A Comparative Aroma Analysis of Intermediate Wheatgrass and Whole Wheat Bread Crusts

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Abstract

Sustainability is a current focus of agronomy research and of growing importance to food consumers. A promising sustainable alternative food crop for the replacement of annual wheat is the perennial, intermediate wheatgrass (*Thinopyrum intermedium*) (IWG). For IWG to be utilized for food production, flavor acceptability is essential. Aroma has long been recognized as an essential part of the flavor profile of foods and thus consumer acceptability.

The objective of this work was to investigate and compare the aroma profiles of IWG and whole wheat (WW) bread crusts in an attempt to better understand the flavor performance of IWG formulated bread and related mechanistic pathways of flavor generation. Bread was chosen for flavor analysis as it is a staple food across the world with large market potential. Gas chromatography -olfactometry-mass spectrometry (GC-O-MS) and aroma extract dilution analysis (AEDA) were utilized to identify aroma fingerprints and compare aroma compounds between WW and IWG bread crusts. Sensory descriptive analysis was performed in tandem on the crusts to characterize aroma attributes of the bread crusts to further understand the influence of specific aroma compounds on aroma perceptions of the crusts.

Nineteen odorants were selected and identified in the bread crusts based on two criteria, compounds that reported a flavor dilution value of $\geq$ 32 and present in at least one of the bread crust extracts. All compounds were positively identified based on comparisons of linear retention indices (LRIs) from two

different column chemistries, mass spectra, and odor descriptors to authentic compounds. Based on the chemical structures of the odorants, the Maillard reaction, lipid oxidation, and yeast fermentation were suggested as the main pathways of flavor generation. Quantification of the identified odorants indicated that 16 compounds were significantly different between the two bread crusts, with the majority significantly higher in WW bread. Specifically, 2-methylpropanal, 3-methylbutanal, 3-methyl-1-butanol, methional, 2-acetylfuran, 2-acetyl-1-pyrroline, 1-octen-3-one, 2-acetylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-diemthylpyrazine, 2,3-diethyl-5-methylpyrazine, ethyl octanoate, and (*E*,*E*)-2,4-decadienal were present in higher concentration in WW than IWG bread crust by 2.5, 2.09, 1.28, 1.41, 4.2, 3.76, 1.39, 4.89, 5.10, 4.47, 6.72, 3.22, and 7.69-fold respectively; while 1-octen-3-ol, 2-acetyl-2-thiazoline, and (*E*,*Z*)-2,6-nonadienal were present in higher concentrations in IWG bread crust than WW by 13.7, 1.50, and 1.22-fold, respectively. The noted lower concentration of Maillard reaction products detected in IWG bread sample suggested this well-known flavor generation pathway was suppressed compared to WW bread, possibly due to higher phenolic content of the flour. The lower concentration of the yeast fermentation products identified in IWG compared to WW also suggest the pathway was inhibited likely due to a decrease in the fermentation activity of the yeast. The higher concentrations of 1-octen-3-ol and (*E*,*Z*)-2,6-nonadienal in IWG, but higher concentrations of 1-octen-3-one and (*E*,*E*)-2,4-deacdienal in WW suggest the lipid oxidation pathways in the bread crusts generate different

end products, which is likely caused by the flours' unique compositions. Descriptive analysis resulted in the identification of eight aroma attributes in both crusts. WW bread crust was rated significantly higher in roasted and toasted attributes, while IWG was rated significantly higher in green, raisin, and bran attributes. The higher roasted and toasted attributes in WW corresponded to the higher concentration of Maillard reaction compounds in WW. The higher green attribute rating in IWG bread corresponded to the higher concentration of 1-octen-3-ol and (*E*,*Z*)-2,6-nonadienal in IWG bread.

This work provided insights into pathways involved in aroma generation of IWG bread and thus a basis for developing processing strategies for flavor optimization. These insights could ultimately facilitate the development of tailored products that more closely mimic the flavor of WW bread, or the improvement of the unique bread flavor of IWG for increased consumer acceptability. Elucidating the intrinsic chemistry of IWG responsible for aroma development may allow for marker selection for breeding strategies for both crop sustainability and flavor acceptability.

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List of Abbreviations

AEDA	Aroma extract dilution analysis
ANOVA	Analysis of variance
BHT	Butylated hydroxytoluene
CI	Chemical ionization
CIS	Cool injection system
CV	Coefficient of variation
DCM	Dicholormethane
EI	Electron ionization
FD	Flavor dilution
GC	Gas chromatographer
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry
GC-O-MS	Gas chromatography-olfactometry-mass spectrometry
HCA	Hydrocinnamic acid
IWG	Intermediate wheatgrass
LRI	Linear retention index
MACH	Modular accelerated column heater
MS	Mass spectrometry
MSD	Mass spectrometry detector
OAV	Odor activity value
PCI	Positive chemical ionization
RF	Response factor
RRF	Relative response factor
SAFE	Solvent assisted flavor evaporation
SD	Standard deviation
SE	Standard error
SIM	Selective ion monitoring
TDF	Total dietary fiber
TOF	Time-of-flight
WW	Whole wheat

CHAPTER 1: INTRODUCTION, OBJECTIVES, AND JUSTIFICATION

1.1 Introduction

Food choice is based on several factors unique to each consumer including price, quality, flavor, convenience, and availability[1,2]. Flavor, traditionally, has been defined as the sensory perception of taste (non-volatile compounds), and odor (volatile compounds)[3]; however, flavor is much more complex. Flavor is a multimodal experience; sensations of sight, sound, texture, taste, and aroma combine to produce an overall flavor impression. The combination of these numerous sensory inputs determines a person's final perception of a food, and liking. Flavor is therefore a driving force for consumer acceptability especially in societies where the amount, safety, and nutrition of a food are adequately provided[4].

Along with flavor, food producers have to adhere to consumer trends such as demands for health conscious food products, with low fat, sugar, and salt formulations that provide high nutritional impact while maintaining an acceptable flavor profile. Whole grain foods have been highlighted as an essential component to a healthy diet as they provide fiber and phytochemicals that are linked to a reduced risk of several chronic diseases such as cardiovascular disease, type 2 diabetes, and obesity[5]. These benefits of whole grain are due to the inclusion of all parts of the grain into a food, especially the bran, which is removed in refined flour formulated products.

This rising consumer trend for healthier foods[4], has led to increase in whole wheat bread consumption by nearly 70%[6], but refined bread is still preferred due to negative flavor attributes with WW bread[7,8]. Significant time and effort has been spent trying to understand, and improve the flavor of WW bread through taste and aroma research[9–17]. This knowledge is important to the baking industry[18], and has led to improvements in understanding WW flavor, but further improvement is still needed to increase consumer liking.

Another growing aspect affecting consumer food choice is crop sustainability[2]. Food system sustainability protects and respects biodiversity and ecosystems, is culturally acceptable, economically fair and affordable, nutritionally adequate, safe and healthy, and optimizes the use of natural and human resources[1]. A sustainable food finds a balance between benefiting people, the environment, and economy[19]. Although not ranked highest in importance, certain consumer groups are willing to pay a premium for such products[1]. In addition to consumers identifying sustainability as an important value, groups like the American Dietetic Association, Food and Agricultural Organization of the Unites Nations, USDA, and American Society of Agronomy encourage sustainability practices[1,19].

Since concepts like healthfulness and sustainability are growing aspects of the consumer market, new sustainable crops are being developed for food use. Intermediate wheatgrass has been identified as a potential sustainable alternative to wheat based products due to its beneficial agronomic and

environmental properties, as well as its rich nutritional profile[20–22]. However, it is essential that IWG products have acceptable flavor quality in order for the grain to be successfully utilized by the food industry. Little is known regarding the flavor profile of IWG as only a hedonic sensory study has been explored prior to this project[23], hindering the development of flavor improvement methods and utilization of the grain as a viable sustainable crop.

Whole grains are widely used for the production of baked goods and cereal products to provide a healthy diet, especially in bread, which is a staple food across the world with large market penetration across the globe and consumer age groups. The creation of IWG bread with acceptable flavor would ideally lead to the replacement of traditional annual wheat (by perennial IWG), resulting in the use of a sustainable crop that could re-shape modern agriculture practices. Therefore, the current research strategy of this project focused on a direct flavor characterization of WW to IWG bread to further advance our understanding of the flavor performance of IWG as a food ingredient.

As previously mentioned flavor is a highly multimodal experience, and aroma and taste are typically considered the most influential factors. Consequently, the aroma of bread is considered one of the most important parameters that influences consumer acceptance[24].Compounds that contribute to the aroma of bread are typically generated from the Maillard reaction, lipid oxidation, and yeast fermentation[29,30]. The crust of bread, when compared to the crumb, is subjected to high processing temperatures resulting in the generation of more thermally

induced flavor active compounds. The additional pathways of aroma generation in the crust, and the elevated concentration of volatile compounds relative to the crumb mean the crust uniquely, and disproportionally, contributes to the breads overall aroma profile, and subsequently has a strong impact on flavor. The aroma profiles of IWG and WW bread crusts, therefore, were chosen for the first aspect of flavor analysis to be investigated.

The three main pathways of flavor formation are dependent on processing conditions and flour composition[13,29-30]. Flour composition impacts the aroma profiles of bread crust by providing unique amounts and types of chemical components like amino acids, fats, and carbohydrates. These chemical components are the source of precursors for reactions, which generate aroma compounds; therefore, different flours can give breads different aroma profiles[31-32]. It is additionally known that volatile compounds present in raw flour are of minor importance to the aroma of bread[9]. Therefore, processing conditions greatly affect flavor formation by changing the conditions of the reaction as well as the physical and chemical properties of the food[27,29–34]. WW and IWG flours are both whole grain flours, so the germ, bran, and endosperm are included. However, if all processing conditions remain constant during breadmaking, any differences determined between the aroma profiles of the crust would likely be due to flour composition

Investigating how the compositions of different flours affect flavor formation in bread provides insight into possibilities for flavor optimization, and improving

consumer acceptability. Therefore, the aim of this work was to investigate the aroma profiles of WW and IWG bread crusts, by generating and comparing the aroma fingerprints of the two crusts in order to identify specific aroma compounds and reaction pathways that contribute to the aroma profile of IWG bread crust. Descriptive analysis was also performed on the two bread crusts to connect analytical results with sensorial perceptions.

1.2 Justifications and Objectives

The goal of this project was to develop foundational knowledge (flavor chemical fingerprint) for future flavor research on IWG bread. Aroma compounds, determined in the study, could lead to the identification of compound origin and their mechanistic pathways of generation. Understanding the origin and mechanistic pathways involved in the aroma formation of IWG bread crust facilitates development of processing and reformulation as well as breeding strategies for optimized aroma generation in the final product.

By performing descriptive analysis, important attributes in the two breads can be elucidated which could serve as a starting point for consumer acceptability. An improved understanding of the aroma profile of IWG bread provides a basis for optimization of IWG bread to more closely mimic WW bread, or lead to the improvement of the unique IWG bread flavor.

Furthermore, the results from this research would be expected to aid in the flavor research of other IWG cereal based products as well as build upon the agronomical, rheological, and compositional research that is completed, or

currently in progress to create a complete characterization of IWG. The complete characterization of IWG would ideally result in feasible use of sustainable crops for food production.

The main research objectives were the following:

1) Investigate, characterize, and compare the aroma fingerprints of WW and IWG bread crusts via GC-O-MS and AEDA.
2) Conduct descriptive sensory analysis on WW and IWG bread crusts to qualitatively and quantitatively determine the major attributes of the two bread crusts.
3) Corroborate results from objectives one and two to provide an understanding of the main aroma attributes of IWG bread crust and the compounds likely responsible.

CHAPTER 2: LITERATURE REVIEW

2.1 Wheat

2.1.1 History of Wheat

In a review of wheat, Shewry identifies wheat among the most harvested cereal crops globally, with nearly 600 million metric tons harvested annually[35]. It is a staple in many diets throughout the world with bread being one of the major consumer products[35]. From its first cultivation nearly 10000 years ago during the Neolithic Revolution to present day, nearly 25000 types of wheat have been identified[35], which illustrates wheat to be a very adaptable crop, suited for a wide variety of temperate environments. Wheat has the ability to produce high yields, can be harvested by mechanical or traditional methods, and provides unique viscoelastic properties to dough[35]. Unfortunately, high inputs of nitrogen and agrochemicals are required to achieve high yields and desired protein content, which negatively affect the environment due to chemical run-off making wheat a less sustainable crop[35].

2.1.2 Composition of Wheat

Wheat grains are comprised of 3 major parts: the bran, the endosperm, and the germ representing 13-17%, 80-85%, and 2-3% of the grain on a mass basis, respectively[36]. The bran is made up of several layers of cells and tissue. The endosperm consists mostly of starch, which serves as an energy source for the grain and also the aleurone layer which is rich in proteins and enzymes[36].

The germ contains protein, lipids, and minerals. The inclusion of all parts of the grain obtained from milling results in whole wheat flour, and bread that contain essential vitamins and minerals, dietary fiber, and starch and protein for energy, unlike refined flour, which does not include the bran and the germ. WW flour contains, on average, 73.9% starch, 15% protein, and 2.57% total dietary fiber (TDF)[37].

The proteins in wheat include: albumins, globulins, gliadins, and glutenins. Albumins and globulins are the smallest proteins concentrated in the seed coats of the bran, the aleurone cells of the endosperm, and also the germ[36]. Gliadins and glutenins are the two proteins that make up gluten, and account for 75% of the total protein in the grain[36]. The starch of wheat is composed of two polysaccharides: amylose and amylopectin. Amylose is a linear polysaccharide made by 1,4-α-glycosidic bonds[38], whereas amylopectin is a highly branched polysaccharide[39].

Fats, or lipids, originate from the grain, or, in the case of bread, are added in the form of other ingredients like shortening. Native lipid content in grains is dependent on genetic characteristics, environment, and milling. Lipids affect texture, flavor, color and nutritional quality of end products[40], and so are an important component of grains. Common lipids in wheat flour and shortening are listed in **Table 1**.

Table 1. Common lipids in wheat flour bread making listed by ingredient source[40]

Wheat Flour Lipids	Shortening
Free fatty acid	Triacylglycerols
Monoacylglycerol	Diacylglycerols
Diacylglycerol	Monoacylglycerols
Triacylglycerol	
(lyso)phosphatidylcholine	
(lyso)phosphatidylethanolamine	
(lyso)phosphatidylglycerol	
(lyso)phosphatidylinositol	
(lyso)phosphatidylserine	
Phosphatidic acid	
N-acyl phosphatidylethanolamine	
N-acyl lysophosphatidylethanolamine	
Digalactosyldiglycerol	
Digalactosylmonoglycerol	
Monogalactosyldiglycerol	
Monogalactosylmonoglycerol	
Trigalactosylmonoglycerol	
Free stearyl	
Steryl ester	
Steryl glycoside	
Acylated steryl glycoside	

2.2 Annual Crops versus Perennial Crops

Even though farms are producing more food than ever, agriculture is viewed as the, “largest threat to biodiversity and ecosystem functions of any single human activity”[41]. In order to improve current agriculture practices, and make it more sustainable, plant breeders, environmental scientists, and agronomists have been investigating the development of perennial grains[23]. Work has been done to make agricultural practices more sustainable through management practices, but grower adoption can be limited due to socioeconomic and biophysicalfactors[42].

Wheat is an annual crop, and thus, provides a steady supply of grains, and helps meet global food needs[20]. Annual crops have some key advantages that allow farmers to quickly change crops to meet market demands. The crops require a short growth time, and have been adapted to deal with environmental factors such as disease without significant losses[20]. These advantages make replacement of annual crops a challenging task. However, there are several disadvantages to annual crops as well. Typically, less than 50% of the nitrogen applied as fertilizer is taken up by current crops with the rest being lost to a variety of factors including: run off, leaching, or volatilization[41], which negatively impact the environment. Annual wheat fields have also been found to need nearly 12 times the energy requirements (nitrogen and field operations) as perennial grass fields[21], implying greater financial and environmental costs for annual wheat than a perennial crop.

Perennial plants provide several benefits over annual crops from a sustainability and environmental stand point. Perennial plants have a longer growing season and extensive root systems that enables them to be “efficient and responsive micromanagers of soil, nutrients, and water”[41] (see **Figure 1**). As a result and relative to annuals, perennials decrease soil erosion, positively influence water and nutrient managements, store more carbon, and are often more resilient to pests and abiotic stresses[41]. In addition perennial plants positively impact the local bio system by better maintaining topsoil, competing

against weeds, and by offering “wildlife benefit from reduced chemical inputs and from greater shelter”[20].

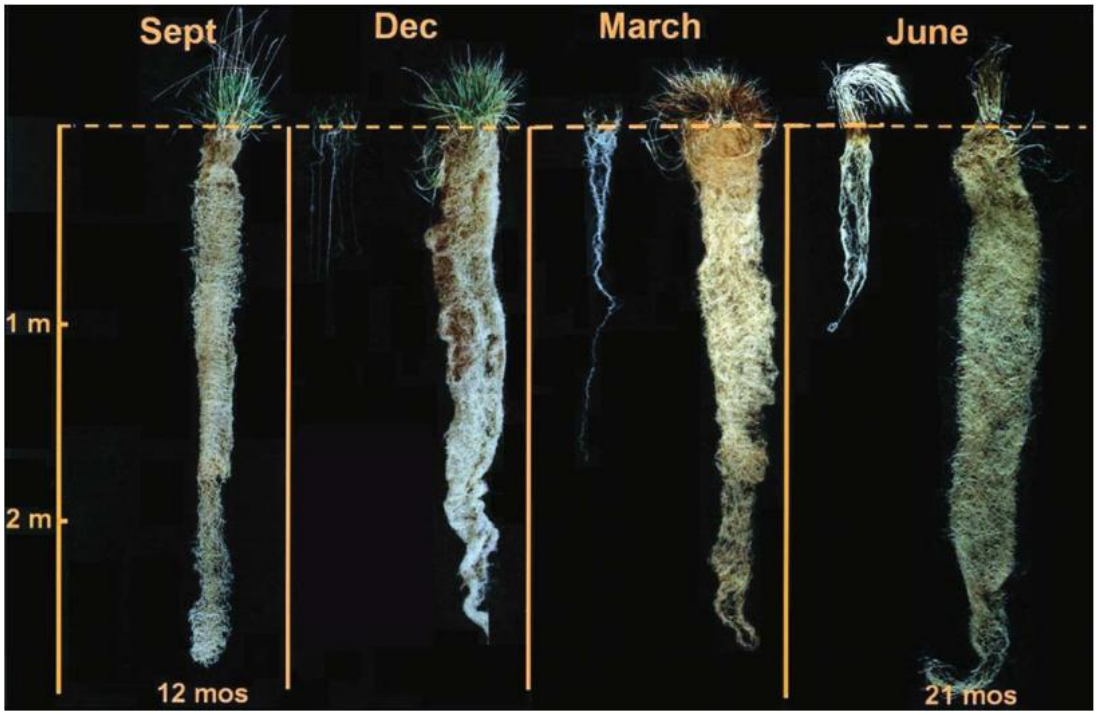

Figure 1. Annual wheat root systems (left) and perennial (right), intermediate wheatgrass, root system[41]

Perennial plants do not leave their crop lands barren after harvest and before planting, allowing for a more efficient use of the land than annual crops[41]. Not only would perennial crops cost less than annual crops, a farmer would also spend less time in the field because they would not have to replant every year, nor apply as much fertilizers or pesticides. Perennials also have the ability to store greater amounts of carbon thus, reduced input requirements can help mitigate global warming[20]. Lastly, perennials could potentially provide greater food security for the future as annuals are predicted to be more detrimentally

impacted by the global temperature increases predicted by climate-change models[41].

The benefits of perennial plants are appealing at a sustainability and environmental level. However, many other production, nutrition, and flavor factors need to be addressed before annual crops are replaced with perennials. Dietary requirements and preferences must also be considered when choosing, or developing a perennial crop[41]. Based on favorable grain qualities, nutrition, and the benefits previously discussed, a promising replacement for annual wheat is perennial intermediate wheatgrass.

2.3 Intermediate Wheatgrass

2.3.1 Intermediate Wheatgrass Current Uses and Desirable Properties

Intermediate wheatgrass (*Thinopyrum intermedium*) is currently used as a forage crop (grazing, pastureland, and hay production), and comes in several cultivars[43]. IWG is a promising perennial alternative, specifically to wheat, because it has good agronomic properties including easy mechanical harvesting, easy threshing, and a resistance to shattering[41]. IWG also has acceptable seed size (with current breeding research focusing on increasing seed size) and is a nutritious grain[37,41].

2.3.2 Intermediate Wheatgrass Flour Composition

IWG flour is composed of 46.3% starch, 20% protein, and 16.87% TDF. IWG groats are smaller than WW groats resulting in higher bran, supported by higher TDF, and lower endosperm content in IWG flour when compared to WW[37]. The ratio of amylose to amylopectin between WW and IWG is similar; however, the low starch content in whole grain IWG flour greatly affects the volume of baked goods[37,44]. IWG shows virtually no gluten network forming abilities, so IWG dough does not exhibit the same viscoelastic properties as WW dough suggesting the gluten profiles of the two flours differ greatly[44]. This was supported by Marti *et. al*.[37] who found IWG gluten protein to be composed mainly of α- and γ-gliadins, and low molecular weight glutenins. WW flour, on the other hand, contains high molecular weight glutenins, which are needed for good gluten-forming ability. Minimal gluten network formation directly impacts the texture of the product by creating a denser crumb.

IWG is a relative of wheat, but the two grains are very different compositionally. IWG flour has been found to have different amounts of essential amino acids than WW flour with the limiting amino acid being lysine in both flours based on one study[23](**Table 2**). Differences in amino acid compositions between flours is important for flavor development as different amino acids provide different precursors for aroma compounds.

Table 2. Protein amino acid composition (g/16 g N) of intermediate wheatgrass, whole wheat flour, and the FAO standard[23]

Amino Acid[a]	Intermediate Wheatgrass[b]	Wheat Flour[c]	FAO Standard[d]
Lysine	2.9 ± 0.2	2.9	5.4
Histidine	2.4 ± 0.2		
Threonine	3.5 ± 0.3	2.9	4.0
Cystine	3.4 ± 0.3	2.5	
Methionine	2.2 ± 0.3	1.5	
Cys + Met	5.6 ± 0.6	4.0	{3.5}
Valine	5.3 ± 0.5	4.4	5.0
Isoleucine	4.0 ± 0.3	3.3	4.0
Leucine	7.2 ± 0.5	6.7	7.0
Tyrosine	3.5 ± 0.3	2.9	
Phenylalanine	4.9 ± 0.4	4.5	{9.6}
Serine	5.4 ± 0.4		
Glycine	4.3 ± 0.2		
Arginine	5.2 ± 0.4		
Alanine	3.8 ± 0.3		
Aspartic Acid	5.1 ± 0.3		
Glutamic Acid	29.6 ± 2.2		
Proline	10.4 ± 0.9		
Limiting Amino Acid	Lys	Lys	

[a] N = 12
[b] Mean ± standard deviation
[c] FAO (1970)
[d] FAO (1973)

However, different cultivars, growing location, and growing conditions affect the amino acid composition of all crops. Therefore the amino acid composition of the IWG and WW flour used in this study were likely slightly different from the averages provided in **Table 2**.

2.4 Breadmaking

The creation of any yeast bread involves the formation of a dough, the manipulation of the formed dough, yeast fermentation, and baking. Dough formation can be divided into three phases. The first is the moistening of flour particles with water through mixing[45], which also helps distribute yeast cells throughout the dough[46]. The second phase involves the solubilization of components in the flour, and the final phase involves the restructuring of gluten proteins. The gluten structure forms through the formation of inter- and intramolecular disulphide bonds creating a “viscoelastic rheological system”[45]. Gluten network formation and interactions are complicated and not yet well understood, but the structure and interactions of the gluten network are dependent on several factors, including amino acid composition.

After the dough is initially mixed it needs to be kneaded. Manipulation of the dough continuously strips away the hydrated layers from the surface of the flour and brings all the components in contact to create a gluten network[46]. The gluten network is formed by gliadins uniting through hydrogen bonds and van der Waals forces that make the dough cohesive, while the glutenins untie to form elongated fibrils that give elasticity to the dough[46]. The air that is incorporated during kneading serves as nucleation sites for the accumulation of carbon dioxide during fermentation[46].

Fermentation begins during mixing and continues through the entire bread making process until baking[45]. In order for fermentation to occur the dough must be proofed at a temperature and humidity that is optimum for the growth of the yeast[46]. During proofing, dough is punched several times to keep the films of gluten from overstretching, break apart large pockets of air in order to create a uniform crumb, and to release the gas formed by the yeast cells[46]. After the dough has proofed, the dough is baked. During the beginning of the baking stage, starch gelatinization occurs giving some rigidity to the dough. As the baking continues flavors are formed, and proteins harden to give the final rigid product[46].

2.5 Bread Ingredients

Flour, liquid, yeast, salt, fat, and sugar are the basic ingredients needed to create yeast breads. Flour provides the starch and protein, with the gluten proteins being the most important in terms of baking properties[31].

The function of yeast in bread is to work as a leavening agent by producing CO_2, but it is also responsible for flavor generation. Yeast can ferment glucose, fructose, sucrose, and maltose[46]. However, yeast will only ferment maltose once glucose and fructose sources are depleted[45]. In addition to producing CO_2, yeast produces acids which lower the pH of the dough closer to the isoelectric point of gluten. As a result the gluten is less sticky and more elastic, making it easier to work with[46].The addition of fat to bread creates a more

tender and larger volume product, and aids in the browning process. Lipids are also essential for the formation of the dough, due to both endogenous lipids and lipids from shortening becoming trapped in the gluten structure, which may add stability to gas cells through the bread making process[40].

Sugar is included primarily to serve as a source of fermentable sugars for the yeast, but it can also aid in browning and add sweetness. Sugar results in reduced tenderness as it inhibits formation of the gluten network. Added sugar, and sugars present in the starch of the flour are necessary for the creation of Maillard reaction products. Salt is added to the dough to improve taste. Salt also moderates yeast fermentation by slowing the production of CO_2, and prevents the dough from rising too quickly. Furthermore, salt inhibits the action of proteases that depolymerize proteins and weaken the gluten network[46]

2.6 Human Olfaction

Approximately 1% of human genes are dedicated to detecting odors, allowing a wide spectrum of odors to not only be detected, but also prompt varied emotional and cognitive responses[47]. Odors are detected upon the inhalation (orthonasal)[48], and by traveling through the mouth (retronasal)[49]. Due to the intricacy and immense complexity of the human olfactory system, a human's sense of smell is not completely understood nor is its relation to flavor perception. Slight changes in structure, or even concentration, can result in a dramatic shift in perceived odor[50] leading to a challenge in determining how a

compound will specifically impact the aroma of a food and in turn overall flavor perception. This complexity indicates the challenges and importance of understanding the impact of volatile flavor compounds on the overall sensory experience.

2.7 Aroma Generation Pathways

The olfactory system is a complex system responsible for human recognition and perception of odor, but aroma development is just as dynamic. A reported 540 volatile compounds have been identified in bread, most of which fall within alcohols, aldehydes, esters, ketones, acids, pyrazines, and pyrrolines; furans, hydrocarbons, and lactones have also been identified. Various odorants found in wheat bread crust are presented in **Figure 2**. Aroma compounds in bread are formed via caramelization, the Maillard reaction, lipid oxidation, yeast fermentation, and enzyme activity.

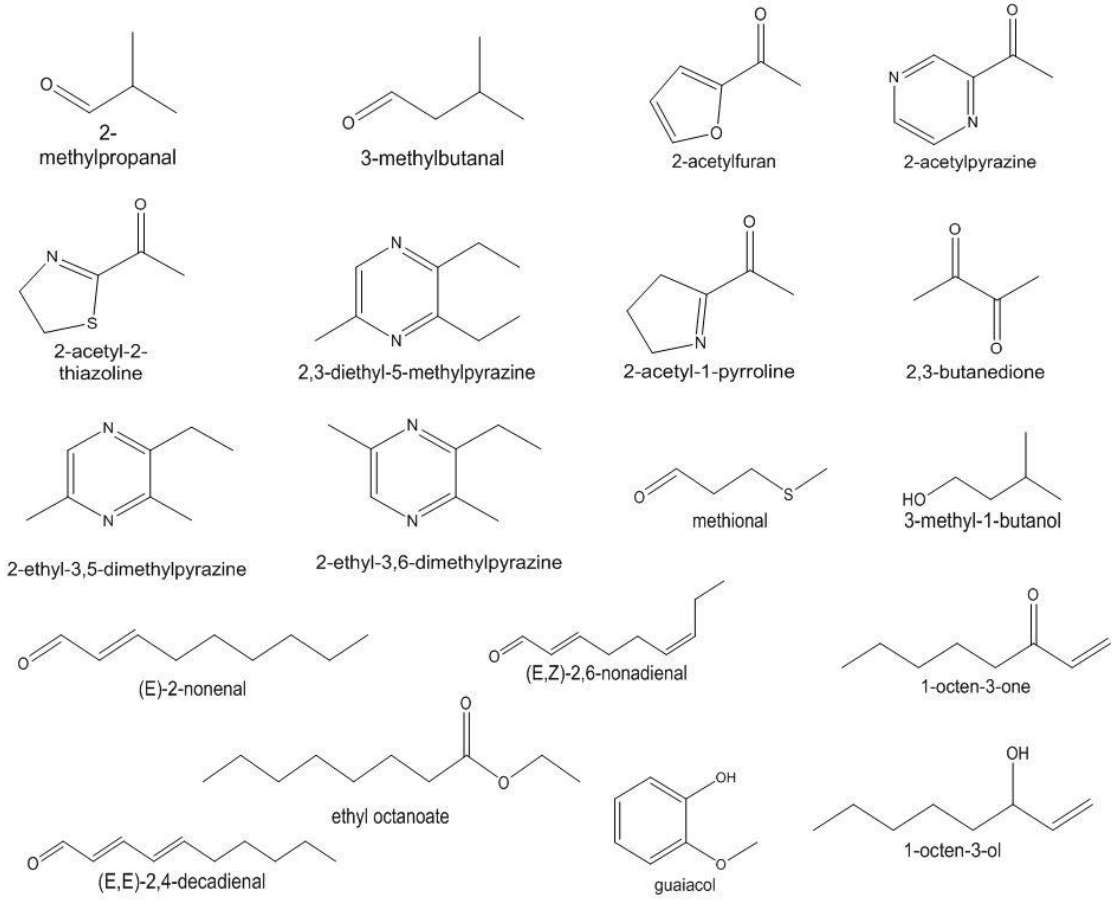

Figure 2. Selected bread aroma compounds

2.7.1 Caramelization

Caramelization is a non-enzymatic browning reaction of reducing sugars, in the absence of amino compounds favored at temperatures greater than 150°C, similar to baking and roasting processes[51]. As caramelization progresses, sugars degrade and undergo intramolecular rearrangements resulting in the formation of volatile compounds and brown pigments as seen in **Figure 3**. Sugar degradation products begin with enolisation, and end with radical reaction. Oxygen

heterocyclic and carbocyclic compounds via aldol condensation are created from the further reaction of aliphatic products formed from sugar degradation[51]. Furans, furanones, pyrones, and carbocyclics, like those presented in **Figure 4**, are typical caramel aroma compounds. Sugar caramelization at the surface/crust of the bread can also produce high molecular weight colored compounds that have acidic and bitter tastes[9].

Caramelization can occur at any pH; however, acidic pH favors the formation of carbonyl cyclic compounds that are reactive, while alkaline pH favors retroaldol reactions resulting in fragmentation products. Although pH affects the compounds formed, temperature is the most influential factor on the generation, and/or proportion, of the compounds formed during caramelization[51].

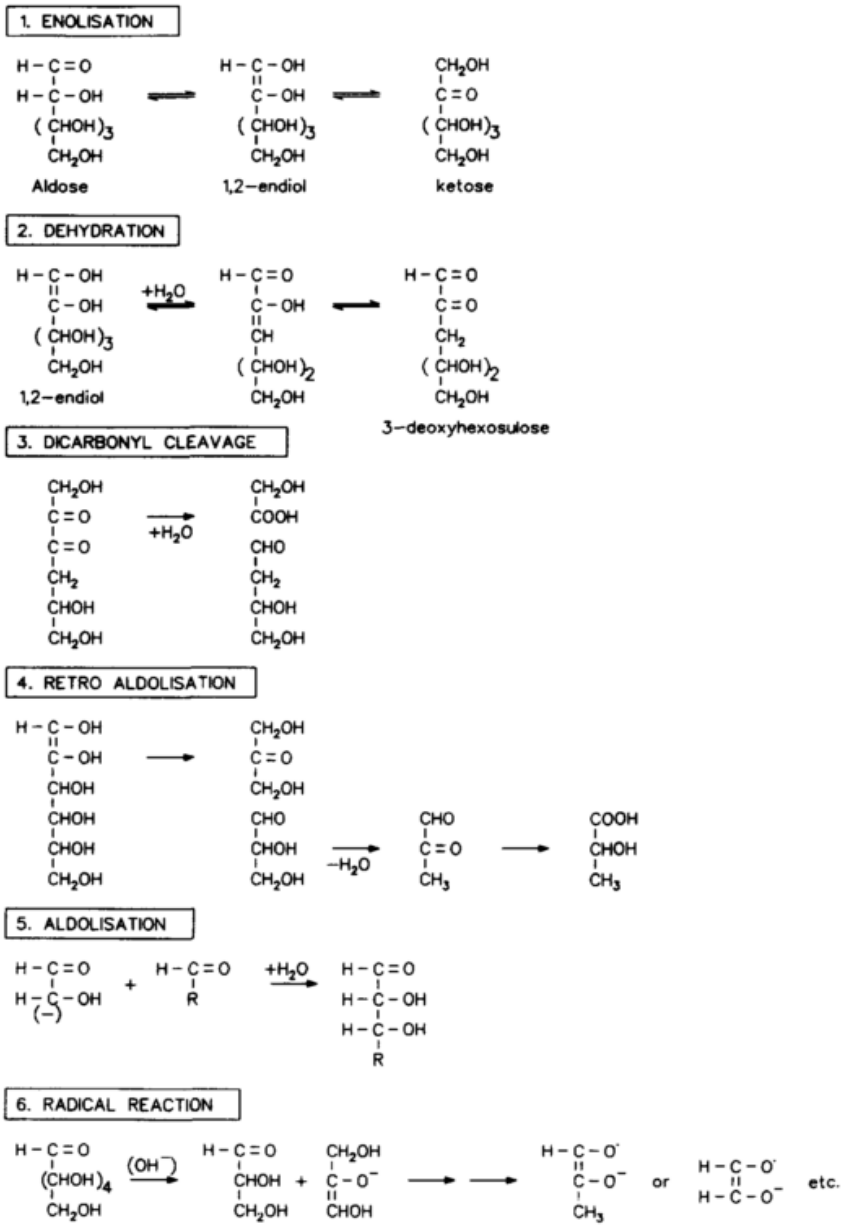

Figure 3. Selected sugar degradation reactions[51]

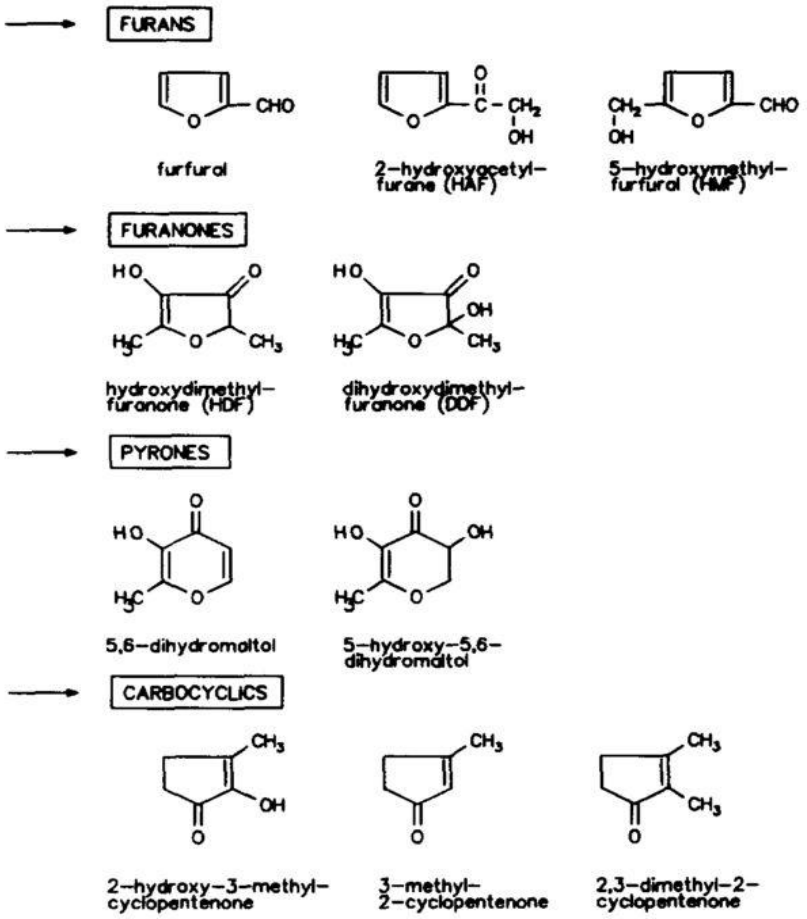

Figure 4. Typical caramel aroma compounds[51]

2.7.2 The Maillard Reaction

The Maillard reaction is the reaction between free amino groups of amino acids, peptides or proteins, and carbonyl groups from reducing sugars [52]. The Maillard reaction plays a very important role in the appearance and taste of foods as it imparts aroma, taste, and color[53]. In addition to flavor and color the significance of the Maillard reaction includes a reduction in nutritional value attributed to decrease of digestibility, destruction and/or biological inactivation of amino acids, and toxicity through the possible formation of imidazoles[53-54]. The

Maillard reaction is a complex and dynamic reaction made up of several consecutive and parallel reactions[53] that is yet to be fully understood. According to Hodge, mechanisms involved in the Maillard reaction can be classified into three stages of development: initial, intermediate, and final[55]. The individual reaction pathway of each stage and their integration within the Maillard reaction can be seen in **Figure 5**.

The initial stage of the Maillard reaction includes sugar-amine condensation and Amadori rearrangement (Reaction A and B in **Figure 5** respectively). Sugar-amine condensation leads to the formation of the Schiff bases, N-substituted glycosylamine (see **Figure 6**) and N-fructosylamines, and is one of the only reversible mechanisms in the Maillard reaction[56,52].

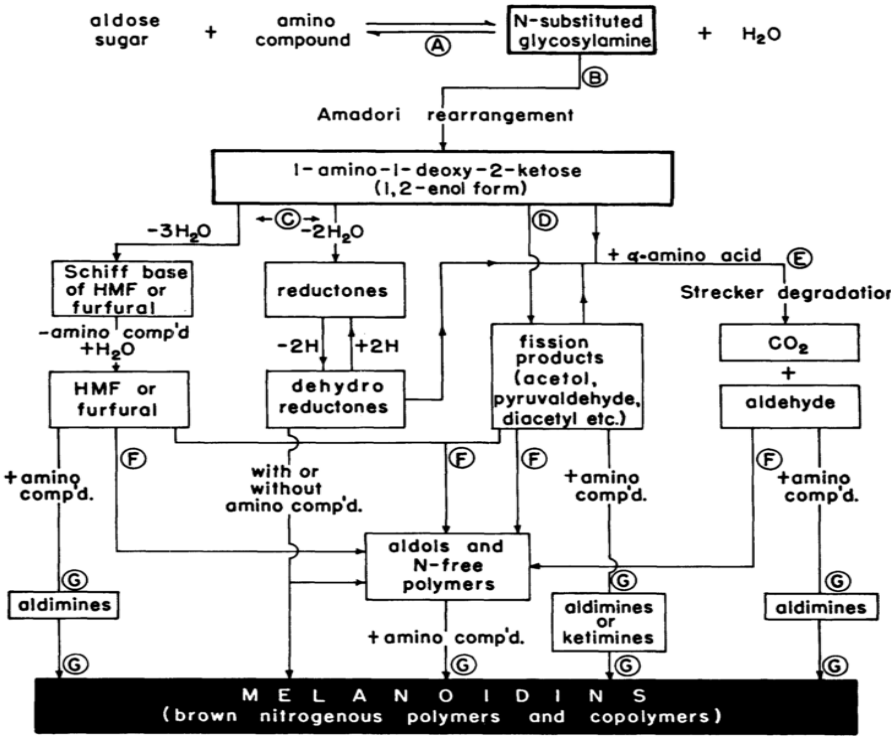

Figure 5. Maillard reaction scheme[55]

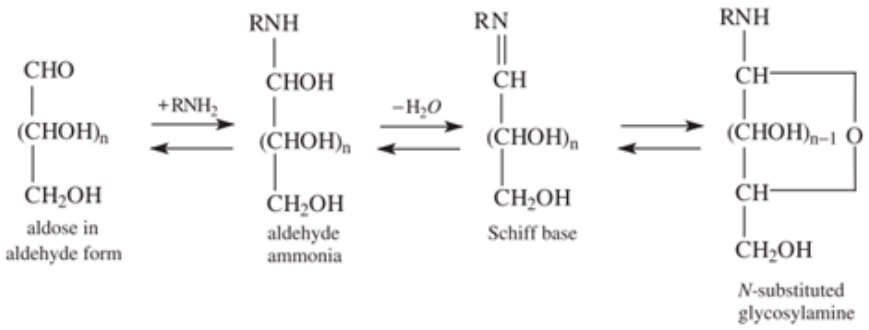

Figure 6. Sugar-amine condensation[56]

Amadori rearrangement involves the Schiff bases from the previous mechanism rearranging to form l-amino-1-deoxy-2-ketoses (Amadori compounds) from aldoses, and 1 -amino-2-deoxy-2-aldoses (Heyns compounds) from ketoses [52,55]; the latter follows a similar reaction to Reaction B seen in **Figure 5**, but is termed Heyns rearrangement[54]. Amadori rearrangement occurs spontaneously at 25°C, but intense conditions, like those in baking, result in the destruction of Amadori compounds. Therefore, any Amadori compounds found in bread after baking most likely formed during storage, not processing[56]. An example of Amadori rearrangement can be seen in **Figure 7**. Amadori compounds react in the following two prominent pathways: 1,2-enolisation via 3-deoxy-1,2-dicarbonyls favored at low pH, and 2,3-enolisation via 1-deoxy-2,3-dicarbonyls favored at high pH which is demonstrated in **Figure 8**.

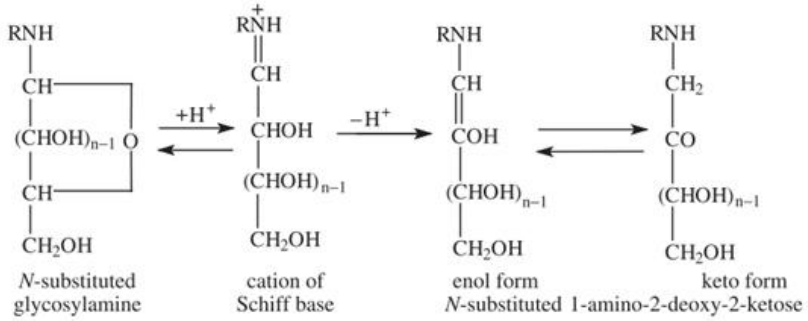

Figure 7. Amadori rearrangement leading to 1-amino-l-deoxy-2-ketose[52]

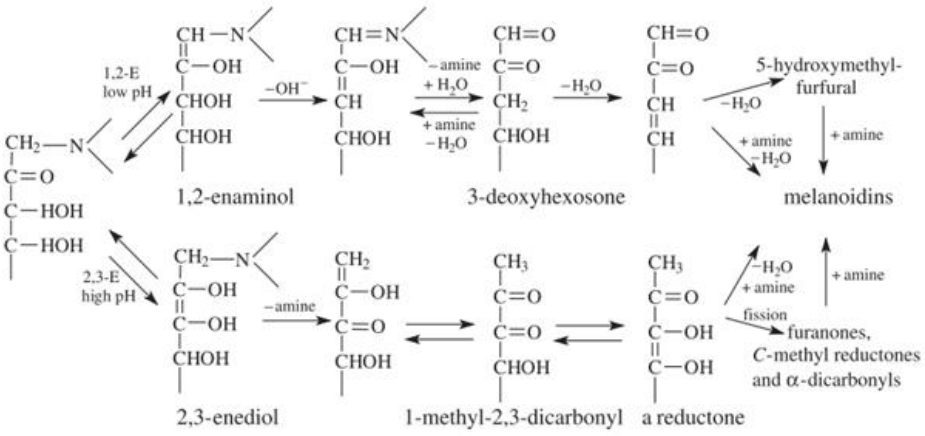

Figure 8. Major pathways from Amadori compounds to melanoidins[56]

The intermediate stage of the Maillard reaction includes: sugar dehydration, sugar fragmentation, and amino acid degradation (Reaction C, D, and E respectively in **Figure 5**). In sugar dehydration reactions of the Maillard reaction, Amadori and Heyns compounds undergo dehydration to form furfural (from pentose) or hydroxymethyl furfural (from hexose)[56]. Furfural and hydroxymethyl furfural are favored under acidic conditions. Amadori and Heyns compounds can also be rearranged into 1-deoxyglycosones or 3-deoxyglycosones (reductones), which are favored at neutral or alkaline conditions[56,52,55].

Sugar fragmentation occurs principally by retroaldolisation (dealdolisation). Fragmentation can occur at C5/C1, C4/C2, or C3/C3 of the hexose derivatives leading to a considerable number of pathways and final products. Key, for aroma generation and highly reactive, products are α-dicarbonyl compounds, such as diacetyl (2,3-butanedione), that are involved in all subsequent pathways[56,51].

Following sugar fragmentation, Strecker degradation occurs between dicarbonyls and α-amino groups. During the reaction, α-amino acids are oxidized to their corresponding aldehydes, releasing carbon dioxide and ammonia[56,33]; α-aminoketones are also formed during Strecker degradation[53]. The aldehydes formed in the Strecker degradation have been speculated to be important contributors to the aroma of food, and are used in many flavoring materials[56,55].

The final stage of the Maillard reaction includes aldol condensation and aldehyde-amine polymerization. These two reactions result in the formation of melanoidins, nitrogen containing brown pigments, and the formation of heterocyclic nitrogen compounds such as: pyrroles and pyrazines[55]. Strecker aldehydes as well as carbonyls from lipid oxidation contribute to aldol condensation resulting in melanoidins[56].

2.7.2.1 Influence of Reaction Conditions on the Maillard Reaction

The mechanisms involved in the Maillard reaction depend on the identity of the precursors (sugars and amino acids) as well as processing conditions and intrinsic properties of the food system such as the temperature, water activity, and pH[56].

Temperature affects the prevailing reaction route, and activities of reactants[57]. An increase in temperature modifies the rate of the Maillard reaction, and consequently, the rate of color formation, and volatile development[58]. During baking, the crust is subjected to higher temperatures than the crumb resulting in more volatile compounds, which leads to a strong impact on the overall flavor and perception of bread[59]. Temperature can also change the aroma profile of a product. A study conducted by Lane and Nursten, showed that aromas were generally formed between 100°C and 180°C and became stronger and more unpleasant as temperature increased with time[58].

Water activity and water content also affect the Maillard reaction as water acts as a solvent and reactant in the system[57]. A lower reaction rate is usually seen with higher water activity due to the dilution of reactants[58].

The pH of a system affects the reaction rate and products formed during the Maillard reaction with a faster reaction rate typically occurring at a higher pH[58]. Several steps in the Maillard reaction are pH dependent, as previously discussed. In addition, the open chain (the reactive form of sugars) concentration of sugar depends on pH as does the active form of the amino reactant[57]. The

active form of amino groups is the un-protonated form, so at a pH above an amino groups pKa the amino group remains not protonated and a more reactive nucleophile[58].

2.7.2.2 Relevant Maillard Reaction Volatiles

Major odor active compounds known to be formed by the Maillard reaction previously reported in bread include: 2-acetyl-1-pyrroline, 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine, 2-methylpropanal, 2-acetyl-2-thiazoline, methional, 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, 3-hydroxy-4,5-dimethyl-3-(5H)-furanone, diethylmethylpyrazine, and acetylpyrazine[9,10,15,17,25,28,34,60–63].

The volatile products of the Maillard reaction can be classified into three groups: simple sugar dehydration/fragmentation products, simple amino acid degradation products, and volatiles produced by further interactions. “Simple sugar” products include: furans, pyrones, cyclopentenes, carbonyls, and acids. “Simple amino acid” products include aldehydes via Strecker degradation that contain characteristic carbon chains, or other functional groups of an amino acid that are sufficiently volatile. Further reaction products are comprised of pyrroles, pyridines, pyrazines, imidazoles, oxazoles, and thiazoles[56].

Heterocyclic nitrogen-containing compounds, namely pyrazines, directly contribute to the roasted flavor of foods[64]. There are over 70 known pyrazines in various thermally processed foods, but only a few ,such as 2-ethyl-3,5-dimethylpyrazine, have a strong impact on aroma profiles because of their low

odor threshold. A mechanism for the formation of a general pyrazine is shown in **Figure 9** below**.**

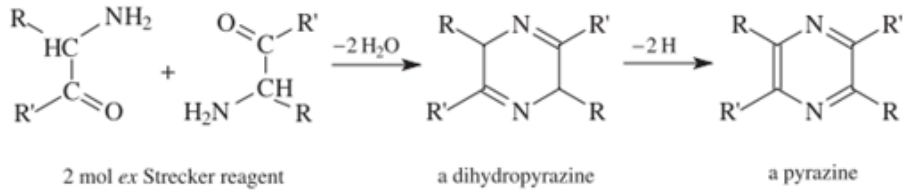

Figure 9. Pyrazine formation from 1,2-aminocarbonyl derived from the Strecker degradation[56]

Alpha-acetyl-N-heterocycles are considered a separate class of Maillard reaction products as they are formed by further reaction. They generally have low to very low odor thresholds, and pleasant roasty, cracker-like aroma, which could be attributed to the α-iminoketone or α-acylenamine structural element in a ring; the sulfur containing 2-acetyl-2-thiazoline and 2-acetyl-1-pyrroline are two of these compounds, and both have been identified as important odorants contributing to the characteristic aroma of bread [9,65].

The aroma compound, 2-acetyl-2-thiazoline has an odor threshold of about 1 ppb (in air) and is formed in small amounts from cysteine and glucose. When cysteamine and 2-oxopropanal react, 2-acetyl-2-thiazoline is formed along with 2-acetylthiazolidine and 2-formyl-2-methylthiazolidine (see **Figure 10**). Proportion of products formed during the reaction are controlled by temperature[56].

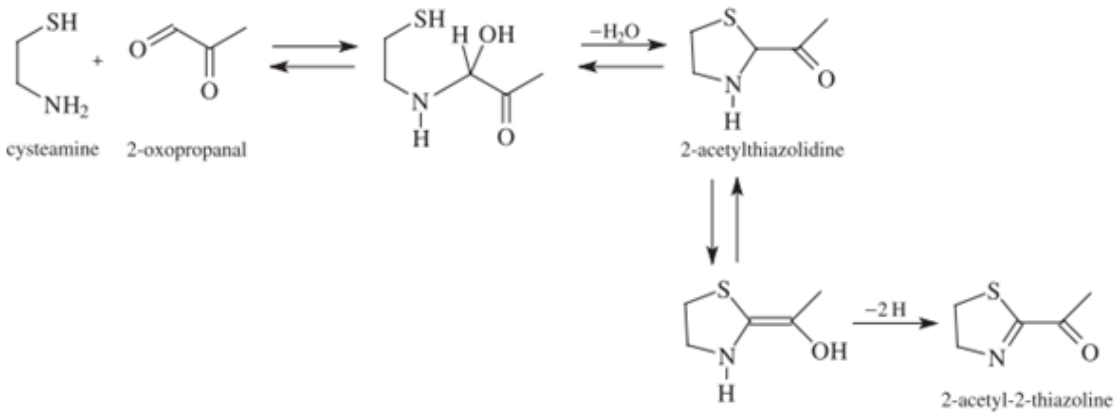

Figure 10. Formation of 2-acetylthiazolidine and 2- acetyl-2-thiazoline from cysteamine and 2-oxopropanal[56]

The compound, 2-acetyl-1-pyrroline has been extensively investigated because of its importance in many food products leading to many hypotheses on methods of formation. One suggested pathway of formation in thermally degraded proline/glucose mixtures is the acylation of 1-pyrroline by a two carbon sugar fragment with 2-oxoaldehydes[65]. It has also been indicated that proline undergoes decarboxylation due to a lack of integration of labeled carboxyl carbon atom in proline into the 2-acetyl-pyrroline structure[57]. Alternatively, it has been shown that in the presence of proline, 3-carbon sugar fragments, like pyruvaldehyde, can yield 2-acetyl-1-pyrroline [57,15,60]. A suggested mechanism of 2-acetyl-1-pyrroline formation involving pyruvaldehyde is proposed in **Figure 11**. Phosphate groups in yeasts are thought to stimulate the generation of pyruvaldehyde, therefore it is not surprising that without the presence of phosphate ions, no 2-acetyl-1-pyrroline was formed in model systems[57,65].

Ornithine in yeast cells also serves as a precursor for 2-acetyl-1-pyrroline. Schieberle hypothesized that the Strecker degradation of ornithine leads to the formation of 4-aminobutyraldehyde as an intermediate, which reacts with 2-oxopropanal, after its immediate cyclization to 1-pyrroline. It is suggested that ornithine is released by the bursting of yeast cells under the high temperature experienced at the crust leading to the formation of 2-acetyl-1-pyrroline[66].

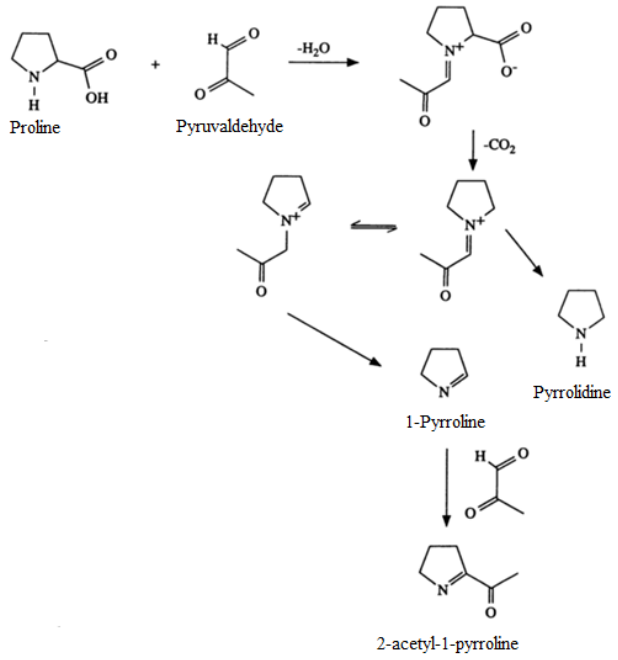

Figure 11. Formation of 2-acetyl-1-pyrroline in the reaction of proline and pyruvaldehyde[60]

2.7.2.3 Impact of Phenolic Content on Whole Wheat Flour and Implications for Intermediate Wheatgrass Flour

Flour composition affects the type of Maillard reaction products that form, as well as the concentration of the products. It has been found that refined breads have higher concentrations of Maillard reaction than WW, and rye breads,

while the highest concentration of specific Maillard reaction compounds vary between WW and rye bread crusts[25,57,62]. Since IWG is related to wheat, it is expected that the concentration of Maillard reaction compounds will be similar to the concentration in WW, and inferred that IWG will have a lower concentration of Maillard reaction compounds than a refined bread crusts. One reason for this lower concentration of Maillard reaction products in whole grain flours is phenolic compounds.

Phenolic compounds have been shown to contribute to the flavor profiles of foods by inhibiting or promoting certain reaction pathways as well as through their inherent flavor attributes. Phenolic compounds are present in the bran of a grain, and so present in whole grain flours[67]. Hydrocinnamic acids (HCAs) are ubiquitous in plant material and are the predominant phenolic compounds in grain[68]. HCAs are known to alter Maillard chemistry, which generate desirable qualities in baked goods. In model systems, HCAs have been reported to form adducts with Maillard reaction flavor precursors as well as amino acids and their reaction products[67-68]. Although these studies investigated the formation of adducts in model systems, the findings are applicable to phenolic compounds forming adducts with Maillard reaction intermediates in bread. Phenolic content, therefore, is likely to directly impact the flavor profile and perception of baked products. In addition, Maillard reaction volatile compounds generated both in model systems and in food systems have been shown to be suppressed in the presence of phenolic compounds such as HCA[67,68,25].

Specifically, ferulic acid, has been shown to suppress the generation of 2-acetyl-1-pyrroline, a compound important in bread aroma, by reacting with an important precursor of the compound[25]. Reduction of this Maillard reaction compound impacts the aroma profile of the bread by reducing desirable toasted notes, especially the crust. Bunzel *et al*.[69] found that ester-linked trans-ferulic acid was the dominant HCA in IWG and two-fold higher than the levels in wheat or rye, suggesting IWG may experience more Maillard reaction inhibition leading to fewer Maillard reaction aroma compounds.

2.7.3 Lipid Oxidation

Lipids provide a reaction medium for interaction, impart flavor, and generate aroma[70], and the lipids present in whole grain flours are likely to oxidize. Lipid oxidation includes autoxidation, thermal oxidation, photo oxidation, and lipoxygenase-derived oxidation. These reaction pathways are influenced by many factors including the composition and content of triglycerides, antioxidants, and metal ions[70-30] (**Figure 12**).

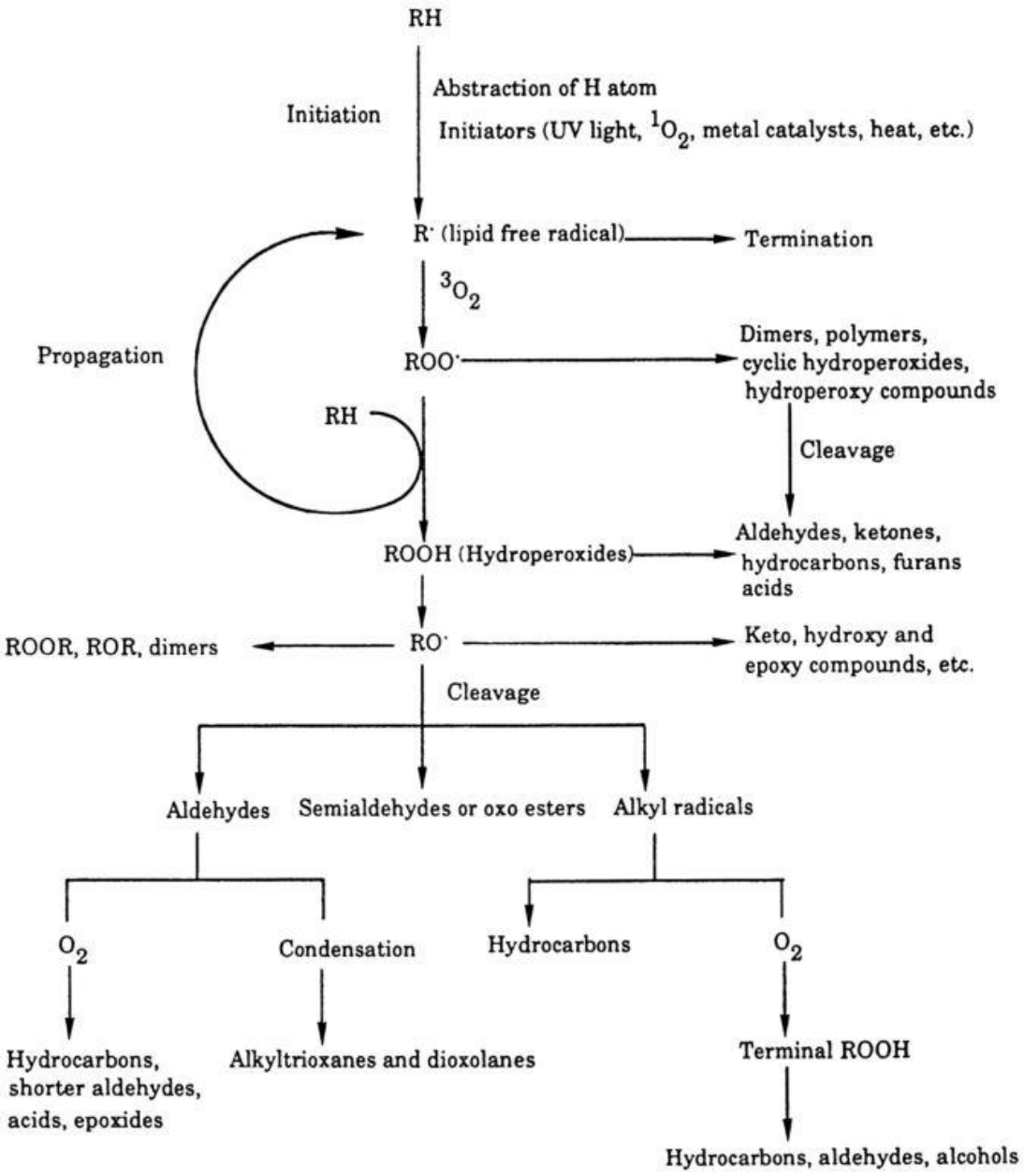

Figure 12. Mechanism of lipid oxidation and formation of primary and secondary degradation products[70]

The variability of lipid oxidation compounds between flours is not as wide as Maillard reaction compounds, but flour composition is still responsible for the products formed by lipid oxidation. As is intuitive, breads made with whole grain flour with higher fat content when compared to refined flour show a higher concentration of lipid oxidation products than refined breads[9,14,25]. The addition of exogenous fats in bread formulations have also been reported to influence the formation of lipid oxidation products. Notably, the use of margarine over butter resulted in a higher degree of lipid oxidation due to a higher level of linoleic acid[11,70].

The primary products of lipid oxidation reactions are tasteless and odorless, but the secondary products are odor-active[30]. In addition, primary and secondary lipid oxidation products may participate in Maillard reactions upon heating, adding complexity to pathways of flavor generation. Interestingly, the flavor qualities of different heated oxidized fats are markedly unique, and provide characteristic attributes to the food[30-71]. There are several classes of oxidation products with various odor thresholds. Classes of oxidation products with odor thresholds at or below 0.04 ppm include 2-alkenals, (*E*,*E*)-2,4-alkadienals, and (*E*,*Z*)-alkadienals. The formation of the specific classes from lipids depends on the heating conditions, nature of the fatty acids involved, presence of enzymes, and presence of moisture to produce free fatty acids, ketones, lactones, acylglycerols (mono and di), and glycerol via hydrolysis[70].

Temperature affects the formation of aroma compounds from lipid oxidation. For instance, at low temperatures hexanal is the major oxidation volatile compound, whereas 2,4-decadienal is the major aldehyde formed at high temperatures. These two compounds come from linoleate hydroperoxides, though different bonds are vulnerable at different temperatures in linoleate hydroperoxides resulting in the favoring formation of one over the other. In addition, saturated fatty acids are extremely stable at low temperatures and rarely contribute to the oxidative decomposition pattern, but they can play a significant role when temperatures exceed 150°C[71].

Enzyme-catalyzed oxidation, involving lipoxygenases and isomerases, of unsaturated fatty acids yield large quantities of carbonyls, some of which are reduced to alcohols and potentially esterified to yield esters. Lipoxygenases form a peroxide of an unsaturated fatty acid by adding molecular oxygen while isomerases rearrange the peroxide resulting in numerous choices in degradation products[72]. The cleavage points and some resultant degradation products of linoleic and linolenic acid oxidation are shown in **Figure 13**.

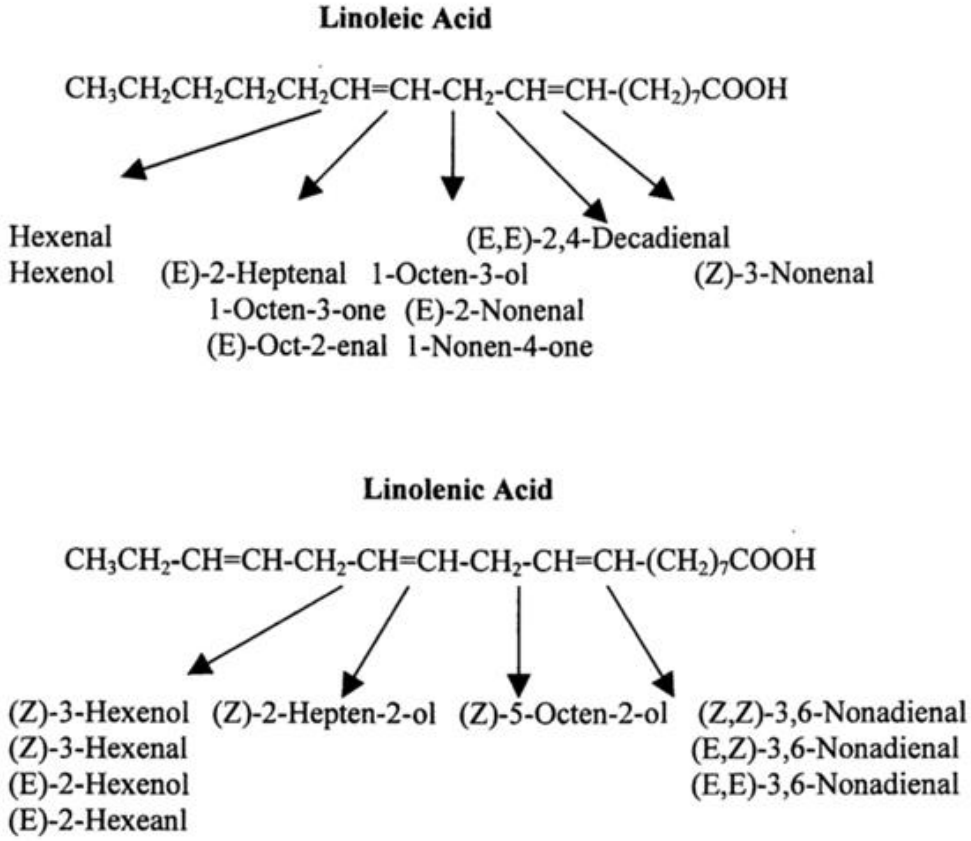

Figure 13. Aroma compounds from the oxidative degradation of linoleic and linolenic acids[72]

Overall, the generation of lipid oxidation products is not the only reason for their impact on the aroma profile of breads; their correlation with loss of freshness is also a factor.

Important odorants to freshness rapidly diminish during storage while less desirable volatiles, like lipid oxidation products, remain relatively unchanged and at times continue to increase, causing the aroma of the bread to be less desirable over time. The volatile compounds important to freshness of bread are lost due to evaporation at the breads surface as well as continued oxidation reactions. Unlike volatile compounds formed by lipid oxidation, formation of

volatile compounds generated by yeast fermentation and the Maillard reaction cease either before or immediately after baking. Diffusion of Maillard reaction compounds from the crust to the crumb and adsorption by the starch and protein contents of the crumb also result in a loss of volatiles important to freshness[14,31]. If lipid oxidation was halted, or chemically unfavorable, the compounds responsible for freshness would still be lost changing the ratio of present compounds, which would likely change the overall perception of the profile as well.

2.7.3.1 Relevant Lipid Oxidation Volatiles

Major odor active compounds formed by lipid oxidation reported in bread include (*E*)-2-nonenal, (*E*,*E*)-2,4-decadienal, (*E*,*Z*)-2,6-nonadienal, 1-octen-3-one, and 1-octen-3-ol[9–11,14,25,70,73]. Many lipid oxidation products form from specific peroxidation of linoleic acid, the major fatty acid among the lipids occurring in wheat flour[34], like (*E*)-2-nonenal and (*E*,*E*)-2,4-decadienal. These compounds generate in the crumb, and then are transferred to the crust[63]. 1-octen-3-one is an additional compound characterized by the peroxidation of linoleic acid that is formed from linoleic acid after it is converted to the 10-hydroperoxide isomer via lipoxygenase activity. An intrinsic lyase enzyme, specific to the 10-position, cleaves the hydroperoxide resulting in the formation of 1-octen-3-ol. 1-octen-3-ol can be transformed into 1-octen-3-one via auto-oxidization (see **Figure 14**)[72]. Even though 1-octen-3-ol has similar aroma and is

often present in much larger amounts than l-octen-3-one, the ketone has a much lower odor threshold value and likely a stronger impact on the aroma of a food.

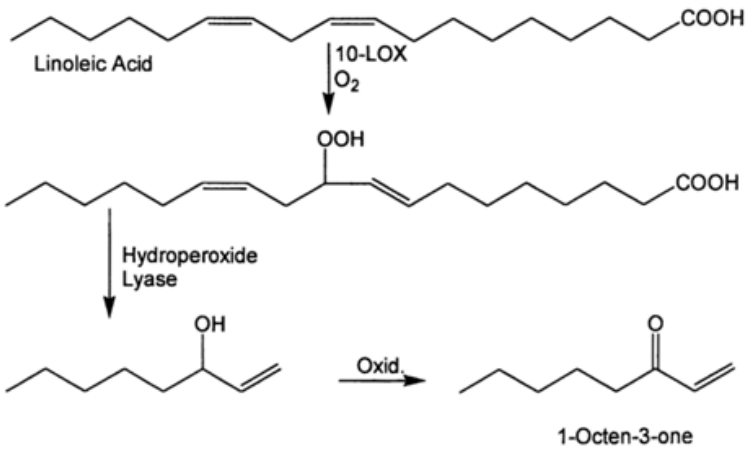

Figure 14. Formation of 1-octen-3-one from linoleic acid[72]

(*E*,*Z*)-2,6-nonadienal is another lipid-derived aroma compound, but instead of its origins stemming from linoleic acid, it originates from linolenic acid. Linolenic acid, liberated from endogenous lipids via acyl hydrolases, is converted to the hydroperoxide isomer by 9-lipoxygenase. Cleavage of the hydroperoxide by lyases forms (*Z*,*Z*)-3,6-nonadienal which can be converted to (*E*,*Z*)-2,6-nonadienal by an isomerase enzyme (see **Figure 15**)[72].

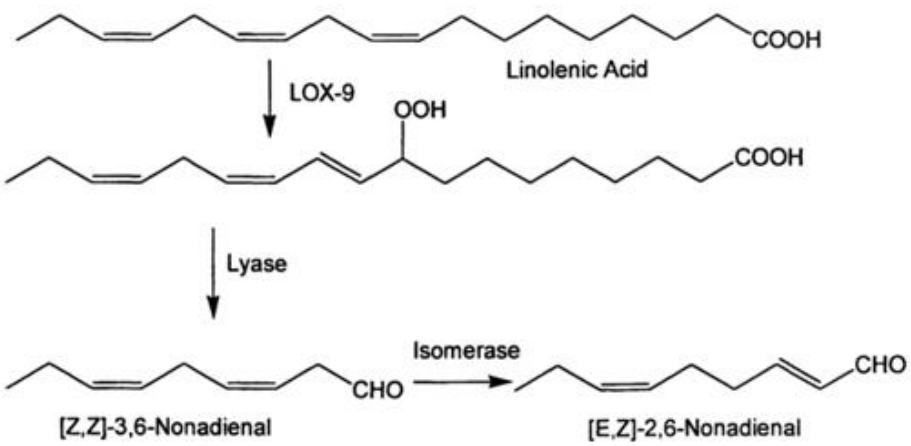

Figure 15. Formation of (*E*,*Z*)-2,6-nonadienal from linolenic acid[72]

2.7.4 Yeast Fermentation

Besides yeast providing a leavening agent metabolite in bread, yeast metabolism contributes to aroma generation. Yeast fermentation has been reported to be an important contributor on the generation of fragrant alcohols and esters like 2-phenylethanol, 3-methyl-1-butanol, 1-hexanol, ethyl octanoate, and ethyl nonanoate[25,32,61,66,74]. Previous studies have found that yeast fermentation provides precursors for some Maillard reaction products, and the concentration of aroma compounds from yeast fermentation is dependent upon the fermentation activity of the yeast and the strain of yeast[32]. The fermentative activity in turn is affected by nitrogen composition, amount of starch, anaerobic conditions, and accumulation of ethanol[75–77].

All baker's yeasts are selected from strains of *Saccharomyces cerevisiae*, with each strain having unique properties that affect bread flavor[32]. During fermentation the alcohol produced, the decrease of pH, and the synthesis of

different metabolites contribute to the development of flavor and aroma of the bread[77]. Fermentation time and temperature affect the type and concentration of aroma compounds formed by yeast metabolism. The fermentation step of breadmaking therefore serves as a source for flavor optimization in yeast breads.

Yeast fermentation is responsible for the majority of the aroma compounds formed in the crumb, dominated by alcohols, aldehydes, 2,3-butanedione, and esters[32]. As previously mentioned, it is possible for the transfer of compounds during crust formation from crumb to crust and vice versa, resulting in volatiles associated with yeast fermentation being detected in the crust and compounds associated with Maillard reactions being detected in the crumb[63]. Aldehydes, or their corresponding alcohols, are formed inside the yeast cell from degradation of the amino acids found in the flour via the Ehrlich pathway. Esters are produced in the yeast cell by an enzymatic reaction between acetyltransferases, acetyl coenzyme A, and various alcohols. 2,3-butanedione, and similar compounds, are formed from acetohydroxy acids leaked from the yeast cell through non-enzymatic chemical reactions outside the yeast cell[32].

2.8 Influences of Enzymes on Bread Aroma

The flavor of bread is influenced by flour composition, enzymatic reactions occurring during dough fermentation by yeasts and/or lactic acid bacteria, and thermal reactions induced by baking. However, other enzymes can also play a role in bread flavor.

The intensity of enzymatic activity varies between breadmaking stages[9]. There are three sources of enzymes available during breadmaking that create positive or negative effects on bread flavor: those existing in flour, those associated with the metabolic activity of yeast and/or lactic acid bacteria, and those purposely included in formulation. There are three main enzymatic systems in breadmaking related to flavor: amylases, proteases, and lipoxygensases. It should be noted that secondary activities of invertases, oxidases, and lipases could also have minor contributions to flavor.

Amylase activity produces reducing sugars, which become fermentable substrates for yeasts and precursors for non-enzymatic browning. Proteases hydrolyze peptidic bonds in proteins, polypeptides, and peptides, producing oligopeptides, peptides, and amino acids. These products can then participate in metabolic and thermal reactions. Lipoxygenases, used in some baking processes, and native in the germ and bran of wheat grain give unstable products that decompose to carbonyl compounds generating lipid oxidation products[78].

2.9 Identification of Odor-Active Compounds

Gas chromatography-olfactometry (GC-O) has become the most widely used technique for evaluation of complex food flavors because it directly provides important information about the presence of compounds with aromatic properties in food[79]. GC-O allows flavor chemists to focus on components that may

contribute to flavor by discriminating the minute fraction of odor-active volatiles from the plethora of odorless volatiles present in a food[80-81]. A sample isolate, or extract, is injected into a GC where the individual compounds are separated on a GC column. The effluent is then split between a detector, commonly a mass spectrometer, and a capillary located inside a heated arm with a humidified nose cone (see **Figure 16**). A panelist continuously analyzes the effluent, orthonasally, documenting any responses.

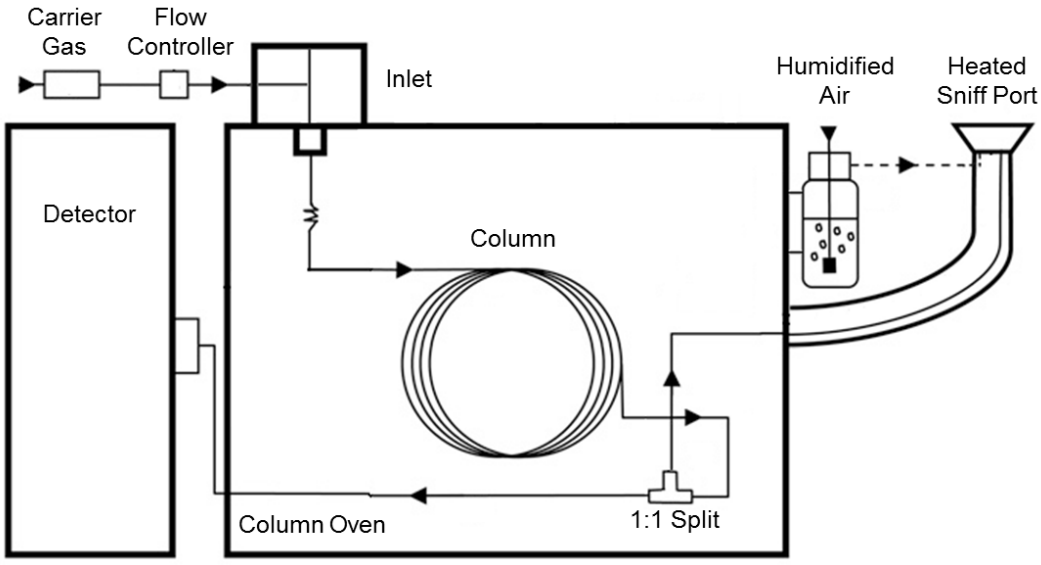

Figure 16. Gas-chromatography-olfactometry-mass spectrometry schematic[57]

2.10 GC-O Techniques

The drawback of GC-effluent sniffing is that it does not allow a differentiation between those odor compounds that contribute intensely to a flavor and those which are only components of the background flavor[28]. The ratio between the concentration of a compound and its threshold in a specific food is

known as its odor activity value (OAV). Historically, OAVs have been used to assign importance to compounds, with a higher value indicating more importance[28,61,82]. More recently, the importance of a compound's OAV has been called into question. For example, Vickers *et al.* found that OAV was a poor estimate of odor intensity across compounds at higher intensities, but was reliable at lower intensities. OAVs also did not accurately predict the relative odor intensities of different odorants. The use of OAVs therefore should only be taken as a suggestion of importance only, and the practice of listing odorants in tables in order of OAV should desist because it unintentionally implies a ranking order. Vickers *et al*. go on to state that any methodologies used to determine which aroma compounds most likely make a contribution to the odor of a food need to perform sensory work to determine which aroma compound are truly contributory[83].

Several other GC-O methods have been developed to elucidate which compounds contribute the most to aroma, and can be classified into 3 broad categories: 1) techniques based on determination of threshold concentration/dilutions, 2) techniques based on the measurement of the frequency of citations, and 3) techniques based on the assessment of intensity[79].

Aroma extract dilution analysis and Charm are two common techniques based on the determination of threshold. AEDA is a simple, semi-quantitative, technique where the flavor extract is sequentially diluted. Each dilution is analyzed using GC-O performed typically by a small number of experienced

judges until no more odors are detected. The flavor dilution (FD) of an odorant corresponds to the maximum dilution at which that odorant can be perceived by at least one of the judges; a larger FD value, therefore, implies more importance[79].

Charm values are based on the area of a chromatographic peak produced from the plot of dilution value (y-axis) versus retention index (x-axis), and differs from AEDA by the inclusion of the duration of the perception[84,85]. In addition to the functionality of dilution techniques as a technique for screening, dilution techniques add significance to GC-O results.

A single GC-O run does not present meaningful results because the perception of aroma substances in the carrier gas stream depends on limiting quantities independent of the aroma value including, the amount of food analyzed, the degree of concentration of the volatile fraction, and the amount of sample separated by GC[73]; however, when used in tandem with dilution techniques the impact of the limiting quantities is reduced. Techniques based on the measurement of frequency of citations will not be discussed as they are not commonly used in determining importance.

Intensity methods are based on magnitude estimation of the odor intensity. McDaniel *et al*. developed a time-intensity method called Osme where a trained assessor(s) directly record the intensity and duration of each odor active compound detected at the sniff port along with a description of the odor[86].

This technique is used to a lesser extent than AEDA, or Charm, likely due to the complexity and difficulty to perform the technique.

2.11 Approach Limitations

Before choosing a GC-O technique one needs to consider the shortcomings and assumptions of each method. It is important to keep in mind that though the human nose is a very sensitive detector it has been shown that the olfactory sensitivity of an individual changes through the day and over longer periods of time which can introduce variability in data collection[87]. In addition, results can also be influenced by genetic sensitivity of the individual assessor to perceive particular aroma substances[84]. Furthermore, assessors can describe the same odor differently from each other due to their unique previous experiences.

It is important to note that GC-O analysis does not mimic the conditions of food consumption. For instance odors detected during GC-O analysis are analyzed individually instead of as a mixture from the sample matirx[88]. By removing the volatiles from the food matrix for analysis, the delivery of the volatile compounds to the olfactory system is significantly changed. The selection of odorants by dilution analysis also does not take into account additive or suppressing effects of compounds because of the GC separation of volatile compounds[73].

Any isolation, extraction, and injection procedures can introduce contaminants, change the partition coefficient of a compound, or create flavor artifacts, respectively[3,80]. Furthermore, it is known that the ratio between compounds is very important in overall perception. If the concentration ratio of volatiles in a vapor phase of an extract is not the same as the ratio in the vapor phase above the food, which is commonly the case when you examine an extract versus a food system, a different perception is experienced when compared to the original sample[3]. Therefore, performing GC-O does not guarantee that all the important flavor compounds will be among the volatiles identified, especially if compounds co-elute[80,84].

Experience shows that many odor active compounds occur at very low concentrations, so the peak profile obtained by any chemical detector does not necessarily reflect the aroma profile of a food, or which compounds are the most impactful. Moreover, deeming a compound perceived at the highest dilution as the most potent in a sample requires the assumption that the response to an odor stimulus is linear with dilution and all compounds have identical response slopes with increasing concentration[85]. To offset some of these shortcomings it is common practice to identify an aroma based on agreement of mass spectra, to examine retention indices on at least two capillary columns of different polarity, and to use multiple panelists[84,73]. As mentioned by Vickers *et al*., it is essential to combine analytical results with sensory evaluation to prove the correct selection of odor active compounds[83,85].

CHAPTER 3: A COMPARATIVE AROMA ANALYSIS OF INTERMEDIATE WHEATGRASS AND WHOLE WHEAT BREAD CRUSTS

3.1 Summary

The aroma profiles of intermediate wheatgrass (IWG) and whole wheat (WW) bread crust were examined and compared by gas-chromatography-olfactometry-mass-spectrometry/aroma extract dilution analysis (GC-O-MS/AEDA). Nineteen odorants were selected based on flavor dilution (FD) values. All compounds were positively identified and consisted of 10 Maillard reaction, six lipid oxidation, three yeast fermentation products, and one known to be formed by all three of the previously mentioned pathways. Quantitative analysis revealed the concentration of 16 odorants were significantly different between both samples, of which 13 were found to be higher in concentration in WW and primarily consisted of Maillard reaction compounds. These findings suggested Maillard-reaction-type pathways were inhibited in IWG likely due to differences in phenolic composition and amino acid composition. A reduction in the fermentation activity of the yeast was also suggested. Different lipid oxidation products were favored between the two crusts indicating that different pathways dominate in each product. Furthermore, sensory descriptive analysis of bread crusts was in agreement with the data confirming the influence of the identified compounds on the flavor development of both products. These findings provide an improved basis to optimize the flavor of IWG bread by processing and breeding strategies.

3.2 Introduction

Food choice is based on several parameters unique to each consumer including flavor, but more recently, healthfulness has become an important factor to consumers[1,2]. Whole grain foods have been highlighted by the USDA as an essential component to a healthy diet as they provide fiber and phytochemicals that are linked to a reduced risk of several chronic diseases[5]. Another growing aspect affecting consumer food choice is crop sustainability[2]. A sustainable food finds a balance between benefiting people, the environment, and the economy[19].

The increased influence of sustainability on consumer purchasing habits, has led to developments in new sustainable crops for food use. Intermediate wheatgrass has been identified as a potential sustainable replacement for annual wheat based on its beneficial agronomic and environmental properties, as well as its rich nutritional profile[20–22]. However, it is essential that IWG products have acceptable flavor quality in order for the crop to be utilized by the food industry.

Bread is a staple food across the world with large market penetration, and the rising consumer trend for healthier foods[4], has lead an increasing number of consumers to purchase WW bread; however, market penetration of WW bread is still hindered due to negative flavor attributes associated with that bread. Significant time and effort has been spent on understanding, and improving the flavor of WW bread through adjustment of ingredients, but also taste and aroma research[9–17]. This knowledge is important to the baking industry[18], and has led to improvements in understanding WW flavor. Therefore, in order for IWG to be

successful in food production, such as breadmaking, similar research into the taste and aroma of IWG bread is required.

Numerous aroma profile studies have been performed on refined, WW, sourdough, and rye breads[17,25,28,34,63,73,74,89]. Major sources of aroma generation in bread were found to be the result of the Maillard reaction, lipid oxidation, and yeast fermentation pathways, and were dependent of flour composition[25,9,26]. The inclusion of all parts of the grain (germ, bran, and endosperm), and variety of grain create variation in the flavor between breads, due to differences in physical and chemical composition that impact the reaction pathways involved in flavor generation. It has been reported that inclusion of the bran in WW flour results in the inhibition the formation of Maillard reaction products in WW bread crust compared to refined bread crust due to phenolic compounds in the bran binding and forming adducts with key Maillard intermediates[25,67,68]. It has also been found that the inclusion of the germ in flour results in the formation of aroma compounds characteristic of lipid oxidation reactions[9,25,40,63]. However, nothing is known about the formation pathways involved in aroma generation, the compounds present in the aroma profile, or the impact of flour composition on IWG bread.

The overall objective of this work was to investigate the aroma profile of IWG to determine the major formation pathways involved in aroma generation and specific compounds involved in the aroma profile of IWG crust with comparison to the aroma profile of WW bread crust. Comparison between the aroma profiles

of IWG and WW bread crusts provides a foundation to develop flavor optimization strategies for IWG products by linking aroma compounds to specific responses of the olfactory system.

3.3 Materials

3.3.1 Chemicals

The following compounds, 2-methylpropanal, 2,3-butanedione, 3-methylbutanal, methional, 2-acetylfuran, 1-octen-3-one, 1-octen-3-ol, (*E*)-2-nonenal, (*E*,*Z*)-2,6-nonadienal, 2-acetylpyrainze, 2-ethyl-3,5 (or 6)-dimetylpyrazine, 2-acetyl-2-thiazoline, (*E*,*E*)-2,4-decadienal, and ethyl octanoate were purchased from Sigma Aldrich (St. Louis, MO, USA). 3-methyl-1-butanol, guaiacol, and 2-methyl-3-heptanone were purchased from Tokyo Chemical Industry (TCI; Portland, OR, USA). 2,3-diethyl-5-methylpyrazine, butylated hydroxytoluene (BHT), ethanol (200 proof) and dichloromethane (DCM) were purchased from Fisher Scientific (Pittsburgh, PA). Sodium bicarbonate and anhydrous sodium sulfate were purchased from Avantor (Center Valley, PA). 2-acetyl-pyrroline (deuterated) was a gift from Pennsylvania State University (State College, PA).

3.3.2 Ingredients

King Arthur Flour premium 100% hard red whole wheat unbleached flour was used for the preparation of WW bread samples, and IWG groats, provided by the Land Institute (Salina, KS), were ground in a whole grain flour cyclone sample mill (UDY, Fort Collins, CO) equipped with a 0.25 mm screen for the preparation of IWG bread samples. Fleischmann's Dry Active Yeast (ConAgra), Crisco vegetable shortening (The J.M. Smucker Co., Orrville, OH), Crystal Sugar (American Crystal Sugar Company, Moorhead, MN) granulated sugar, and Morton Salt (Chicago, IL) iodized salt were purchased from local markets and used in both bread samples.

3.4 Methods

3.4.1 Breadmaking

Bread samples were prepared using an adjusted[25] AACC method 10-10B[90] with some adjustments made to the amount of ingredients and baking time. Briefly, one beaker of sugar (72 g) and salt (18 g), and another of yeast (24 g), were each mixed with 150 g of heated (39°C) distilled water. Flour (1200 g of either WW or IWG) was added to a mixing bowl and was combined with the sugar/salt and yeast solutions as well as vegetable shortening (36 g) that had been melted at 60°C. The total amount of distilled water was then brought to 873.6 g. The dough was mixed with a bread hook for a total of seven minutes using a 10-speed stand mixer (KitchenAid Artisan KSM150PSWH, St. Joseph,

MI). Batches of dough were scaled to 100 g of flour, placed in lightly greased fermentation bowl, and covered with saran wrap. The fermentation bowls were placed in a fermentation cabinet/proofing oven (Baxter PW2E, East Orting, WA) held at 30°C and 85% relative humidity. A sheeter performed first punch with 3 in roll width and 3/16 in roll spacing at 52 min, the second punch occurred 25 min after the first, and the final punch occurred 13 min after the second. After the final punch, the dough was sheeted and molded into loaves and placed in loaf pans. The loaf pans were put back in the proofing oven for 33 min, and then placed in a rotary oven, primed with 1 L of water, at 215°C for 17 min. The loaves were immediately removed from the pans, and prepped for solvent extraction.

3.4.2 Preparation of Bread Crust Solvent Extracts for Volatile Identification and Quantification

The crust was immediately separated from the bread crumb after baking, frozen with liquid nitrogen, and ground using a food processor. For identification, 625 g of crust was extracted with 1.25 L of DCM spiked with 8 µg of 2-methyl-3-heptanone as an internal standard, and 8 µg of BHT to serve as an antioxidant. The extraction was performed under a blanket of nitrogen gas and agitation using an orbital shaking table (Thermo Scientific MaxQ™ HP Tabletop Orbital Shaker, Waltham, MA) at room temperature for 15 h. The solvent was removed by filtering through glass wool, and filter paper (Whatman 4). The crust slurry/residue was extracted under the same conditions with an additional 1.25 L of DCM for 3 h. The solvent was removed, filtered, and pooled with the previous

extract. The combined pooled extract was dried with anhydrous sodium sulfate, and then concentrated to approximately 100 mL using a Vigreux distillation column (60 cm). The extract was then fractionated by solvent assisted flavor evaporation (SAFE)[91]. The volatile isolate recovered by SAFE was neutralized by washing three times with a 1:1 volume of 0.5 M sodium bicarbonate and once with a half volume equivalent of saturated sodium chloride[3]. The isolate was dried with anhydrous sodium sulfate, and stored at -20°C overnight. The isolate was removed from the freezer and allowed to thaw to room temperature prior to a final distillation and concentration volume of 1 mL[25]. For quantification, the crust slurry was extracted for an additional 3 h with DCM in order to increase the recovery of aroma compounds.

3.4.3 Gas Chromatography-Olfactometry-Mass Spectrometry/ Aroma Extract Dilution Analysis

GC-O-MS analysis was performed on an Agilent GC G1530A (Agilent Technologies, Santa Clara, CA) equipped with an HP-5 column (30m x 0.25mm ID x 0.25µm film thickness; Agilent Technologies), a CombiPAL auto sampler (PAL System, Lake Elmo, MN), and coupled with a Hewlett Packard 6890 mass spectrometer detector (MSD) (Hewlett Packard, Palo Alto, CA), and a sniffing port (Hillesheim, heated transfer line with nose cone, Waghäusel, DE). The effluent was split 1:1 after separation, between the MSD and sniffing port. Purified air was bubbled through distilled water and purged at the end of the sniffing arm at a rate of 20 mL/min to reduce nasal dehydration. One µL of

volatile extract was injected using splitless injection. Inlet temperature was set at 250°C, helium carrier gas set to a constant flow of 1.5 mL/min, and the GC temperature gradient program was as follows: 40°C was initially held for 1 min and then ramped at a rate of 3°C/min until 150°C, followed by 30°C/min ramp to 250°C and held for 5 min. Each sample was serially diluted by 1/2 volume in dichloromethane and analyzed until no further aromas were detected. Each dilution was analyzed in duplicate by three panelists (one female, two males). The largest dilution, at which each aroma was detected, by at least one panelist, was defined as its FD value.

All samples were run on two columns of different polarities; an Agilent HP-5 (30m x 0.25 mm x 0.25 µm film thickness) and an Agilent DB-Wax (30m x 0.25 mm x 0.25 µm film thickness). The GC conditions for the DB-Wax were as follows: GC oven initial temperature was 40 °C and held for 1 min, ramped at 3°C/min to 180°C, then ramped at 30°C/min to 240°C and held for 5 min. The MSD conditions for both columns were as follows: capillary transfer line was set at 250°C, source temperature 143°C, mass range 29-350 amu at 2.35 scans/sec operated in electron ionization (EI) mode.

3.4.4 Identification of Compounds of Interest

Compounds were positively identified using mass spectra, odor descriptors, and linear retention index (LRI) comparison to authentic compounds. LRI values were calculated with an *n*-alkane from C_6-C_{25}.

Five compounds of interest required variation from the GC-MS method described above due to co-elution and/or low sensitivity. 2-acetyl-2-thiazoline, was separated and positively identified using an Agilent DB-1 (x 0.25 mm x 1 µm film thickness) on an Agilent 6890N GC (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 5973 Network MSD (Agilent Technologies, Santa Clara, CA), a Gerstel sniffing port (Gerstel, Inc, ODP 2, Linthicum, MD), a Gertsel modular accelerated column heater (MACH) system, an Agilent 7683B injector, and a Gerstel cool injection system (CIS). The helium carrier gas was set to a constant pressure of 357 pKa and 1 µL of each isolate was injected by splitless injection. The CIS initial temperature of 40°C for 0.2 min was obtained by cryo-cooling; the CIS was then followed a ramp of 12°C/min to 250°C for 2 min. The GC temperature gradient program was the same as previously described. 2-acetyl-2-thiazoline MSD parameters were: capillary transfer line set at 250°C, source temperature set at 230°C, mass range 29-350 amu at 4.37 scans/sec operated in EI mode.

Two other compounds of interest, 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine, were separated and identified using an Agilent DB-5MS (30m x 0.25 mm x 0.25 µm film thickness) on an Agilent 7890A GC (Agilent Technologies, Santa Clara, CA) equipped with a LECO Pegasus TOF-MS (time-of-flight) (LECO, St. Joseph, MI), and a Hewlett Packard 7683 automatic sampler (Hewlett Packard, Palo Alto, CA) . As before, 1 µL of each isolate was injected using splitless injection. The inlet temperature was set to 250°C, with the

hydrogen carrier gas set to a constant flow of 1.1 mL/min. The GC oven as program was as follows: initial temperature set at 40°C and held for 1 min, then ramped at a rate of 3°C/min until 150°C, then raised by 30°C/min to 300°C and held for 5 min. The TOF-MS parameters for the identification conditions of 2-ethyl-3,5 or 6-dimethylpyrazine isomers were as follows: capillary transfer line set at 250°C, source temperature set at 250°C, mass range 29-350 amu at 20 spectra/sec operated in EI mode.

The two remaining compounds of interest, 2-acetyl-1-pyrroline and 2,3-diethyl-5-methylpyrazine, were positively identified with an Agilent DB-5MS (60 m x 0.25 mm x 0.25 µm film thickness) on an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA), equipped with an Agilent 5977A MSD (Agilent Technologies, Santa Clara, CA) operated in positive chemical ionization (PCI) mode using methane as a reagent gas under selective ion monitoring (SIM) mode for maximum sensitivity, and a Hewlett Packard 7683 automatic sampler (Hewlett Packard, Palo Alto, CA). One µL of the standard was injected using splitless injection. Inlet temperature was set at 250°C, helium carrier gas set to a constant flow of 1.0 mL/min, and the GC temperature gradient program was as follows: 32°C was initially held for 1 min and then ramped at a rate of 3°C/min until 150°C, followed by 50°C/min ramp to 300°C and held for 5 min. The MSD conditions were as follows: transfer line set at 250°C analysis, the ion source at 300°C, and mass range of 70-220 amu at 4.6 scans/sec.

After identification and analysis by AEDA, 19 compounds were selected for quantification based on FD values $\geq$ 32 and presence in at least one of the bread crust extracts. The selected compounds are presented in **Table 3**.

Table 3. GC/MS-CI quantification parameters for select aroma compounds

Compound[a]	MW	CI Ion[b]
2-methylpropanal	72	73
diacetyl	86	87
3-methylbutanal	86	85
3-methyl-1-butanol	88	71
methional	104	105
2-acetylfuran	110	111
2-acetyl-1-pyrroline	111	112
2-methyl-3-heptanone (IS)	128	129
1-octen-3-one	126	127
1-octen-3-ol	128	111
2-acetylpyrazine	122	123
2-ethyl-3,6-dimethylpyrazine	136	137
2-ethyl-3,5-dimethylpyrazine	136	137
guaiacol	124	125
2-acetyl-2-thiazoline	129	130
2,3-diethyl-5-methylpyrazine	150	151
(*E*,*Z*)-2,6-nonadienal	138	121
(*E*)-2-noneal	140	123
ethyl octanoate	172	145
(*E*,*E*)-2,4-decadienal	152	153

[a] Compound positively identified (LRI, MS, and authentic compound)
[b] Select ion monitored
IS (internal standard)

3.4.5 Quantification of Aroma Compounds

The selected compounds (**Table 3**) were quantified by relative response factors (RRF)[92]. The standard mixture for calculating the RRFs was created by adding 8 µg of the internal standard, 2-methyl-3-heptanone, and 8 µg of all selected compounds to 100 mL of DCM. The standard was subjected to SAFE followed by concentration to a final volume of 0.25 mL. An in-house deuterated analog was used for quantification of 2-acetyl-1-pyrroline. The standard

mixture(s) were analyzed in triplicate on an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 5977A MSD (Agilent Technologies, Santa Clara, CA) operated in PCI mode using methane as a reagent gas under SIM mode for maximum sensitivity, and a Hewlett Packard 7683 automatic sampler (Hewlett Packard, Palo Alto, CA). An Agilent DB-5MS (60 m x 0.25 mm x 0.25 µm film thickness) was used for the calculation of RRFs and quantification. Ions monitored are listed in **Table 3**. The calculation of the RRFs can be found in the **Appendix Table 9**. The relative response factor was calculated using equations1-3[92]:

(1) Response Factor = $\frac{Peak\ area}{Concentration}$

(2) Relative Response Factor = $\frac{Response\ factor\ A}{Response\ factor\ IS}$

(3) Concentration of A = $\frac{Peak\ area\ A}{Peak\ area\ IS} \times \frac{1}{RRF} \times Concentration\ IS$

One µL of the standard was injected using splitless injection. Inlet temperature was set at 250°C, helium carrier gas set to a constant flow of 1.0 mL/min, and the GC temperature gradient program was as follows: 32°C was initially held for 1 min and then ramped at a rate of 3°C/min until 150°C, followed by 50°C/min ramp to 300°C and held for 5 min. The MSD analysis was performed with the ion source at 300°C, and a mass range of 70-220 amu at 4.6 scans/sec. The linearity of all compounds was confirmed by constructing five point calibration curves ($r^2 > 0.957$ for all compounds) for each compound in concentration ranges expected in bread crust. Each bread sample was extracted in triplicate, and each resulting extract was analyzed in triplicate.

3.4.6 Measurement of pH and Water Activity

The pH and water activity values of the bread crusts were measured after removal from the crumb. The water activity was measured using an AquaLab Vapor Sorption Analyzer (Decagon Devices, Pullman, WA). A disposable plastic sample cup was filled approximately half way with WW or IWG ground bread crust; the crust was ensured to be level and to fully cover the bottom of the sample cup. The sample cup was then placed into the vapor sorption analyzer for water activity measurement. Each crust was measured in triplicate between 24.46 and 24.88 °C.

The pH of the ground WW and IWG bread crusts was measured using a SympHony SB70P pH meter (VWR Radnor, PA). A slurry of each bread crust was created by mixing 5 g of each bread crust with 20 g of DI water. The pH probe was immersed in the slurry, and the pH reading was recorded after approximately 1 min. Each crust pH was measured in triplicate.

3.4.7 Sensory Evaluation

Descriptive analysis was used to describe the key aroma attributes and their intensities in WW and IWG crust[93]. The panel consisted of two females and four males, ages 24 to 32, from the University of Minnesota Flavor Research and Education Center (Saint Paul, MN). All panelists had previously received extensive training in descriptive analysis techniques within the Flavor Research and Education, and had at least one year of experience performing as a sensory panelist. In addition to the panelist's previous training and experience they were

screened for their ability to differentiate the aroma of the bread crusts. Ballot development and panelist training occurred over 10, 1-hr sessions. The descriptive terms developed for each aroma attribute included earthy, roasted, oxidized, toasted, raisin, green, bran, and mushroom. The attributes, descriptors, and reference samples chosen by the panel is presented in **Table 4**.

WW and IWG bread samples for sensory evaluation were prepared as previously described (see section **3.4.1**). Immediately after baking, the crust was separated from the bread, frozen in liquid nitrogen, and finely ground. The crust of each sample was then stored in glass jars with Teflon-lined lids at -80 °C under a blanket of nitrogen until sensory evaluation. Prior to sensory testing samples were placed in room temperature for equilibration (2 h). Both samples were evaluated by the panelists within 24 h after baking. For evaluation, 5 g of each sample (ground) was presented in an amber bottle (60 mL) labeled with a three digit code and fitted with polytetrafluoroethylene (PTFE) lids.

Table 4. Odor descriptor lexicon development by panelists for attribute identification and reference samples for sensory evaluation of WW and IWG bread crusts

Attribute	Descriptors	Reference Sample
Earthy	Soil, dirt, earthy	Canned chickpeas (La Preferida; 5 g)
Roasted	Slightly nutty, roasted, browned	Roasted sunflower nuts (Good Sense; no salt; 5 g)
Oxidized	Musty, wet cardboard, oil	Steel cut oats (Bob's Red Mill; 5 g)
Toasted	Toasted, lightly browned	Organic whole grain buckwheat flour (Bob's Red Mill; 5 g)
Raisin	Raisin, dark fruit, warmed jam	Baby Bella mushrooms (3 g)
Green	Green, herb, grassy	Toasted white bread (Cub white enriched bread; crumb only; 3 g)
Bran	Sweetened cereal, grain, processed cereal	Bran cereal (Fiber One Original; General Mills; 10 g)
Mushroom	Raw mushroom	California raisins (Essential Everyday; 2.5 g)

Descriptive analysis on the WW and IWG bread crusts was performed in triplicate over three days. During each session, panelists evaluated two samples in random order, with a mandatory 10 s wait between samples. The aroma intensities were rated using a 13 categorized box scale (0 = not present; 1 = threshold; 5 = weak; 9 = moderate; 13 = strong). Data was collected using Compusense Cloud Software (Compusense Inc., Guleph, Ontario). Statistical analysis was performed using Statistix 10 (Analytical Software, Tallahassee, FL, USA). Analysis of variance (ANOVA) was conducted on each attribute followed by pairwise comparisons of individual attribute means from the bread crusts using Tukey's honest significant difference (HSD) method ($\alpha = 0.05$).

3.5 Results and Discussion

3.5.1 GC-O-MS/AEDA

Nineteen aroma compounds from bread crusts formulated with WW and IWG flour were characterized and identified by comparing odor descriptors with high FD-values in each sample. Compounds that had an FD-value $\geq$ 32 in at least one of the bread crust extracts were selected for further investigation and identified (shown in **Table 5**) and subsequently quantified (**Table 6**). All compounds listed in **Table 5** have been previously identified in WW bread[63,60,64].

Table 5. Odorants with FD-value ≥ 32 identified in at least one of the WW and IWG bread crusts after GC-O-MS/AEDA analysis

Compound[a]	Odorant Descriptor[b]	LRI DB-Wax	LRI DB-5	FD-value WW[c]	FD-value IWG[c]	FD Ratio (WW/IWG)
2-methylpropanal	malty, grain	< 1000	< 800	64	8	8.00
diacetyl	butter	<1000	< 800	128	128	1.00
3-methylbutanal	pungent, grain	<1000	< 800	128	32	4.00
3-methyl-1-butanol	musty, grain, fruity	1216	< 800	64	32	2.00
methional	potato,earthy, roasted	1451	907	128	128	1.00
2-acetylfuran	yeasty, fermented	1508	913	8	64	0.13
2-acety-1-pyrroline	crust, bread, corn chip	1333	924	128	128	1.00
1-octen-3-one	green, metallic, sharp	1308	980	128	128	1.00
1-octen-3-ol	green, herbs	1462	986	128	256	0.50
2-acetylpyrazine	baked, bread	1625	1028	64	16	4.00
2-ethyl-3,6-dimethylpyrazine	roasted, chocolate, earthy	1457	1090	64	64	1.00
2-ethyl-3,5-dimethylpyrazine	sweet, burnt, roasted	1465	1094	128	128	1.00
guaiacol	sweet, cream	1855	1096	128	64	2.00
2-acetyl-2-thiazoline	crust, corn chip	1756	1114	128	256	0.50
2,3-diethyl-5-methylpyrazine	earthy, roasted, green	1499	1155	32	16	2.00
(E,Z)-2,6-nonadienal	green, fresh	1584	1157	32	16	2.00
(E)-2-nonenal	green, oil, beany	1538	1167	128	32	4.00
Ethyl octanoate	green, wine	1442	1204	128	32	4.00
(E,E)-2,4-decadienal	cooked oil	1806	1316	64	32	2.00

[a] Compound positively identified (LRI, MS, and authentic compound)
[b] Odor described by panelist using GC sniffing port
[c] Flavor dilution (FD)-value based on the highest value from three panelists

Table 6. Quantification of select aroma compounds in WW and IWG bread crust solvent extracts presented as mean concentrations and standard error (SE) calculated from triplicate analysis of each sample

Compound	Mean WW[a] ± SE (µg/kg)	Mean IWG[a] ± SE (µg/kg)	Concentration Ratio (WW/IWG)
2-methylpropanal	370 ± 20.2	148 ± 15.2	2.50
diacetyl	329 ± 12.8	309 ± 3.27	1.06
3-methylbutanal	351 ± 11.7	168 ± 11.3	2.09
3-methyl-1-butanol	5330 ± 256	4150 ± 114	1.28
methional	78.7 ± 2.23	55.9 ± 1.69	1.41
2-acetylfuran	51.9 ± 3.32	12.4 ± 1.23	4.20
2-acetylpyrroline	11.5 ± 0.800	3.04 ± 0.339	3.76
1-octen-3-one	27.5 ± 0.829	19.8 ± 0.491	1.39
1-octen-3-ol	13.3 ± 1.18	182 ± 2.96	0.07
2-acetylpyrazine	3.64 ± 0.218	0.745 ± 0.0520	4.89
2-ethyl-3,6-dimethylpyrazine	29.2 ± 3.17	5.71 ± 1.28	5.10
2-ethyl-3,5-dimethylpyrazine	6.03 ± 0.481	1.35 ± 0.136	4.47
guaiacol	8.35 ± 0.946	7.76 ± 0.284	1.08
2-acetyl-2-thiazoline	4.01 ± 0.225	6.00 ± 0.153	0.67
2,3-diethyl-5-methylpyrazine	1.36 ± 0.126	0.202 ± 0.0499	6.72
(E,Z)-2,6-nonadienal	4.76 ± 0.315	5.79 ± 0.362	0.82
(E)-2-noneal	78.2 ± 5.56	83.7 ± 4.98	0.93
ethyl octanoate	517 ± 46.6	160 ± 0.599	3.22
(E,E)-2,4-decadienal	329 ± 61.5	42.8 ± 4.40	7.69

[a] Mean concentrations and standard error for both samples were calculated based on nine readings (triplicate analysis on three replicates)

Of the 19 identified compounds of interest 2-methylpropanal, 3-methylbutanal, 3-methyl-1-butanol, methional, 2-acetylfuran, 2-acetyl-1-pyrroline, 1-octen-3-one, 2-acetylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-diemthylpyrazine, 2,3-diethyl-5-methylpyrazine, ethyl octanoate, and (*E*,*E*)-2,4-decadienal were higher in concentration in WW than IWG bread crust by 2.5, 2.09, 1.28, 1.41, 4.2, 3.76, 1.39, 4.89, 5.10, 4.47, 6.72, 3.22, and 7.69-fold respectively; while 1-octen-3-ol, 2-acetyl-2-thiazoline, and (*E*,*Z*)-2,6-nonadienal were present in higher concentrations in IWG bread crust than WW by 13.7, 1.50, and 1.22-fold respectively. Three of the identified compounds, diacetyl, guaiacol, and (*E*)-2-nonenal, were not observed to be significantly different between each bread crust based on $p \geq 0.05$, using a two-sided t-test. The significance ($p \leq 0.05$) between concentrations of each compound in the two bread crusts is presented in the **Appendix Table 10**.

The largest concentration reduction from WW to IWG was observed for (*E*,*E*)-2,4-decadienal while the largest concentration reduction from IWG to WW was 1-octen-3-ol. Both of the compounds are known to be formed by lipid oxidation[30]. Based on identification of compounds with high FD values and high odor impact in WW and IWG bread crust extracts three main reaction pathways were identified as important for aroma generation in bread crust, the Maillard reaction, lipid oxidation and yeast fermentation.

Due to the low number of compounds with high FD values and odor impact generated via yeast fermentation it was suggested that the pathway has

limited influence on the generation of bread crust aroma profiles. Comparison between the concentrations of all compounds between the bread crusts are presented in **Figures 17-19** with calculations of means and standard error presented in **Appendix Tables 7-8**.

3.5.1.1 Maillard Reaction

Ten known Maillard derived compounds were identified as important odorants in the two bread crusts namely, 2-methylpropanal, diacetyl, 3-methylbutanal, methional, 2-acetylfuran, 2-acetyl-1-pyrroline, 2-acetylpyrazine, 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2-acetyl-2-thiazoline, and 2,3-diethyl-5-methylpyrazine [29,91]. Quantification results revealed that WW bread crust extracts had higher concentrations of all Maillard reaction compounds identified, except 2-acetyl-2-thiazoline, which was significantly higher in IWG bread crust. Maillard reaction compounds are responsible for the baked, corn chip, and roasted odors, which are commonly desirable attributes in baked products.

These findings suggested Maillard reaction pathways were either less reactive or the flavor pathways were suppressed in IWG bread crusts, which was hypothesized to be, in part, the due to a higher concentration of phenolic compounds present in IWG flour based on the higher TDF in IWG. Phenolic compounds have been shown to inhibit the Maillard pathways by forming adducts with reaction intermediates as well as amino acids and their reaction products

thus leading to decreased formation of Maillard derived aroma compounds[67,68]. As water activity and pH are known to influence the reaction pathways of the Maillard reaction, their values were determined in each bread crust[56–58]. The mean water activity values for WW and IWG were 0.843 and 0.876, respectively, and the mean pH values for WW and IWG were 6.52 and 6.64 respectively; the measurements for water activity and pH are presented in **Appendix Table 13**. The similarity in the water activity and pH values between the two crusts suggests they were likely minor factors in the inhibition of the Maillard reaction pathway in IWG.

The observed higher concentration of 2-acetyl-2-thiazoline in IWG is most likely attributed to a difference in the amount of cysteine present in the two flours[37,23], as cysteine is known to be precursor of 2-acetyl-2-thiazoline[53]. However, more research is needed to determine the amount of cysteine present in the flours used in the study for a conclusion.

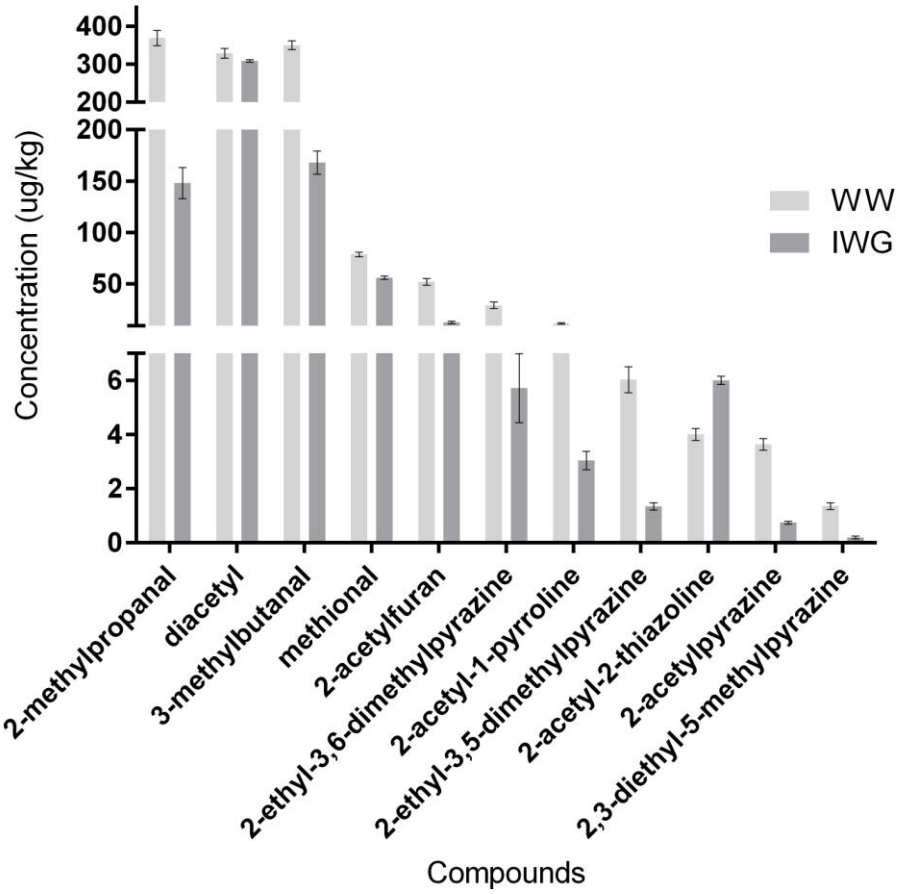

Figure 17. Mean concentration ± standard error of identified compounds of triplicate results from analysis of WW and IWG bread crust extracts known to be formed by the Maillard reaction

3.5.1.2 Lipid Oxidation

The identified aroma compounds, (E,E)-2,4-decadienal, 1-octen-3-ol, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, and 1-octen-3-one, are known to be formed by lipid oxidation. Both, (*E*,*Z*)-2,6-nonadienal and 1-octen-3-ol were higher in IWG crusts, (*E*,*E*)-2,4-decadienal and 1-octen-3-one were higher in WW, and

(*E*)-2-nonenal was not found to be significantly different between the bread crusts.

Lipid oxidation products in whole grain breads are higher in concentration due to the inclusion of the germ in the flour[11], which was included in both WW and IWG flours. Notably, both crusts contained the same lipid oxidation products. The observed change in the lipid oxidation product profile suggests different pathways of lipid oxidation are favored in the two crusts. The favoring of specific lipid oxidation compounds in WW and IWG bread is likely the result of different fatty acid composition in the flours. As lipoxygenase activity is involved in the production of volatile lipid oxidation products, including 1-octen-3-ol and (*E*,*Z*)-2,6-nonadienal, the flours may have had different amounts of lipoxygenase also resulting in favored lipid oxidation pathways; but, more work is needed to support these hypotheses as there is limited information regarding the fatty acid composition or the amount of lipoxygenase present in IWG flour.

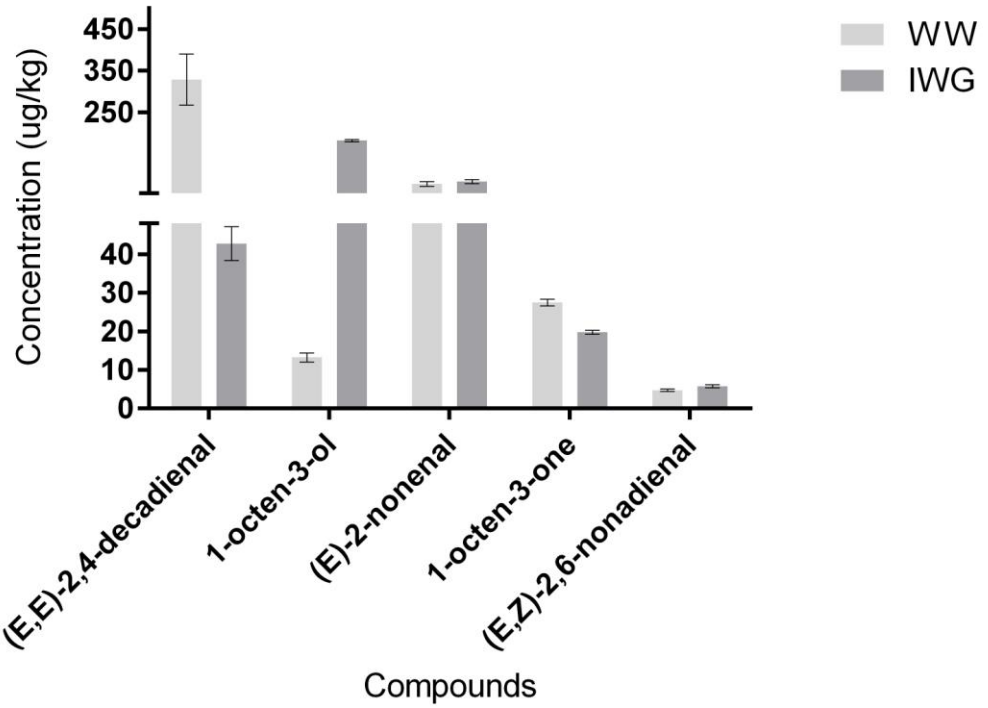

Figure 18. Mean concentration ± standard error of identified compounds of triplicate results from analysis of WW and IWG bread crust extracts known to be formed by lipid oxidation

3.5.1.3 Yeast Fermentation

Yeast fermentation was responsible for the formation of three out of the 19 compounds identified by GC-O-MS/AEDA, 3-methyl-1-butanol, guaiacol, and ethyl octanoate. 3-methyl-1-butanol and ethyl octanoate were present in higher concentrations in WW than IWG, while the concentration of guaiacol was not significantly different between the two crusts. The lower concentrations of the yeast fermentation products in IWG compared to WW suggest the pathways were inhibited, likely due to a decrease in the fermentation activity of the yeast, or difference in flour composition. The gluten network is responsible for the

capacity of the dough to retain the air incorporated during kneading and the CO_2 produced by the yeast, and so gives an indication on the fermentative activity of the yeast[77]. If yeast fermentation is inhibited, low loaf volume would be expected, which was observed in the IWG bread. The low loaf volume therefore supports the findings of fewer yeast fermentation products in IWG compared to WW, and suggests the pathway was inhibited.

Saccharomyces cerevisiae produces different concentrations of aroma compounds as a function of fermentation conditions including the composition of the media in which it is added[75]. Research specifically highlights nitrogen content available to the yeast as a key factor in the fermentation ability of the yeast with slow fermentation related to nitrogen deficiency[75]. Knowledge on the amount of protein in IWG suggests higher amounts of nitrogen in IWG; however the knowledge of nitrogen availability in IWG is still limited, and requires further investigation. Additionally, the amount of glucose available for yeast fermentation is less in IWG compared to WW, as it contains less starch, which could result in the inhibition of yeast fermentation and fewer aroma compounds formed by the pathway.

The inhibition of the yeast fermentation pathway can be caused by premature cessation of fermentation due to high anaerobic conditions[76]. Although yeast can grow under anaerobic conditions, growth is limited because the available sugars can only be converted to ethanol and CO_2, which produces less energy than aerobic conditions[94]. The accumulation of ethanol from the

anaerobic respiration also limits yeast growth as high levels of ethanol is toxic to yeast[76]. Therefore, IWG may provide a more anaerobic environment to the yeast than WW resulting in lower formation of yeast fermentation compounds and higher concentrations of ethanol in IWG. Lafon-Lafourcade *et. al* also determined that yeast survival and growth was affected by the presence of octanoic and decanoic acids, which are believed to act in synergy with ethanol. IWG may have a higher content of octanoic and decanoic acids than WW leading to greater toxicity effects on the yeast in IWG, but more research is needed on the composition of IWG.

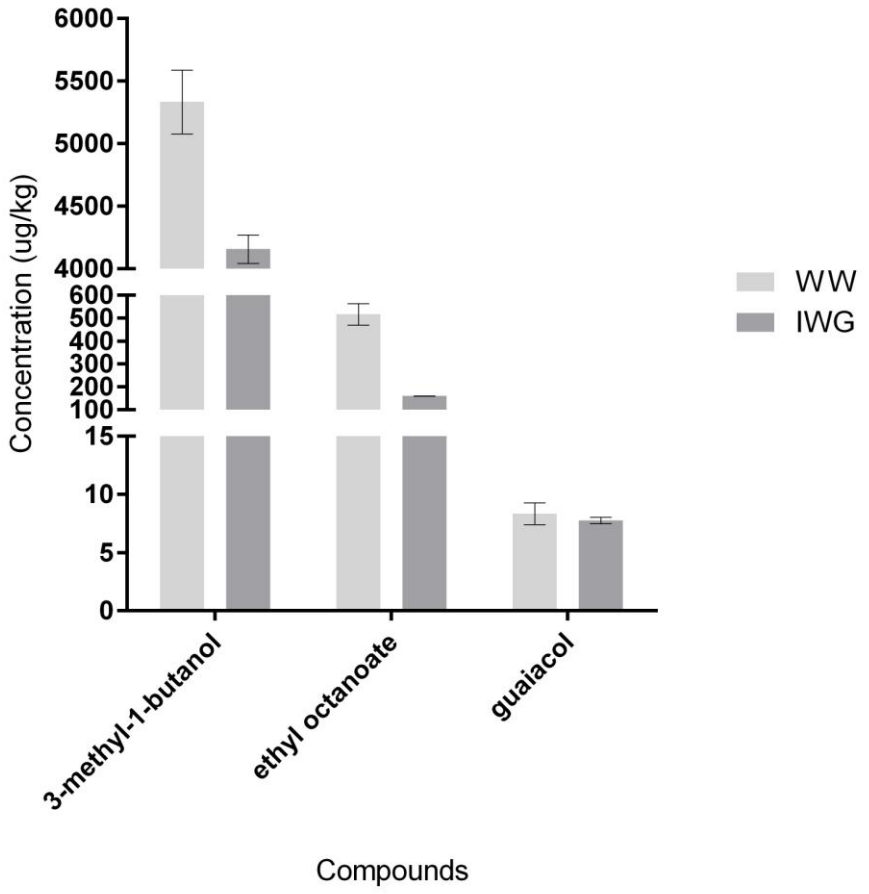

Figure 19. Mean concentration ± standard error of identified compounds of triplicate results from analysis of WW and IWG bread crust extracts known to be formed by yeast fermentation

3.5.2 Sensory Evaluation - Descriptive Analysis

Eight aroma attributes were identified in both bread crust (earthy, oxidized, roasted, toasted, raisin, green, bran and mushroom) and their intensities were evaluated by a trained sensory panel (shown in **Figure 20**). Five aroma attributes were found to be significantly different between the WW and IWG bread samples including roasted, toasted, raisin, green, and bran. The roasted and toasted attributes were found to be higher in WW than IWG with mean intensity ratings of

3.83 and 5.56 versus 2.89 and 4.17, respectively. Raisin, green, and bran attributes were found to be higher in IWG than WW with mean intensity ratings of 4.06, 3.44, and 4.06 versus 1.22, 2.22, and 3.22, respectively. The oxidized, earthy, and mushroom attributes were found to not be significantly different in intensity between the two bread crust samples. No attributes were unique to one bread crust. The results of Tukey's HSD of the mean scores of each attribute between the two bread crusts are listed in **Appendix Table 14**. ANOVA summary tables for each attribute are presented in **Appendix Tables 17-24**.

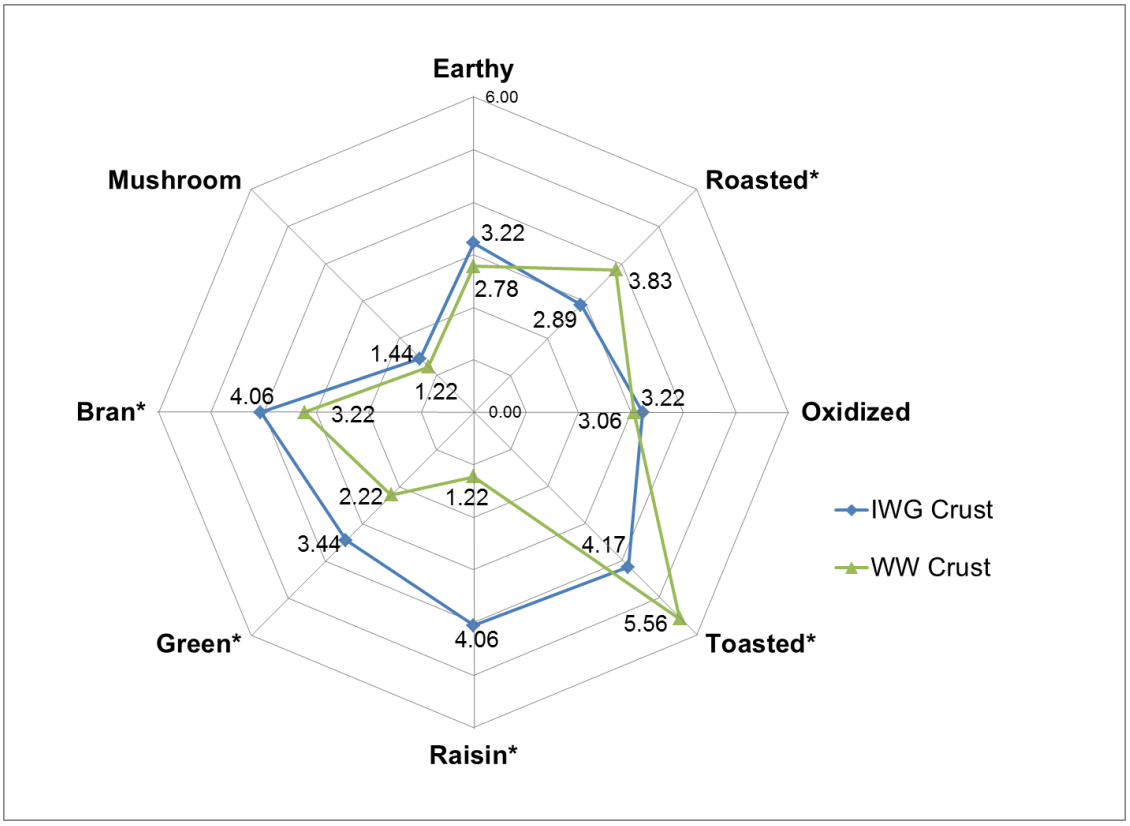

Figure 20. Radar plot of mean intensity ratings of three replicates from descriptive analysis descriptors generated by six sensory for WW and IWG bread crusts; * indicates means that are significantly different ($p < 0.05$)

Direct comparison of the sensory aroma profile (**Figure 20**) to the GC-O-MS/AEDA data (**Figures 17-19**) provided further insight to corroborate sensory attributes of each bread sample with specific identified aroma compounds. The WW bread profile was reported to have a significantly higher perceived roasted and toasted intensity and was in agreement with the aroma characterization data. The WW bread had higher concentrations for nine of the 10 Maillard reactions products identified in both products, all of which can contribute to these sensory attributes. Based on odor descriptions from GC-O analysis, methional, 2-ethyl-3,6 or 5-dimethylpyrazine(s), and 2,3-diethyl-5-methylpyrazine were likely strong contributors to the roasted attribute, while 2-acetyl-1-pyrroline, 2-acetylpyrazine, 2-acetyl-2-thiazoline, and diacetyl were likely strong contributors to the toasted attribute identified in the bread crusts (**Table 5**). Additionally, the lipid oxidation product, (*E*,*E*)-2,4-decadienal is also associated with fried-type notes and would be expected to contribute to these attributes (roasted/toasted). The concentration of (*E*,*E*)-2,4-decadienal was found to be significantly higher in the WW crust extract compared to IWG, like the majority of Maillard reaction compounds, confirming the increased intensity of roasted/toasted aromas in WW bread. The roasted and toasted attributes were the highest rated sensory attributes in WW (**Figure 20**), with toasted additionally rated as the highest sensory attribute in IWG, suggesting that the Maillard reaction was influential in both breads aroma development, but more influential in WW aroma development.

The higher perceived green attribute intensity in the IWG bread profile (**Figure 20**) was possibly related to changes in the lipid oxidation profile. Volatiles generated via lipid oxidation are known to produce green, cardboard, and/or oxidized notes in baked products[31]. Based on the GC-O odor descriptors (**Table 5**) and the concentration profile (**Table 6**), it is likely that (*E,Z*)-2,6-nonadienal and 1-octen-3-ol are responsible for the noted higher perceived green attribute in IWG bread. Ethyl octanoate was also reported to have a green-like character (**Table 6**), however, it was likely a minor contributor of the green character compared to (*E*,*Z*)-2,6-nonadienal and 1-octen-3-ol, as ethyl octanoate was present at higher concentrations in WW, which does not match the higher perceived intensity of the green attribute in IWG. The green attribute was a high rated sensory attribute in IWG (**Figure 20**) suggesting that lipid oxidation was influential in IWG aroma development. However, the decreased concentration of Maillard reaction compounds in IWG may also play an influential role in the higher intensity of green in its aroma profile. Fewer Maillard reaction products present in IWG crust and noted lower roasted and toasted aroma could allow the green notes to become prominent, and shape the aroma profile of IWG bread crusts.

The fourth attribute found to be significantly different between WW and IWG bread profiles was raisin (**Figure 20**). The raisin attribute had an important influence on the overall aroma of IWG bread crust as it was the second highest rated attribute in IWG with an intensity rating of 4.06 compared to 1.22 in the WW

bread crust. There were no odors described as raisin-like during GC-O analysis (**Table 5)** suggesting the attribute was likely the result of a combination of odors rather than a single compound, or the compounds responsible were reported at a FD value of < 32. The compound 3-methyl-1-butanol had an odor description from GC-O analysis (**Table 5**) that suggested it may contribute to the raisin note, but it would be minor constituent due to a higher concentration noted in the WW sample (**Table 6**). Guaiacol may also be a minor contributor due to odor description, and similar concentrations in both samples (**Table 5 and 6**).

The lack of major possible contributors for the raisin attribute in the bread crusts from **Table 5** lead to a more extensive examination of the collected data for both breast crust extracts**.** In order to identify compounds possibly responsible for the raisin attribute present in the bread crust profiles compounds detected below an FD value of 32 were investigated utilizing GC-O descriptors, mass spectra, and DB-5/DB-Wax LRIs. Any probable compound was compared to a list of odor active volatiles identified in raisins[95-96], resulting in 1-hexanol, 2-phenylethanol, and ethyl nonanoate being identified as potential contributors to the raisin attribute in IWG bread crust (**Appendix Table 23**). In addition to these three compounds having appropriate GC-O descriptors, and being previously identified in raisins, all three compounds were higher in concentration in IWG compared to WW based on peak areas, which is likely required for a significant higher intensity rating to be observed in IWG than WW. All three compounds, 1-hexanol, 2-phenylethanol, and ethyl nonanoate had peak areas 14.6%, 30.1%,

and 17.9% larger in IWG than WW, respectively (**Appendix Table 25**). In addition to the observed differences in concentration between bread crust extracts, the suppression of the Maillard reaction and strong attributes such as roasted and toasted could have played a role in the raisin attribute perception and its more dominant role in IWG bread.

The final attribute significantly different between the WW and IWG bread profiles was bran. The bran attribute was found to be higher in IWG than WW, and like the raisin attribute no compounds were described as bran during GC-O analysis (**Table 5**). However, 2-methylpropanal, 3-methylbutanal, and 3-methyl-1-butanol during GC-O analysis were labeled with same descriptors used for the bran attribute (**Table 4 and 5**)**.** All three compounds were found to be higher in concentration in WW than IWG, suggesting the compounds were minor contributors to the bran attribute, or the suppression of Maillard chemistry allowed the bran attribute to be perceived more intensely in IWG than WW. Similarly, based on the lack of major possible contributors for the brans notes in the bread crusts from **Table 5**, further examination of the collected data for both breast crust extracts followed to include compounds detected below an FD value of 32 while utilizing GC-O descriptors, mass spectra, and DB-5/DB-Wax LRIs. Any probable compounds were compared to a list of odor active volatiles identified in breads and flours[10,17,97], resulting in 2-methoxy-4-vinylphenol and salicylaldehyde being identified as potential compounds contributing to the bran attribute (see **Appendix Table 24**). In addition to having appropriate GC-O

descriptors, and being previously identified in breads and flours, both compounds were higher in concentration in IWG compared to WW based on peak areas, which is likely required for a significant higher intensity rating in IWG than WW bread crust profiles. 2-methoxy-4-vinylphenol and salicylaldehyde had peaks areas 163.0% and 175.4% larger in IWG than WW, respectively.

Three aroma attributes from descriptive analysis were found to not be significantly different in intensity between the two bread crust profiles: oxidized, earthy, and mushroom. Based on GC-O odor descriptions (**Table 5**) it is likely that (*E*)-2-nonenal was responsible for the oxidized attribute. The concentration of (*E*)-2-nonenal (**Table 6**) was found to not be significantly different between the extracts, therefore, the similar perceived intensity of the oxidized attribute between the two bread crusts is supported. 1-octen-3-one was likely responsible for the mushroom attribute based on data in **Table 5**. This compound was found to be significantly higher in concentration in WW than IWG (**Table 6**), but did not result in a significantly higher sensory response between the two bread crusts. Methional, 2-ethyl-3,6-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine in addition to contributing to the roasted attribute, may also contribute to the earthy attribute based on GC-descriptors in **Table 5**. If so, the significant difference in concentration between these compounds in the crust extracts (**Table 6**) did not result in a significant difference in perceived intensity of earthy between the two crusts.

3.6 Conclusions

The Maillard reaction, lipid oxidation, and yeast fermentation were identified as the main reaction pathways for the flavor of IWG bread. In comparison to WW bread, the unique flavor profile of IWG bread was related to lower amounts of Maillard products, and unique lipid oxidation and fermentation profiles. The identity of the 19 aroma compounds characterized and quantified in the IWG bread crust aroma profile provided a molecular fingerprint for targeted flavor optimization strategies. Mapping the pathways of these aroma compounds afforded insights to mechanistic approaches for flavor optimization that can facilitate the development of unique IWG flavored products with specific nuances known to have positive sensory impact. The identification of the key aroma compounds can also facilitate breeding strategies for flavor development. Breeders could create, or select, varieties of IWG with desirable amounts of specific flavor precursors, like amino acids, to aid in the creation of IWG bread that would be accepted by consumers. For example, creating IWG varieties with higher concentrations of proline and cysteine could lead to the increase of concentration of the desirable Maillard reaction compounds 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline respectively. It may also be beneficial to increase the amount of lysine and phenylalanine in IWG as they are responsible for caramel, toast, buttery, and floral flavor descriptors[29]. Breeders could increase seed size resulting in more starch, and less bran, which could lead to less inhibition of the Maillard reaction, and increase in yeast fermentation products.

Further flavor optimization of IWG bread could be accomplished with the addition of selected ingredients to the dough, such as initial reactants that might result in increased generation of Maillard derived compounds, changing the level and type of reducing sugars, increasing baking time and/or temperature, and control of pH. For example, adjusting the pH of the dough to above 7 would likely increase the formation of flavor compounds[53]. Another approach would be to target and decrease the formation of lipid oxidation products, which can be detrimental to the aroma profile of baked goods. Decreasing the amount of added fat, changing the type of fat added, and/or the addition of antioxidants to the formulation provide possible ways to decrease lipid oxidation products in IWG bread. Fat, either endogenous or added, could also be removed, but the Maillard reaction products may also be affected by their removal[44,71]. In terms of augmenting yeast fermentation a different strain of yeast could be chosen as an ingredient, more or less yeast could be added, or the proofing oven temperature and humidity could be adjusted. Ultimately, flavor optimization could lead to improved acceptability of IWG bread and provide insight for optimization of other IWG based products, and support the use of IWG as a sustainable replacement to wheat.

CHAPTER 4: FUTURE WORK

4.1 Future Concepts for Investigation

The aroma characterization of IWG bread crusts serves as a foundation for future flavor work on IWG bread, and opens doors for future IWG flavor research. To further this work, a more complete characterization of aroma fingerprints should be performed including identification of compounds with FD values below 32. This could allow characterization of raisin and bran attributes identified in crusts and reveal further mechanistic pathways of interest for flavor optimization. Following the quantification of new compounds, recombination models should be made of all GC-O-MS/AEDA identified aroma compounds in WW and IWG bread crusts to confirm the descriptive analysis and GC-O conclusions made from the current work.

As aroma is only one aspect of flavor and crust only one aspect of bread, the aroma profile of the crumb, and taste profile of IWG bread should be investigated to create a complete flavor profile of IWG bread. After the completion of the flavor profile of IWG bread crust, it would be interesting to examine the flavor profiles of blended WW and IWG breads as Marti *et al*. determined the rheological properties of IWG dough are improved when blended with WW flour in a 50/50 ratio. The flavor profiles of other cereal products, such as cookies or pasta, made from IWG should also be investigated as there are many other potential applications for IWG in the food industry as well as a comparison to other flours, such as rye. In addition, as flavor optimization

strategies continue to be developed, interdisciplinary research combining agronomy and engineering could come together to yield a product with both good flavor, and rheological and baking qualities.

4.2 Alternative Approaches to Identifying Characteristic Compounds in IWG Bread Crust

4.2.1 Dynamic Headspace (Purge and Trap)

There are additional, or alternative, extraction techniques that could be used in the aroma profile characterization of IWG bread crust. The extraction and isolation method used in this experiment provided a neutral/basic isolate that was concentrated by nearly 4000 times. This concentration increased the likelihood of detection for compounds that otherwise would be below an instruments level of detection under other techniques. However, the isolate after extraction and concentration, as previously discussed, is not what is experienced when smelling native bread crust. There are less severe extraction techniques and analysis methods, like purge and trap dynamic headspace, which better replicate the orthonasal experience of smelling a native sample, but still lack the retronasal experience. Purge and trap dynamic headspace analysis involves passing a gas flow onto the headspace of a sample contained in a vial, which can be heated, cooled, or agitated. The gas flow then passes through a trap containing an adsorbent matrix that is used to trap the aroma compounds removed from the headspace of the sample. Unlike static headspace analysis, purge and trap allows the analysis of potentially liter quantities of headspace leading to an

increase in sensitivity. The trapped volatiles are then released for detection by thermal desorption and transferred to a GC-MS system for analysis. An olfactometry technique would then be used to identify which of the hundreds of volatile compounds are odor-active. By performing purge and trap, volatiles will be extracted differently compared to liquid extraction. This difference could result in a subtlety different aroma profile of IWG bread crust than the one characterized in this work. A different profile may result in the identification of compounds directly responsible for the raisin and bran attribute identified by descriptive analysis as headspace analysis more closely mimics smelling of the native sample. Purge and trap, however, is not without its limitations which include: head space bias, single use extraction, affinity of traps to preferentially trap different compounds, and saturation of traps preventing the trapping of all compounds.

This technique was not initially chosen due to concerns of not being able to identify high impact odorants, like 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline, and the limitations mentioned previously. The concentration of such compounds were determined in this study, providing an opportunity to combine the results of the current study with results of a purge and trap extraction in order to continue to discover the aroma profile of IWG bread crust.

4.2.2 Use of Osme in Characterization of IWG Bread Crust Aroma

A different olfactometry method could also be used in the comparison of the aroma profiles of WW and IWG bread crust. One option could be to use a modified Osme technique. With a modified Osme technique, the isolate would not be serially diluted, and the perceived intensity of an odorant would be used as the determining factor for character odorants. It is likely that the compounds identified by AEDA would still be determined as impactful since the dilutions of AEDA measure intensity as well, but additional compounds could also be identified. By instead rating the intensity of compounds, new compounds may be selected as impactful that directly correlate to the descriptive analysis results of the IWG and WW bread crusts. The use of Osme on IWG and WW bread crust isolates could also be used to test the theory that the lower concentration of Maillard reaction compounds in IWG allow other odorants to become dominant in the IWG aroma profile that are masked in the WW aroma profile.

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CHAPTER 6: APPENDIX

Figure 21. Whole wheat bread crust extract chromatogram SIM DB-5 column

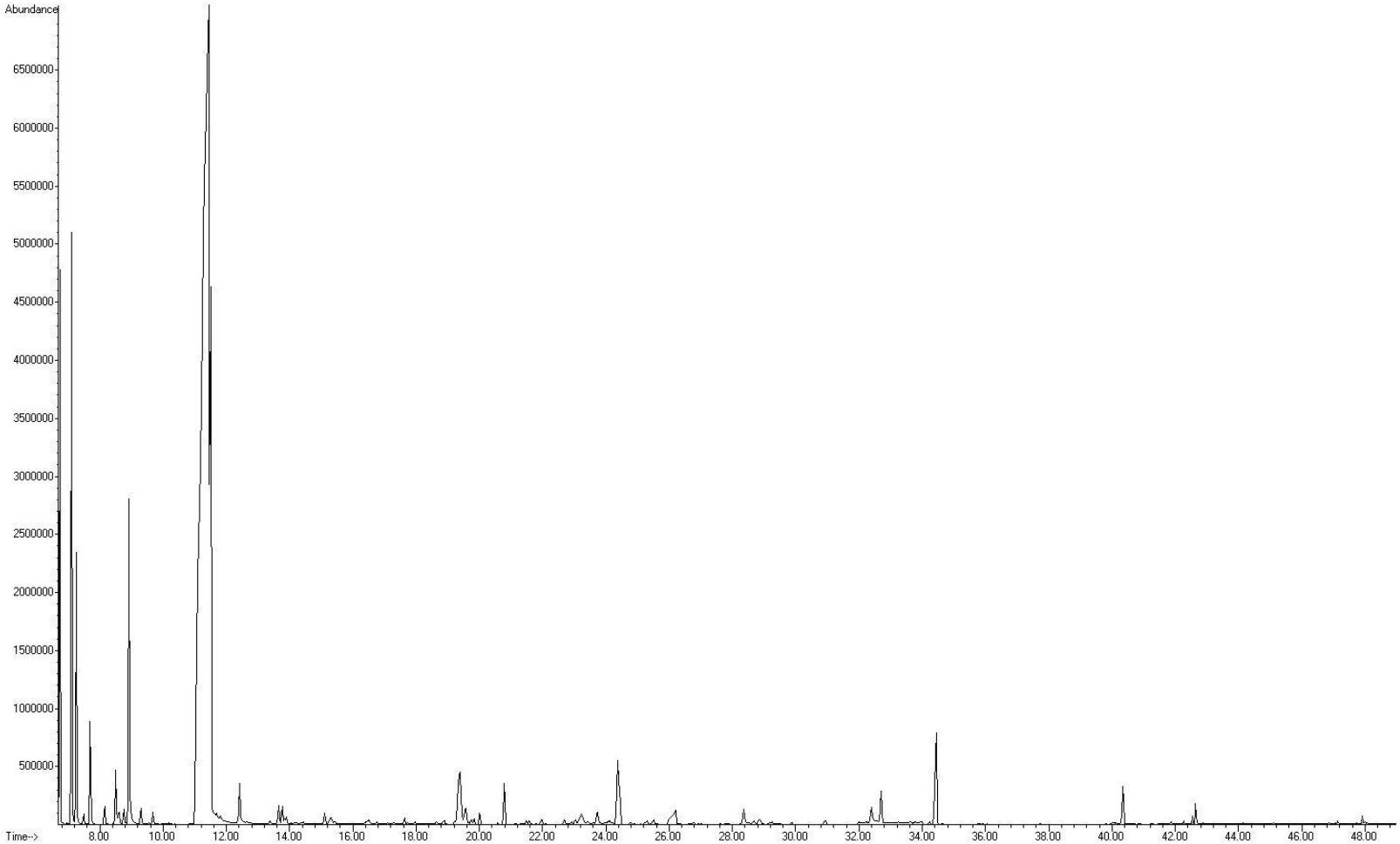

Figure 22. Intermediate wheatgrass bread crust extract chromatogram SIM DB-5 column

Table 7. Concentration of 19 odorants identified by high FD and odor description in WW bread crusts with average concentration, standard deviation (SD), coefficient of variation (CV), and standard error (SE) from triplicate analysis of three samples

	WW I			WW II			WW III						
Compound	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**Mean[a] (µg/kg)**	**SD[a]**	**CV[a]**	**SE[a]**
2-methylpropanal	284	307	308	444	419	444	350	395	380	370	60.7	0.164	20.2
diacetyl	278	288	287	370	365	379	320	339	337	329	38.3	0.116	12.8
3-methylbutanal	337	289	317	371	326	360	385	382	388	351	35.1	0.0100	11.7
3-methyl-1-butanol	4280	4470	4620	6300	6100	6230	5240	5410	5340	5330	767	0.144	256
methional	76.8	69.5	74.0	85.9	70.2	76.8	85.5	86.1	83.3	78.7	6.70	0.0852	2.23
2-acetylfuran	46.9	42.4	43.8	46.7	46.4	46.1	64.6	66.0	64.6	51.9	9.96	0.192	3.32
2-acetylpyrroline	8.97	8.83	8.59	14.5	14.4	14.0	11.5	11.1	11.1	11.5	2.40	0.210	0.800
1-octen-3-one	24.8	24.3	24.7	30.6	30.0	29.3	29.6	27.1	27.2	27.5	2.48	0.0903	0.828
1-octen-3-ol	9.63	9.73	9.72	18.1	17.8	17.0	13.3	12.9	11.2	13.3	3.55	0.268	1.18
2-acetylpyrazine	3.13	3.69	3.12	4.61	4.36	4.44	2.96	3.22	3.25	3.64	0.654	0.179	0.218
2-ethyl-3,6-dimethylpyrazine	29.0	30.0	29.0	39.5	41.3	39.3	18.2	18.1	18.1	29.2	9.50	0.326	3.17
2-ethyl-3,5-dimethylpyrazine	6.38	6.52	6.55	7.29	7.55	7.43	4.20	4.16	4.20	6.03	1.44	0.239	0.481
guaiacol	8.69	8.27	8.24	11.7	11.6	11.4	5.35	4.88	4.92	8.35	2.84	0.340	0.946
2-acetyl-2-thiazoline	4.13	4.12	4.12	4.60	4.85	4.70	3.18	3.15	3.22	4.01	0.675	0.168	0.225
2,3-diethyl-5-methylpyrazine	1.25	1.25	1.26	1.86	1.84	1.82	1.01	0.976	0.978	1.36	0.38	0.278	0.126
(E,Z)-2,6-nonadienal	3.20	3.95	3.85	5.73	5.96	5.68	4.85	4.76	4.82	4.76	0.945	0.199	0.315
(E)-2-noneal	74.0	71.6	72.4	99.4	100	99.2	62.5	61.9	62.6	78.2	16.70	0.213	5.56
ethyl octanoate	569	569	574	653	641	638	329	340	337	517	140	0.271	46.6
(E,E)-2,4-decadienal	502	503	507	390	390	391	91.9	90.8	92.5	329	184.00	0.561	61.5

[a] Means concentration, SD, CV, and SE were calculated based on nine readings (triplicate analysis on three replicates).

Table 8. Concentration of 19 odorants identified by high FD and odor descriptions in WW bread crusts with average concentration, standard deviation (SD), coefficient of variation (CV), and standard error (SE) from triplicate analysis of three samples

	IWG I			IWG II			IWG III						
Compound	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**µg/kg**	**Mean (µg/kg)**	**SD[a]**	**CV[a]**	**SE[a]**
2-methylpropanal	178	183	178	172	185	171	85.9	91.1	85.8	148	45.5	0.308	15.2
diacetyl	297	302	298	311	328	313	310	317	307	309	9.81	0.0317	3.27
3-methylbutanal	155	187	167	207	205	204	131	131	126	168	34.0	0.202	11.32
3-methyl-1-butanol	4620	4600	4530	3800	3890	3780	4090	4110	3970	4150	341	0.0820	114
methional	60.1	56.1	55.0	62.8	61.8	56.7	51.2	51.6	48.0	55.9	5.06	0.0906	1.69
2-acetylfuran	16.6	16.5	16.3	12.6	12.7	12.6	8.02	8.02	7.92	12.4	3.68	0.298	1.23
2-acetylpyrroline	4.35	4.32	4.31	2.80	2.80	2.77	2.03	2.02	2.00	3.04	1.02	0.34	0.34
1-octen-3-one	18.1	17.9	17.7	20.4	20.6	20.5	20.3	21.3	21.3	19.8	1.47	0.0744	0.491
1-octen-3-ol	176	178	173	180	177	175	195	195	191	182	8.89	0.0489	2.96
2-acetylpyrazine	0.959	0.940	0.928	0.615	0.620	0.612	0.733	0.713	0.584	0.745	0.156	0.210	0.0520
2-ethyl-3,6-dimethylpyrazine	10.9	10.8	10.8	3.35	3.31	3.31	3.31	2.83	2.86	5.71	3.83	0.671	1.28
2-ethyl-3,5-dimethylpyrazine	1.92	1.91	1.80	1.18	1.20	1.15	0.948	1.02	1.00	1.35	0.408	0.302	0.136
guaiacol	8.79	8.42	8.24	6.97	6.50	6.55	8.25	8.00	8.11	7.76	0.853	0.110	0.284
2-acetyl-2-thiazoline	6.03	6.11	6.21	5.43	5.40	5.46	6.31	6.47	6.57	6.00	0.458	0.0763	0.153
2,3-diethyl-5-methylpyrazine	0.409	0.402	0.394	0.106	0.11	0.105	0.109	0.0925	0.0962	0.202	0.150	0.740	0.050
(E,Z)-2,6-nonadienal	5.64	5.38	5.52	4.73	4.65	4.72	7.13	7.11	7.22	5.79	1.08	0.187	0.362
(E)-2-noneal	72.1	72.2	72.6	76.3	75.40	73.9	104	103	104	83.7	14.9	0.179	4.98
ethyl octanoate	159	161	161	159	159.00	158	161	163	162	160	1.80	0.0112	0.599
(E,E)-2,4-decadienal	40.3	40.2	41.1	28.9	28.30	29.1	59.1	58.8	59.0	42.8	13.2	0.308	4.40

[a] Means concentration, SD, CV, and SE were calculated based on nine readings (triplicate analysis on three replicates).

Table 9. Calculation of response factor (RF) and relative response factor (RRF) of 19 odorants identified by high FD and odor descriptions with the average, standard deviation (SD), coefficient of variation (CV), and standard error (SE) of RRFs based on comparison to 2-methyl-3-heptanone from triplicate analysis

Compound	Amount (µg)	Concentration (µg/mL)	RF 1	RRF 1	RF 2	RFF 2	RF 3	RFF 3	RFF Avg.[a]	RRF SD[a]	RRF CV[a]	RRF SE[a]
2-methylpropanal	8.05	32.2	479449	0.975	496654	1.01	514562	1.01	0.999	0.0204	0.0205	0.0118
diacetyl	7.96	31.8	730707	1.49	742656	1.51	770987	1.51	1.50	0.0153	0.0101	0.00881
3-methylbutanal	8.09	32.4	48788	0.0992	50356	0.103	53061	0.104	0.102	0.00251	0.0246	0.00145
3-methyl-1-butanol	8.12	32.5	555003	1.13	555181	1.13	576385	1.13	1.13	0.00127	0.00113	0.000735
methional	8.13	32.5	35133	0.0715	35013	0.0713	36287	0.0712	0.0713	0.000122	0.00170	7.02E-05
2-acetylfuran	8.12	32.5	1288516	2.60	1288230	2.62	1337102	2.62	2.61	0.0146	0.00557	0.00841
2-acetylpyrroline	8.00	32.0	1122444	1.41	1285716	1.61	1429367	1.36	1.46	0.132	0.0901	0.0760
1-octen-3-one	8.01	32.0	115009	0.234	113422	0.231	118142	0.232	0.232	0.00154	0.00662	0.000888
1-octen-3-ol	8.01	32.0	1402856	0.285	140918	0.287	145609	0.286	0.286	0.000791	0.00277	0.000457
2-acetylpyrazine	8.07	32.3	1370113	2.79	1375996	2.80	1431898	2.81	2.80	0.0121	0.00431	0.00697
2-ethyl-3,5-dimethylpyrazine	3.57	14.3	700522	1.42	699579	1.42	727164	1.43	1.43	0.00167	0.00117	0.000965
2-ethyl-3,6-dimethylpyrazine	4.70	18.8	702254	1.43	702468	1.43	730374	1.43	1.43	0.00271	0.00189	0.00156
guaiacol	8.41	33.6	663995	1.35	591033	1.20	565833	1.11	1.22	0.121	0.0990	0.0698
2-acetyl-2-thiazoline	8.26	33.0	508225	1.03	510957	1.04	531328	1.04	1.04	0.00475	0.00457	0.00274
2,3-diethyl-5-methylpyrazine	8.17	32.7	630389	1.28	633605	1.29	658335	1.29	1.29	0.00527	0.00409	0.00304
(E,Z)-2,6-nonadienal	8.03	32.1	99694	0.203	99069	0.202	102703	0.202	0.202	0.000655	0.00325	0.00378
(E)-2-noneal	8.25	33.0	217756	0.443	217884	0.444	226344	0.444	0.443	0.000694	0.00156	0.000401
ethyl octanoate	8.08	32.3	191943	0.390	193384	0.394	201107	0.395	0.393	0.00227	0.00577	0.00131
(E,E)-2,4-decadienal	8.21	32.8	269942	0.549	261283	0.532	272359	0.535	0.538	0.00921	0.0171	0.00532

[a] Average value, SD, CV, and SE were calculated based three replicates of the standard mixture(s)

Table 10. Odor activity values (OAVs) and significance using a two-sided t-test of 19 odorants identified by high FD and odor descriptions in WW and IWG bread crust extracts

Compound	OAV WW[a] (µg/kg)	OAV IWG[a] (µg/kg)	Significance[b]
2-methylpropanal[73]	370.00	148	2.92E-07
diacetyl[73]	22.0	20.6	1.66E-01
3-methylbutanal[73]	1750	840	5.45E-09
3-methyl-1-butanol[73]	21.3	16.6	1.44E-03
methional[73]	393	280	7.35E-07
2-acetylfuran[98]	0.0100	0.00100	4.98E-07
2-acetylpyrroline[73]	115	30.5	1.20E-06
1-octen-3-one[99]	5502	3960	2.19E-06
1-octen-3-ol[100]	13.3	182	4.49E-14
2-acetylpyrazine[101]	0.0600	0.0100	4.46E-07
2-ethyl-3,6-dimethylpyrazine[102]	3	0.640	3.35E-05
2-ethyl-3,5-dimethylpyrazine[102]	151	34	4.99E-06
guaiacol[73]	8	8	5.66E-01
2-acetyl-2-thiazoline[73]	4	6.00	3.63E-06
2,3-diethyl-5-methylpyrazine[73]	15.1	2	4.85E-06
(E,Z)-2,6-nonadienal[103]	476	579	4.72E-02
(E)-2-noneal[73]	978	1050	4.75E-01
ethyl octanoate[73]	103	32	6.01E-05
(E,E)-2,4-decadienal[96]	4670	611	1.62E-03

[a] OAVs were calculated using odor thresholds (in water) found in literature 73, 98-103
[b] Significance was determined by use of a standard two sided t-test on the mean concentrations of each compound in WW and IWG bread crust extracts in Excel

Table 11. Odor description, retention time, and linear retention index value of odorants detected from a 1 µL injection of the concentrated WW bread crust extract on a DB-1 column

WW Bread Crust Extract		
Avg. Time (min)	**Odor Description**	**Avg. LRI**
5.08	butter	<600
6.41	off, malty, musty	630
7.67	chemical, glycerin	676
9.13	oil	719
9.44	baked	726
10.49	green	751
11.52	floral, grass	775
14.54	earthy, green	837
15.54	baked, earthy	856
16.36	dog food	871
16.82	fruity, citrus	880
19.54	sweet	929
20.98	floral	954
21.14	green, clean	957
22.21	floral, sterile	976
22.46	earthy, citrus	981
24.14	floral, pollen	1011
25.36	earthy, crust	1033
25.52	earthy, roasted	1036
26.93	roasted, cocoa	1061
27.14	earthy	1065
27.33	roasted, seasoning	1068
27.77	baked, green	1076
28.56	floral, fruity	1090
28.76	floral, sweet, malty	1094
29.99	old fashioned candy	1117
30.42	clean	1125
32.24	earthy, baked, sweet	1159
33.89	corn chips, stale	1190
36.66	cooked, beany	1257
38.24	corn chips, warmed over	1297
39.09	banana	1388
39.38	wine/grape, fruity	1423

Table 12. Odor description, retention time, and linear retention index value of odorants detected from a 1 µL injection of the concentrated WW bread crust extract on a DB-1 column

IWG Bread Crust Extract		
Avg. Time (min)	**Aroma**	**Avg. LRI**
5.01	malty	<600
5.09	butter	<600
5.24	fat	<600
5.96	musty	614
6.42	popcorn, green	630
7.25	earthy, fresh, green	661
7.42	fat	667
7.70	chemical, glycerin	677
7.86	fat	683
9.12	baked, earthy, oil	718
9.27	off, rancid, musty	722
10.60	chemical, soap	753
11.64	fruity, grass	777
15.20	caramel, baked, bread	849
15.50	green, floral, earthy	855
15.63	cereal, baked, sweet	857
16.18	musty, earthy	868
16.87	citrus	881
17.26	fruity	888
17.50	baked	892
17.62	spices, seasonings	895
20.63	chemical	948
20.77	dirty	951
21.06	floral, green	956
22.62	citrus	983
24.26	floral	1013
25.16	caramel/sweet, baked	1029
26.53	clean	1054
27.05	roasted, cocoa	1063
28.08	earthy, baked	1082
28.24	roasted, cocoa	1085
28.39	fruity	1087
28.62	floral, sweet, malty	1092
29.53	earthy, floral	1108
31.18	green ,oil, fat	1139
35.45	black licorice	1227
36.54	citronella	1254
39.06	fruity, tropical	1385

Table 13. Measurement of the moisture content of WW and IWG flour, and measurement of the water activity and pH of WW and IWG bread crusts in triplacte with the mean ± standard deviation (SD) moisture content of WW and IWG flour, and the mean ± standard deviation (SD) water activity and mean ± standard deviation (SD) pH of WW and IWG bread crust

Sample	Moisture Content (%)[104]	Water Activity (a_w)	pH	Mean Moisture Content ± SD	Mean Water Activity ± SD	Mean pH ± SD
WW	12.2	0.843	6.52	12.1 ± 0.1	0.843 ± 0.002	6.52 ± 0.00
	12.2	0.841	6.52			
	12.0	0.840	6.52			
IWG	10.4	0.873	6.63	10.4 ± 0.1	0.876 ± 0.003	6.64 ± 0.01
	10.3	0.877	6.63			
	10.4	0.879	6.65			

Table 14. Mean scores ± standard deviation for three replicates of descriptors generated by six sensory panelists and significance based on Tukey's HSD between IWG and WW bread crusts

Attribute	IWG Crust Avg. Intensity	WW Crust Avg. Intensity
Earthy	3.22 ± 0.299[a]	2.78 ± 0.385[a]
Roasted	2.89 ± 0.402[b]	3.83 ± 0.368[a]
Oxidized	3.22 ± 0.410[a]	3.06 ± 0.453[a]
Toasted	4.17 ± 0.320[b]	5.56 ± 0.448[a]
Raisin	4.06 ± 0.376[a]	1.22 ± 0.476[b]
Green	3.44 ± 0.383[a]	2.22 ± 0.433[b]
Bran	4.06 ± 0.383[a]	3.22 ± 0.359[b]
Mushroom	1.44 ± 0.448[a]	1.22 ± 0.385[a]

Significance analyzed by row
[a/a] Attribute is not significantly different between crusts; higher mean
[a/b] Attribute is significantly different between crusts; lower mean

Table 15. ANOVA summary table for intensity of earthy between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	10.667	2.133	5.120	0.010
Sample	1	1.778	1.778	4.270	0.061
Rep	2	0.000	0.000	0.000	1.000
Panelist*Rep	10	6.333	0.633	1.520	0.243
Panelist*Sample	5	2.222	0.444	1.070	0.425
Error	12	5.000	0.417		
Total	35	26.000			

Table 16. ANOVA summary table for intensity of roasted between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	12.139	2.428	3.240	0.044
Sample	1	8.028	8.028	10.700	0.007
Rep	2	0.389	0.194	0.260	0.776
Panelist*Rep	10	5.278	0.528	0.700	0.707
Panelist*Sample	5	3.472	0.694	0.930	0.497
Error	12	9.000	0.750		
Total	35	38.306			

Table 17. ANOVA summary table for intensity of oxidized between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	16.472	3.294	3.830	0.026
Sample	1	0.250	0.250	0.290	0.600
Rep	2	5.056	2.528	2.940	0.092
Panelist*Rep	10	3.278	0.328	0.380	0.932
Panelist*Sample	5	2.917	0.583	0.680	0.649
Error	12	10.333	0.861		
Total	35	38.306			

Table 18. ANOVA summary table for intensity of toasted between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	14.472	2.894	14.890	0.000
Sample	1	17.361	17.361	89.290	0.000
Rep	2	3.389	1.694	8.710	0.005
Panelist*Rep	10	4.944	0.494	2.540	0.064
Panelist*Sample	5	5.806	1.161	5.970	0.005
Error	12	2.333	0.194		
Total	35	48.306			

Table 19. ANOVA summary table for intensity of raisin between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	12.472	2.494	7.480	0.002
Sample	1	72.250	72.250	216.750	0.000
Rep	2	1.556	0.778	2.330	0.139
Panelist*Rep	10	5.778	0.578	1.730	0.182
Panelist*Sample	5	16.250	3.250	9.750	0.001
Error	12	4.000	0.333		
Total	35	112.306			

Table 20. ANOVA summary table for intensity of green between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	12.667	2.533	3.970	0.024
Sample	1	13.444	13.444	21.040	0.001
Rep	2	7.167	3.583	5.610	0.019
Panelist*Rep	10	3.167	0.317	0.500	0.863
Panelist*Sample	5	2.889	0.578	0.900	0.509
Error	12	7.667	0.639		
Total	35	47.000			

Table 21. ANOVA summary table for intensity of bran between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	4.472	0.894	1.460	0.272
Sample	1	6.250	6.250	10.230	0.008
Rep	2	0.222	0.111	0.180	0.836
Panelist*Rep	10	7.111	0.711	1.160	0.396
Panelist*Sample	5	8.917	1.783	2.920	0.060
Error	12	7.333	0.611		
Total	35	34.306			

Table 22. ANOVA summary table for intensity of mushroom between WW and IWG bread crusts

Source	DF	SS	MS	F	P
Panelist	5	22.667	4.533	13.600	0.000
Sample	1	0.444	0.444	1.330	0.271
Rep	2	0.167	0.083	0.250	0.783
Panelist*Rep	10	5.167	0.517	1.550	0.233
Panelist*Sample	5	3.556	0.711	2.130	0.131
Error	12	4.000	0.333		
Total	35	36.000			

Table 23. Additional compounds from literature and GC-O to describe raisin attribute found in WW and IWG bread crusts

Compound	Odorant Descriptor	LRI DB-Wax	LRI DB-5	FD-Value WW	FD-Value IWG
1-hexanol	bready, fruity	1360	870	16	16
2-phenylethanol	floral, sweet, *yeasty*	1934	1132	8	16
ethyl nonanoate	tropical fruity	1542	1295	16	16

Table 24. Additional compounds from literature and GC-O to describe bran attribute found in WW and IWG bread crusts

Compound	Odorant Descriptor	LRI DB-Wax	LRI DB-5	FD-Value WW	FD-Value IWG
2-methoxy-4-vinylphenol	cooked, sweet, pasta	2224	1281	16	16
salicylaldehyde	pungent, grain, cooked	1693	1020	16	16

Table 25. Peak areas for suggested compounds for raisin and bran attribute in WW and IWG bread crusts

Compound	Peak Area IWG	Peak Area WW
1-hexanol	38813536	33538821
2-phenylethanol	1894601517	1399560326
ethyl nonanoate	14442856	12066461
2-methoxy-4-vinylphenol	107220224	10922629
salicylaldehyde	20753423	1361751