
Snowbird Crumb	0.18444	-1.36406	-1.2858	-1.19051	-1.08278	-0.10733	0.780368	0.55251	0.22522	0.539568	0.764593	0.782937
Snowbird Crumb	0.207246	-1.45078	-1.36524	-1.26492	-1.15107	-0.1056	0.801455	0.576827	0.244024	0.569089	0.790163	0.797902
Snowbird Crumb	0.213319	-1.45504	-1.36239	-1.26277	-1.15261	-0.10489	0.806251	0.579582	0.251592	0.575388	0.795609	0.79686
Snowbird Crumb	0.20626	-1.47905	-1.40613	-1.2956	-1.19484	-0.10798	0.802178	0.585212	0.239628	0.571239	0.791933	0.812972
Snowbird Crumb	0.1929	-1.40614	-1.33953	-1.24316	-1.13016	-0.10927	0.78901	0.5473	0.229	0.53711	0.77042	0.800762
Snowbird Crumb	0.193913	-1.41431	-1.34091	-1.2439	-1.12966	-0.10123	0.79942	0.55301	0.230787	0.542007	0.76601	0.798862
Mean	0.213085	-1.51836	-1.44509	-1.32053	-1.21801	-0.11354	0.806276	0.593672	0.240086	0.57921	0.793871	0.817202
STDEV	0.021299	0.123766	0.141631	0.106221	0.105166	0.011184	0.018293	0.034949	0.011648	0.030646	0.021756	0.028164
% RSD	9.99563	-8.15132	-9.8008	-8.04387	-8.63429	-9.85079	2.268825	5.886839	4.851739	5.291013	2.740457	3.446424

Table 2. 4. Sensor data for whole Platte crumb (corresponding to Fig. 4.3).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Platte Crumb	0.251891	-1.77148	-1.73064	-1.55265	-1.42822	-0.13328	0.833355	0.652015	0.266597	0.63172	0.832743	0.865306
Platte Crumb	0.246365	-1.76915	-1.71874	-1.53955	-1.41747	-0.13016	0.830965	0.649045	0.262686	0.628894	0.829879	0.862363
Platte Crumb	0.24941	-1.73044	-1.67236	-1.50359	-1.38023	-0.12656	0.826673	0.64132	0.260106	0.623533	0.824879	0.854331
Platte Crumb	0.261988	-1.78909	-1.7369	-1.55548	-1.42457	-0.13193	0.836574	0.652581	0.266711	0.634917	0.835341	0.862912
Platte Crumb	0.247198	-1.7304	-1.67294	-1.49807	-1.37638	-0.12724	0.827597	0.640424	0.258204	0.622162	0.825703	0.854398
Platte Crumb	0.24027	-1.665	-1.74983	-1.57689	-1.45772	-0.13583	0.840654	0.658073	0.275991	0.640136	0.840908	0.868705
Platte Crumb	0.243462	-1.6706	-1.61957	-1.44724	-1.33104	-0.12355	0.823482	0.631218	0.218481	0.602747	0.809187	0.853559
Platte Crumb	0.243906	-1.65104	-1.58146	-1.45029	-1.33757	-0.12327	0.8216	0.634131	0.219302	0.605663	0.80825	0.853646
Platte Crumb	0.192959	-1.39748	-1.3057	-1.21366	-1.1234	-0.09006	0.757686	0.559653	0.210334	0.513784	0.768233	0.786284
Platte Crumb	0.192691	-1.4181	-1.34441	-1.23744	-1.132	-0.1011	0.789129	0.56799	0.231186	0.553274	0.776306	0.796782
Platte Crumb	0.223961	-1.54063	-1.46253	-1.34416	-1.22945	-0.11146	0.815224	0.597745	0.250438	0.586554	0.806669	0.818338
Platte Crumb	0.212408	-1.46359	-1.38026	-1.27562	-1.16399	-0.10472	0.806971	0.580093	0.246968	0.572814	0.795594	0.800698
Platte Crumb	0.199275	-1.39063	-1.3025	-1.20786	-1.10174	-0.09937	0.795967	0.563268	0.244444	0.559332	0.782526	0.78342
Platte Crumb	0.194537	-1.42486	-1.35514	-1.24842	-1.1482	-0.10258	0.791861	0.571786	0.232236	0.557223	0.779859	0.80198
Platte Crumb	0.220742	-1.50807	-1.44459	-1.39414	-1.17115	-0.11351	0.828698	0.542882	0.227987	0.615976	0.816742	0.856597
Platte Crumb	0.213961	-1.34063	-1.26253	-1.4634	-1.19852	-0.12504	0.80543	0.55452	0.2242	0.55654	0.7667	0.78314
Platte Crumb	0.162959	-1.27479	-1.2057	-1.11366	-1.01199	-0.09006	0.757686	0.529653	0.210334	0.513784	0.738233	0.766284
Platte Crumb	0.192691	-1.4181	-1.34441	-1.23744	-1.132	-0.1011	0.789129	0.56799	0.231186	0.553274	0.776306	0.796782
Platte Crumb	0.199453	-1.48877	-1.37765	-1.23822	-1.15297	-0.1197	0.791976	0.585434	0.26758	0.574	0.79006	0.80086
Platte Crumb	0.192134	-1.4097	-1.32307	-1.23381	-1.10832	-0.1009	0.785437	0.5589	0.23003	0.554434	0.777866	0.782143
Platte Crumb	0.213961	-1.54063	-1.46253	-1.34416	-1.22945	-0.11146	0.815224	0.597745	0.250438	0.586554	0.806669	0.818338
Platte Crumb	0.212408	-1.46359	-1.38026	-1.27562	-1.16399	-0.10472	0.806971	0.580093	0.246968	0.572814	0.795594	0.800698
Platte Crumb	0.199275	-1.39063	-1.3025	-1.20786	-1.10174	-0.09937	0.795967	0.563268	0.244444	0.559332	0.782526	0.78342
Platte Crumb	0.194537	-1.42486	-1.35514	-1.24842	-1.1482	-0.10258	0.791861	0.571786	0.232236	0.557223	0.779859	0.80198
Mean	0.216768	-1.52801	-1.46214	-1.35032	-1.22793	-0.1129	0.806922	0.593817	0.242045	0.582362	0.797776	0.818874
STDEV	0.025874	0.154044	0.17392	0.13964	0.129903	0.01413	0.022913	0.03998	0.019113	0.036846	0.026106	0.033831
% RSD	11.93613	-10.0814	-11.8949	-10.3413	-10.579	-12.5155	2.83961	6.732703	7.89652	6.327022	3.272395	4.13143

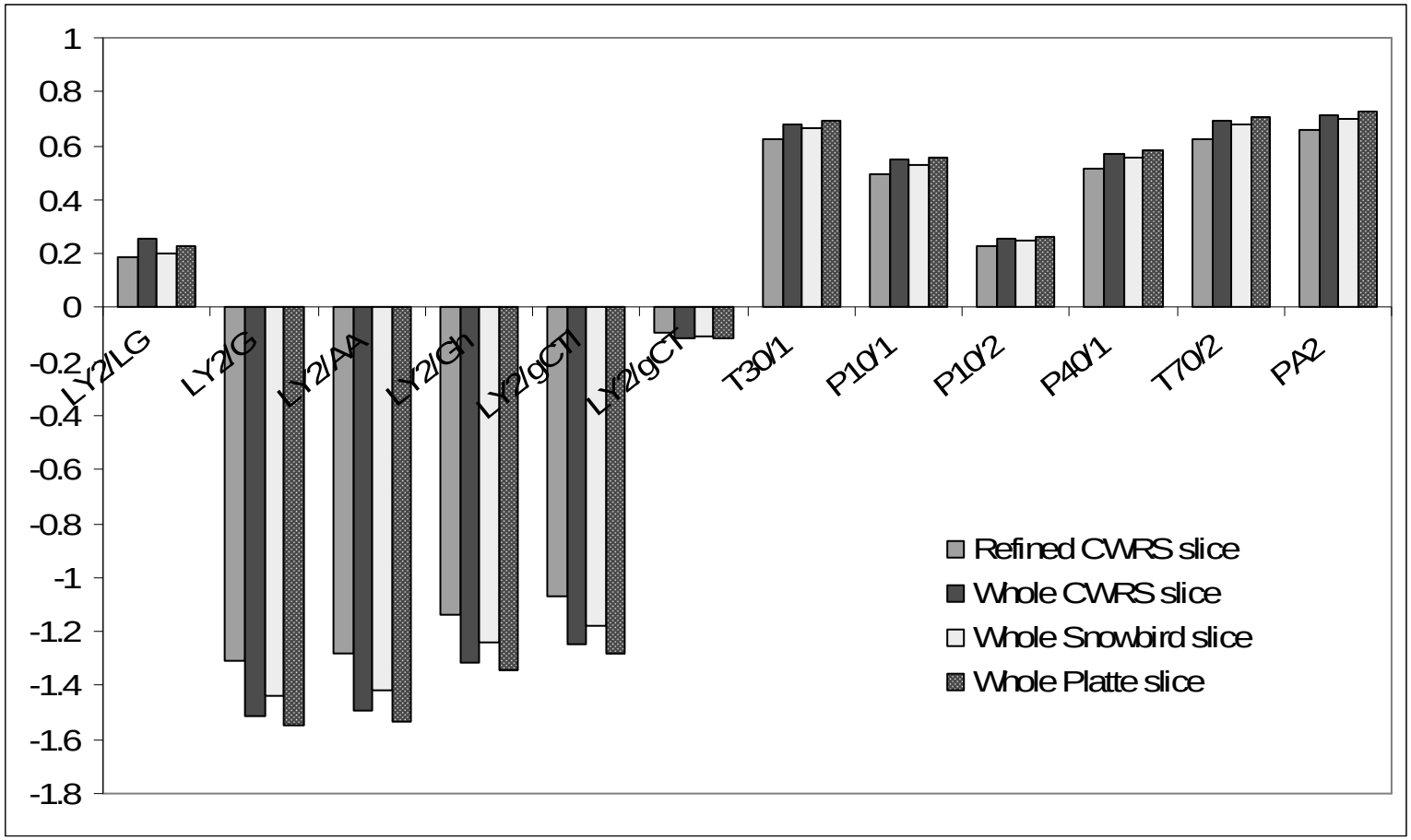


Figure. 3. Average E-nose sensor output for whole slices of indicated breads corresponding to Fig. 4.4. Data follows in Tables 3.1-3.4. Based on sensor output, whole slices of refined CWRS bread had the lowest concentration of volatiles (all sensors); Platte whole wheat bread slices tended to have the highest sensor output.

Table 3.1. Sensor data for refined (R) CWRS whole slice (WS) (corresponding to Fig. 4.4).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
RCWRS WS	0.194262	-1.52041	-1.4905	-1.32242	-1.23196	-0.11128	0.789771	0.604293	0.22218	0.574837	0.780977	0.831374
R CWRS WS	0.181922	-1.39486	-1.35652	-1.22212	-1.142	-0.10133	0.776547	0.575328	0.213072	0.547438	0.762933	0.81262
R CWRS WS	0.185851	-1.48871	-1.45521	-1.28986	-1.19816	-0.10653	0.780905	0.596299	0.215066	0.565075	0.770878	0.824178
R CWRS WS	0.185163	-1.51085	-1.47723	-1.30901	-1.21423	-0.1087	0.783	0.600854	0.214038	0.568113	0.772506	0.828968
R CWRS WS	0.179394	-1.49403	-1.46537	-1.29099	-1.19446	-0.10543	0.777462	0.59702	0.208627	0.562457	0.765993	0.826811
R CWRS WS	0.185819	-1.53584	-1.51071	-1.33036	-1.23358	-0.10922	0.784229	0.608307	0.213777	0.574098	0.774494	0.834999
R CWRS WS	0.150246	-1.36485	-1.33016	-1.1758	-1.08115	-0.09457	0.74432	0.564387	0.187697	0.526334	0.727148	0.800613
R CWRS WS	0.170819	-1.38092	-1.34775	-1.20424	-1.11586	-0.09961	0.763744	0.569661	0.204927	0.539254	0.750167	0.806363
R CWRS WS	0.29458	-1.46978	-1.37502	-1.27531	-1.19467	-0.10951	0.701605	0.573509	0.327676	0.621371	0.722346	0.732433
R CWRS WS	0.279323	-1.35529	-1.24975	-1.17415	-1.08896	-0.10044	0.691053	0.55259	0.319932	0.605544	0.707216	0.718985
R CWRS WS	0.272648	-1.38705	-1.29235	-1.20194	-1.12574	-0.10339	0.663331	0.537732	0.312054	0.586561	0.680142	0.700798
R CWRS WS	0.307591	-1.48633	-1.40629	-1.29741	-1.2251	-0.11293	0.674395	0.552027	0.319759	0.596512	0.694143	0.718263
R CWRS WS	0.29469	-1.44431	-1.36181	-1.25848	-1.19283	-0.11005	0.68424	0.564985	0.321314	0.61296	0.704801	0.723117
R CWRS WS	0.270463	-1.36888	-1.28389	-1.19177	-1.129	-0.10367	0.6553	0.538526	0.309217	0.586947	0.67364	0.693646
R CWRS WS	0.273012	-1.36788	-1.28104	-1.19343	-1.13485	-0.10478	0.66658	0.558063	0.313159	0.605365	0.687842	0.69793
R CWRS WS	0.317283	-1.53235	-1.43211	-1.32955	-1.24602	-0.11382	0.724757	0.596737	0.339034	0.643377	0.747294	0.753088
R CWRS WS	0.11201	-1.11573	-1.13173	-0.98337	-0.93901	-0.07833	0.471819	0.390687	0.140351	0.337762	0.37405	0.390357
R CWRS WS	0.106183	-1.09804	-1.1152	-0.92744	-0.89538	-0.07402	0.434218	0.343175	0.163409	0.395607	0.443156	0.453552
R CWRS WS	0.089835	-1.03078	-1.06618	-0.88546	-0.854	-0.06873	0.417394	0.33046	0.157159	0.380641	0.424018	0.437078
R CWRS WS	0.086308	-1.0864	-1.12926	-0.93615	-0.9045	-0.07321	0.437323	0.348544	0.164044	0.39964	0.446617	0.459163
R CWRS WS	0.082116	-1.06005	-1.10029	-0.91002	-0.87875	-0.07089	0.42543	0.339467	0.158886	0.388848	0.433608	0.446822
R CWRS WS	0.088892	-1.03887	-1.07569	-0.89128	-0.86156	-0.06858	0.41691	0.33301	0.155747	0.382286	0.423946	0.43917
R CWRS WS	0.087767	-0.92972	-0.9514	-0.79463	-0.75741	-0.05971	0.376729	0.295347	0.141045	0.343112	0.379839	0.396361
R CWRS WS	0.089248	-1.00988	-1.0412	-0.86806	-0.83436	-0.06571	0.409592	0.321377	0.154054	0.372528	0.415871	0.428457
Mean	0.186893	-1.31132	-1.28028	-1.13597	-1.06973	-0.09393	0.627111	0.495516	0.228176	0.513194	0.627651	0.656464
STDEV	0.082995	0.20182	0.165105	0.179702	0.156719	0.018154	0.153363	0.116482	0.071047	0.103722	0.155395	0.169295
%RSD	44.40782	-15.3906	-12.8961	-15.8193	-14.6503	-19.3259	24.45555	23.50715	31.13712	20.2111	24.75826	25.78898

Table 3.2. Sensor data for whole CWRS whole slice (WS) (corresponding to Fig. 4.4).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
CWRS WS	0.217391	-1.60779	-1.57906	-1.40324	-1.31534	-0.11907	0.806965	0.625299	0.237256	0.598512	0.801964	0.84609
CWRS WS	0.218149	-1.63326	-1.60506	-1.42029	-1.32743	-0.11897	0.806839	0.62935	0.236644	0.601615	0.802022	0.847402
CWRS WS	0.187462	-1.49437	-1.46	-1.2954	-1.20319	-0.10559	0.780582	0.597785	0.21553	0.566162	0.770987	0.825709
CWRS WS	0.206478	-1.51358	-1.47566	-1.32541	-1.23537	-0.11146	0.796346	0.604077	0.230515	0.577554	0.78815	0.832107
CWRS WS	0.216758	-1.66646	-1.64619	-1.44739	-1.34864	-0.12267	0.808604	0.636843	0.2355	0.606812	0.804033	0.853893
CWRS WS	1	-1.69418	-1.67369	-1.46748	-1.36997	-0.12248	0.811487	0.642692	0.238281	0.612046	0.807218	0.857354
CWRS WS	0.225231	-1.72067	-1.70284	-1.49034	-1.38947	-0.12578	0.813588	0.64854	0.240047	0.617505	0.80999	0.85976
CWRS WS	0.217498	-1.69591	-1.67816	-1.46924	-1.37158	-0.12403	0.809786	0.644312	0.236177	0.611964	0.805126	0.857682
CWRS WS	0.34718	-1.63602	-1.53668	-1.41365	-1.33123	-0.12305	0.745127	0.620307	0.351962	0.6642	0.769812	0.773452
CWRS WS	0.358624	-1.67105	-1.58298	-1.44671	-1.36234	-0.1262	0.741069	0.618676	0.350892	0.661605	0.766542	0.773802
CWRS WS	0.360793	-1.66946	-1.59044	-1.45012	-1.37532	-0.12679	0.732393	0.613032	0.348754	0.656293	0.758083	0.770017
CWRS WS	0.359175	-1.62182	-1.51739	-1.40597	-1.3214	-0.1229	0.74609	0.616988	0.352017	0.661315	0.77035	0.774206
CWRS WS	0.34375	-1.60765	-1.52963	-1.40105	-1.32976	-0.1237	0.698035	0.577689	0.333024	0.619986	0.721078	0.743384
CWRS WS	0.34843	-1.62001	-1.54558	-1.41045	-1.3425	-0.1252	0.72713	0.612467	0.34447	0.656597	0.752771	0.76453
CWRS WS	0.325269	-1.52548	-1.44694	-1.32837	-1.2655	-0.11767	0.696425	0.582243	0.329973	0.627806	0.720506	0.734149
CWRS WS	0.247516	-1.2661	-1.17443	-1.10547	-1.04353	-0.09725	0.637403	0.529577	0.299908	0.579574	0.654916	0.667591
CWRS WS	0.130027	-1.34712	-1.40685	-1.16179	-1.14441	-0.09592	0.527014	0.418589	0.205389	0.477504	0.546839	0.542089
CWRS WS	0.118483	-1.27784	-1.33219	-1.10534	-1.08278	-0.09063	0.506106	0.402315	0.195712	0.458671	0.523845	0.522243
CWRS WS	0.103354	-1.19549	-1.24842	-1.03062	-1.0052	-0.0822	0.476189	0.381092	0.181351	0.434424	0.490004	0.497058
CWRS WS	0.143949	-1.38006	-1.42391	-1.19774	-1.17676	-0.09834	0.539327	0.426777	0.213462	0.486497	0.560508	0.551026
CWRS WS	0.129275	-1.3573	-1.42369	-1.17314	-1.15407	-0.09743	0.530967	0.424439	0.207848	0.481909	0.551215	0.546416
CWRS WS	0.119203	-1.29914	-1.36165	-1.12299	-1.09858	-0.09201	0.510332	0.408553	0.197725	0.464764	0.527861	0.528528
CWRS WS	0.139157	-1.42426	-1.49856	-1.23267	-1.21634	-0.10415	0.54986	0.443415	0.216915	0.501306	0.572216	0.56847
CWRS WS	0.135705	-1.36282	-1.42361	-1.1814	-1.16051	-0.09834	0.536785	0.424056	0.211634	0.483656	0.557964	0.547489
Mean	0.258286	-1.51199	-1.49432	-1.31193	-1.2488	-0.11133	0.680602	0.547046	0.258791	0.571178	0.693083	0.711852
STDEV	0.182086	0.163707	0.133243	0.143304	0.116931	0.01408	0.122918	0.098187	0.060502	0.075982	0.115638	0.134287
%RSD	70.49784	-10.8272	-8.91666	-10.9232	-9.36346	-12.6473	18.06016	17.94862	23.37879	13.30265	16.68453	18.86448

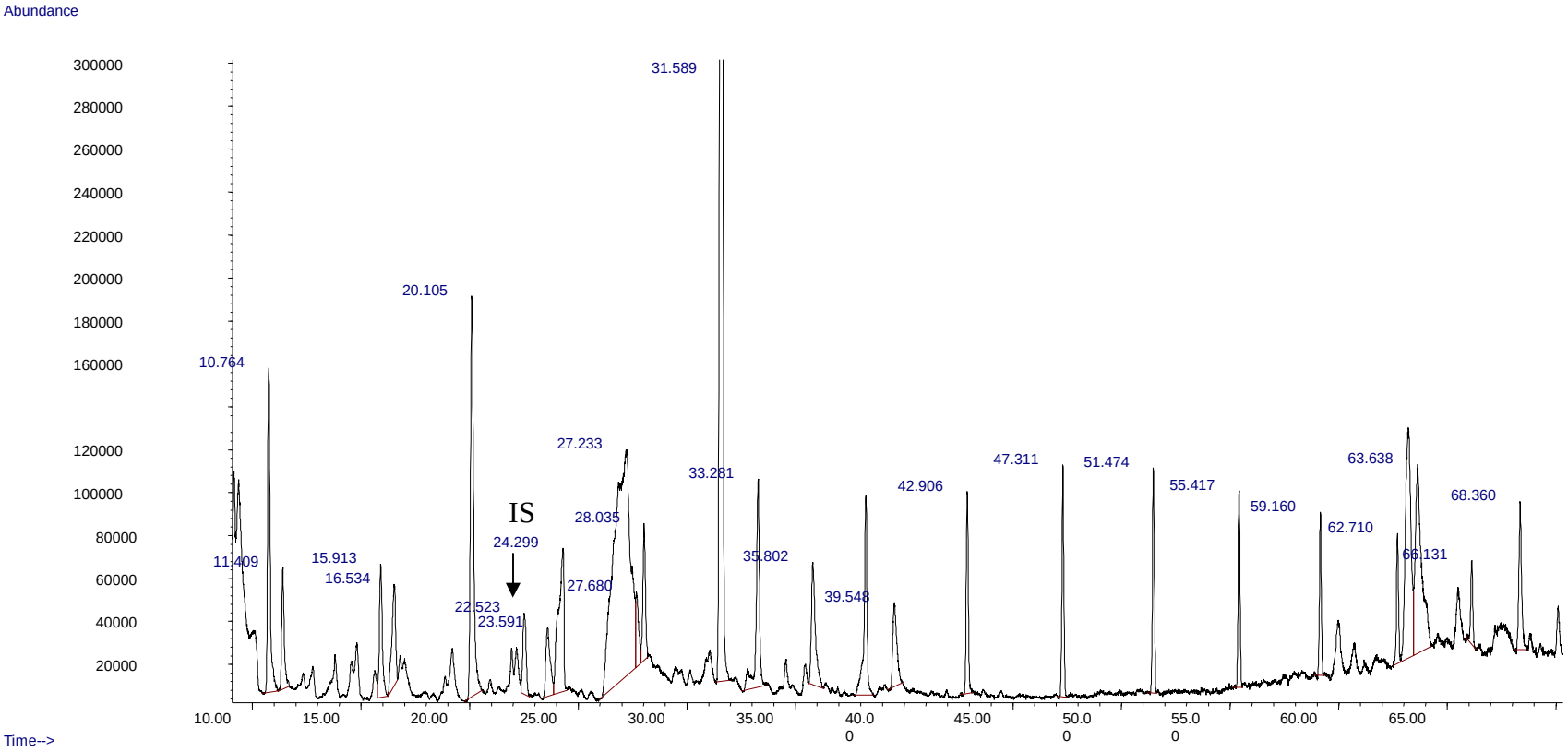
Table 3.3. Sensor data for whole Snowbird whole slice (WS) (corresponding to Fig. 4.4).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Snowbird WS	0.195195	-1.50606	-1.4749	-1.31482	-1.22595	-0.10887	0.787375	0.602899	0.222678	0.573913	0.779187	0.828916
Snowbird WS	0.173309	-1.42588	-1.39392	-1.23623	-1.1493	-0.10264	0.766514	0.580518	0.205994	0.548238	0.754647	0.813933
Snowbird WS	0.183364	-1.4869	-1.45256	-1.28727	-1.19778	-0.10723	0.777646	0.595987	0.213196	0.56389	0.767538	0.824425
Snowbird WS	0.174847	-1.46249	-1.428	-1.26616	-1.17258	-0.10473	0.768441	0.589543	0.205677	0.555857	0.75701	0.819617
Snowbird WS	0.143298	-1.30531	-1.27315	-1.12653	-1.03444	-0.0907	0.729933	0.548298	0.181065	0.511073	0.71099	0.789397
Snowbird WS	0.179901	-1.51029	-1.48117	-1.30545	-1.21314	-0.10775	0.7757	0.602231	0.209741	0.566747	0.765442	0.82821
Snowbird WS	0.187685	-1.4482	-1.40792	-1.26562	-1.1801	-0.10506	0.779151	0.588464	0.218067	0.559975	0.768947	0.819508
Snowbird WS	0.178882	-1.50916	-1.47939	-1.30491	-1.21018	-0.10698	0.775689	0.602835	0.209412	0.567437	0.765527	0.828372
Snowbird WS	0.278396	-1.42429	-1.32323	-1.23351	-1.15283	-0.10552	0.70051	0.570399	0.325193	0.620399	0.719561	0.727872
Snowbird WS	0.261841	-1.36536	-1.26699	-1.18252	-1.10419	-0.10104	0.654437	0.52631	0.307624	0.574935	0.669274	0.691849
Snowbird WS	0.269612	-1.38872	-1.29778	-1.20607	-1.13651	-0.10419	0.671412	0.546222	0.314286	0.595676	0.689688	0.705581
Snowbird WS	0.25941	-1.34184	-1.25434	-1.16881	-1.10298	-0.10172	0.642894	0.520151	0.302723	0.568286	0.657872	0.684475
Snowbird WS	0.288113	-1.43325	-1.35188	-1.25159	-1.18693	-0.10925	0.692431	0.575453	0.323596	0.622934	0.713921	0.724467
Snowbird WS	0.267914	-1.36244	-1.28011	-1.18993	-1.1322	-0.10319	0.664294	0.550835	0.311641	0.598781	0.684303	0.697497
Snowbird WS	0.27601	-1.38069	-1.27511	-1.19963	-1.11539	-0.1028	0.693949	0.560376	0.321398	0.610957	0.711552	0.71993
Snowbird WS	0.271403	-1.36836	-1.2772	-1.19417	-1.1369	-0.1051	0.665129	0.558428	0.313019	0.605831	0.686564	0.695849
Snowbird WS	0.170611	-1.56932	-1.54696	-1.27665	-1.2598	-0.10717	0.563813	0.453484	0.224009	0.513139	0.588466	0.580024
Snowbird WS	0.171185	-1.6232	-1.59683	-1.23172	-1.22116	-0.10415	0.55074	0.443301	0.21746	0.502222	0.573723	0.568614
Snowbird WS	0.158658	-1.40908	-1.48065	-1.22122	-1.20269	-0.10098	0.546241	0.43956	0.214314	0.497686	0.568491	0.563872
Snowbird WS	0.159062	-1.47843	-1.55862	-1.28075	-1.26824	-0.10843	0.56444	0.457655	0.223497	0.515935	0.588905	0.583282
Snowbird WS	0.14965	-1.49196	-1.57584	-1.28912	-1.28098	-0.10919	0.565525	0.460881	0.224262	0.519077	0.590731	0.586875
Snowbird WS	0.155106	-1.51592	-1.5969	-1.31124	-1.30118	-0.10994	0.57339	0.465103	0.22847	0.524629	0.598573	0.592456
Snowbird WS	0.133905	-1.34661	-1.39342	-1.16673	-1.14692	-0.09599	0.528779	0.418013	0.207883	0.477462	0.54845	0.541756
Snowbird WS	0.148322	-1.43584	-1.50121	-1.24636	-1.23228	-0.10506	0.556257	0.442328	0.220849	0.502787	0.580177	0.568194
Mean	0.201487	-1.44123	-1.41534	-1.23988	-1.18186	-0.10449	0.666445	0.529136	0.247752	0.554078	0.676647	0.699374
STDEV	0.052781	0.077426	0.115568	0.05226	0.064026	0.0044	0.0909	0.063335	0.049586	0.042612	0.077731	0.103691
%RSD	26.19599	-5.37224	-8.16544	-4.21495	-5.41736	-4.21092	13.63956	11.96955	20.0145	7.690533	11.48763	14.82632

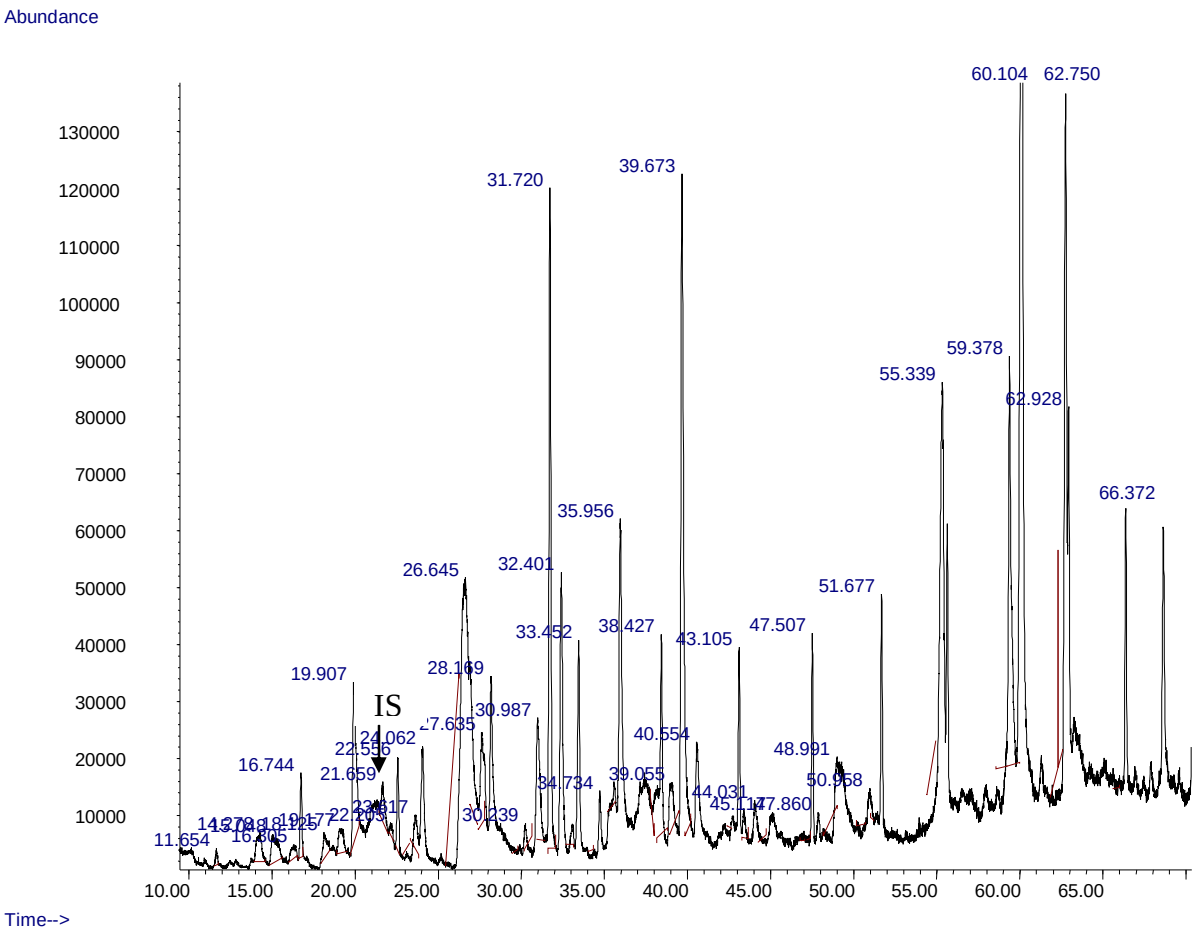
Table 3.4. Sensor data for whole Platte whole slice (WS) (corresponding to Fig. 4.4).

Bread Type	LY2/LG	LY2/G	LY2/AA	LY2/Gh	LY2/gCTl	LY2/gCT	T30/1	P10/1	P10/2	P40/1	T70/2	PA2
Platte WS	0.222953	-1.65032	-1.62603	-1.43579	-1.34313	-0.12131	0.811958	0.633884	0.241236	0.606805	0.807535	0.851688
Platte WS	0.206587	-1.58148	-1.55099	-1.37293	-1.27841	-0.11518	0.798821	0.61828	0.228604	0.589326	0.792084	0.839413
Platte WS	0.208459	-1.6096	-1.5849	-1.39852	-1.30452	-0.11646	0.801488	0.625169	0.230298	0.594886	0.79497	0.844922
Platte WS	0.187576	-1.52565	-1.49612	-1.32151	-1.22664	-0.10861	0.783254	0.605716	0.215146	0.572641	0.773826	0.831824
Platte WS	0.195719	-1.58149	-1.55527	-1.36834	-1.27226	-0.11318	0.792765	0.618967	0.222266	0.586045	0.784931	0.841272
Platte WS	0.190652	-1.56204	-1.53462	-1.35058	-1.25419	-0.11077	0.78737	0.614757	0.217698	0.580754	0.778908	0.837163
Platte WS	0.212084	-1.67579	-1.65639	-1.45054	-1.35497	-0.12248	0.807318	0.640482	0.233033	0.607644	0.80172	0.854684
Platte WS	0.2123	-1.57771	-1.54883	-1.37685	-1.28874	-0.11682	0.80342	0.619077	0.235557	0.592164	0.797718	0.84216
Platte WS	0.343069	-1.63672	-1.54227	-1.41398	-1.33101	-0.12225	0.743646	0.620026	0.349485	0.663094	0.768372	0.773035
Platte WS	0.333333	-1.59355	-1.50632	-1.38373	-1.30262	-0.12061	0.713238	0.589295	0.337489	0.633647	0.736455	0.751711
Platte WS	0.31796	-1.54	-1.4549	-1.33828	-1.26844	-0.11781	0.692762	0.570573	0.329097	0.61532	0.714703	0.734324
Platte WS	0.336889	-1.58862	-1.51454	-1.38556	-1.31616	-0.12291	0.705294	0.58648	0.335165	0.631143	0.72897	0.748072
Platte WS	0.320054	-1.5346	-1.45744	-1.33821	-1.2736	-0.11713	0.711056	0.596338	0.334542	0.640984	0.736075	0.745942
Platte WS	0.281488	-1.40293	-1.32032	-1.22316	-1.1608	-0.10766	0.675079	0.564237	0.317062	0.611501	0.696938	0.707401
Platte WS	0.335297	-1.57349	-1.49237	-1.37936	-1.32129	-0.1242	0.713297	0.611127	0.339526	0.653732	0.741524	0.747451
Platte WS	0.318903	-1.5325	-1.42643	-1.32829	-1.24635	-0.11431	0.723437	0.596462	0.339744	0.643842	0.74601	0.75162
Platte WS	0.158816	-1.50067	-1.57515	-1.30296	-1.29065	-0.10952	0.575595	0.460207	0.230354	0.521993	0.600667	0.587497
Platte WS	0.154422	-1.49048	-1.57294	-1.29216	-1.2833	-0.10927	0.56935	0.460381	0.226519	0.519295	0.594432	0.588001
Platte WS	0.166752	-1.55597	-1.64428	-1.35124	-1.34269	-0.11614	0.587962	0.476659	0.235871	0.536372	0.614702	0.60498
Platte WS	0.156486	-1.51635	-1.59897	-1.31184	-1.30097	-0.11237	0.575384	0.466839	0.229314	0.525887	0.60084	0.593608
Platte WS	0.141873	-1.43437	-1.51179	-1.23866	-1.22785	-0.10399	0.551139	0.44612	0.21722	0.503757	0.573798	0.570662
Platte WS	0.154545	-1.4492	-1.4947	-1.25925	-1.24445	-0.1059	0.559815	0.443932	0.223795	0.505694	0.583187	0.570226
Platte WS	0.154139	-1.51142	-1.59357	-1.30996	-1.29323	-0.11161	0.573378	0.464872	0.229354	0.524768	0.599334	0.591787
Platte WS	0.146471	-1.43227	-1.50004	-1.24232	-1.22783	-0.10431	0.55567	0.443265	0.220588	0.503381	0.579022	0.569658
Mean	0.227368	-1.54405	-1.53163	-1.34058	-1.28142	-0.11437	0.692187	0.557214	0.26329	0.581861	0.706113	0.724129
STDEV	0.073664	0.069731	0.074287	0.060839	0.045122	0.006088	0.09768	0.0742	0.052585	0.0519	0.086382	0.109576
%RSD	32.39839	-4.51612	-4.85019	-4.53824	-3.52128	-5.32365	14.11178	13.31622	19.97232	8.919688	12.2335	15.13204

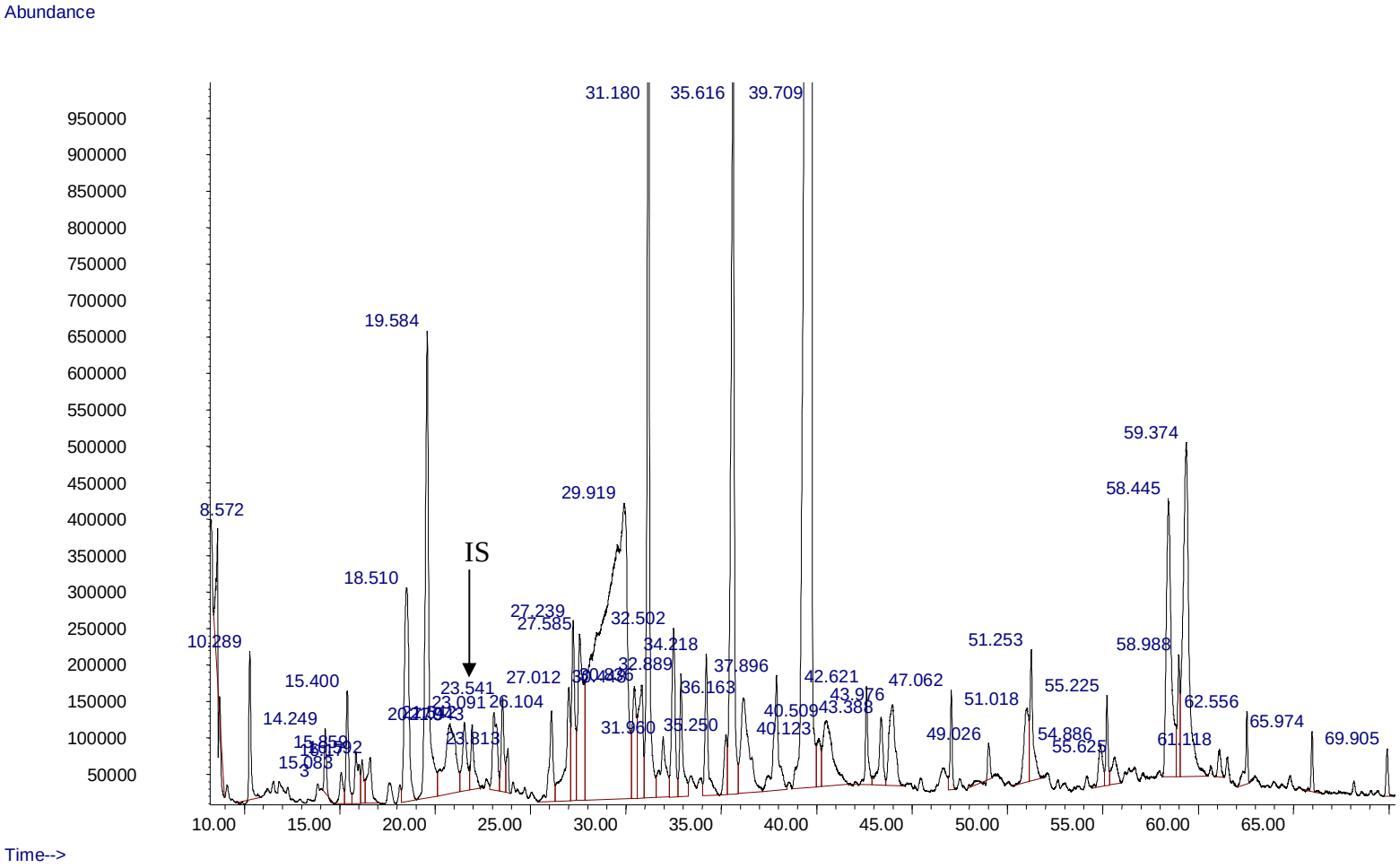
Appendix F: Gas chromatograms of crust and crumb samples of all bread types



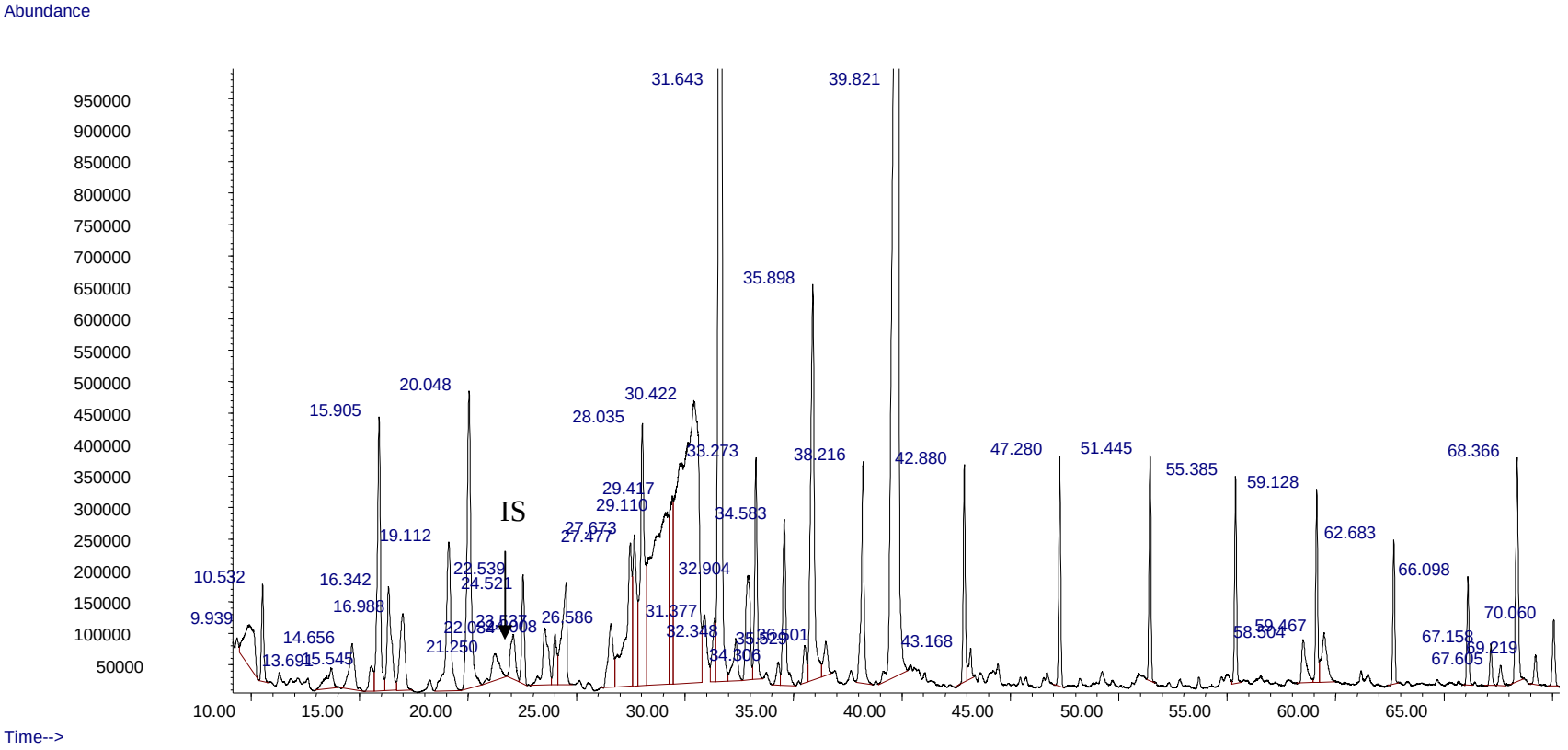
REFINED CWRS CRUMB



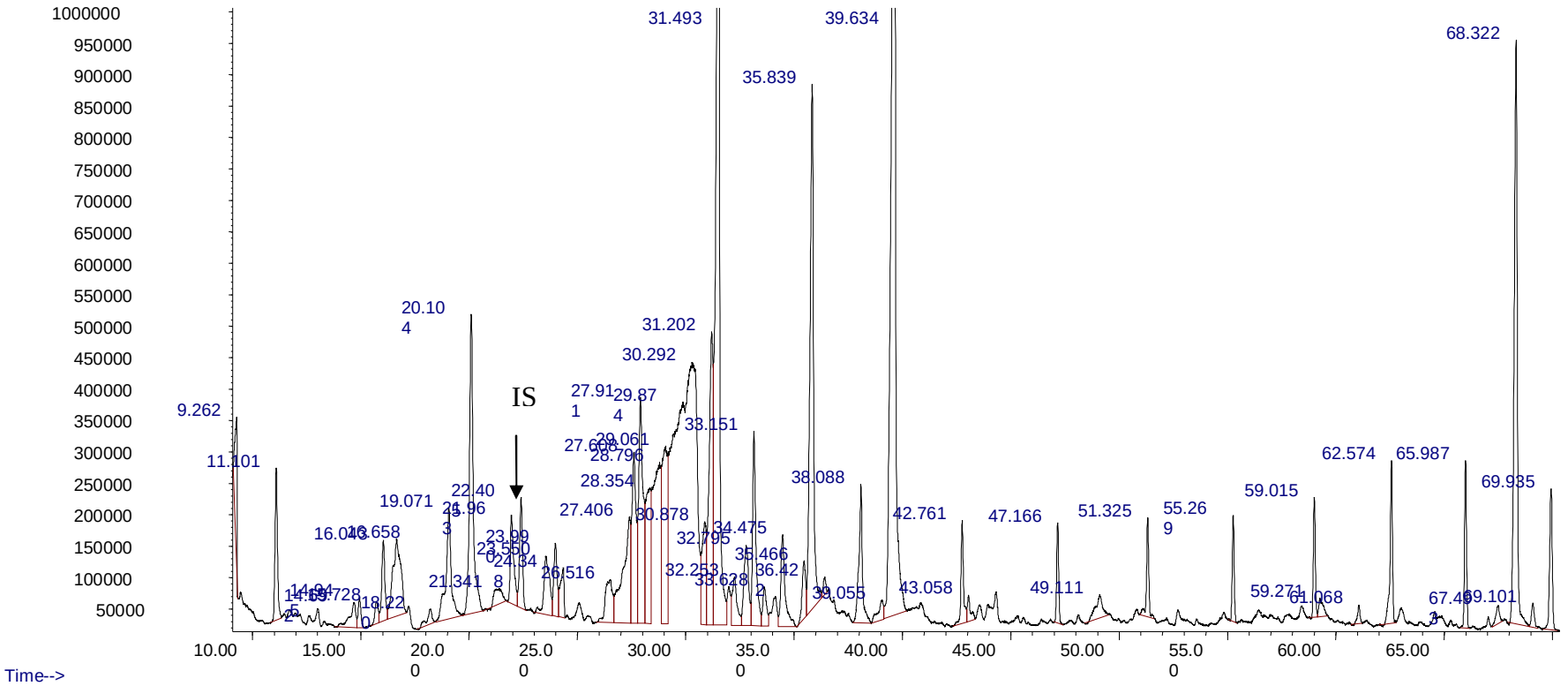
REFINED CWRS CRUST



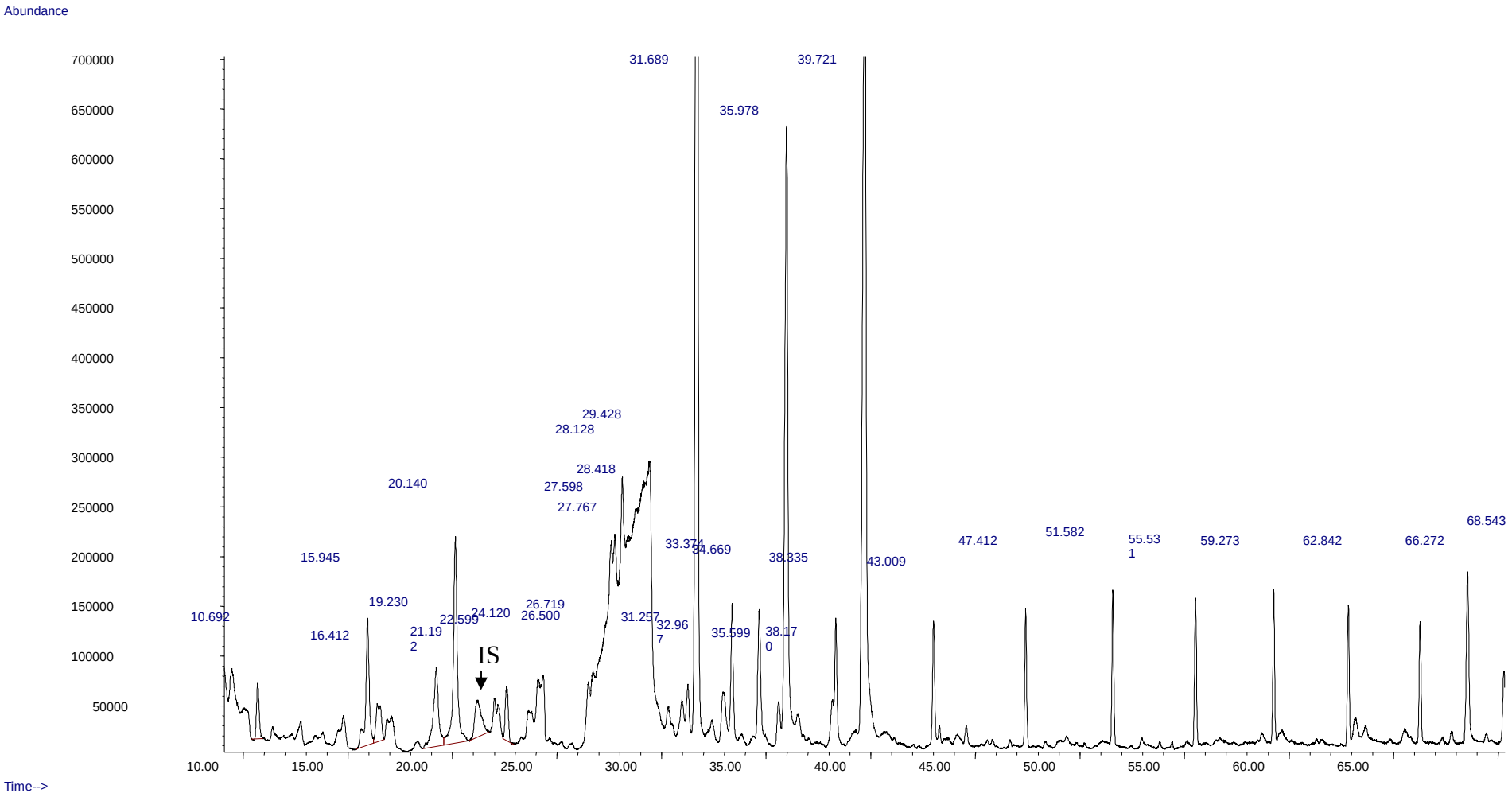
WHOLE CWRS CRUMB



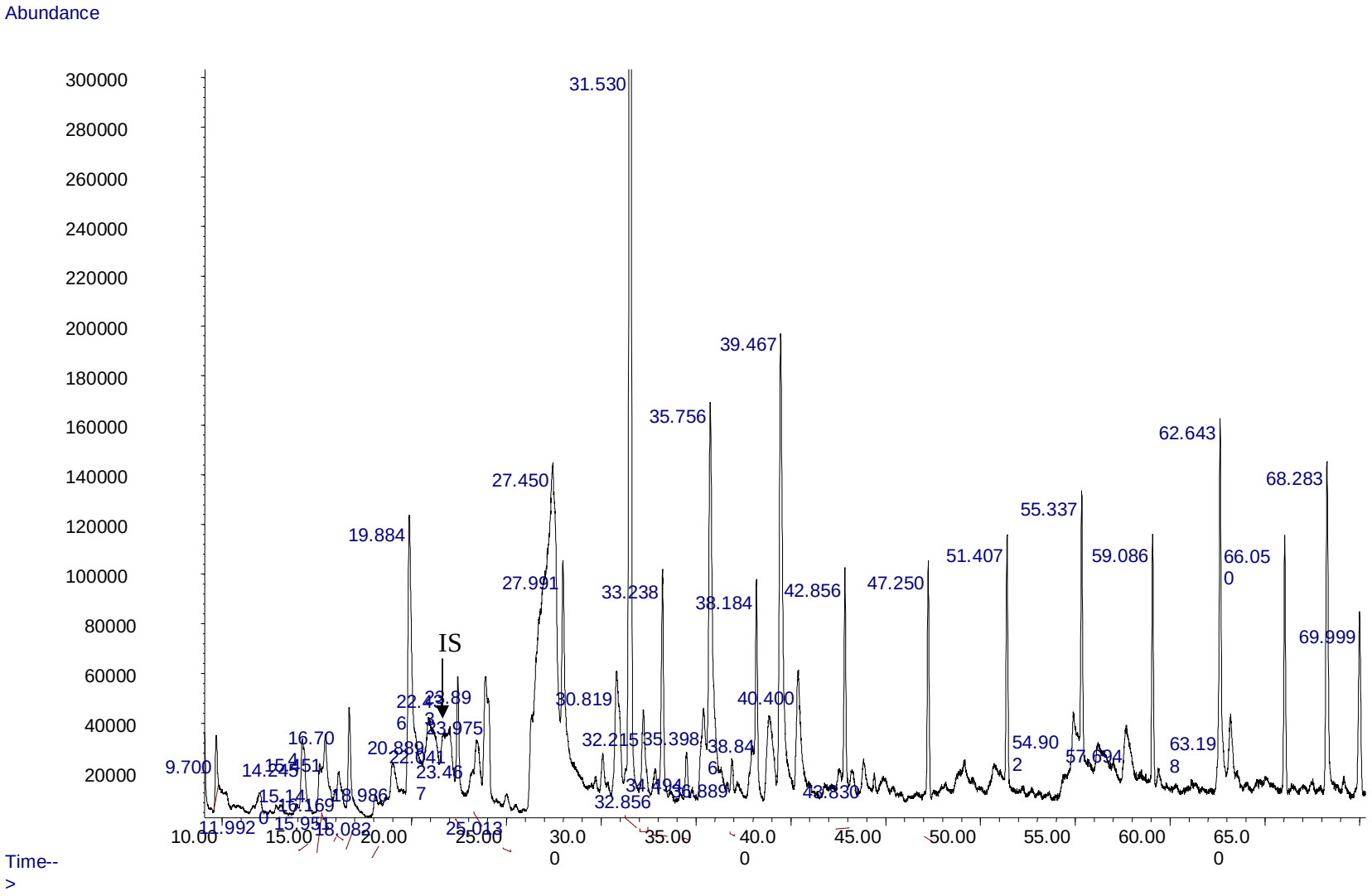
WHOLE CWRS CRUST

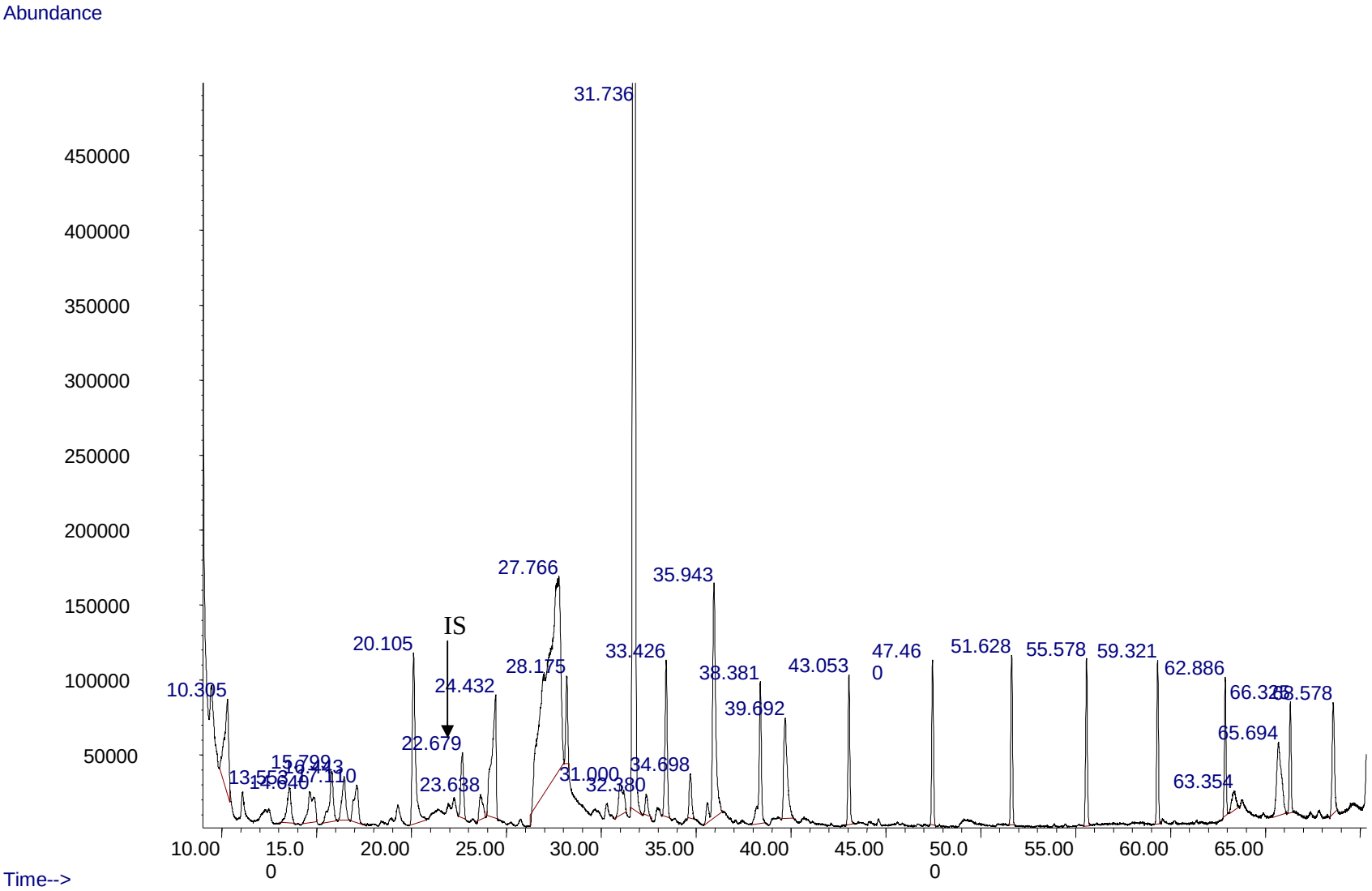


WHOLE SNOWBIRD CRUMB



WHOLE SNOWBIRD CRUST

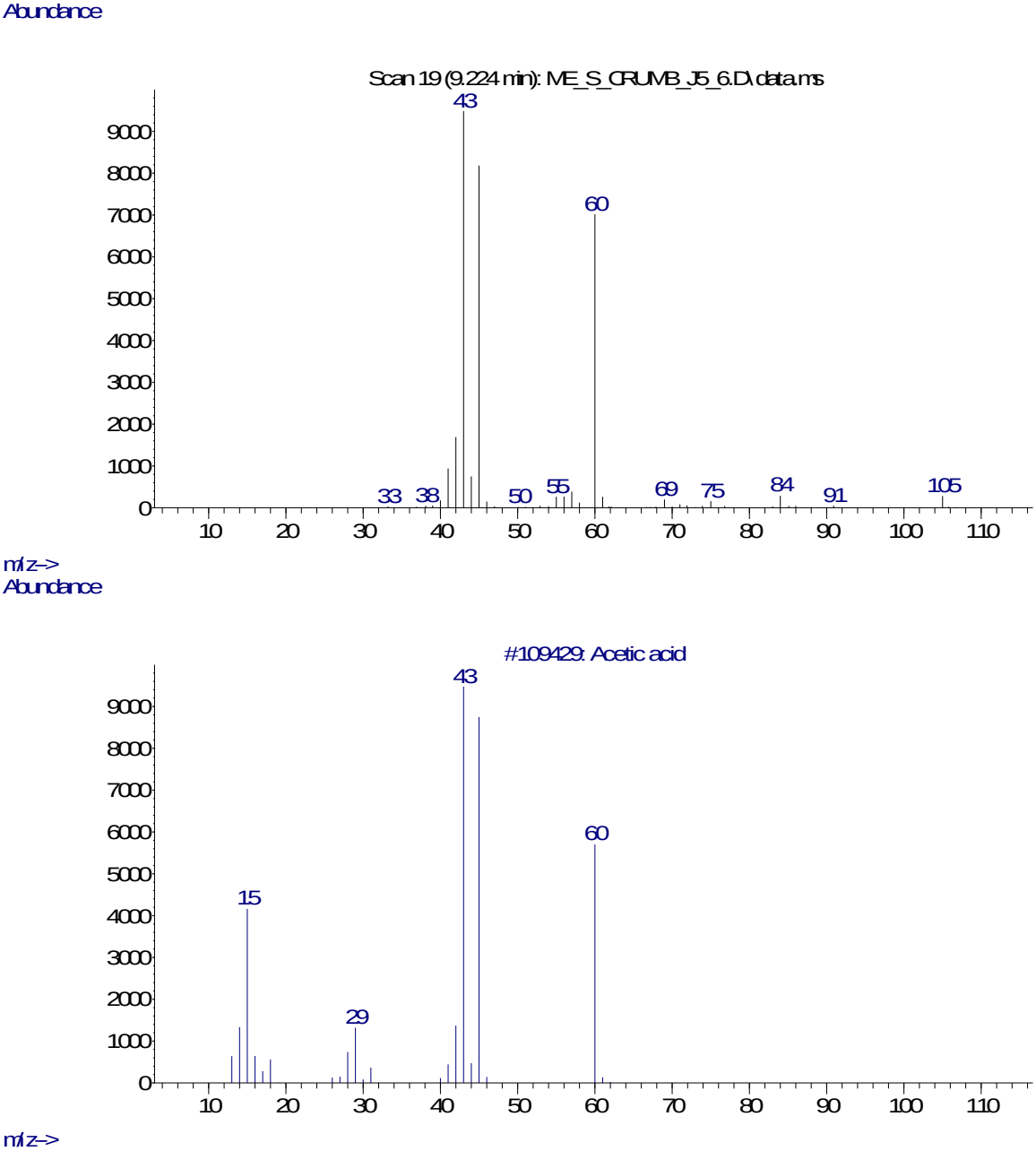

Abundance
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
31.530
39.467
35.756
62.643
68.283
27.450
55.337
19.884
51.407
59.086
66.050
27.991
33.238
38.184
42.856
47.250
69.999
IS
30.819
40.400
9.700
16.704
54.902
63.198
57.694
32.856
11.992
25.013
10.000
15.000
20.000
25.000
30.000
35.000
40.000
45.000
50.000
55.000
60.000
65.000
Time-->
WHOLE PLATTE CRUMB



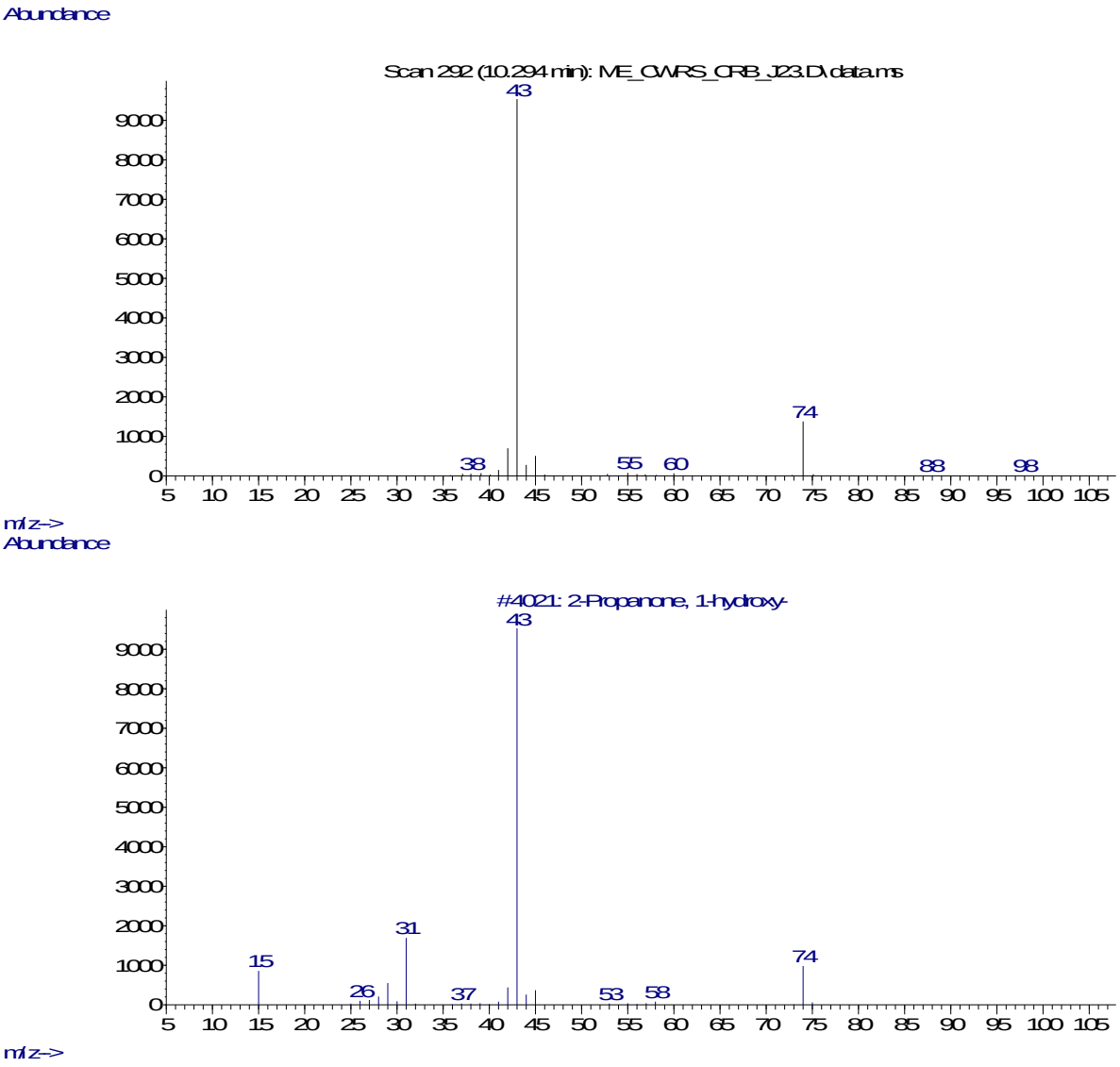
WHOLE PLATTE CRUST

Appendix G: Mass spectrum of possible identified compounds (MS of compound generated followed by MS of compound in NIST 98 library: as listed in Table 5.2)

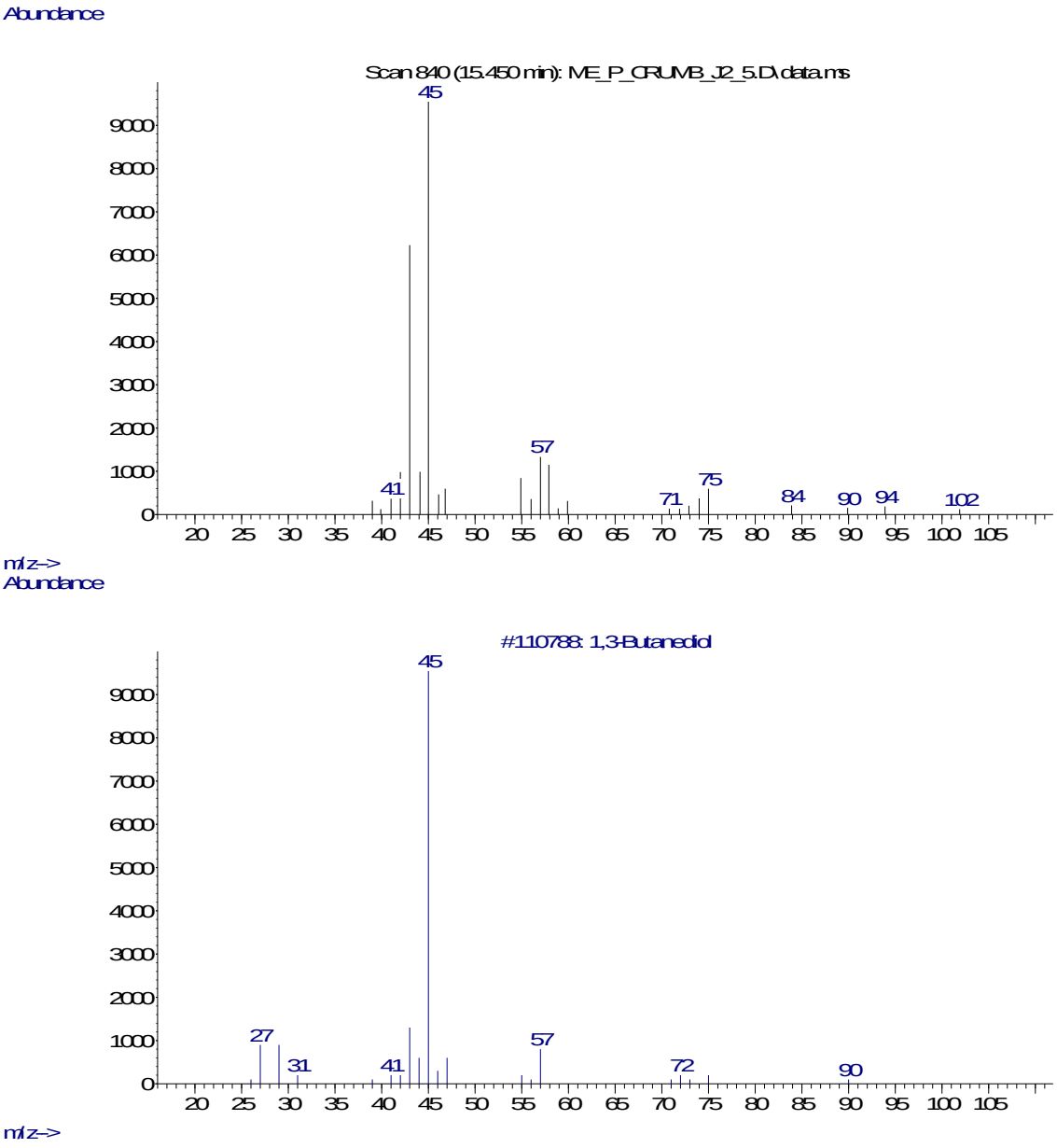
Acetic acid



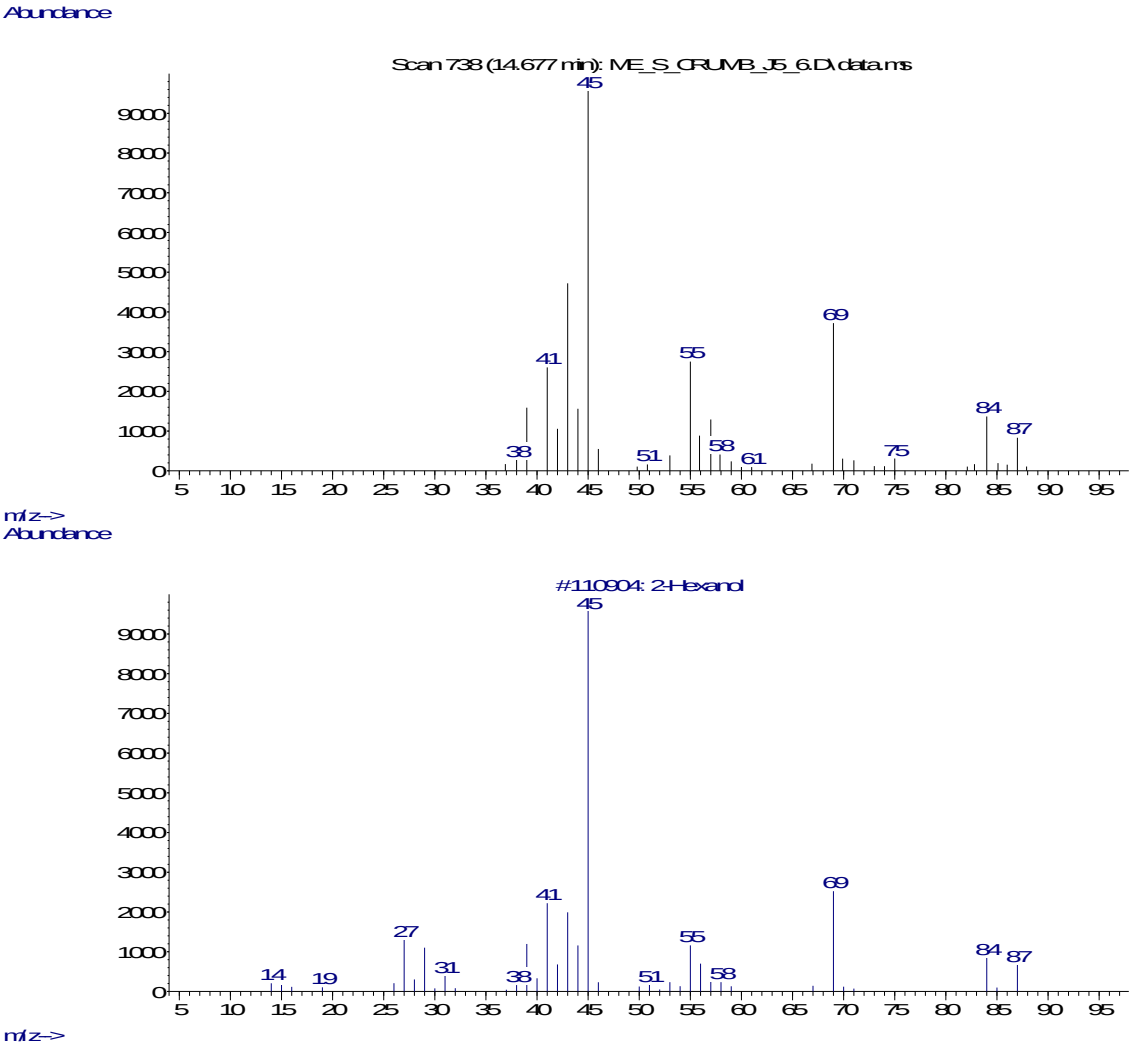
1-hydroxy, 2-propanone



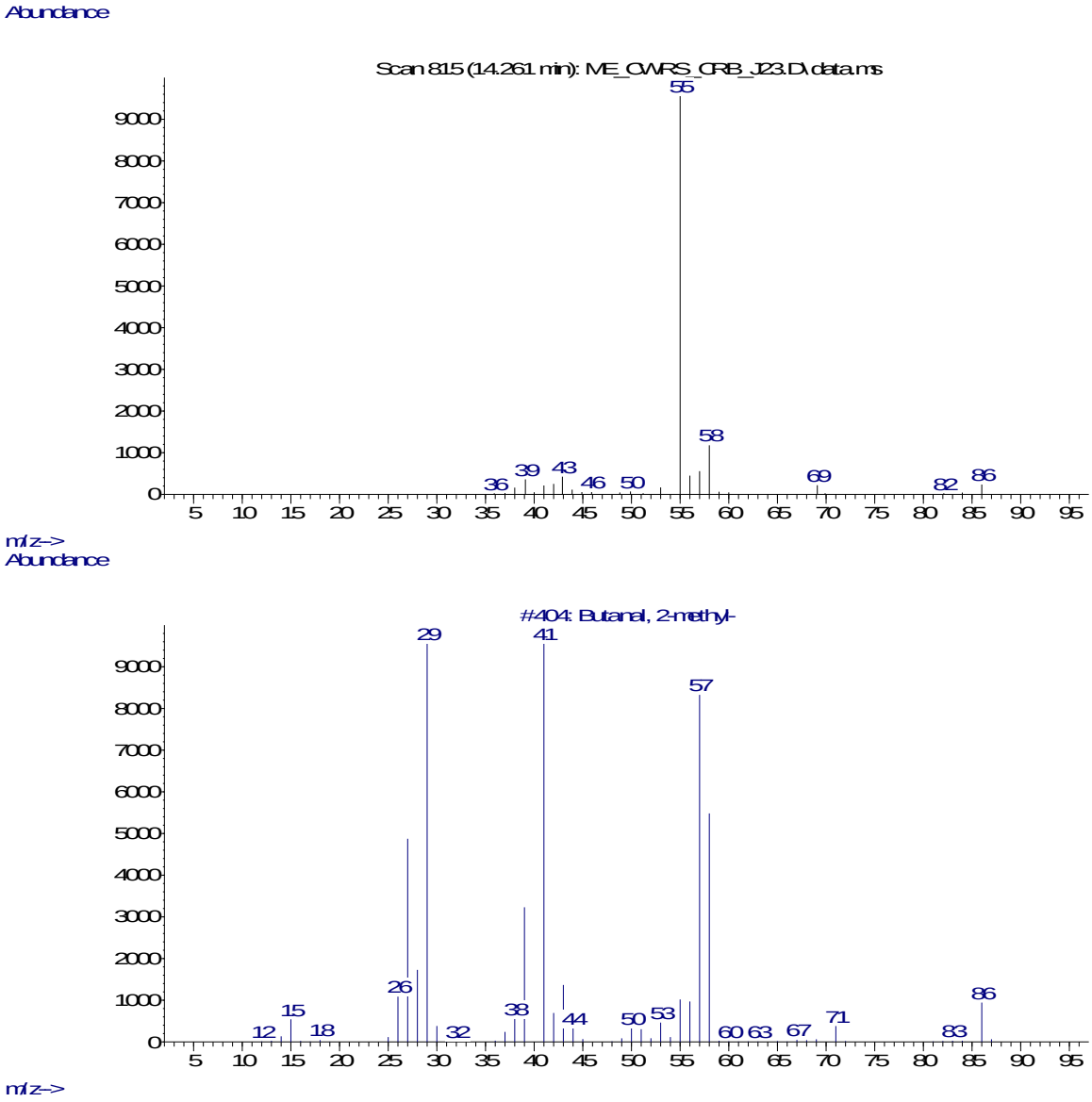
1, 3-butanediol



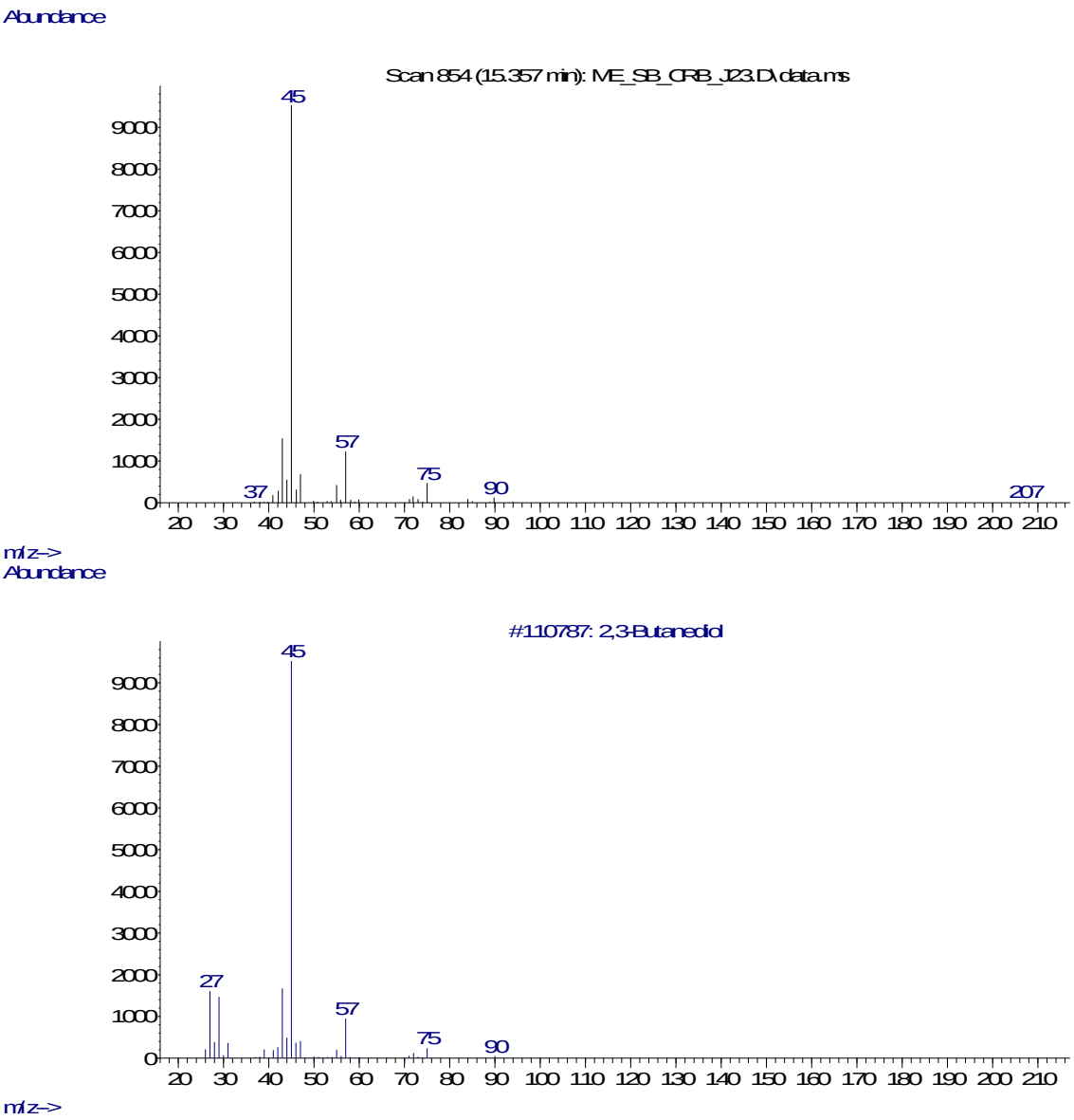
2-hexanol


Abundance
Scan 738 (14.677 min): ME_S_CRUMB_J5_6.D\data.ms
45
69
41
55
84
87
38
51
58
61
75
m/z-->
Abundance
#110904: 2-Hexanol
45
69
41
27
55
84
87
14
19
31
38
51
58
m/z-->

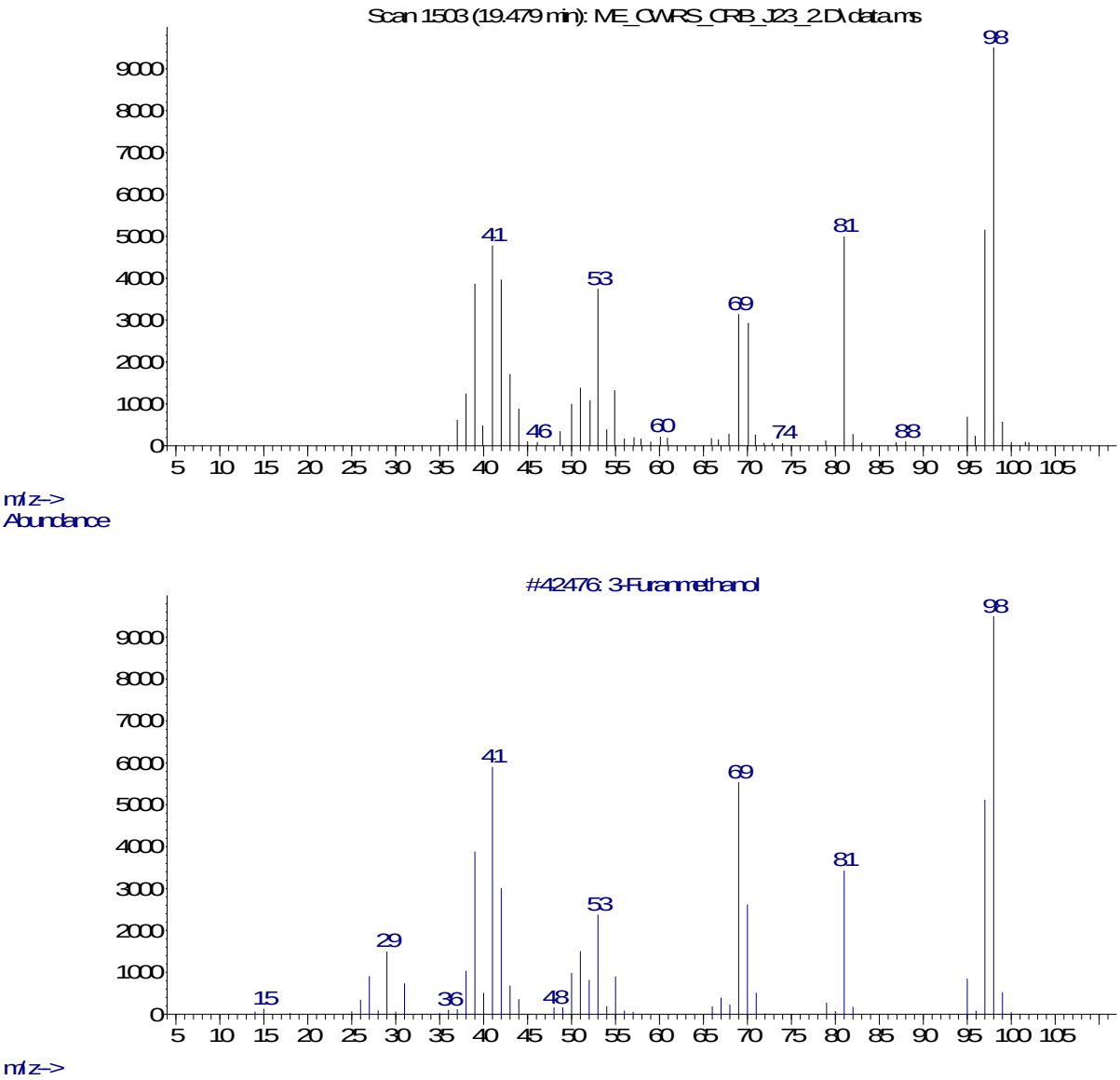
2-methylbutanal



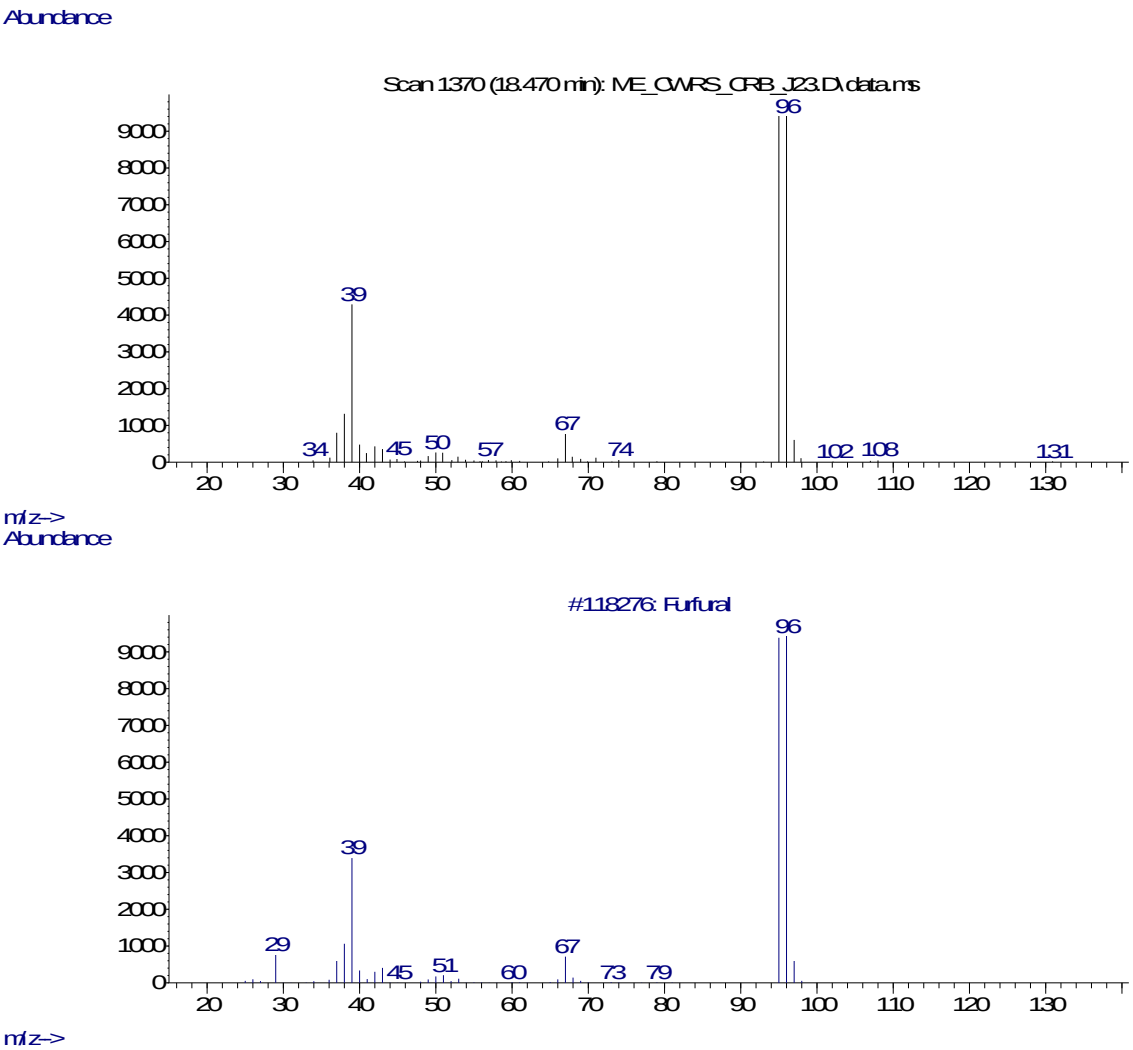
2, 3-butanediol



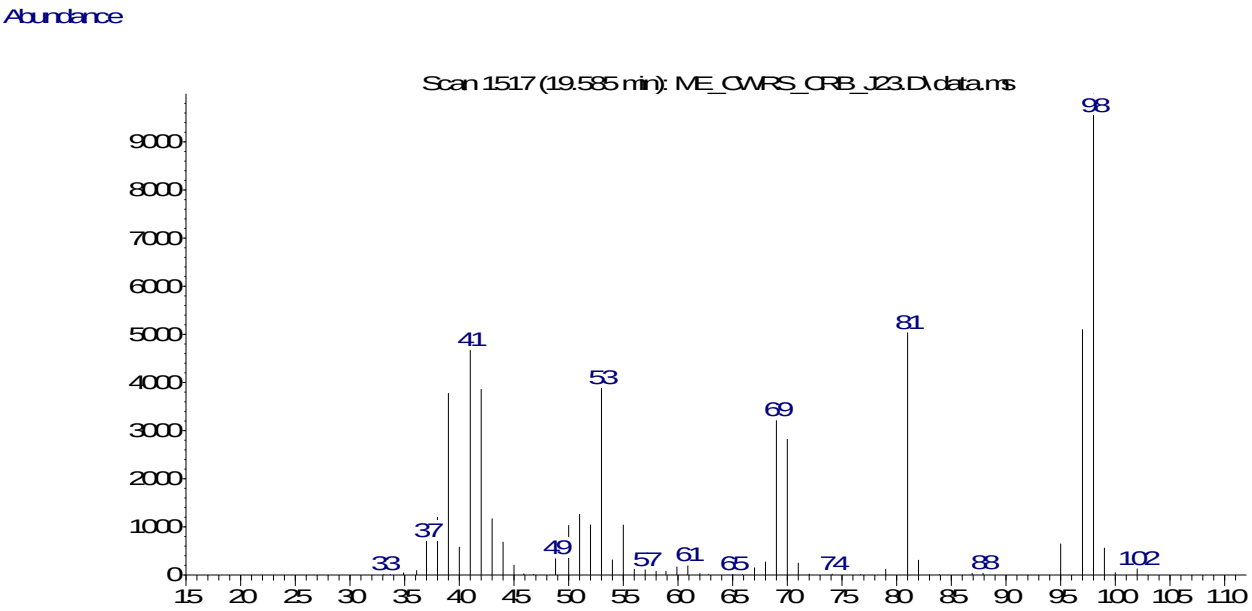
3-furanmethanol

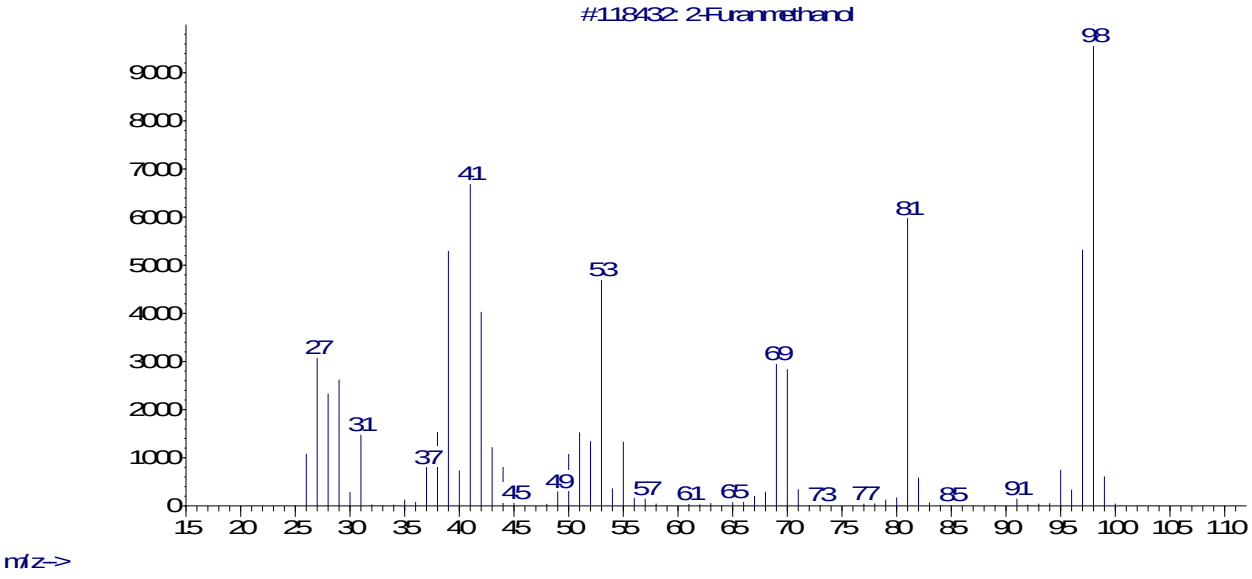


Furfural

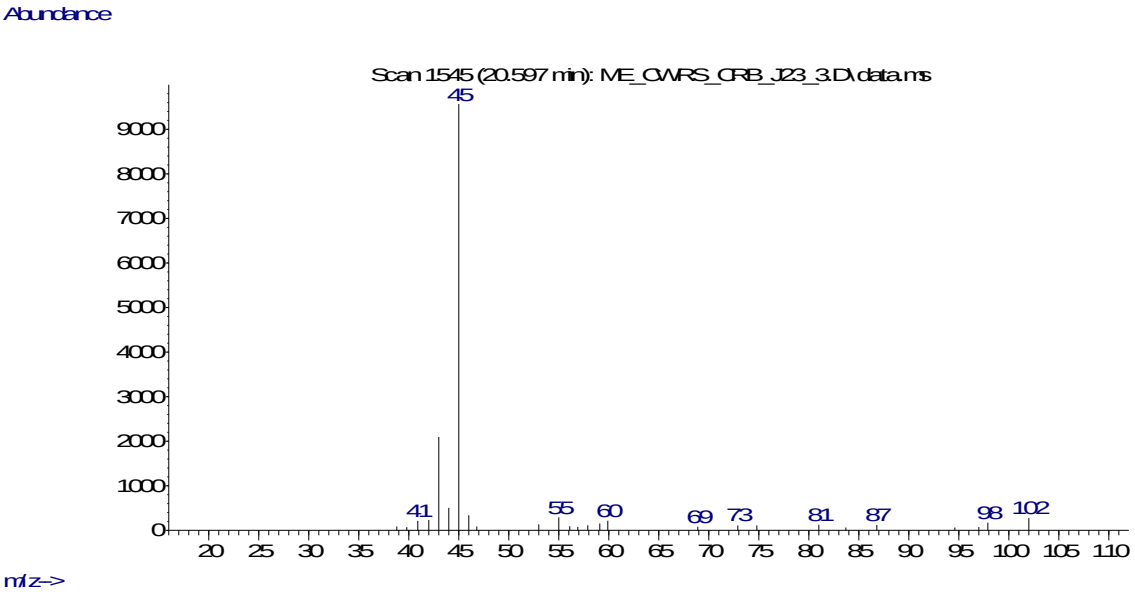

Abundance
Scan 1370 (18.470 min): ME_CWRS_CRB_J23.D\data.ms
96
39
67
34
45
50
57
74
102
108
131
m/z-->
Abundance
#118276: Furfural
96
39
29
67
45
51
60
73
79
m/z-->

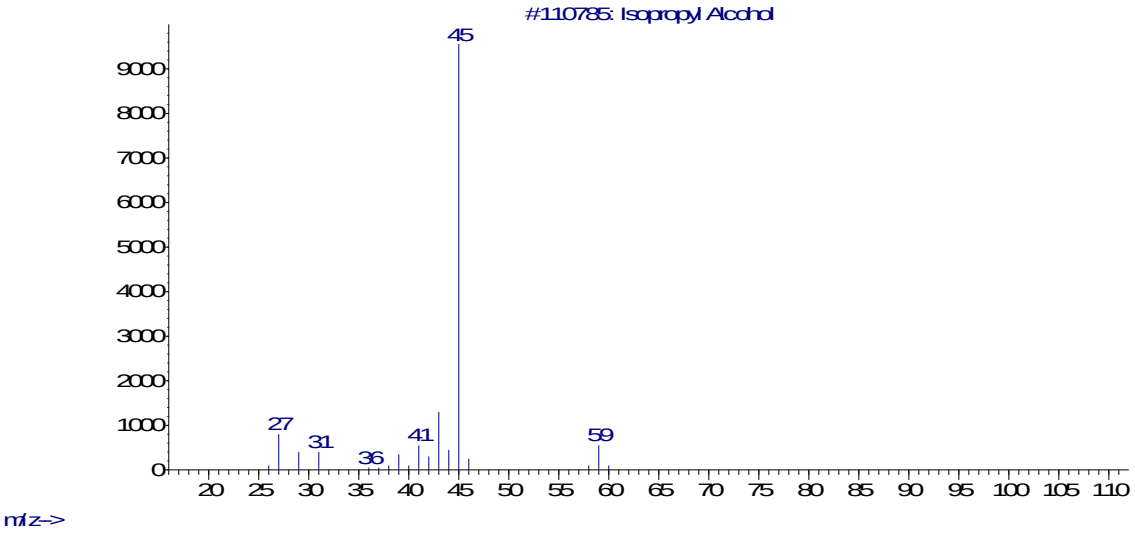
2-furanmethanol



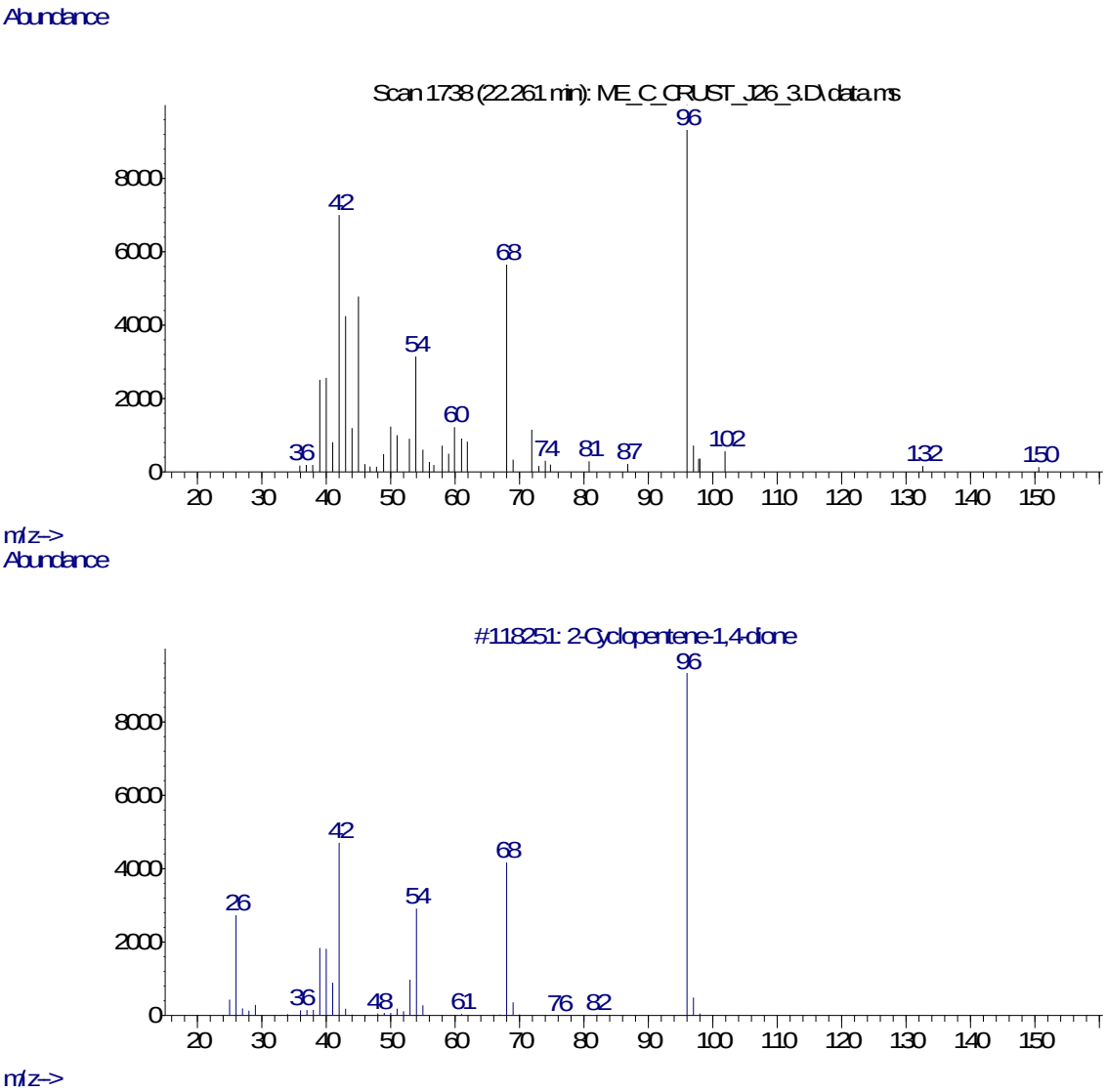


Isopropyl alcohol

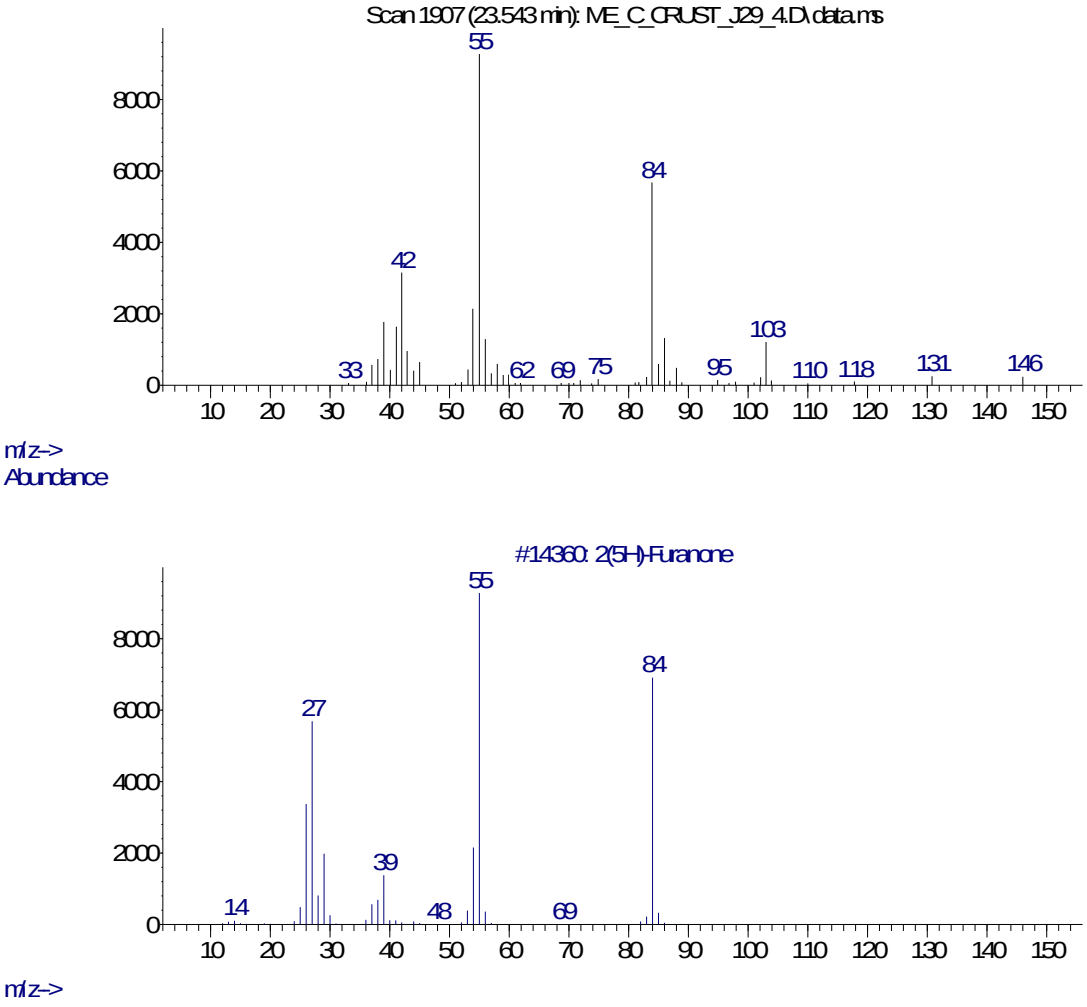




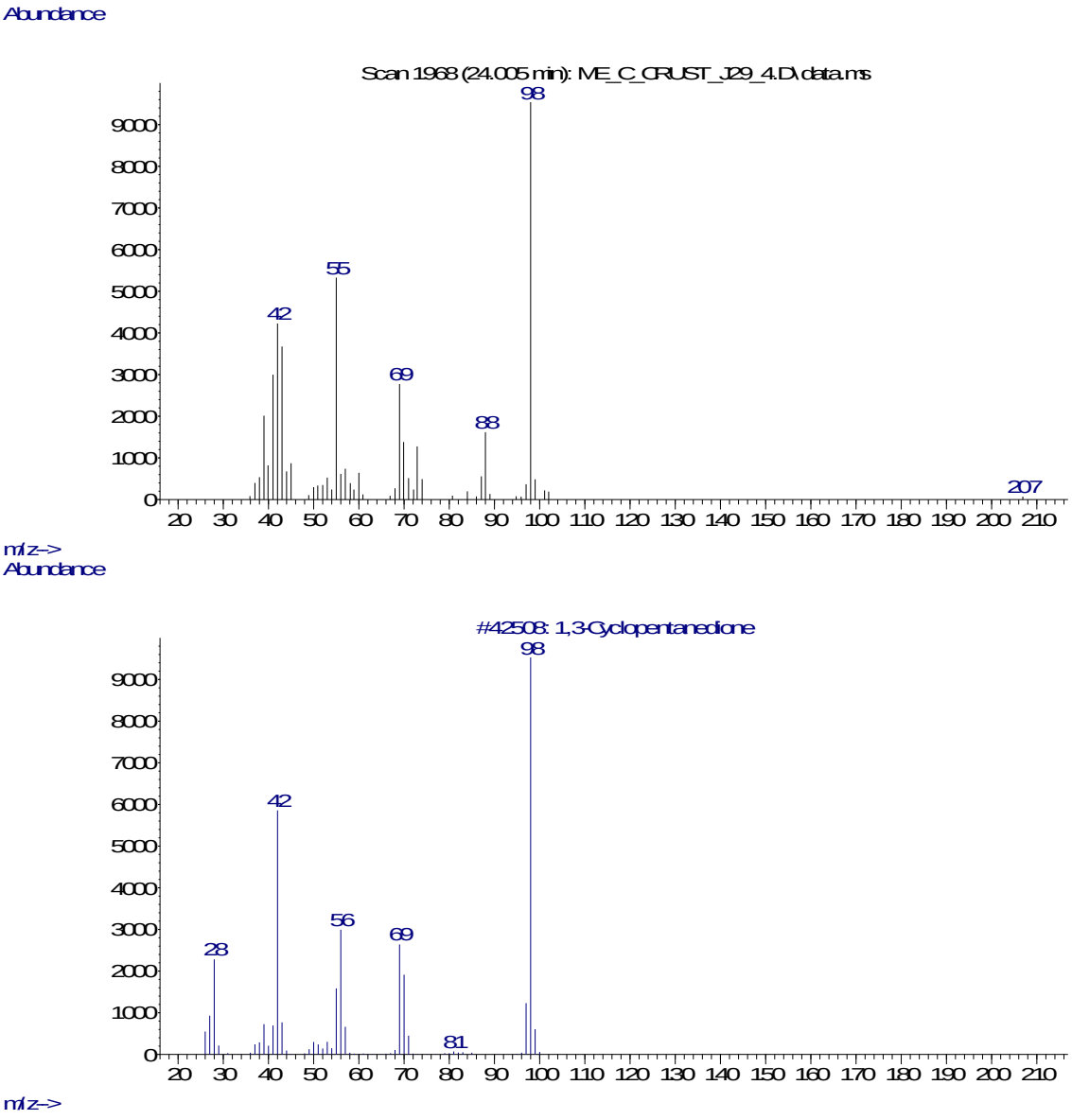
2-cyclopentene-1,4-dione



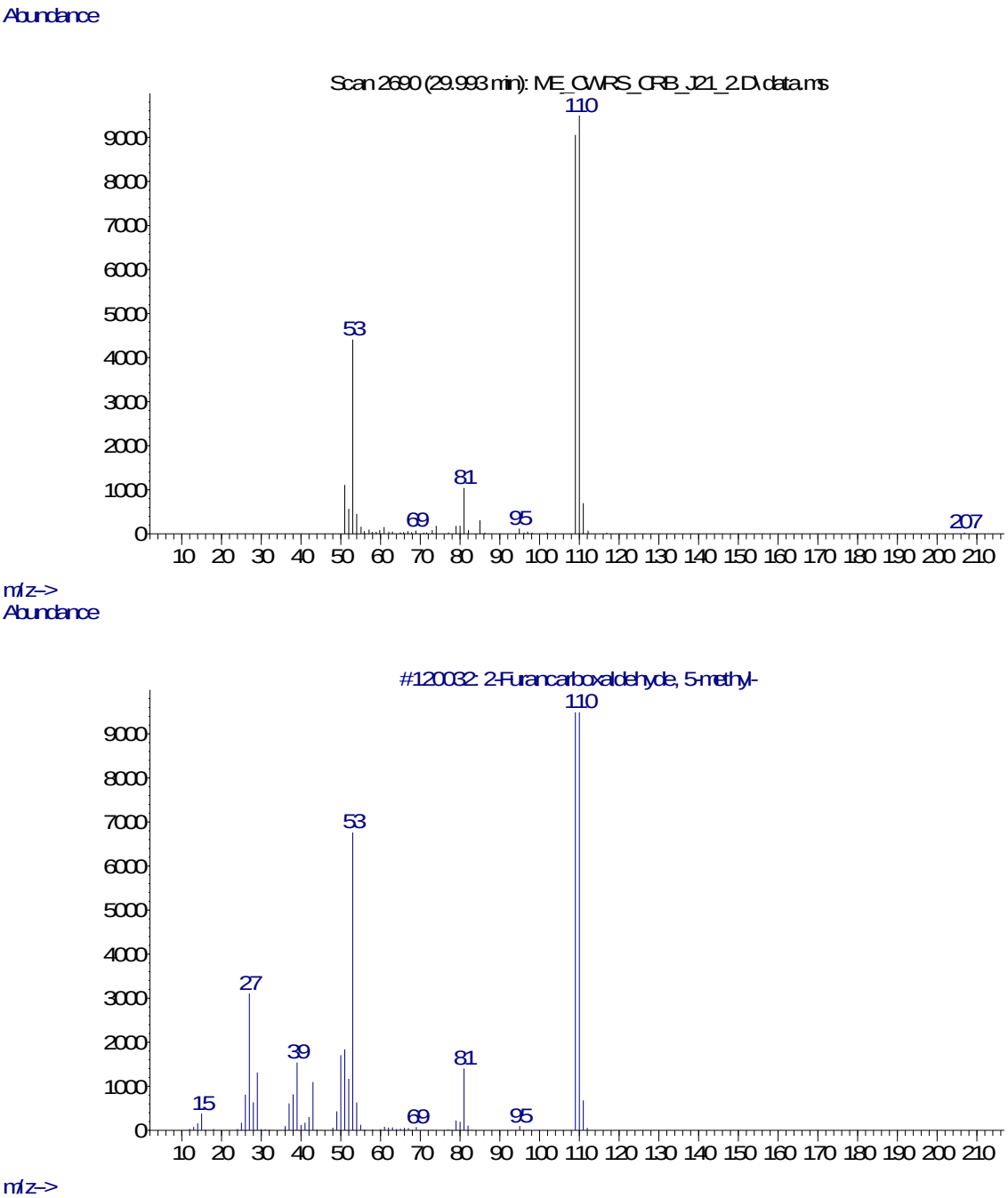
2 (5H)-furanone



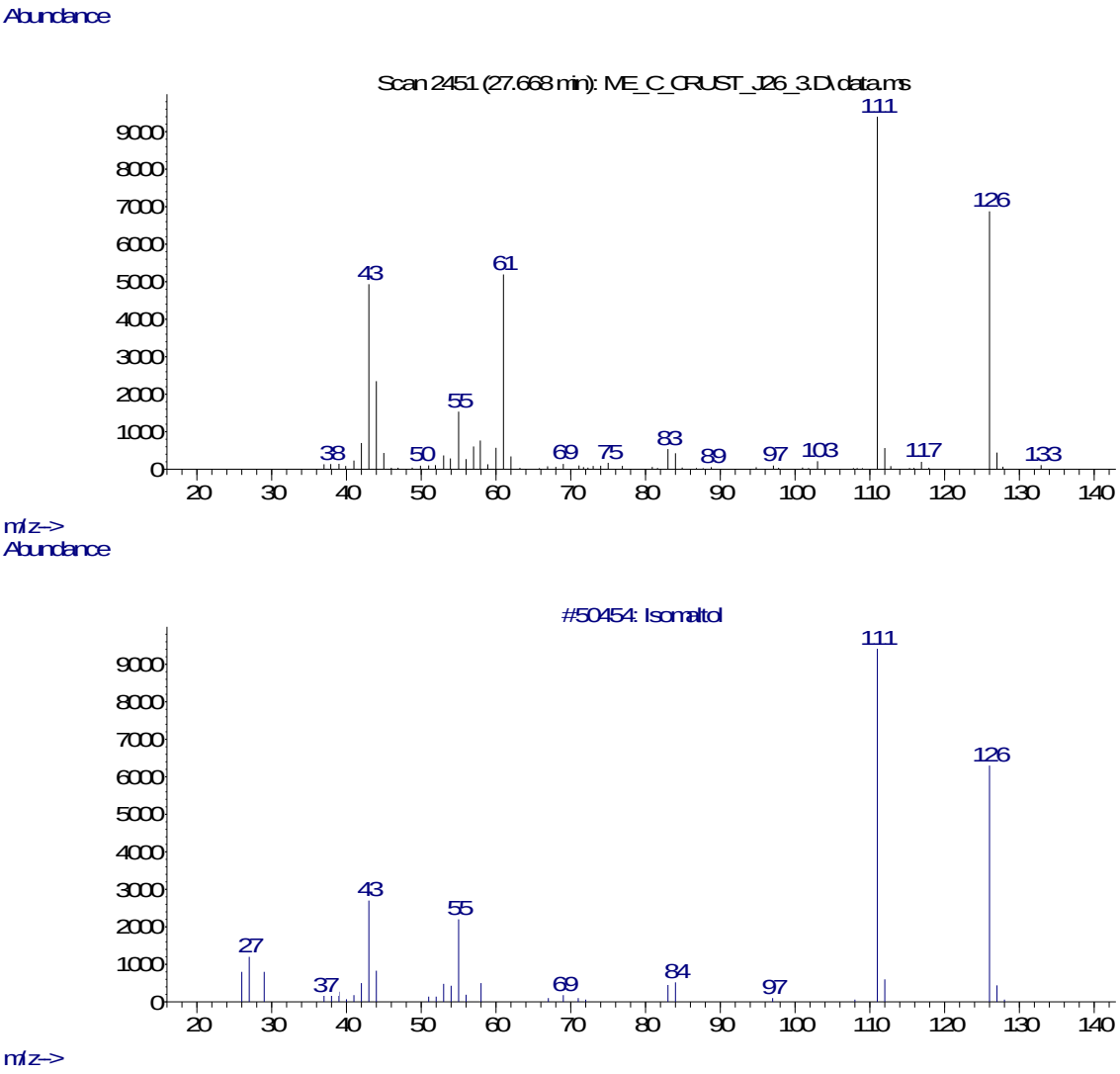
1, 3-cyclopentanedione



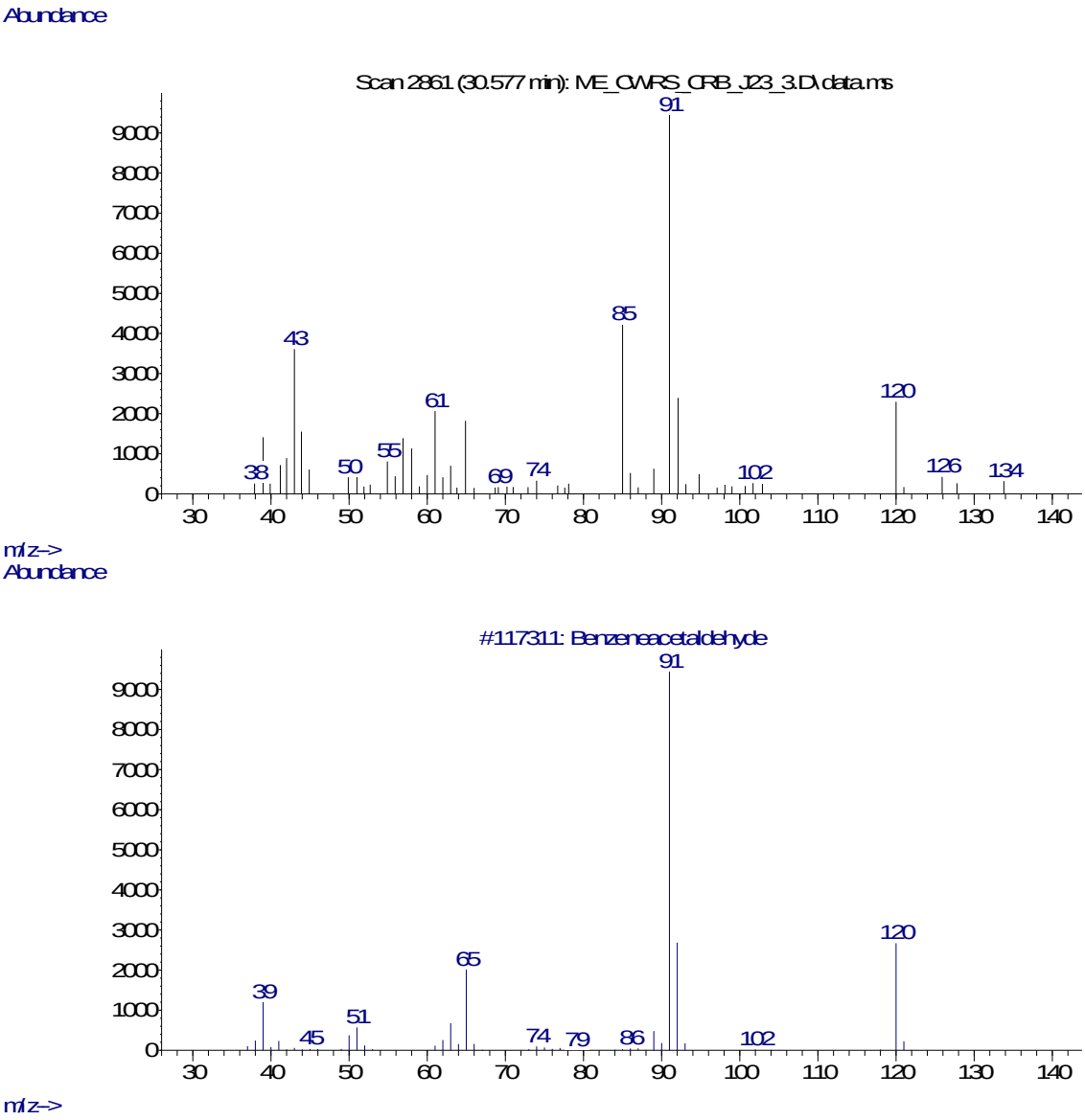
5-methyl, 2-furancarboxaldehyde



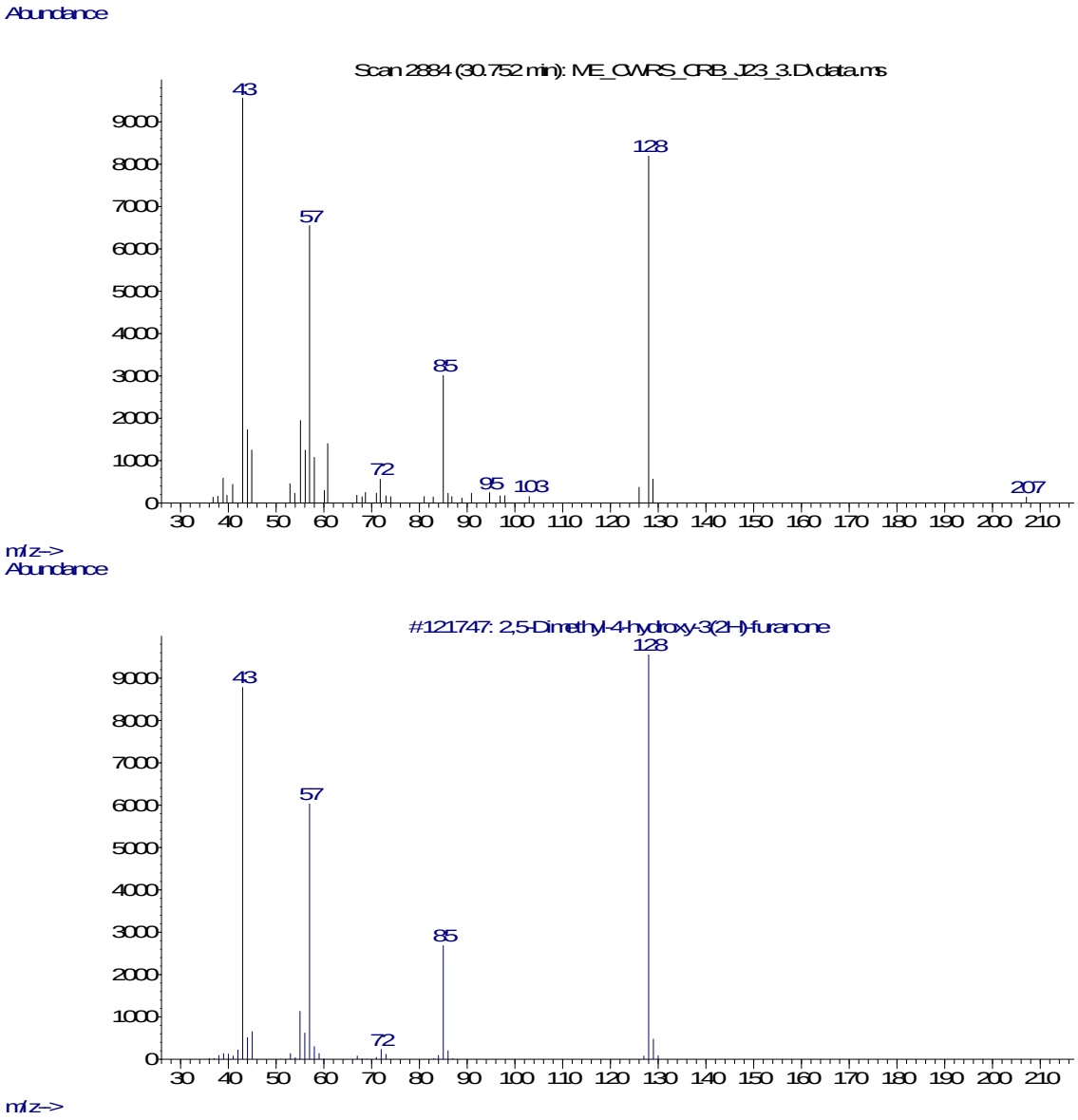
Isomaltol


Abundance
Scan 2451 (27.668 min): ME_C_CRUST_J26_3.D\data.ms
111
126
43
61
55
38
50
69
75
83
89
97
103
117
133
m/z-->
Abundance
#50454: Isomaltol
111
126
43
55
27
37
69
84
97
m/z-->

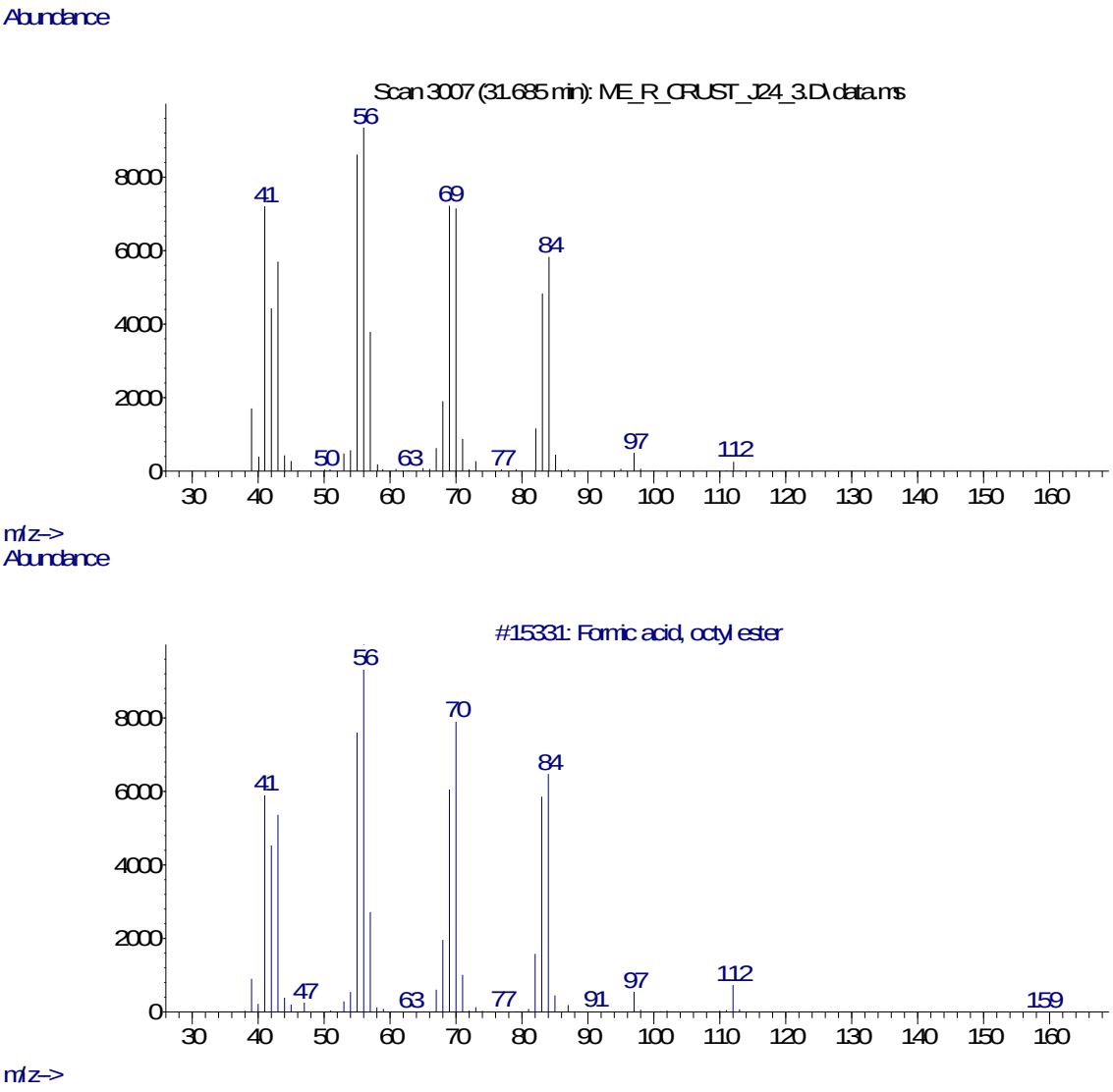
Benzeneacetaldehyde



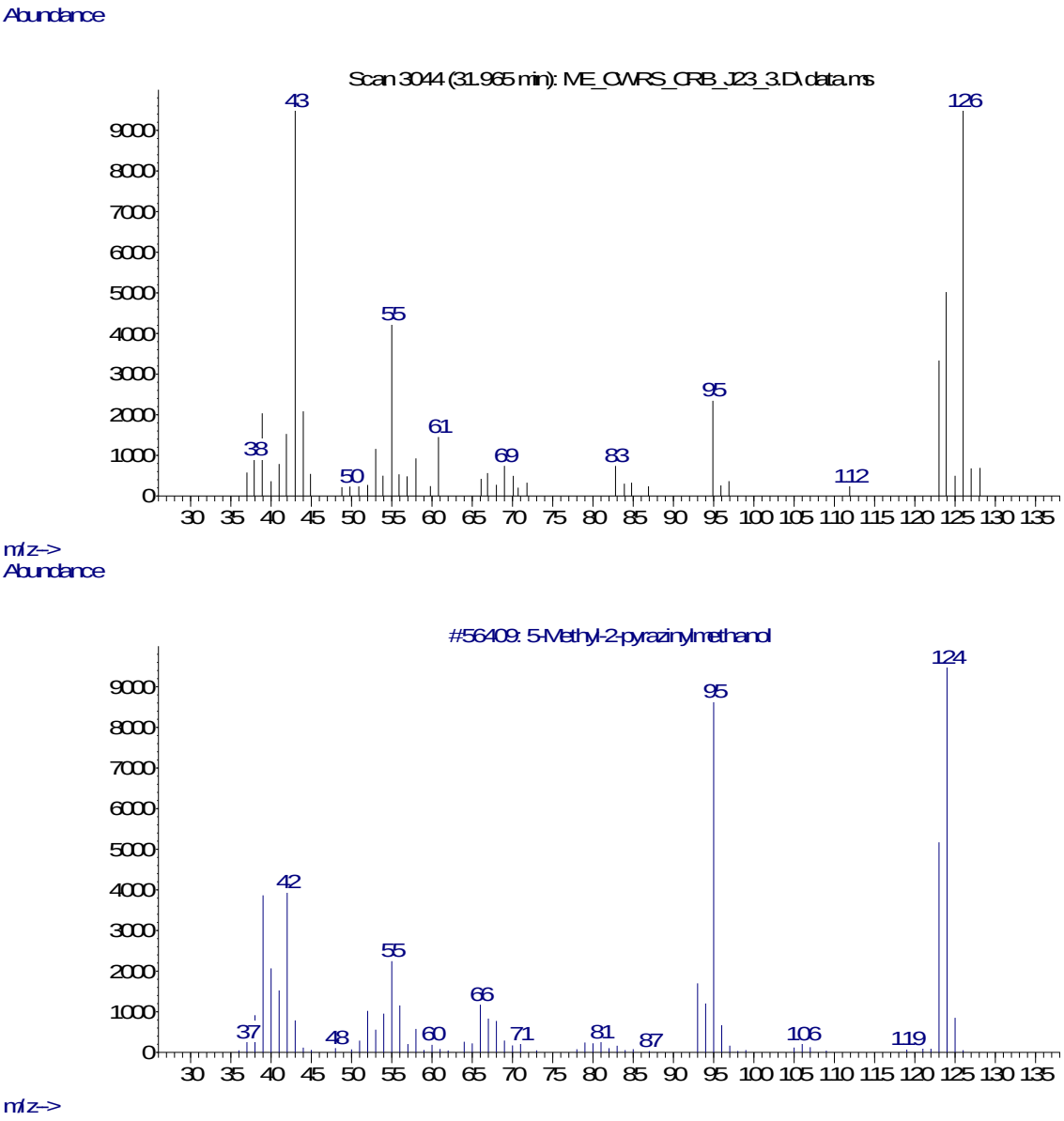
4-hydroxy, 2,5-dimethyl, 3(2H)-furanone



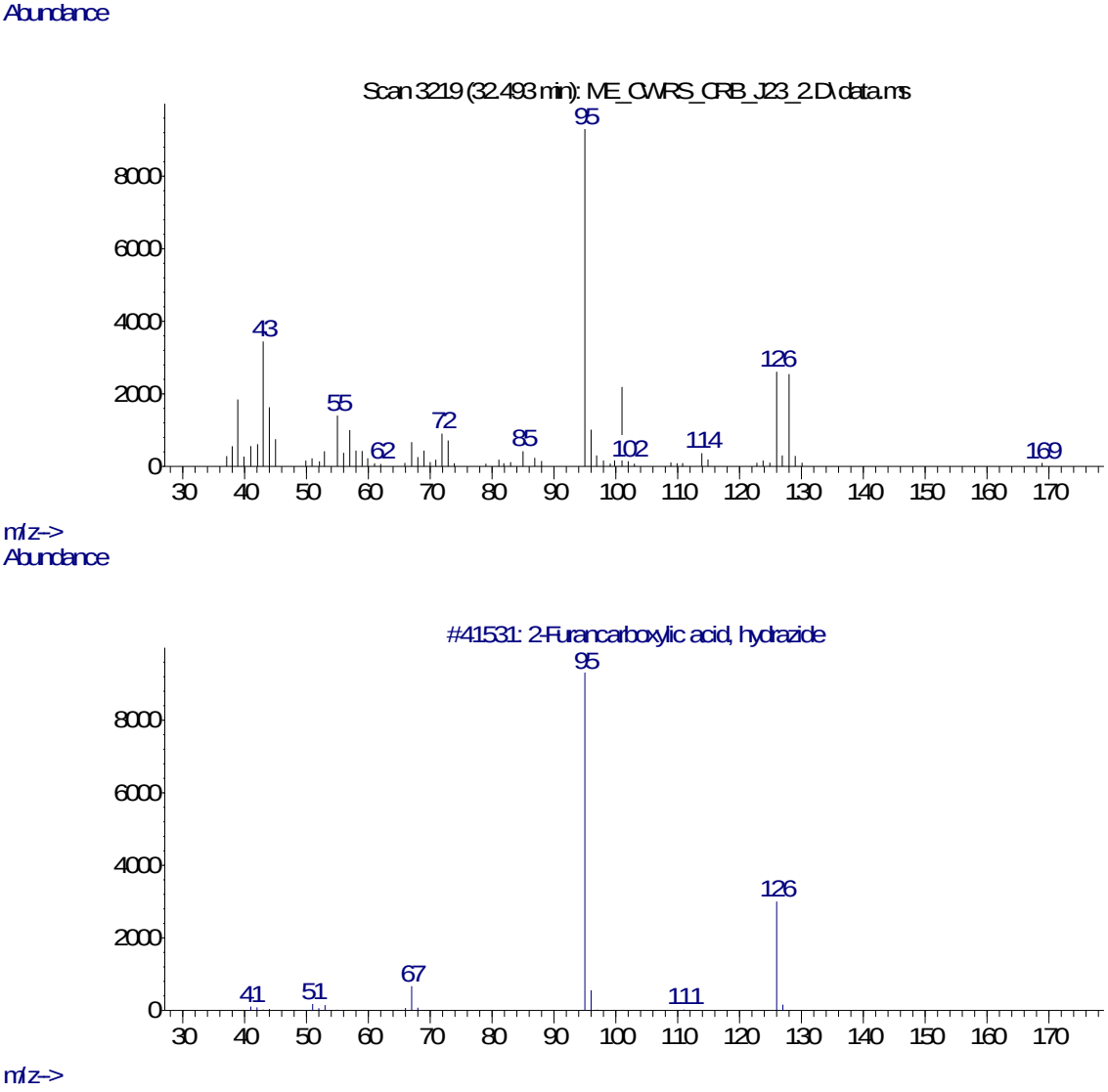
Formic acid


Abundance
Scan 3007 (31.685 min): ME_R_CRUST_J24_3.D\data.ms
56
41
69
84
97
112
50
63
77
8000
6000
4000
2000
0
30 40 50 60 70 80 90 100 110 120 130 140 150 160
m/z-->
Abundance
#15331: Formic acid, octyl ester
56
70
84
41
47
63
77
91
97
112
159
8000
6000
4000
2000
0
30 40 50 60 70 80 90 100 110 120 130 140 150 160
m/z-->

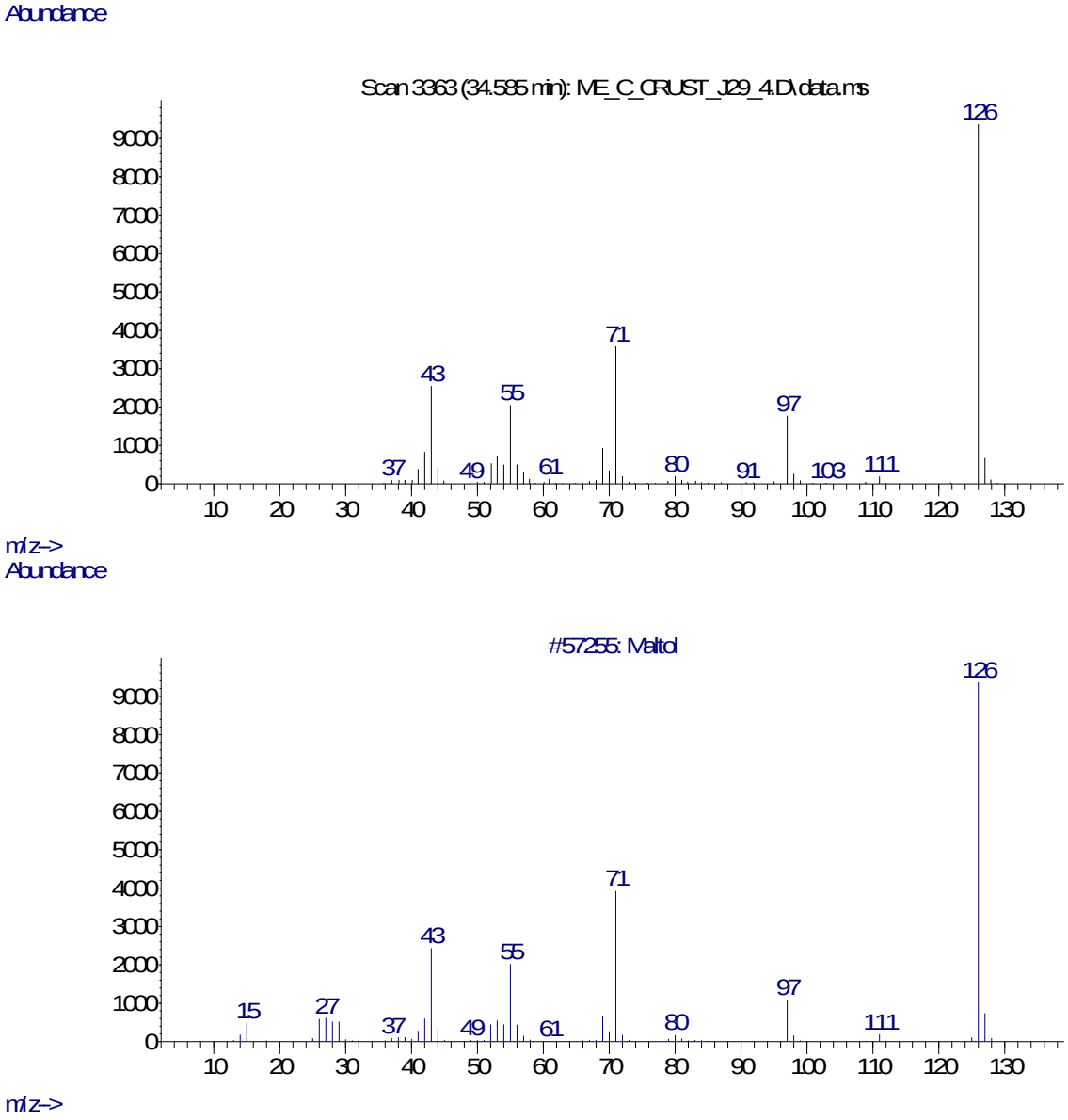
5-methyl, 2-pyrazinylmethanol



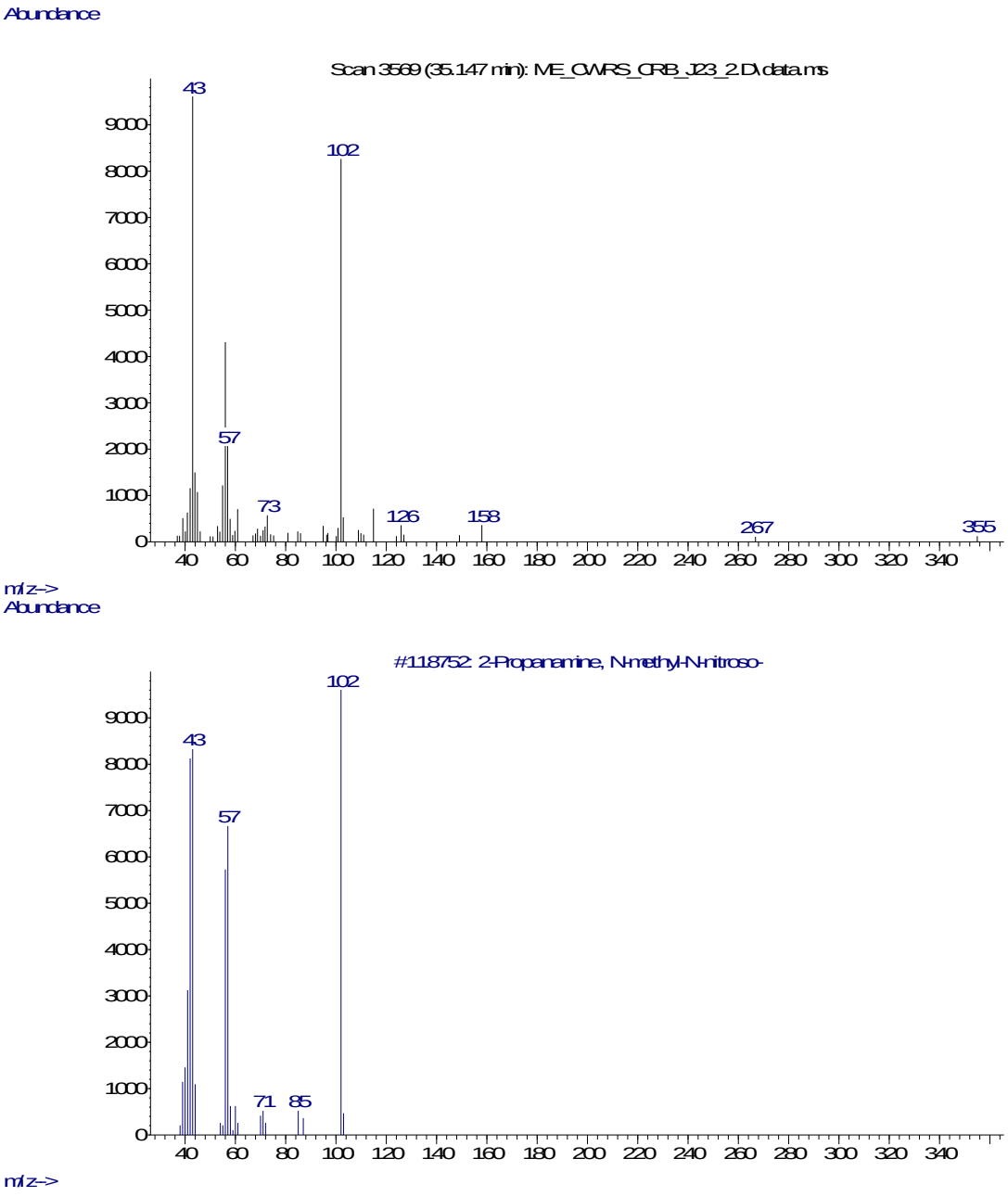
2-furancarboxylic acid



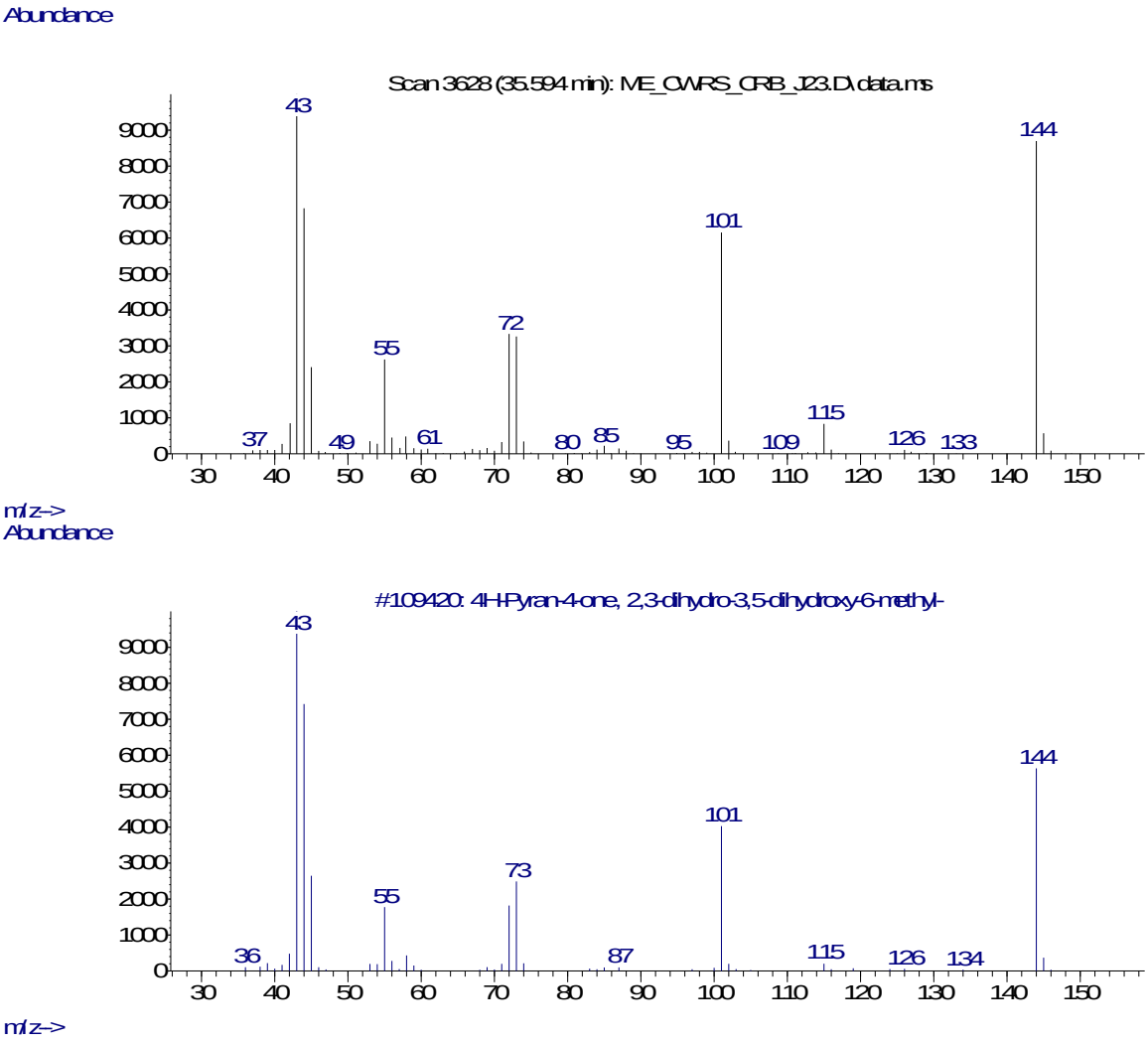
Maltol


Abundance
Scan 3363 (34.585 min): ME_C_CRUST_J29_4.D\data.ms
126
71
43
55
97
37
49
61
80
91
103
111
m/z-->
Abundance
#57256: Maltol
126
71
43
55
97
15
27
37
49
61
80
111
m/z-->

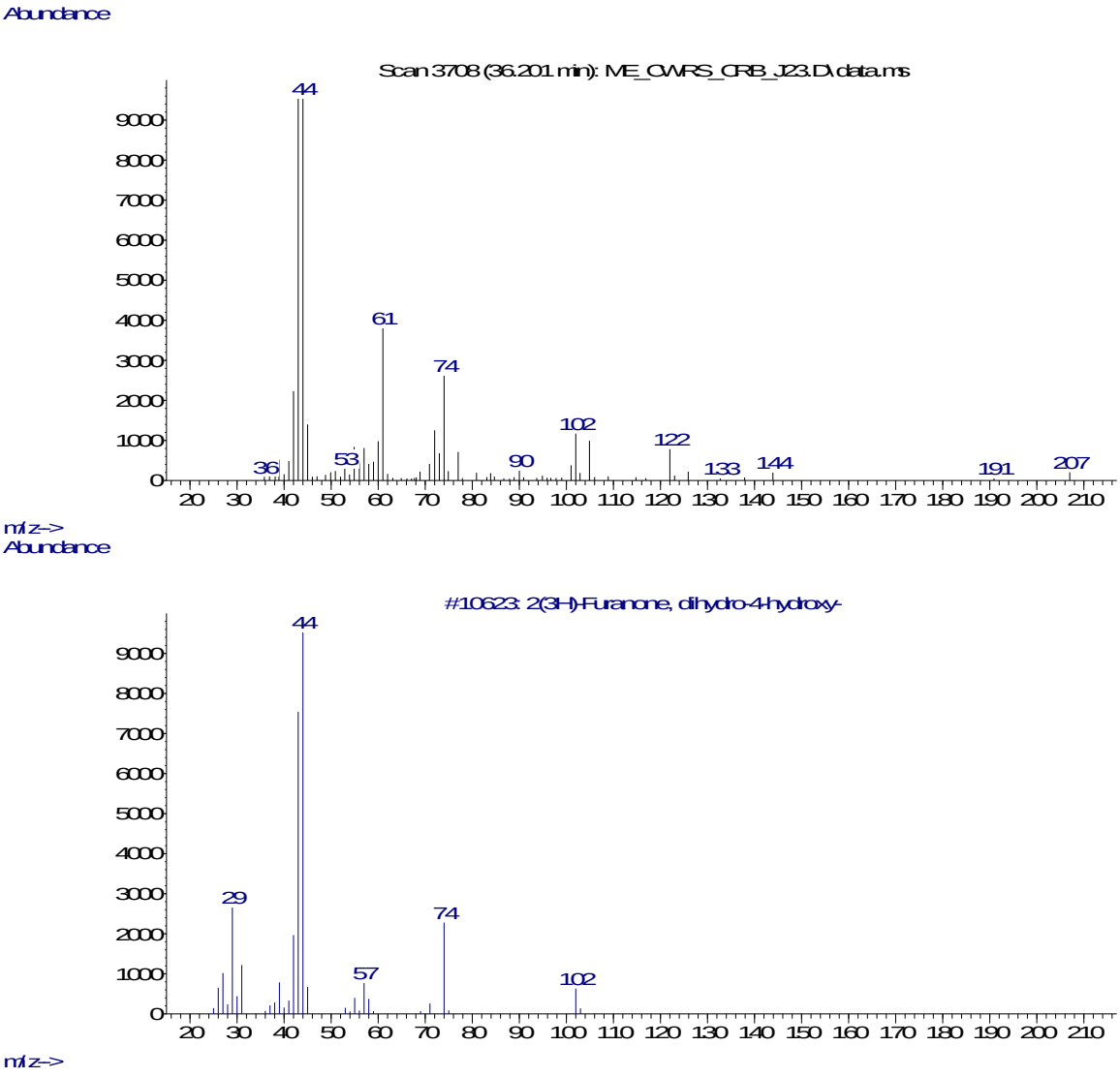
N-methyl-N-nitroso-2-propamine



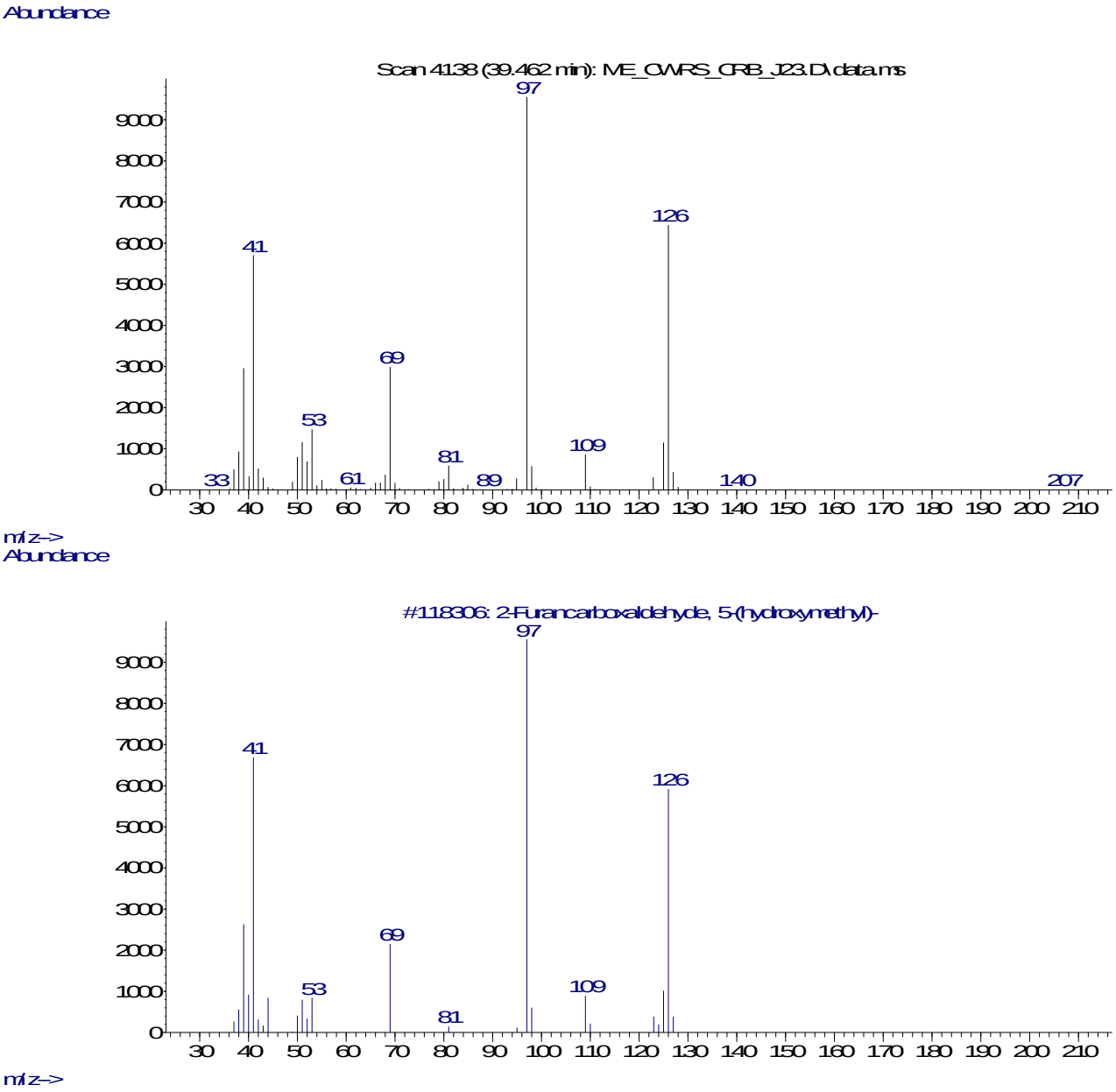
2, 3-dihydro-3,5-dihydroxy,6-methyl-4H-pyran-4-one (pyranone)



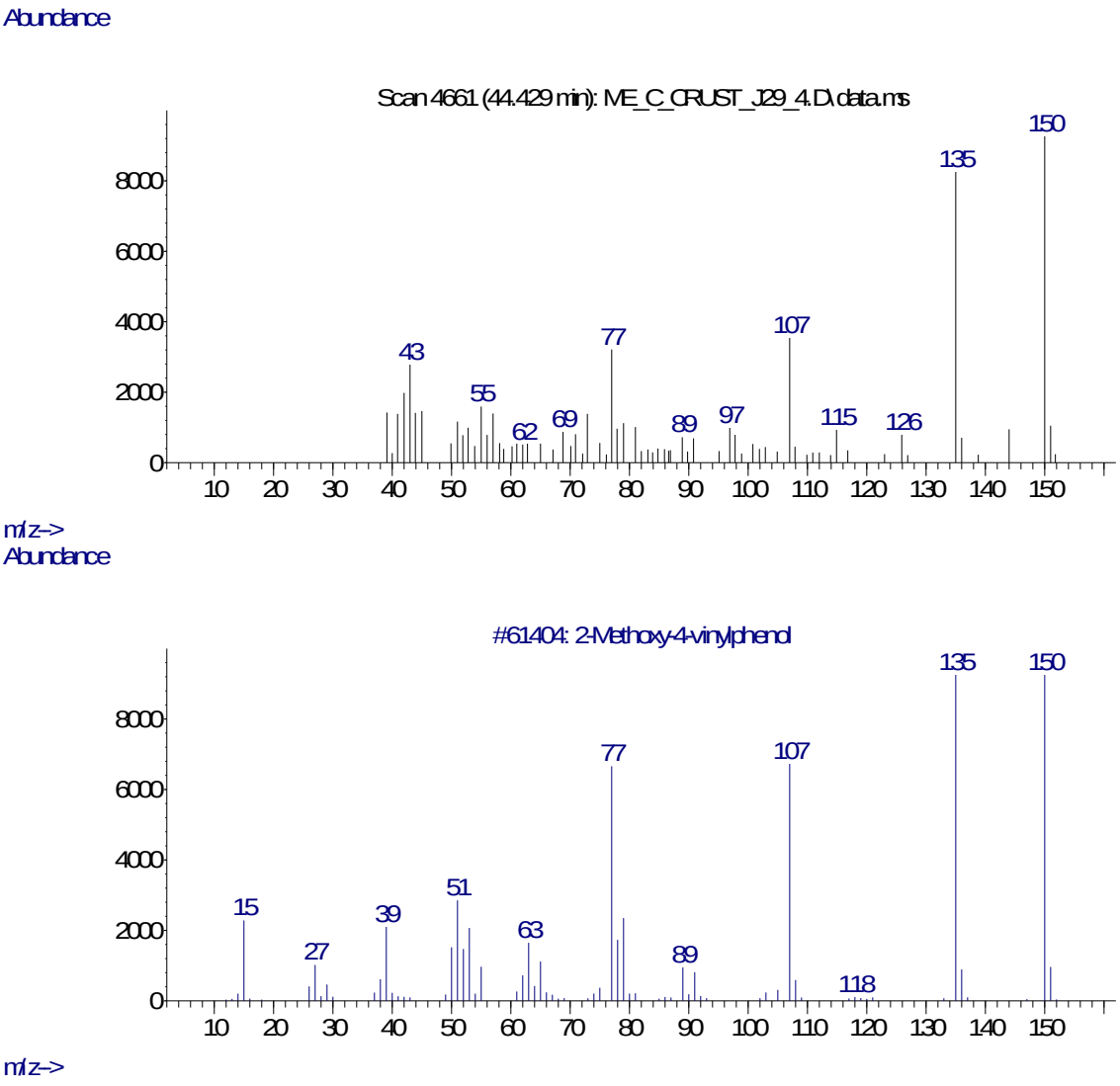
2 3(H)-furanone



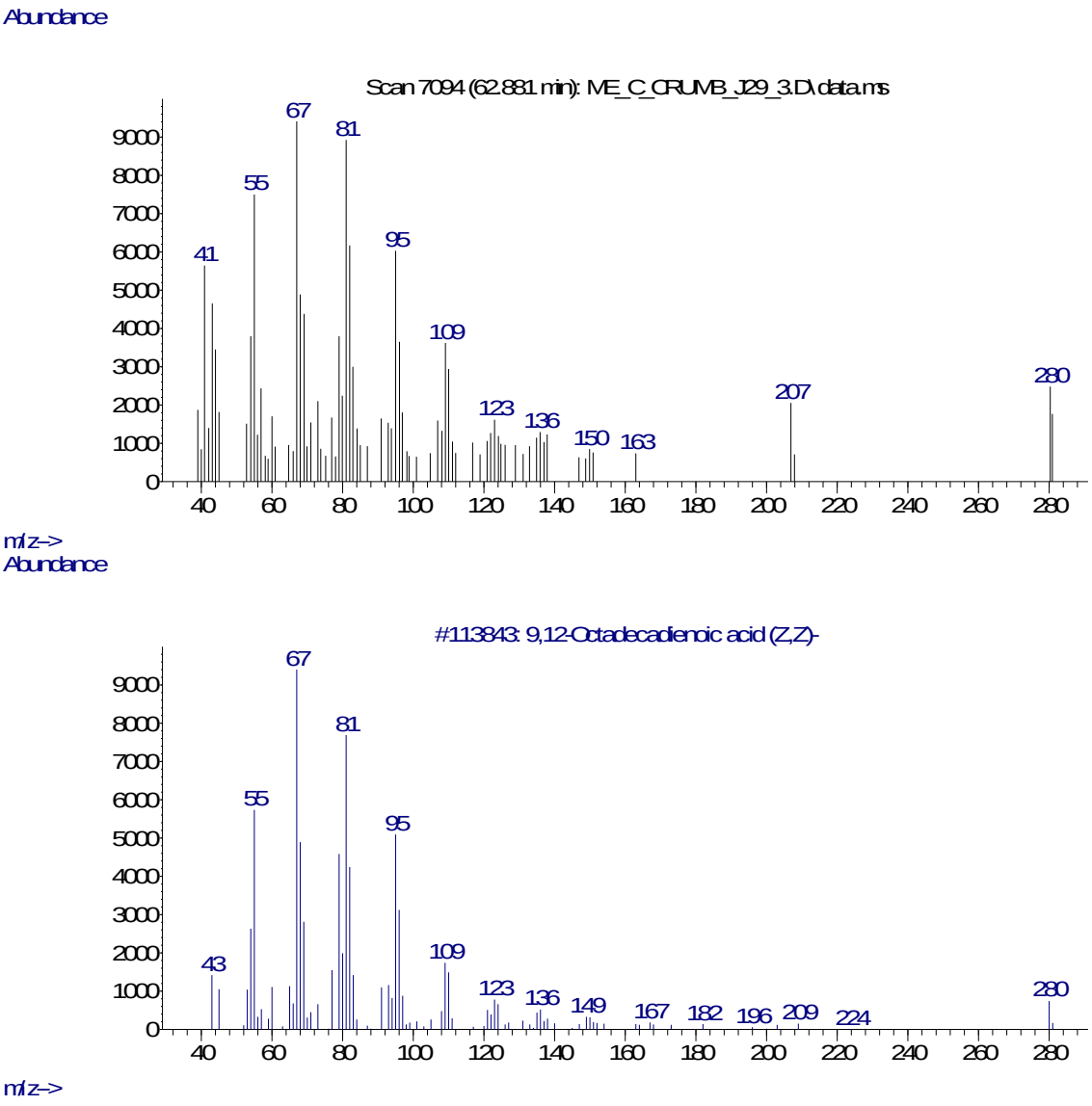
5-hydroxymethyl, 2-furancarboxaldehyde (HMF)



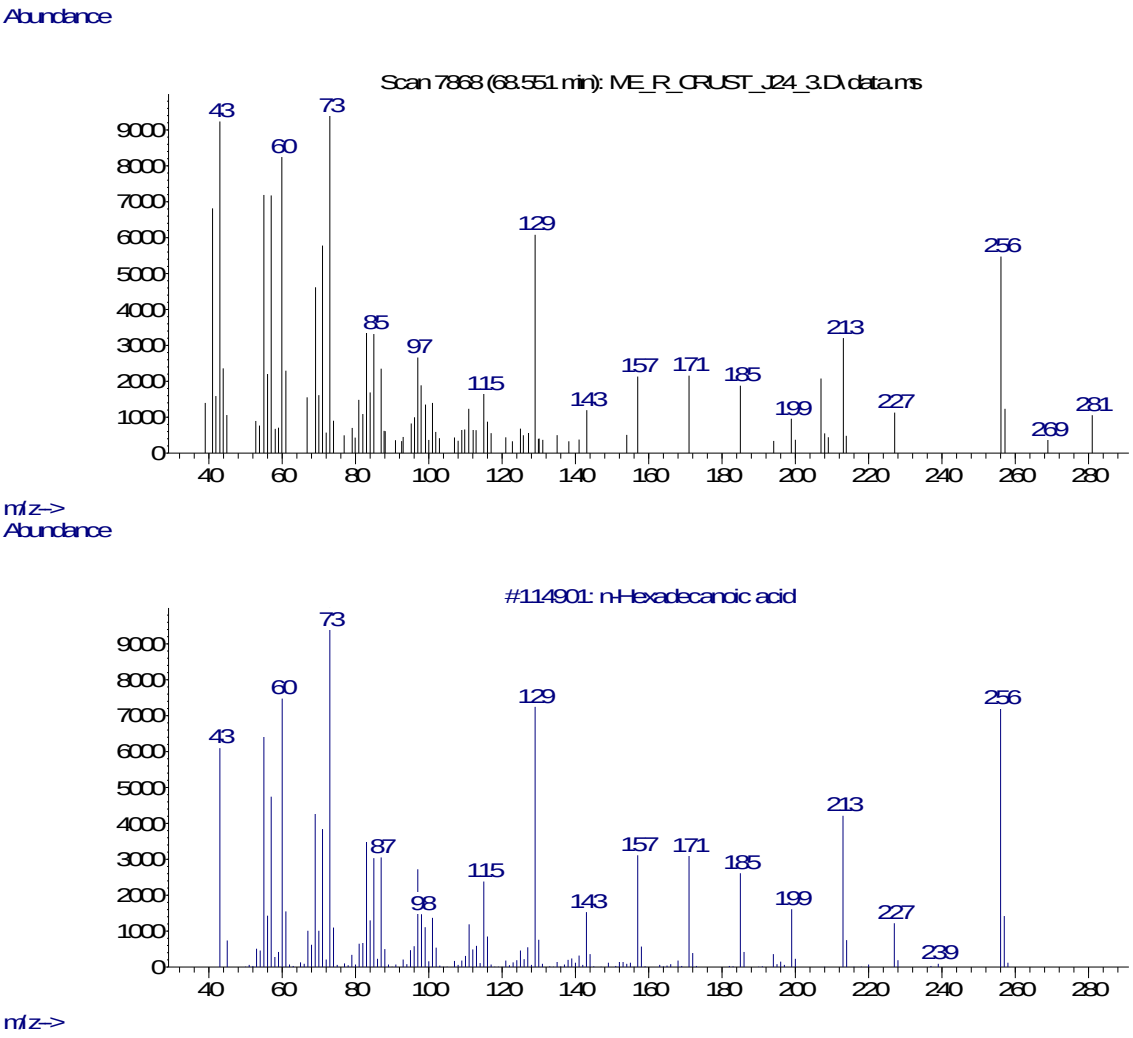
2-methoxy, 4-vinylphenol



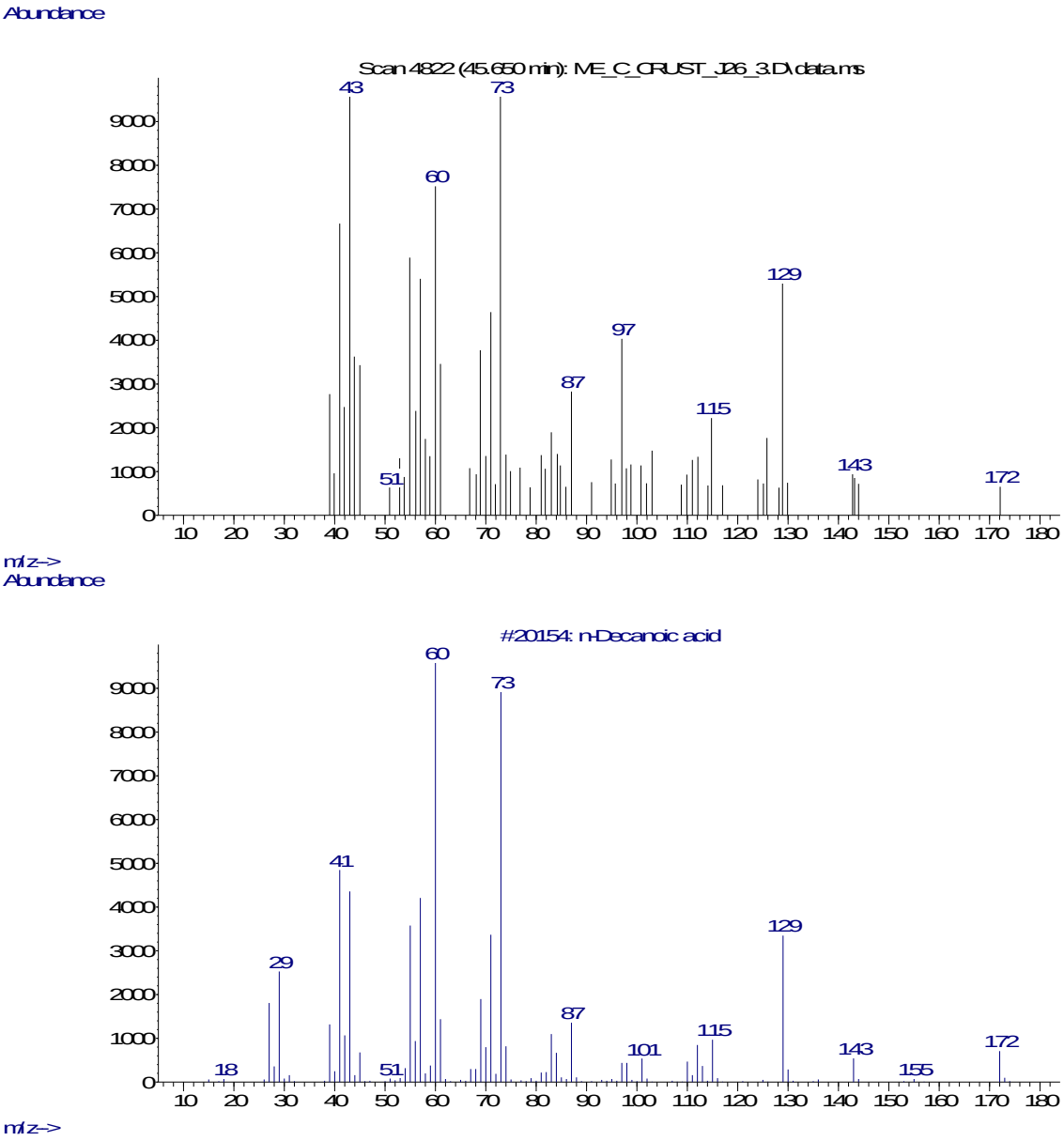
(Z,Z)- 9, 12, octadecadienoic acid



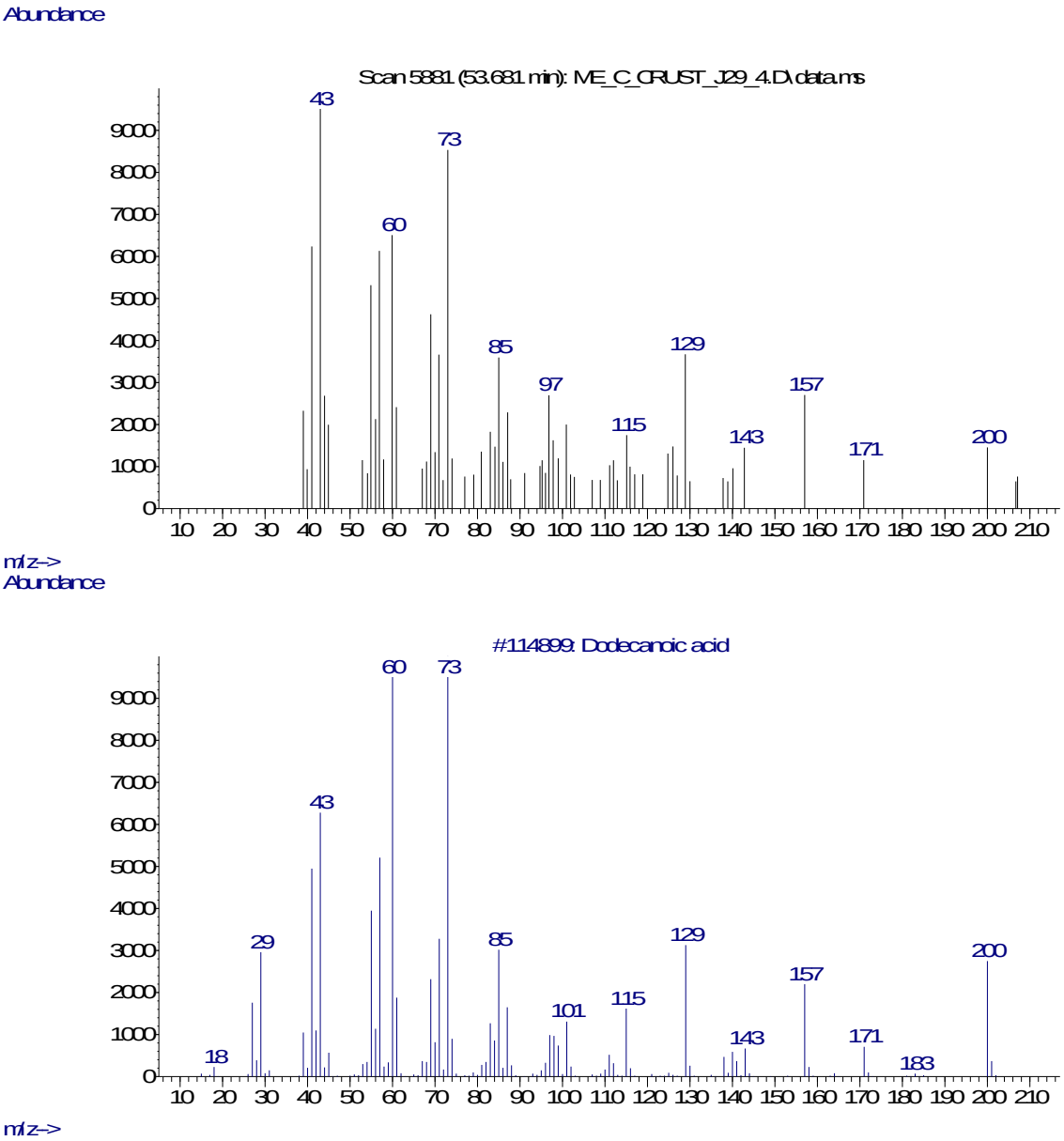
n-hexadecanoic acid



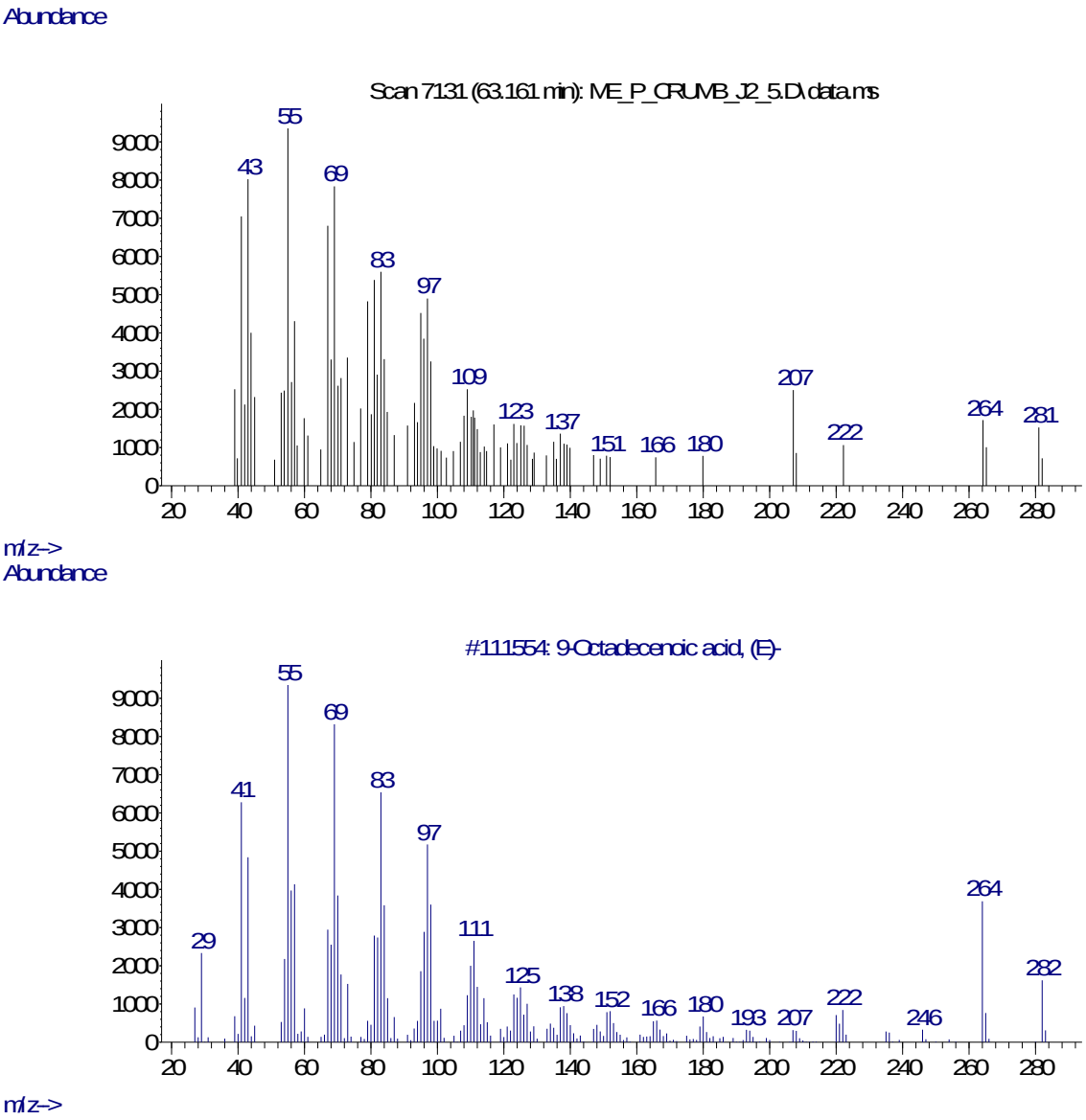
n-decanoic acid



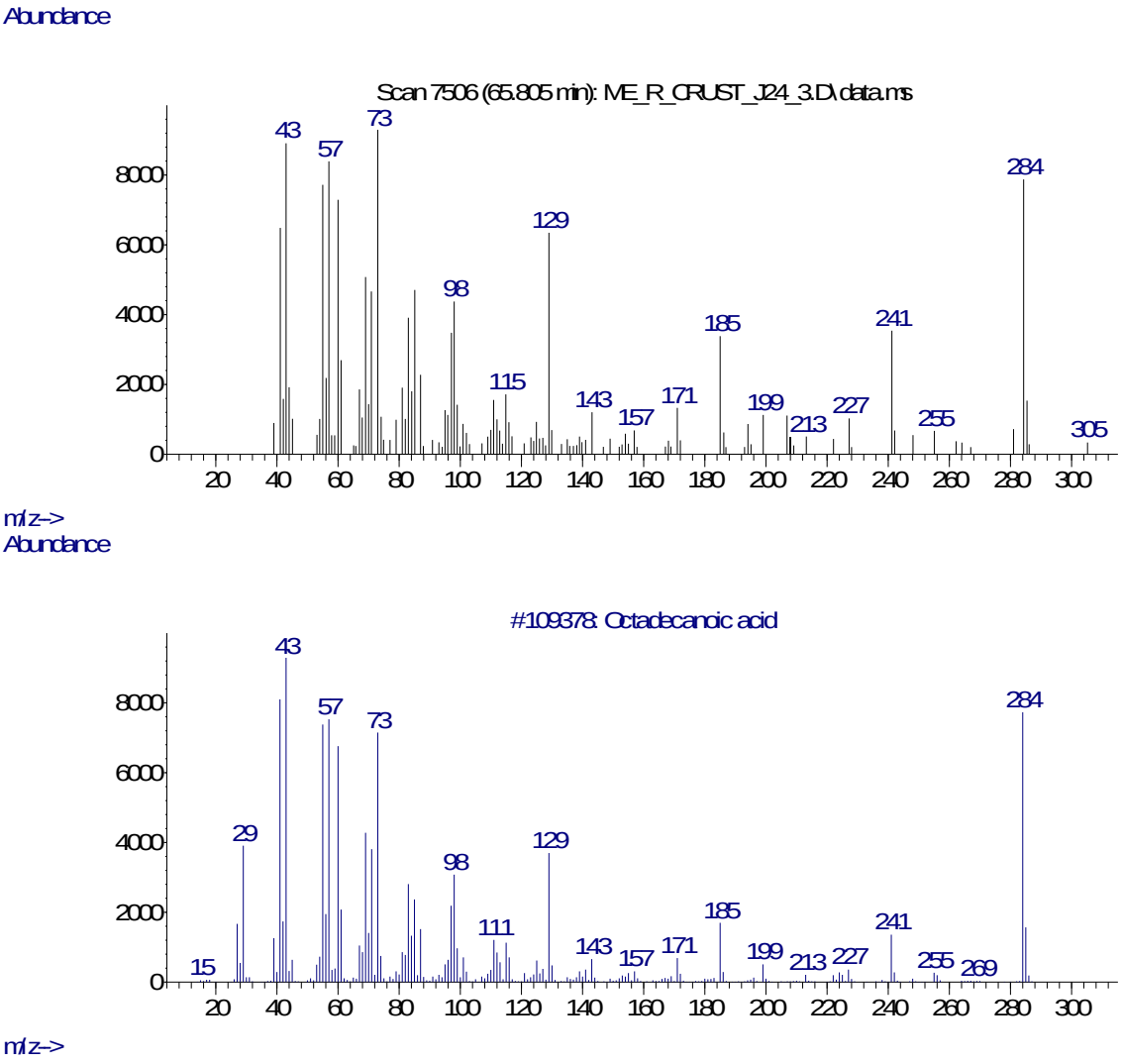
Dodecanoic acid



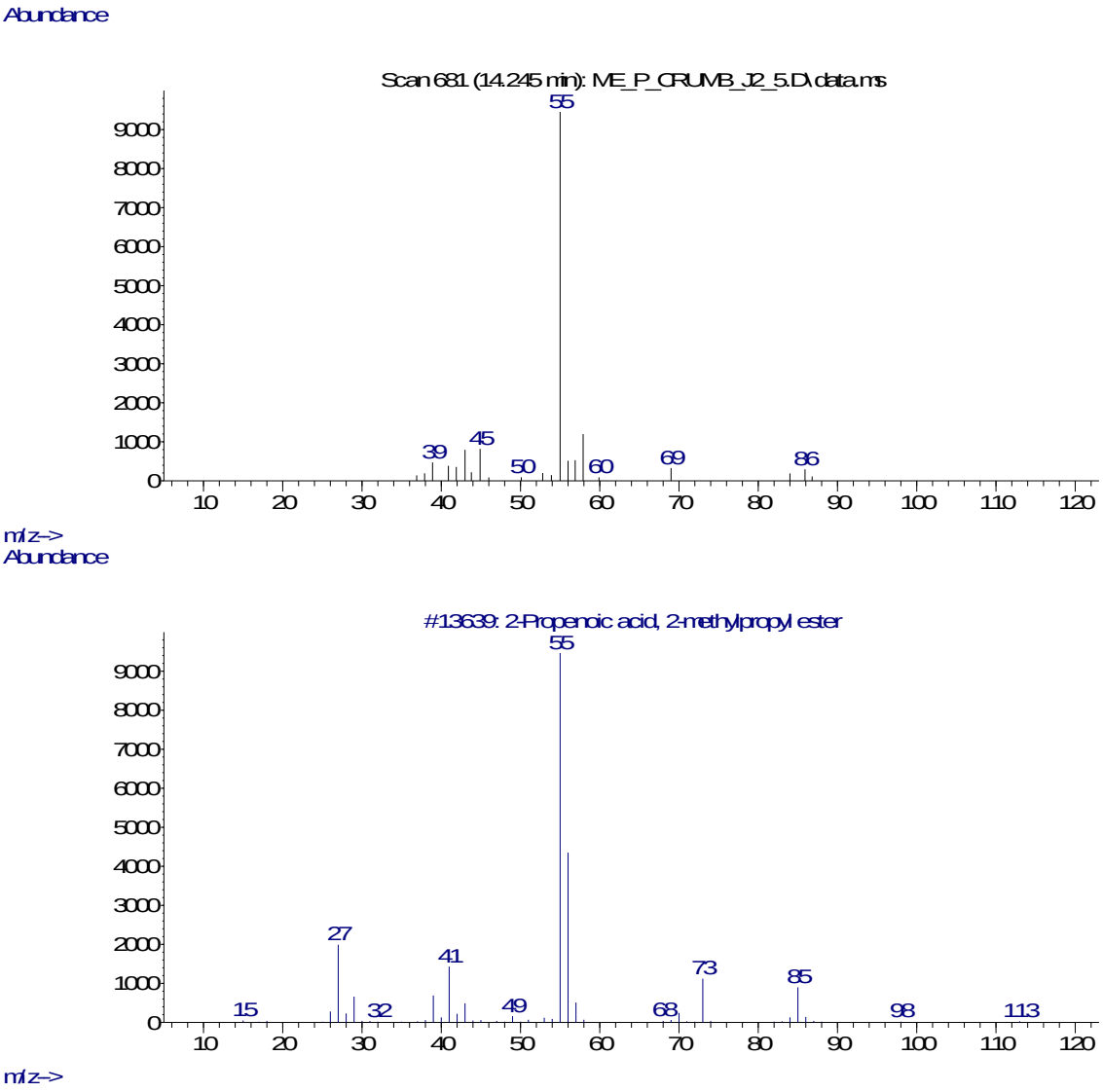
(E)- 9-octadecenoic acid



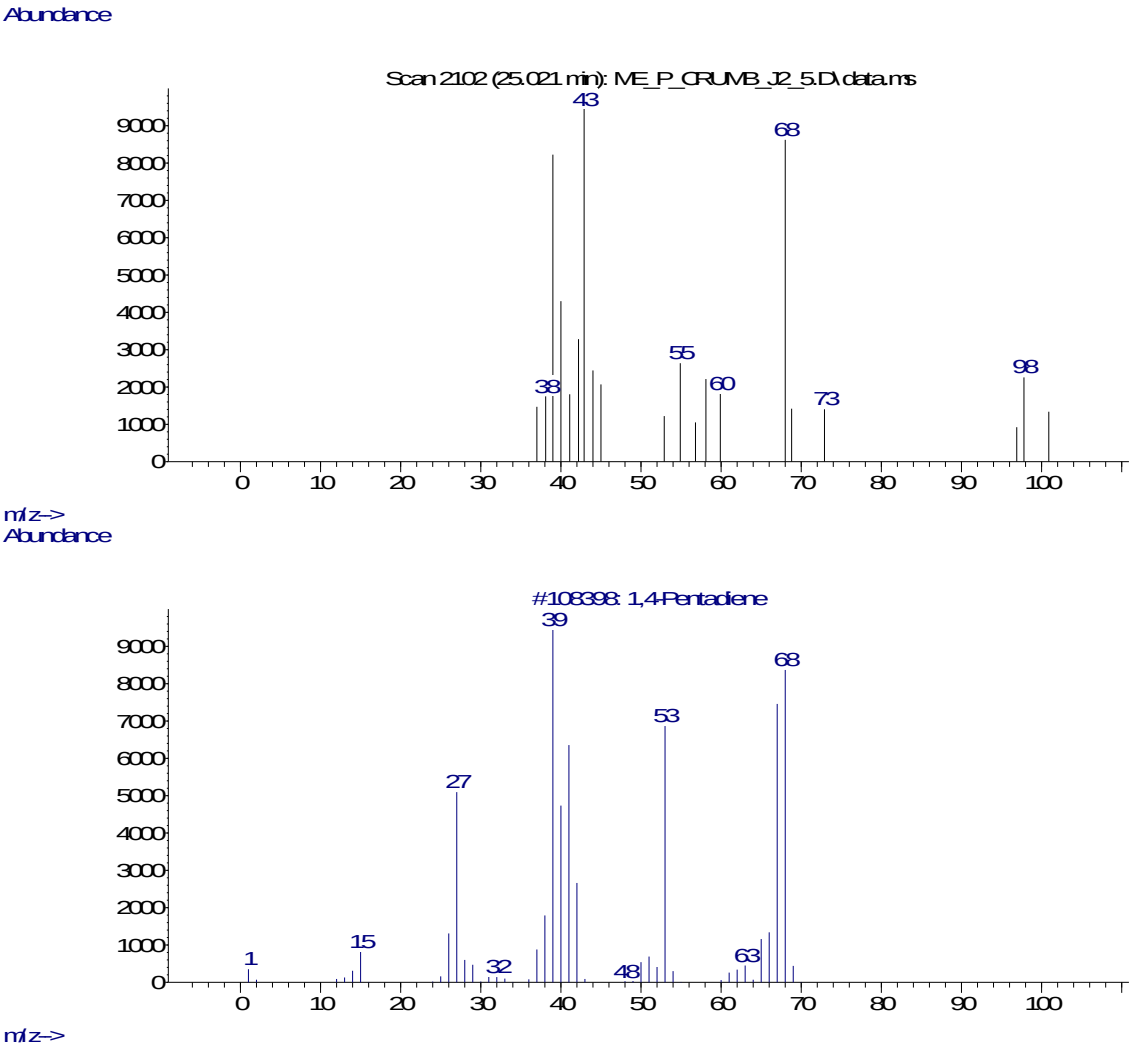
Octadecanoic acid



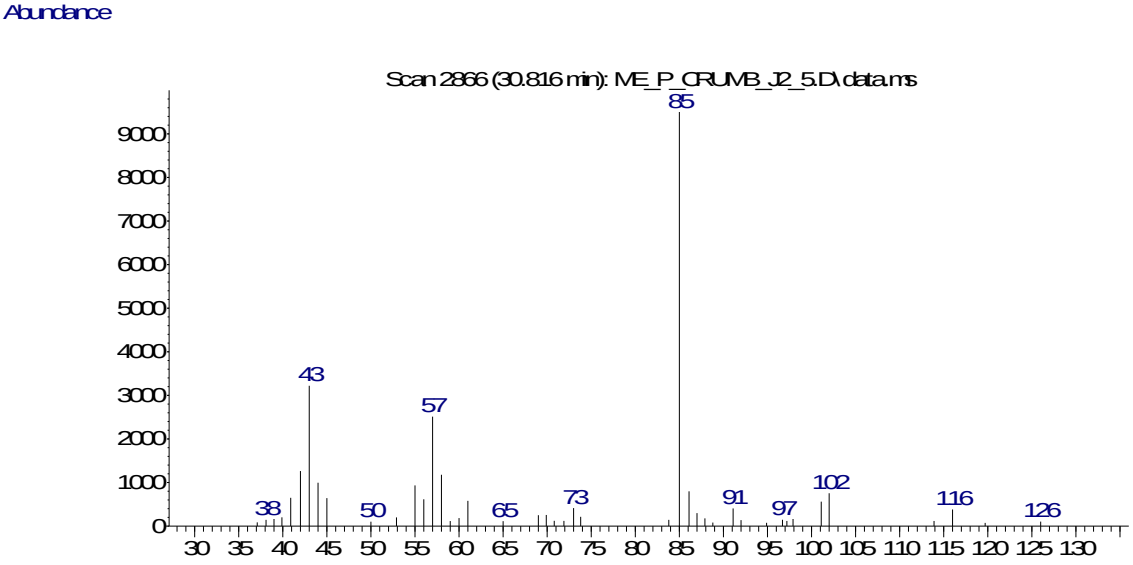
2-methylpropyl ester, 2-propenoic acid



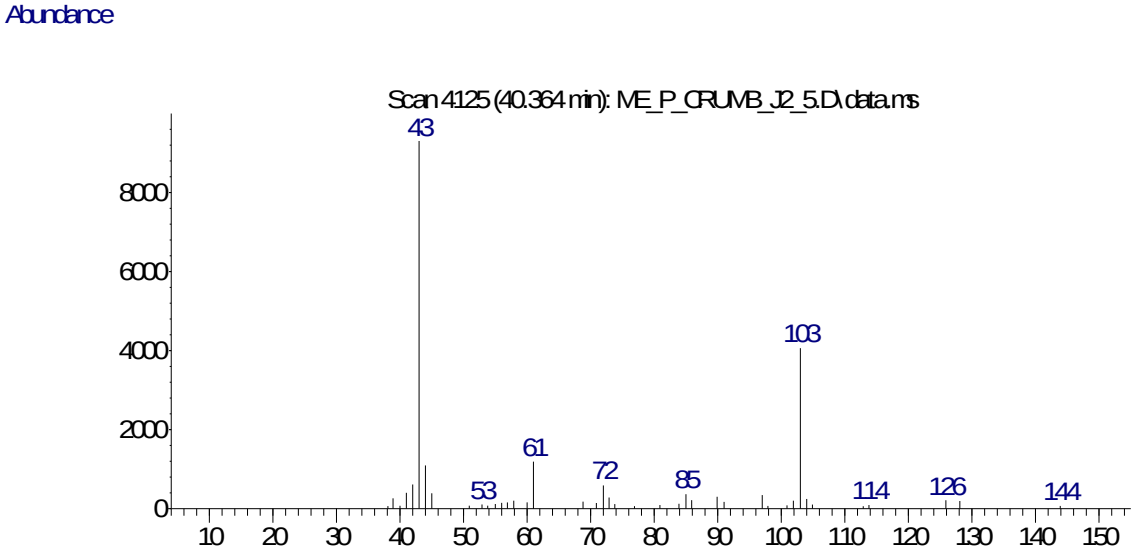
1,4-pentadiene

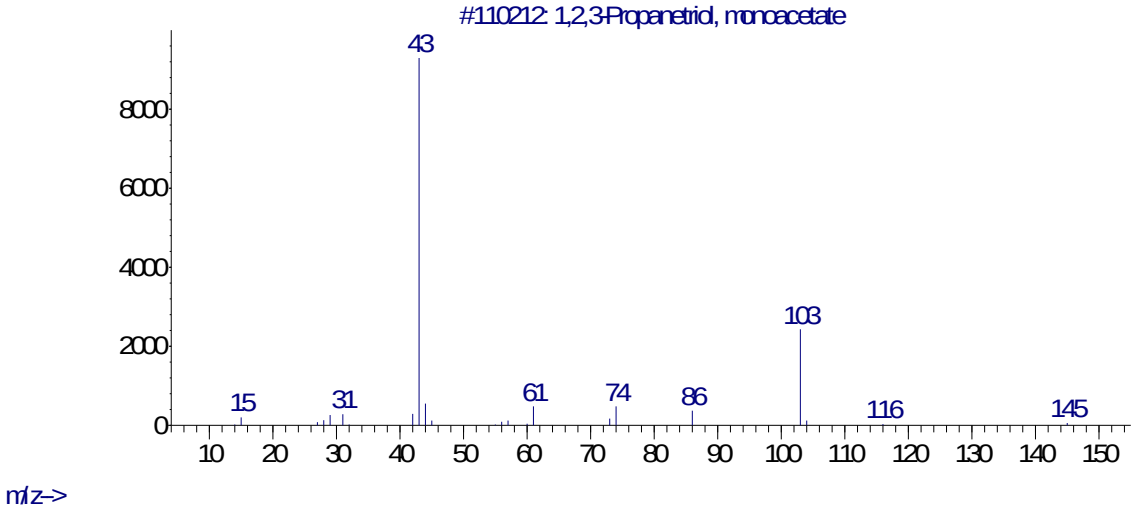


Trans-tetrahydro-5-methyl, 2-furanmethanol

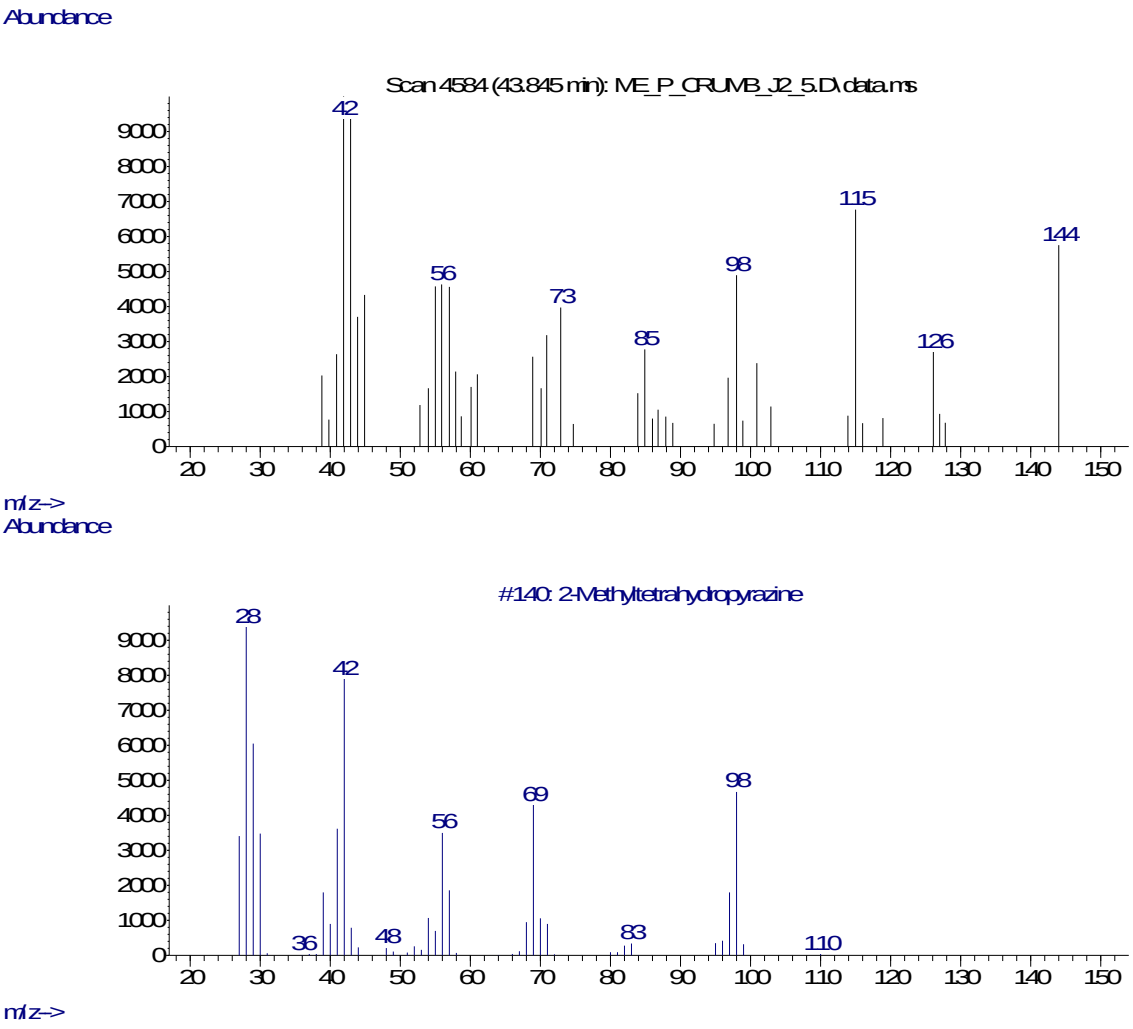


1,2,3-propanetriol, monoacetate

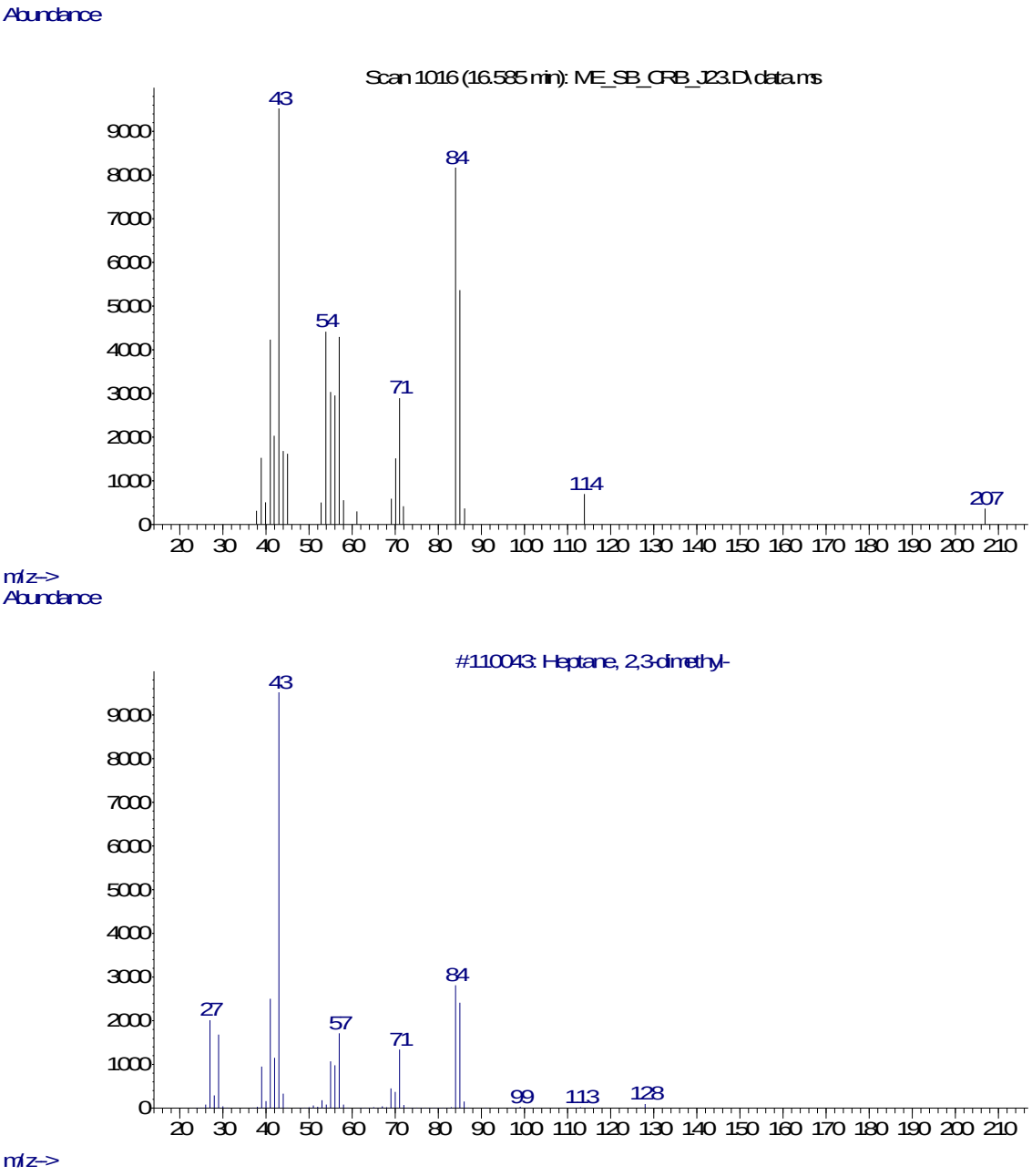




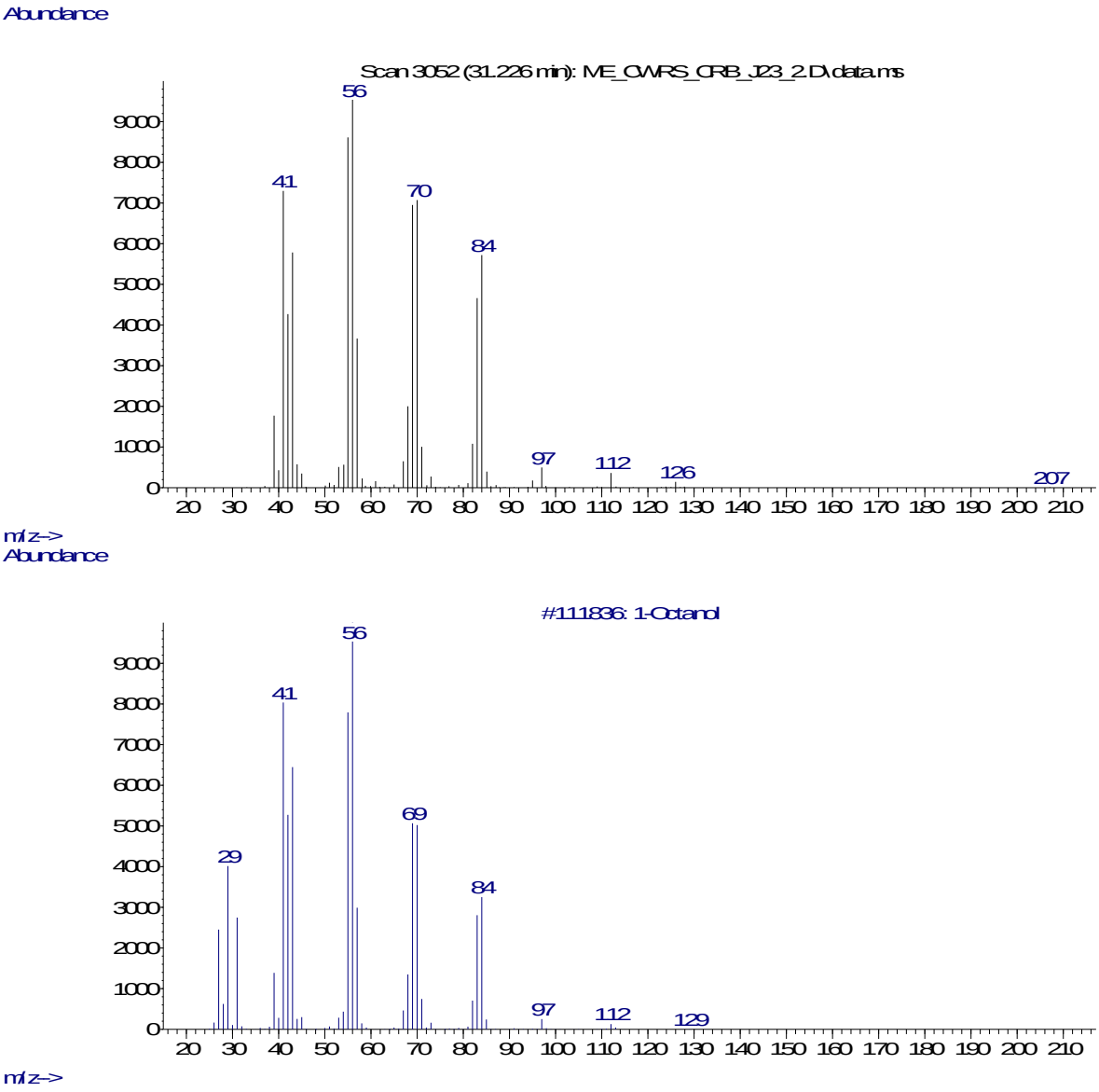
2-methyltetrahydropyrazine



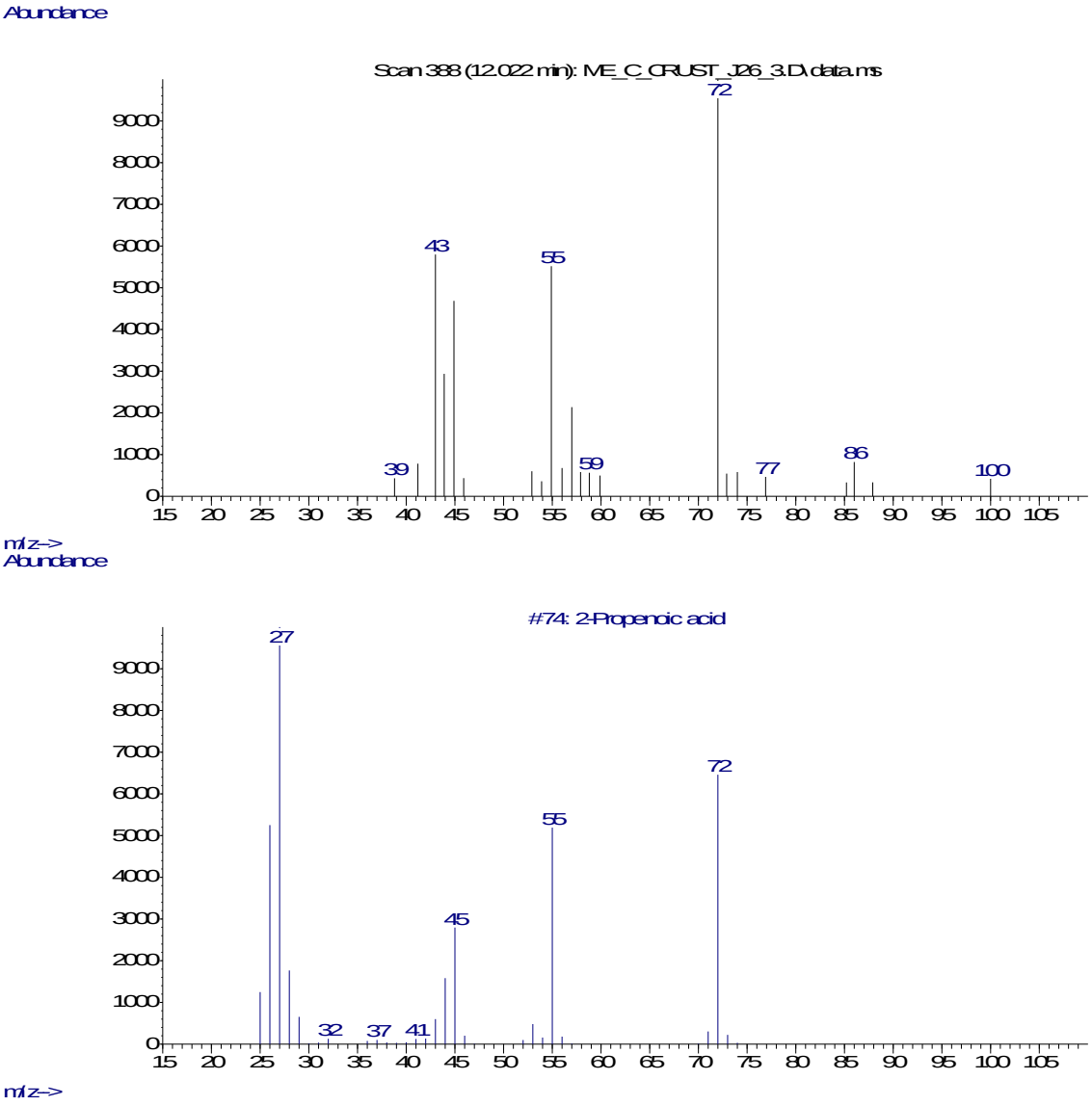
2, 3-dimethyl, heptane



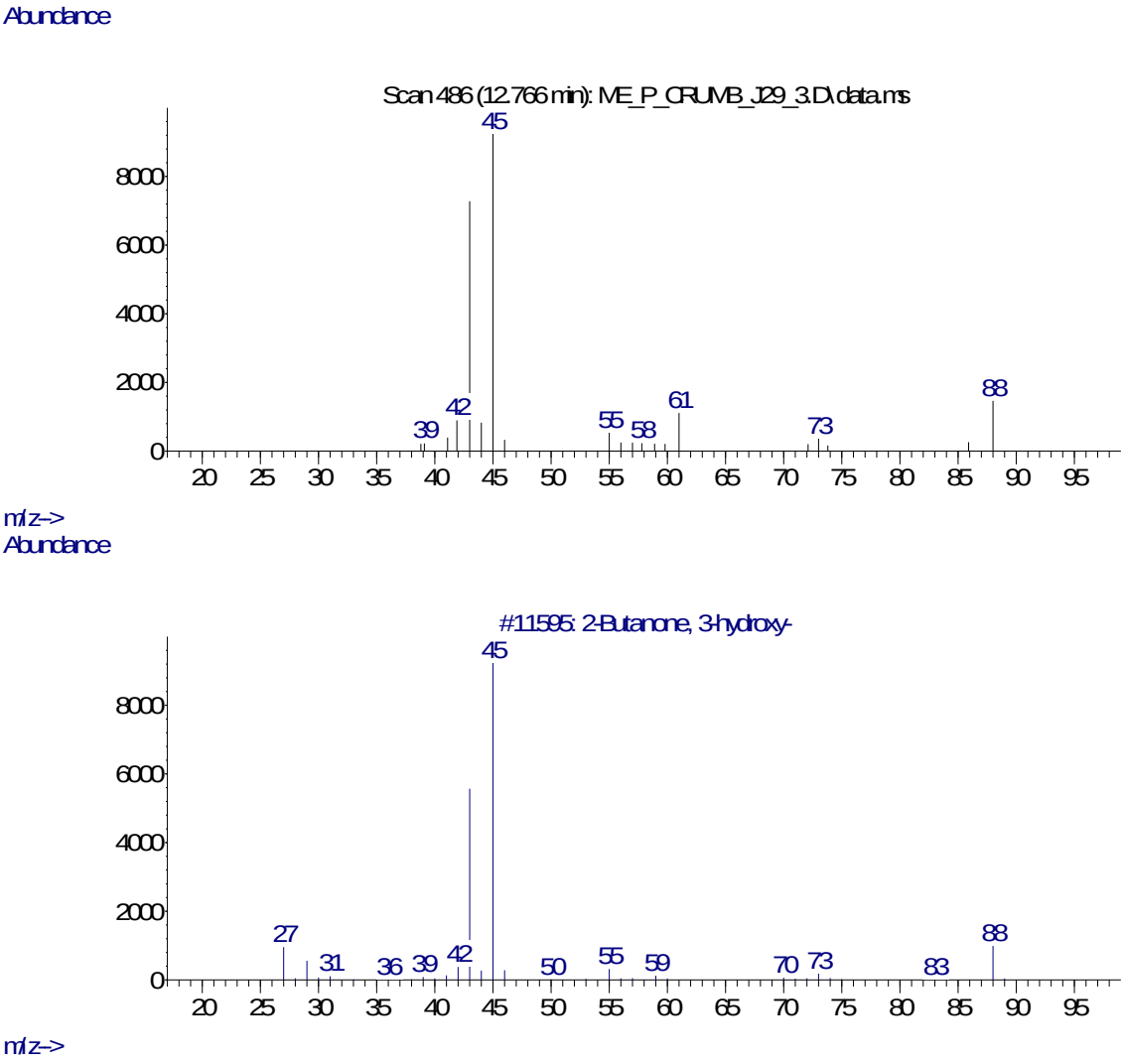
1-octanol


Abundance
Scan 3052 (31.226 min): ME_CWRS_CRB_J23_2.D\data.ms
56
41
70
84
97
112
126
207
m/z-->
Abundance
#111836: 1-Octanol
56
41
69
29
84
97
112
129
m/z-->

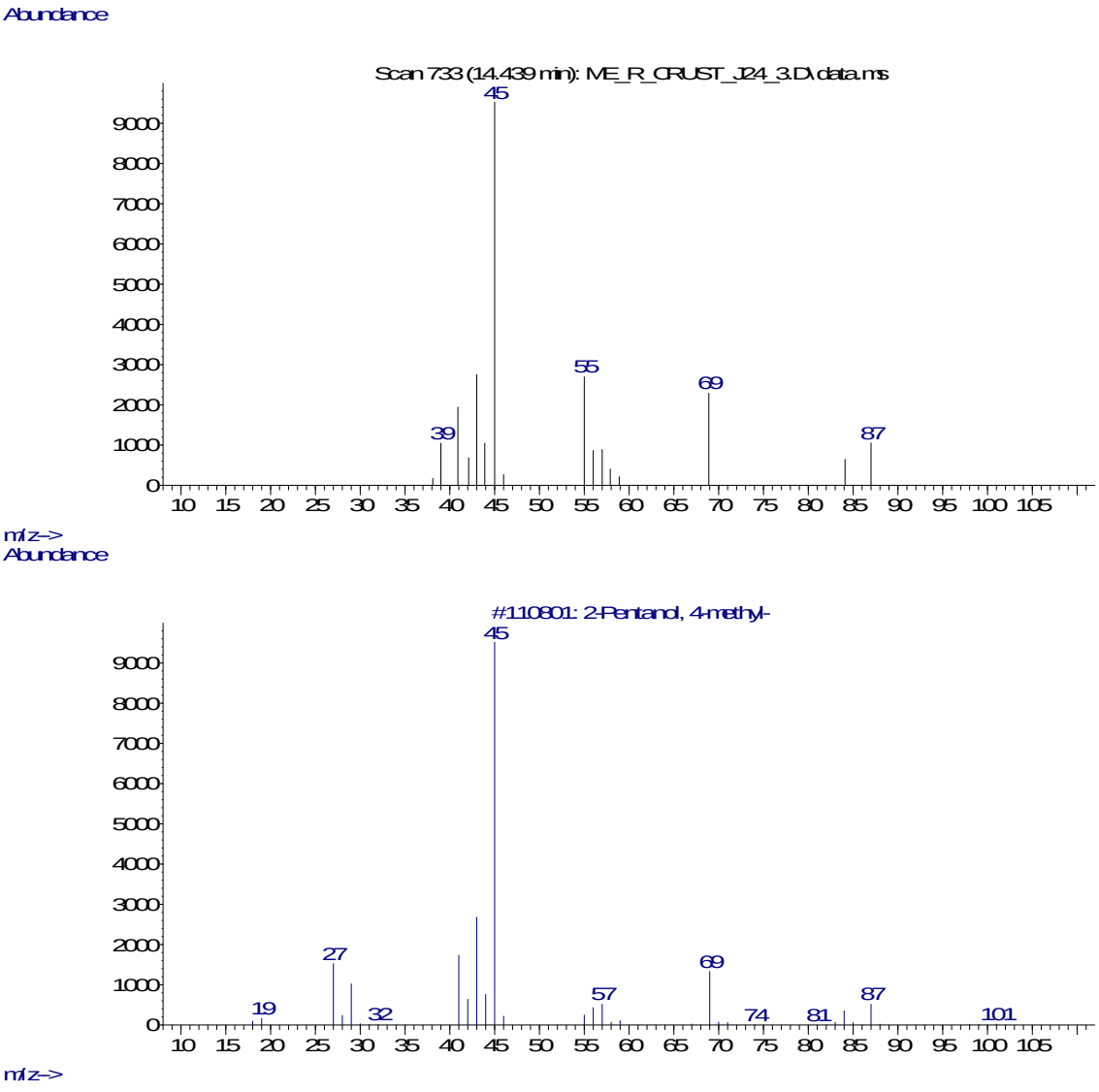
2-propenoic acid



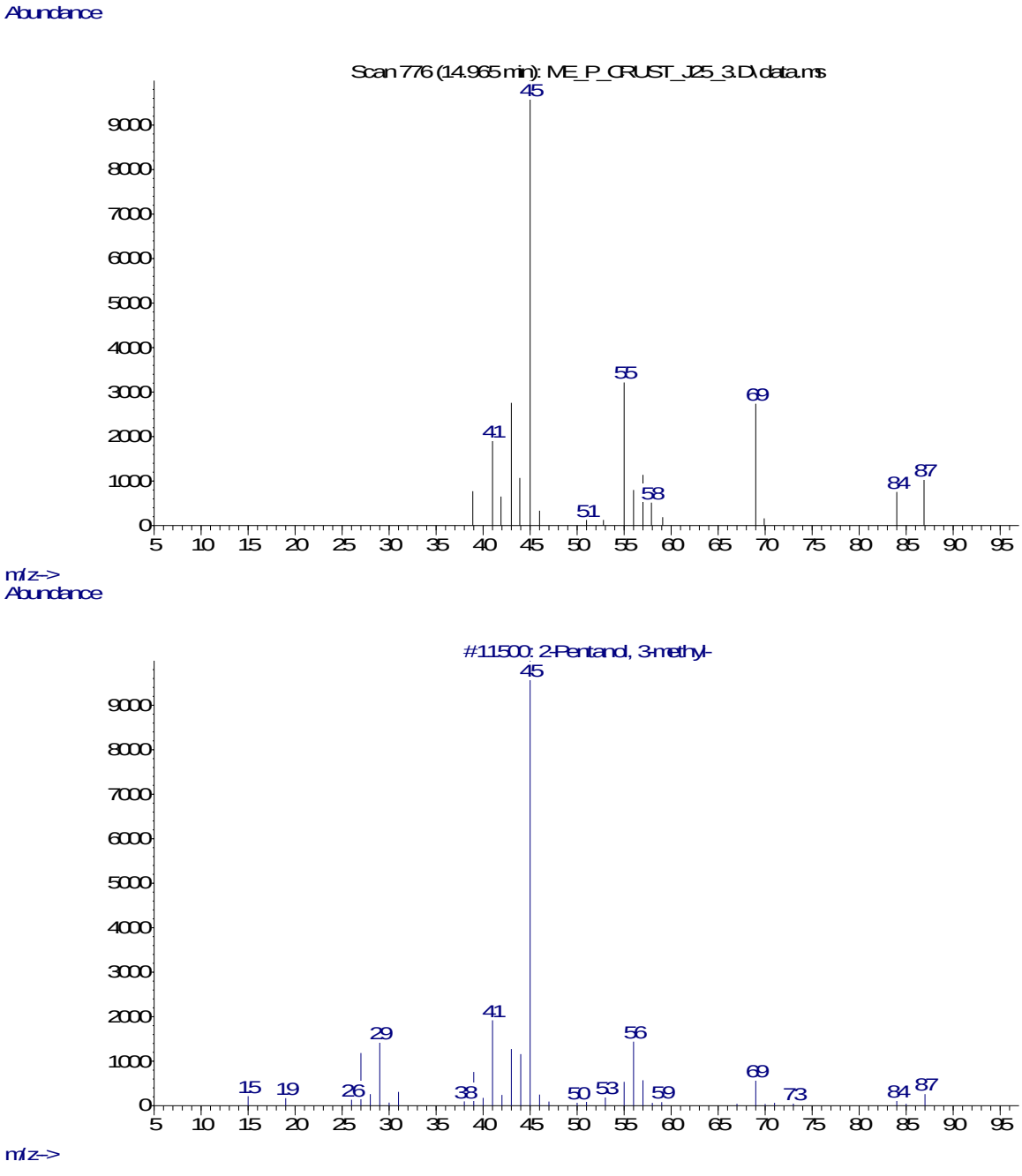
3-hydroxy, 2-butanone



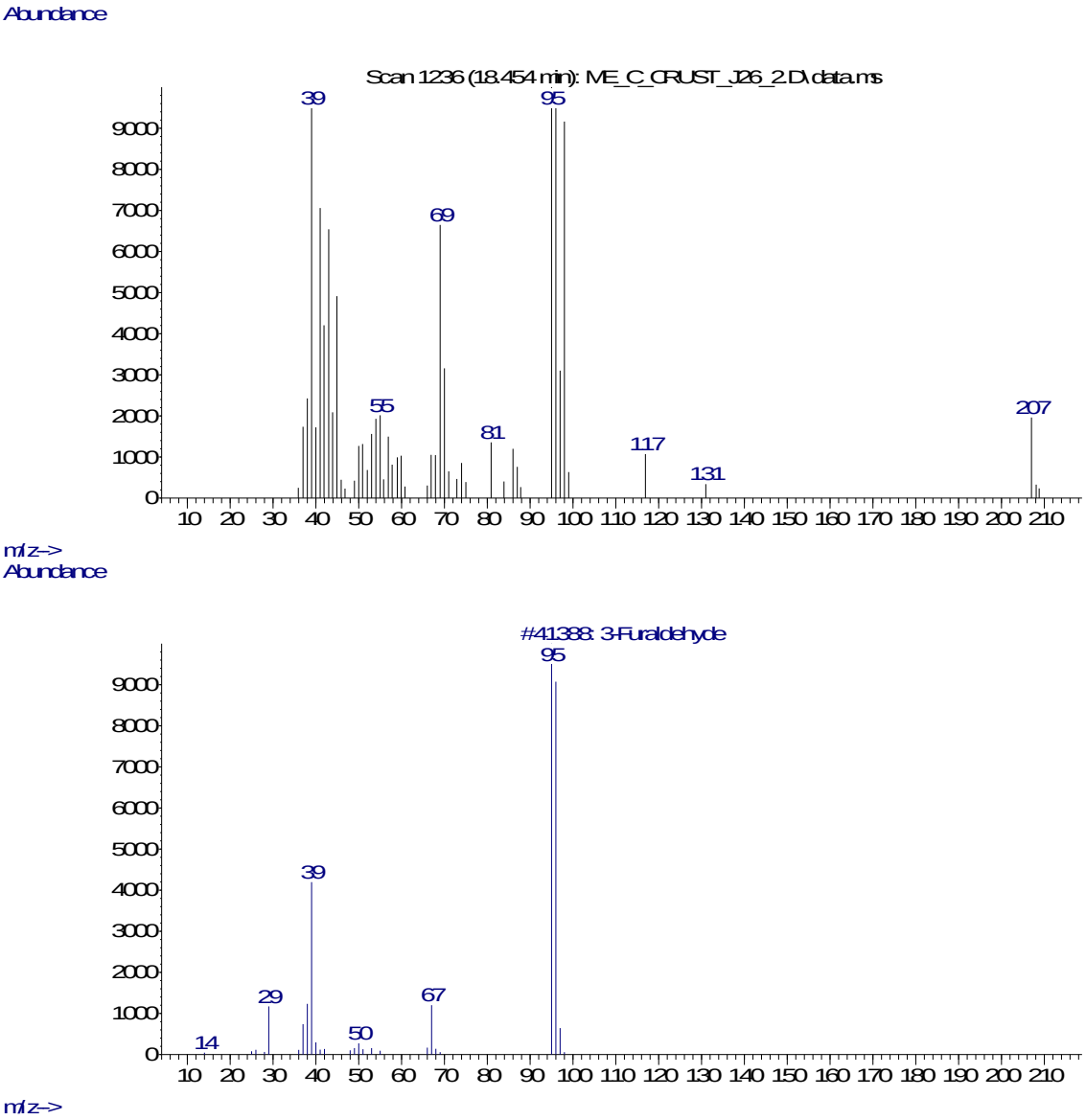
4-methyl, 2-pentanol



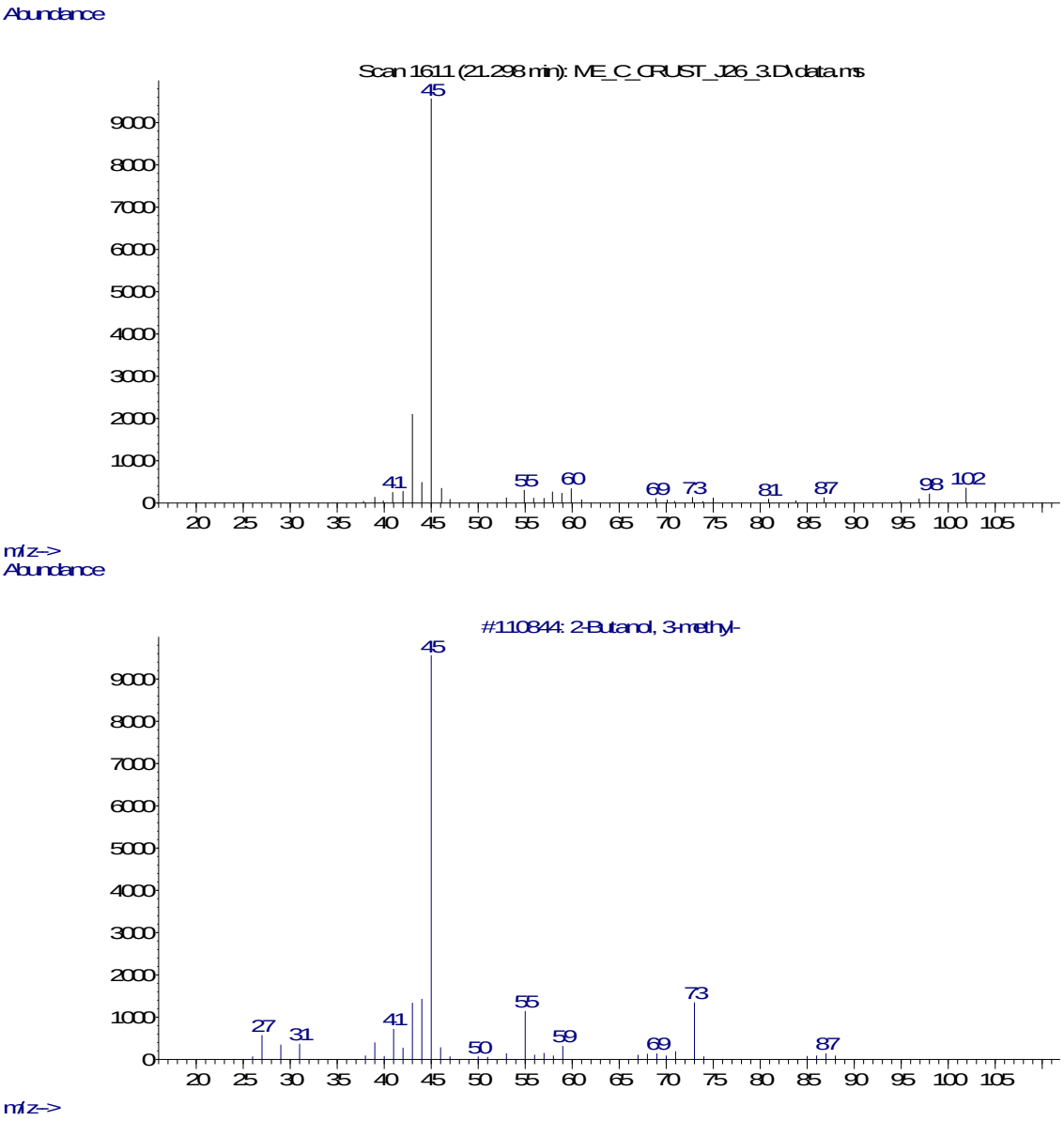
3-methyl, 2-pentanol



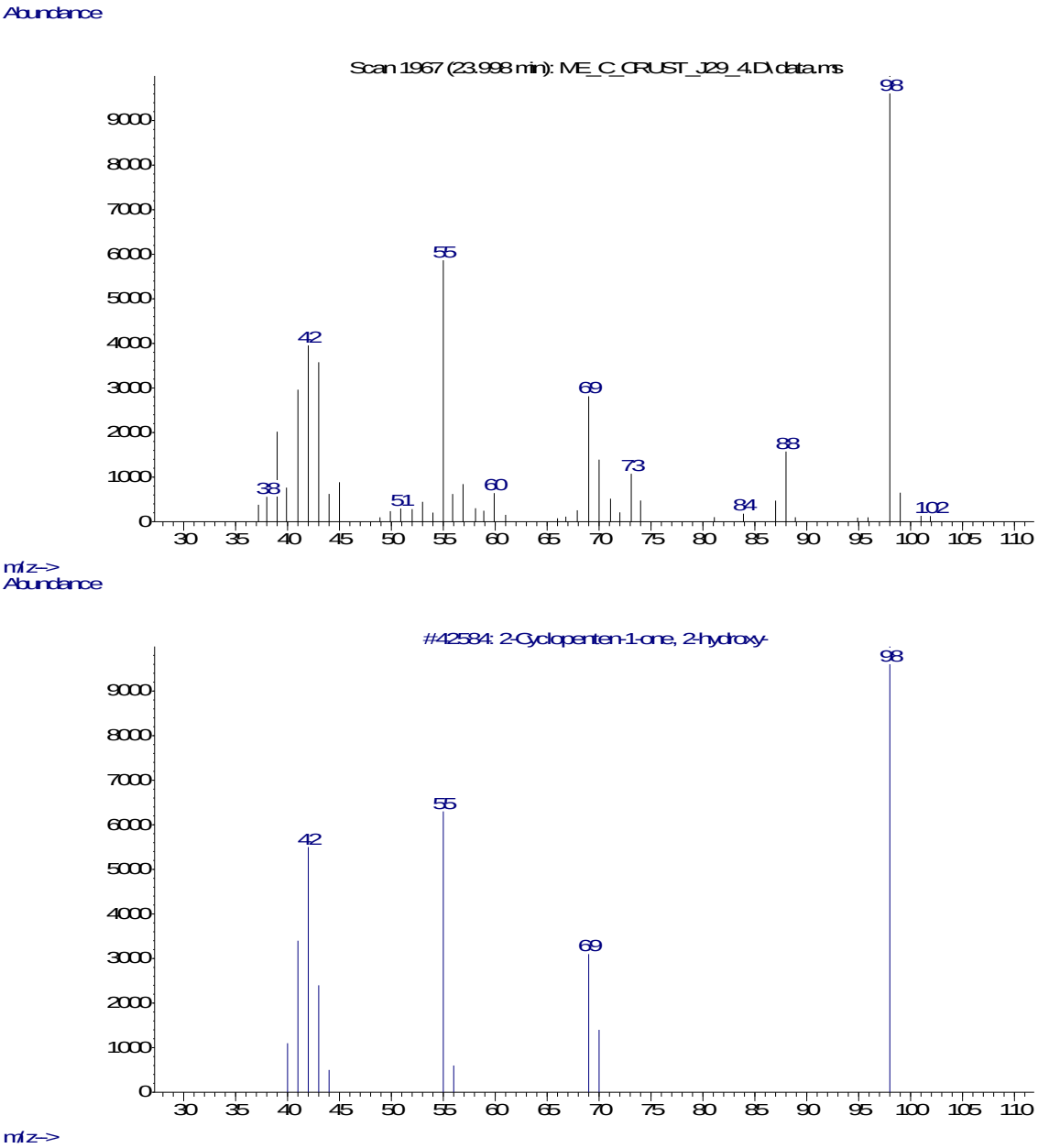
3-furaldehyde



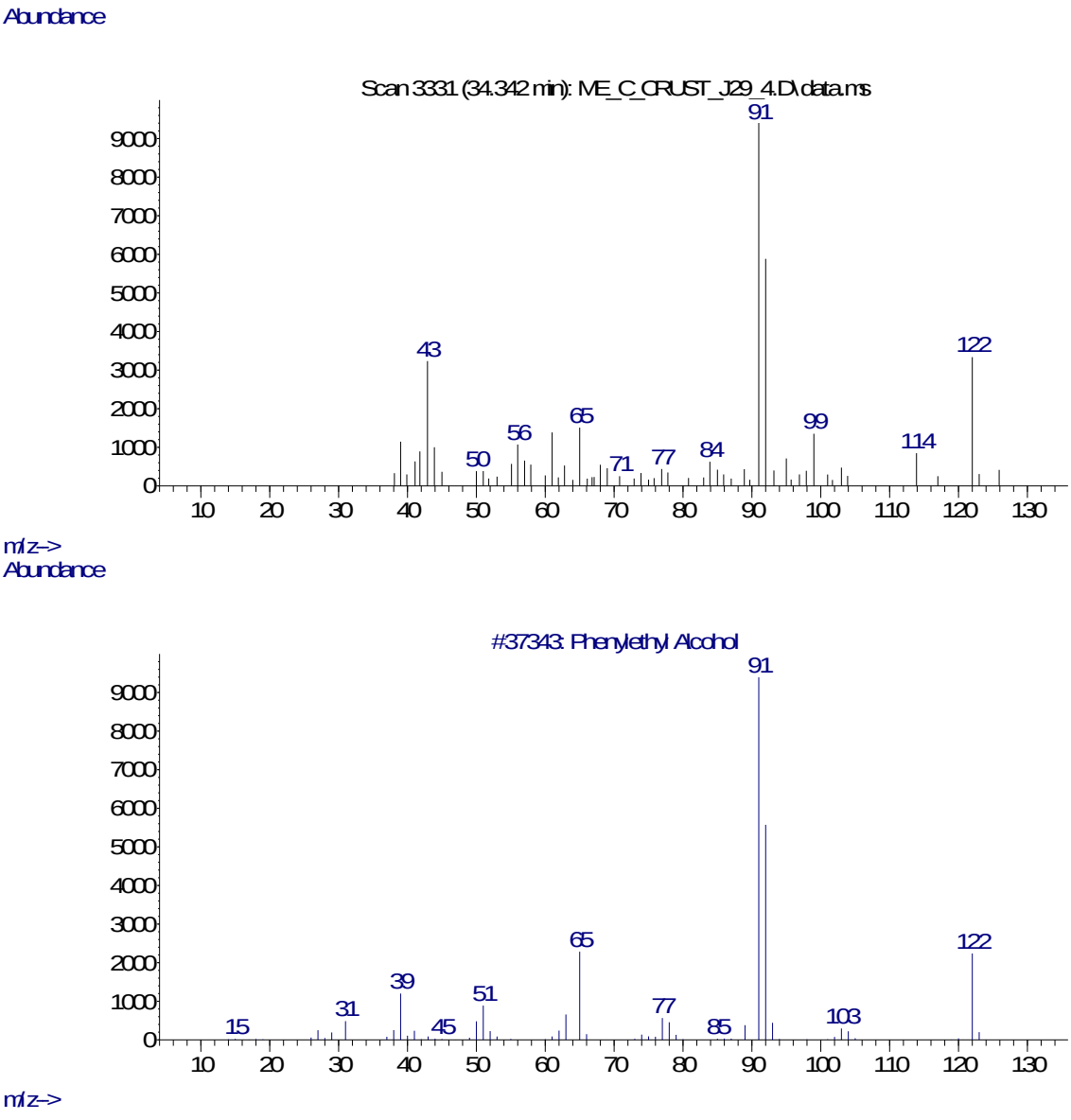
3-methyl, 2-butanol



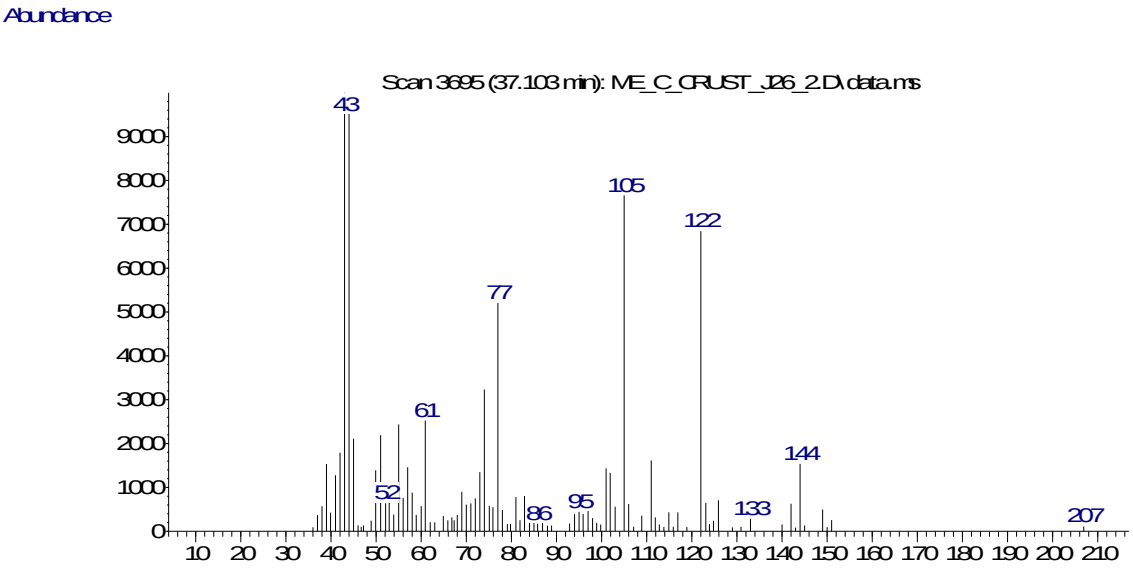
2-hydroxy, 2-cyclopenten-1-one



Phenylethyl alcohol (2-phenylethanol)



Benzoic acid



Hexanoic acid