VALUE-ADDED PRODUCT DEVELOPMENT UTILIZING WASHINGTON STATE GRAPE SEED FLOUR

By

CLIFFORD HOYE

A thesis submitted in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE

WASHINGTON STATE UNIVERSITY
School of Food Science

December 2009

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of CLIFFORD L. HOYE Jr. find it satisfactory and recommend that it be accepted.

_____
Carolyn F. Ross, Ph.D., Chair

_____
Joseph R. Powers, Ph.D.

_____
Jeffri C. Bohlscheid, Ph.D.

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to my major advisor, Dr. Carolyn Ross, whose supervision, encouragement, and willingness to go out of her way to provide insight truly helped to shape my ideas and research. I would also like to thank my committee members, Dr. Joe Powers and Dr. Jeffri Bohlscheid for their support and guidance throughout the writing of this thesis. Without their knowledge and challenging suggestions, this study would not have been successful.

To AprèsVin for their support and flour samples. Without AprèsVin, this study would not have made it past its concept stages.

My sincere thanks to Doug Engle and all of the staff at the USDA Western Wheat Quality Lab, who provided key suggestions into the methodology of my thesis and bent over backwards to make sure that I felt welcome.

I would like to thank all faculty, staff and colleagues in the School of Food Science who contributed to my study here. I would especially like to thank Marsha, Jodi, Carolee and Rich for all of their help and willingness to brighten my days.

I am eternally grateful for the continual love and encouragement of my wife, parents, and sisters who helped me to pursue my advanced education. Finally, I would like to take this opportunity to thank all those who have helped me journey through graduate school and made this whole experience a memorable one.

VALUE-ADDED PRODUCT DEVELOPMENT UTILIZING WASHINGTON STATE GRAPE SEED FLOUR

Abstract

By Clifford Hoye, M.S.
Washington State University
December 2009

Chair: Carolyn F. Ross

The production of grape seed flour (GSF) from grape pomace, a waste product generated during winemaking, has been explored for use in product development due to its potential health benefits. However, research on the stability of GSF during processing and heating is limited. The overall objective of this study was to assess changes in phenolic content and antioxidant activity of GSF used as an ingredient in bread making. To determine the impact of heating time and temperature on the antioxidant activity of GSF, Merlot GSF from two harvest years (2007 and 2008) was heated at five different temperatures (120 to 240°C) for 0 to 90 mins. Total phenolic content (TPC), total flavan content (TFC), Trolox equivalent antioxidant capacity (TEAC), 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and ferric ion reducing antioxidant power (FRAP) of the extracts were determined. Thermal treatment of Merlot GSF caused significant decreases in the TPC and antioxidant activity when heated ≥180°C. A strong correlation between antioxidant properties and TPC was found, indicating that phenolic compounds were primarily responsible for the antioxidant properties measured by the DPPH, TEAC, and FRAP assays.

Doughs and breads were made using replacements of wheat flour with GSF (0 to 10 g/100g flour) and stored for 0, 2 or 6 weeks at -20°C. Replacement of wheat flour with GSF resulted in a dose dependent increase in the TPC and antioxidant activity of the breads. However, recovery of phenolics was low due to phenolic complexation with proteins during mixing. Consumer acceptance and instrumental analyses were used to investigate the changes in sensory and texture properties due to GSF replacement. An increase in GSF replacement above 5g/100g flour caused the brightness and loaf volume to significantly decrease, while increasing the hardness and number of cells/mm^2 in the crumb. GSF replacement did not significantly impact overall consumer acceptance of the breads. However, consumer acceptance of astringency and sweetness significantly decreased as GSF exceeded 5g/100g flour. Based on these results, the replacement of ≤ 5g GSF/100g flour is recommended for the production of fortified breads with acceptable instrumental and sensory properties, and antioxidant activity comparable to antioxidative fruits.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Dedication

This thesis is dedicated to my wonderful wife, Elizabeth, and to my parents, Clifford and Irene, who have supported me every step of the way. Thank you for all of your love, guidance, and words of wisdom throughout this immense undertaking. Thank you for helping me to succeed and instilling in me the faith and confidence to know that I am able to succeed in the face of adversity.

Thank you for everything, and I love you with all my heart!

CHAPTER ONE

INTRODUCTION

Grapes are Washington's fourth largest fruit crop behind apples, sweet cherries, and pears (NASS 2009). Accounting for approximately 30% of the value of all fruits grown in the United States, grapes are the highest value fruit crop in the nation and the sixth largest crop overall (Nelson 2009). Washington's wine and grape industries contribute $3 billion to the state's economy and over $4.7 billion to the national economy each year. A study commissioned by the Washington Association of Wine Grape Growers and the Washington Wine Commission found that between 1999 and 2006, the number of wine tourists in Washington State jumped 385%, from 350,000 to 1.7 million, correlating to a 1200% increase in spending from wine tourism.

The purchase of a bottle of wine produced from a WA State winery helps the local economy while resulting in tax contributions to both the state and the federal government. In 2006, the Washington wine industry contributed $145.2 million to the state, and $196.9 million to the federal government. In addition, the number of wineries grew from 19 in 1981 to over 650 in 2009. Between 1981 and 2006, the number of vineyards has also increased 30%, for a new total of approximately 57,000 acres of grapes (NASS 2009).

Washington is the second largest grape producer in the United States based on annual tons produced. Currently, 57,000 grape-bearing acres produce more than 350,000 tons of grapes with a total market value of over $201 million (NASS 2009). In 2008, 145,000 tons of grapes were processed for wine, resulting in a new Washington State record (NASS 2009).

Due to the decrease in wine consumption in Europe in 2008, United States topped Italy, Germany, and France as the world's largest consumer of wine, for the first time in history (Keller 2009). American wine consumption continues to rise, growing more than 14% between 2004-

2008, with consumption forecast to continue increasing over the next five years (IWSR 2009). Red wine is the driving force behind consumption in the United States. In 2008, Americans were estimated to have consumed more than 1.47 billion bottles of red wine, 565 million bottles of rosé, and 1.3 billion bottles of white wine (IWSR 2009).

If American wine consumption continues to increase at a 12% pace, over the next five years, consumption will reach 307 million cases (IWSR 2009). Each 750 mL bottle contains approximately 2.5 pounds of grapes, equaling 30 pounds of grapes per case. Of the 350,000 tons of grapes grown in Washington State, approximately 20% of their total weight is waste, including stems, seeds, and skins (Mazza and Miniati 1993). This translates into 70,000 tons of grape waste annually for WA State and over 13 million tons worldwide, creating a significant waste disposal hurdle.

Disposal of grape pomace, the waste generated during winemaking, has posed a major challenge for wineries. During wine production, wine grapes are harvested and pressed to extract juice for fermentation. As a result of pressing, the skins, stems, and seeds are left behind as waste. Removal of this pomace is costly and if the pomace is not treated effectively, it can initiate a number of environmental hazards, ranging from surface and ground water contamination to foul odors (Bonilla and others 1999). Winery waste can also have an environmental impact through the increase of the chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) within wastewater streams. The high COD and BOD_5 levels of the grape pomace originate from their high pollution loads, and high content of lipids and other organic substances such as sugars, tannins, polyphenols, polyalcohols, and pectins (Bonilla and others 1999; Schieber and others 2001). Due to the environmental problems that

these high COD and BOD_5 cause, it is beneficial for wineries to find other applications for their grape pomace waste other than animal feeds or fertilizers (Inbar and others 1988).

To help alleviate the issues associated with grape pomace, its use in alternative applications has been explored. Applications have included the production of value-added products such as dietary supplements for disease prevention (Shrikhande 2000), grappa (grape pomace alcohol) production (Hang and Woodams 2008), laccase production (Moldes and others 2003), and pullulan production (Israilides and others 1998). In addition to finding a productive use for a waste product, these products have been produced in response to a changing consumer demand for naturally processed, additive-free, and safe products (Bianco and Uccella 2000). Consumers tend to prefer safe, traditional products, which are promoted as "natural" and without other additives (Bianco and Uccella 2000). Thus, the substitution of currently used synthetic food antioxidants by ones pereceived as "natural" by consumers interests the research community. The market demand for natural antioxidants rather than chemical antioxidants added to baked products has directly increased the demand for novel polyphenolic containing ingredients. As part of this trend, the formation of antioxidant-rich flours milled from dried grape waste and the subsequent incorporation of these flours into baked foods is a promising option.

The food and beverage industry is a multi-billion dollar industry composed of several markets, including bakery, beverage, dairy, and processed foods. The baking market is an important segment within the food and beverage industry. The major products marketed within this industry include bread, morning goods, biscuits, cakes, and pastries (Gale 2009). Consumers are increasingly concerned about their health and are aware of the relationship between nutritious food and optimal health. Increasing consumer demand for healthy and convenient food has

caused the baking industry to search for innovative yet functional ingredients, which will allow them to capitalize on the current market trends.

Various concerns have caused consumers to closely monitor their dietary requirements. The rising incidence of health conditions such as obesity, diabetes, and cardiac problems, concerns over physical appearance, and the increasing price of health care have all contributed to the demand for healthier bakery products (Lempert 2008). One way to create a healthier bakery product with an enhanced nutritional profile is through the addition of functional ingredients such as phytosterols, multigrain, prebiotics, multivitamins, and polyphenolics. Polyphenolic compounds are also known to have beneficial health effects related to their antioxidative capabilities.

Grape seed flour has been shown to be rich in polyphenolic compounds, and because of this profile, a growing demand exists for the inclusion of this flour into processed foods. Proanthocyanidins are the major polyphenols found in red wine and grape seeds with grape seeds being rich sources of monomeric phenolic compounds such as (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-o-gallate, and dimeric, trimeric, and oligomeric procyanidins (Saito and others 1998). The discovery of health benefits associated with procyanidins such as cardioprotection, antimutagenicity, and antioxidant activity, has led to their use as a dietary supplement, with potential applications in the food industry. However, there is concern that food preparation processes such as baking cause a loss of some of the health benefits through phytochemical loss (Wang and Zhou 2004). Thus it is critical to consider the chemical changes which occur during food preparation.

Incorporation of antioxidant containing grape seed flour into specialty breads provides a novel vehicle to increase consumers' antioxidant intake. Sales of specialty and fresh bakery

breads in the U.S. has grown steadily from $5.9 billion in 2004 to $6.7 billion in 2008, equivalent to a growth of 13.5% (Lempert 2008). In total dollar sales of breads categorized by their label claims, whole grain bread has grown from $881.9 million in 2004 to $1.62 billion in 2008 (Lempert 2008). Natural bread, defined as containing no artificial flavors or preservatives, no high fructose corn syrup or trans fatty acids, has lead all segments in percent growth over the last two years, increasing by 34.3% in 2007 and 23.4% in 2008. Breads containing grape seed flour could be marketed as either whole grain or all natural, falling into two of the fastest growing specialty bread categories. Thus, the incorporation of grape seed flour into specialty breads not only follows consumer demands, and adds antioxidant activity, but seems to be corporately fiscally responsible.

As described above, wine grape pomace represents a rich source of antioxidants, demonstrated to show antioxidative, anti-carcinogenic, and anti-microbial properties. The antioxidative properties of grape pomace, coupled with an increased interest by consumers in wheat-based products containing value-added ingredients, present a significant opportunity for research and development. Since there have been no systematic studies on the interaction between grape seed flour and dough matrices, a study of grape seed flour in the breadmaking process would add to the existing literature while providing useful information to the industry.

The objective of this study was to assess changes in the phenolic content and antioxidant activity of grape seed flour used as an ingredient in bread making. Specific aims of this research include:

1) To determine the impact of heating time and temperature on the antioxidant activity of commercially produced grape seed flour extracts. Based on previous research, the hypothesis is that a significant change in the antioxidant activity will result from thermal

processing, with a predicted decrease in antioxidant activity with an increase in heating time and temperature.

2) To evaluate and compare the performance of various single electron transfer antioxidant assays on grape seed flour extracts. These assays will include: 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, Trolox equivalent antioxidant capacity (TEAC), and ferric ion reducing antioxidant power (FRAP) reducing capacity. While the different assays provide slightly different information regarding antioxidant activity, the hypothesis is that no significant difference in Trolox equivalent antioxidant activity will be observed across DPPH, FRAP, and TEAC single electron transport assays.
3) To measure the antioxidant activity and stability of polyphenolic compounds in bread produced using increasing concentrations of grape seed flour and stored under different conditions. Previous research has suggested that no change in antioxidant activity or polyphenolic compounds will occur due to frozen storage. However, through bread production, a significant loss of both antioxidant activity and polyphenolic compounds will be observed.
4) To assess consumer acceptance and antioxidant activity of breads baked with increasing replacement of wheat flour with grape seed flour and subsequently stored under different conditions. It is hypothesized that an increase in grape seed flour concentration will be associated with an increase in antioxidant activity but a decrease in consumer acceptability.

CHAPTER TWO

LITERATURE REVIEW

A. WINE GRAPES (*Vitis vinifera)*

1. Botanical Features

Wine grapes, *Vitis vinifera*, are part of the *Vitaceae* family, which comprises 17 genera of mostly woody or herbaceous lianas (tree-climbing plants) or shrubs with liana-like stems. Morphologically, *Vitaceae* are characterized by the occurrence of tendrils and inflorescences (cluster of flowers in complex branches on a main stem) opposite the leaves (Mullins and others 1992a). Only one of the 17 *Vitaceae* genera, *Vitis*, produces edible berries (Mullins and others 1992a).

2. Fruit

Grapes, the edible fruit of the grapevine, are a prime example of a true berry. A berry in botanical terms is a class of fleshy fruit lacking a stony layer, with the fruit wall being fleshy or pulpy (Robinson 2006). Grapes grow in bunches which vary in size and shape depending on the grape variety (Galet 2002). Grape color varies from green to yellow, pink, crimson, dark blue, and black, with the majority of grapes being yellow or very dark purple (Robinson 2006). The significant parts of the berry are the flesh, skin, and seeds (Robinson 2006). The flesh or pulp is the bulk of the berry (Figure 1). The pulp contains the juice in vacuoles of pericarp cells (Mullins and others 1992b). A central core of vascular strands connects to a mesh of veins that encircles the outer edge of the flesh like a "chicken-wire" cage. The veins contain the xylem, which transports water and minerals from the roots, and phloem, the pathway for sugar from the leaves (Robinson 2006). The grape skin is a tough enveloping layer around the grape that holds it together. The outside layer, or bloom, consists of waxy plates and cutin, which

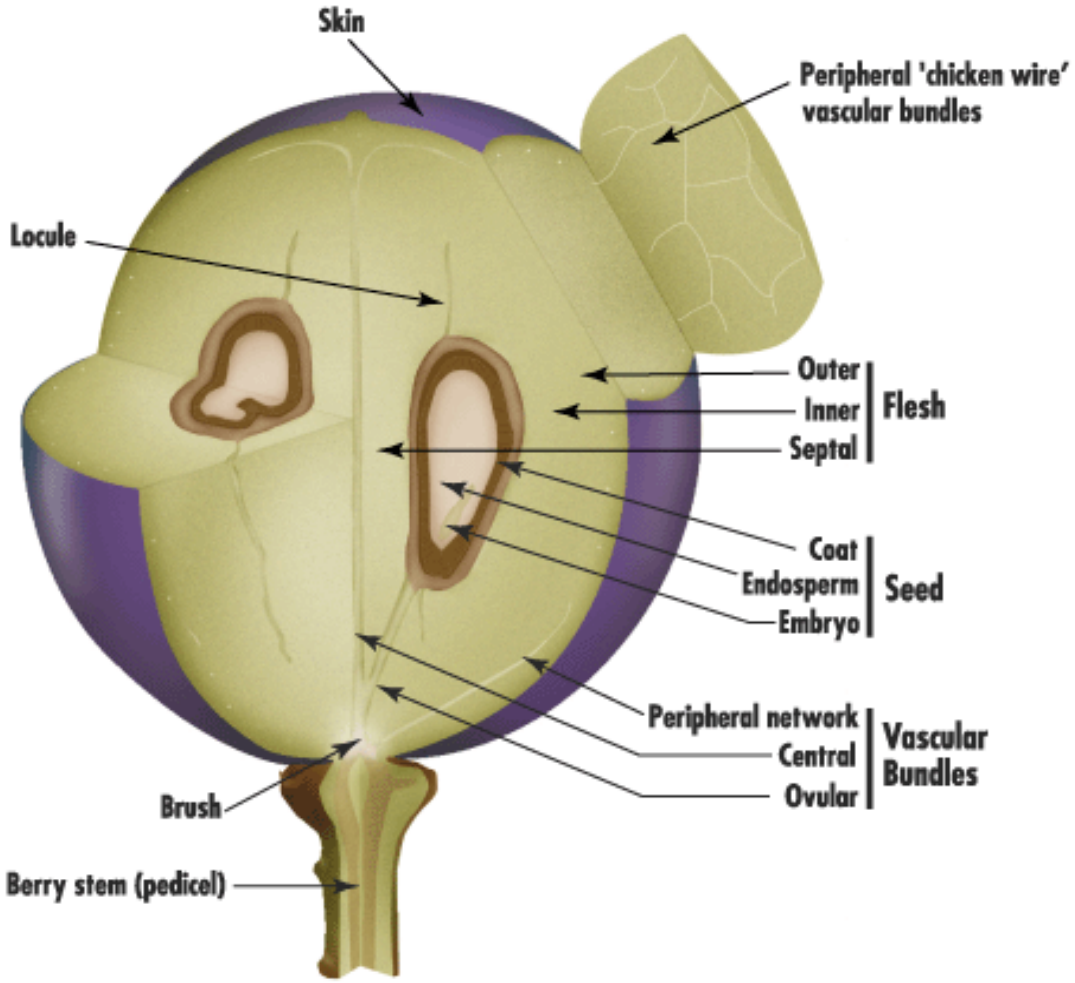

Figure 1. Grape berry structural anatomy partially sectioned. Illustration reproduced from Kennedy (2002).

resist water, fungal spore growth, and other biological infections. Below the bloom are the cell layers that form the skin and contained within these layers are concentrated carotenoids, xanthophylls, and anthocyanins (Mullins and others 1992b). Tannins, along with a significant amount of the grapes' flavor compounds, are also located in the skin (Robinson 2006). Seeds contain tannins, mostly proanthocyanidins, which if crushed, confer a bitter taste (Robinson 2006).

3. Varieties of Wine Grape

Many indigenous species of grapes are present worldwide but the majority of wine is produced using *Vitis vinifera*. Of Washington's 30 wine grape varieties, 52% or 74,800 tons of the grapes utilized are white and 48% or 70,200 tons are red (Table 1). The two major red varieties, accounting for 73% of the total state red wine production, are Cabernet Sauvignon and Merlot. These two varieties will be discussed in greater detail below.

a. Washington's Top Red Wine Grape Varieties

i. Cabernet Sauvignon

The classic variety from Bordeaux, Cabernet Sauvignon, remains the world's most renown grape variety for the production of red wines (Robinson 2006), and has a 37% stake in Washington's red wine grape production. Cabernet Sauvignon grape bunches are small, conical, and loose, with a small wing (Goode 2005). The grapes are small, black and spherical with a thick coating of bloom making them resistant to bunch rot (Goode 2005; Henderson and Rex 2007). Washington State Cabernet Sauvignon has a character that emerges in the wine as black currants, cherry, berry, chocolate, mint, herbs, or bell pepper (Bjornholm 2007).

Table 1. Washington Wine Grapes by Variety as measured by ton from 2004 to 2008 (NASS 2009).

Variety by Color	Quantity Utilized				
	2004	**2005**	**2006**	**2007**	**2008**
	Tons				
White Riesling	16,500	18,800	23,800	26,000	28,500
Chardonnay	28,400	26,000	28,600	26,700	28,000
Sauvignon Blanc	2,800	3,900	4,300	4,200	5,100
Pinot Gris	1,700	1,600	2,100	2,600	4,100
Gewurztraminer	3,000	3,700	4,000	4,100	4,000
Viognier	1,200	1,000	1,100	1,200	1,300
Chenin Blanc	700	1,000	1,100	1,100	1,200
Others[b]	1,700	1,900	1,700	1,800	2,600
TOTAL White Varieties	**39,500**	**39,100**	**42,900**	**41,700**	**74,800**
Cabernet Sauvignon	18,900	17,800	20,000	21,800	26,100
Merlot	20,400	20,500	19,100	21,300	25,400
Syrah	5,900	7,900	8,200	9,300	10,700
Cabernet Franc	2,800	2,300	2,400	2,700	2,500
Malbec	a	a	a	700	1,000
Pinot Noir	1,200	900	900	800	800
Sangiovese	500	600	700	700	800
Others[c]	1300	2,100	2,000	2,000	2,900
TOTAL Red Varieties	**51,000**	**52,100**	**53,300**	**59,300**	**70,200**
STATE TOTAL	**90,500**	**91,200**	**96,200**	**101,000**	**145,000**

a - Included in " red others."
b - Includes Semillon, Mueller-Thurgau, Madeline Angevine, Siegerrebe, Rousanne Muscat Ottonel, Orange Muscat, etc.
c - Includes Lemberger, pink varieties, Grenache, Zinfandel, Barbera, Petit Verdot, Nebbiolo, Mouverdre, Petit Syrah, etc.

ii. Merlot

Merlot bud burst occurs early, exposing it to the risk of frost damage; however, it is a vigorous variety that prefers cool moisture laden terrain (Goode 2005). Washington Merlot, imbibed with cherry flavors and aromas, tends to be more full-bodied, moderately tannic, and slightly higher in alcohol than its Bordeaux counterparts (Bjornholm 2007). Merlot constitutes 18% of the state's total production and 36% of the red wine grape market. The grape bunches, sometimes winged, are packed with blue-black spherical grapes that have a moderately thick skin and a juice laden flesh (Goode 2005).

4. Market and Production

Production of wine in WA State has more than doubled over the past decade, making winegrapes the state's fourth largest fruit crop. The ten American Viticulture Areas (AVAs) currently located in Washington are the Yakima Valley, Columbia Valley, Walla Walla Valley, Puget Sound, Red Mountain, Columbia Gorge, Horse Heaven Hills, Wahluke Slope, Rattlesnake Hills, and Snipes Mountain. These ten AVAs contain more than 600 wineries and 350 wine grape growers, with more than 57,000 bearing acres (NASS 2009).

Washington's wine grape production continues to increase each year (Table 2). Washington's 2008 wine grape production totaled 145,000 tons, a 14% increase over 2007 (NASS 2009). The top four major Washington varieties (Cabernet Sauvignon, Merlot, Riesling, and Chardonnay) made up 74% of total state production (NASS 2009). The production of each of the top varieties continues to increase. From 2007 to 2008, the use of Cabernet Sauvignon grapes increased 20% while Merlot increased by 19%.

Table 2. Production of wine grapes (tons) between 2005 and 2008 by the top five U.S. States. Data were reproduced from NASS-USDA Annual Fruit Survey Study (2009).

State Rank	State	2005	2006	2007	2008
1	California	3,200,000	3,176,000	3,288,000	3,400,000
2	Washington	110,000	120,000	127,000	145,000
3	New York	40,000	38,000	41,000	45,000
4	Pennsylvania	15,100	16,200	14,500	16,400
5	Michigan	4,600	4,300	5,400	5,300

With American wine consumption predicted to increase at a 12% rate over the next five years, consumption is projected to reach 307 million cases by 2014 (IWSR 2009). Each 750 mL bottle contained inside those cases contains approximately 2.5 pounds of grapes, equaling 30 pounds of grapes per case. Of the 350,000 tons of grapes grown in Washington State, approximately 20% of their total weight results in waste from the winemaking process in the form of stems, seeds, and skins (Mazza and Miniati 1993). In Washington State alone, this translates into 70,000 tons of grape waste each year. Removal of this waste is costly, and if not treated effectively, the waste can initiate a number of environmental hazards ranging from surface and ground water contamination to foul odors (Bonilla and others 1999). Due to these potential environmental hazards, it would be beneficial for wineries to find applications other than animal feeds or fertilizers for their grape pomace waste (Inbar and others 1988)

Incorporation of antioxidant-containing grape seed flour into specialty breads provides a novel vehicle to solve the grape pomace waste disposal hurdle. Sales of specialty and fresh bakery breads has grown steadily from $5.9 billion in the 2004 period to $6.7 billion in 2008 (Lempert 2008) (Table 3). The antioxidative properties of grape pomace, coupled with an increased interest by consumers in wheat-based products containing value-added ingredients, present a significant opportunity for research and development.

Table 3. U.S. Bread sales in 2008 expressed in dollar amount as a function of label claim[a].

Label Claim	**Dollar Sales (2008)**	**Dollar sales % change compared to 2007 sales**
Fat Presence	$1,983,445,194	+ 7.90%
Whole Grain	**$1,616,383,448**	**+ 11.20%**
Cholesterol Presence	$1,399,368,257	+ 8.20%
Fiber Presence	$829,091,554	+ 5.10%
Preservative Presence	$550,676,893	+ 8.90%
Calcium Presence	$467,881,508	+ 0.30%
Multigrain	$434,127,980	+ 3.00%
Vitamin/Mineral Presence	$330,204,721	-1.10%
Natural	**$303,810,199**	**+ 23.40%**
Carbohydrate Conscious	$200,310,267	+ 2.40%
Total Specialty and Fresh Bread	**$6,706,513,992**	**+ 6.10%**

a – Table was adapted from Lempert (2008). Data include the total U.S.–Food/Drug/Mass Merchandiser (excluding Walmart), UPC items only. Aug. 09, 2008

B. PHENOLIC COMPOUNDS

1. Plant Phenolics

Phenolic compounds or polyphenols in food originate from one of the main classes of secondary metabolites in plants derived from phenylalanine and, to a lesser extent, tyrosine (Figure 2).

Chemically, phenolics are defined as substances possessing an aromatic ring bearing one or more hydroxyl groups (-OH), including their functional derivatives. Polyphenols exist as simple phenolic molecules, such as phenolic acids or polymerized into larger molecules including proanthocyanidins (condensed tannins) and lignins (Bravo 1998). The presence, type, and content of phenolic compounds often determine the properties of plant products including health benefits, astringency, and antinutritional properties (Shahidi and Naczk 2004). Natural polyphenols are primarily found conjugated with one or more sugar residues linked to the hydroxyl groups. Phenolic compounds can be classified into several subclasses as illustrated in Table 4.

Flavonoids compose the most important and widely distributed single group of phenolics, with more than 4,000 flavonoids identified in fruits, vegetables, seeds, nuts, roots, and flowers (Macheix and others 1990). Flavonoids are low molecular weight compounds formed via the condensation of a phenylpropane (C_6-C_3) with three molecules of malonyl coenzyme A, ultimately leading to the formation of chalcones, which cyclize under acidic conditions (Figure 3).

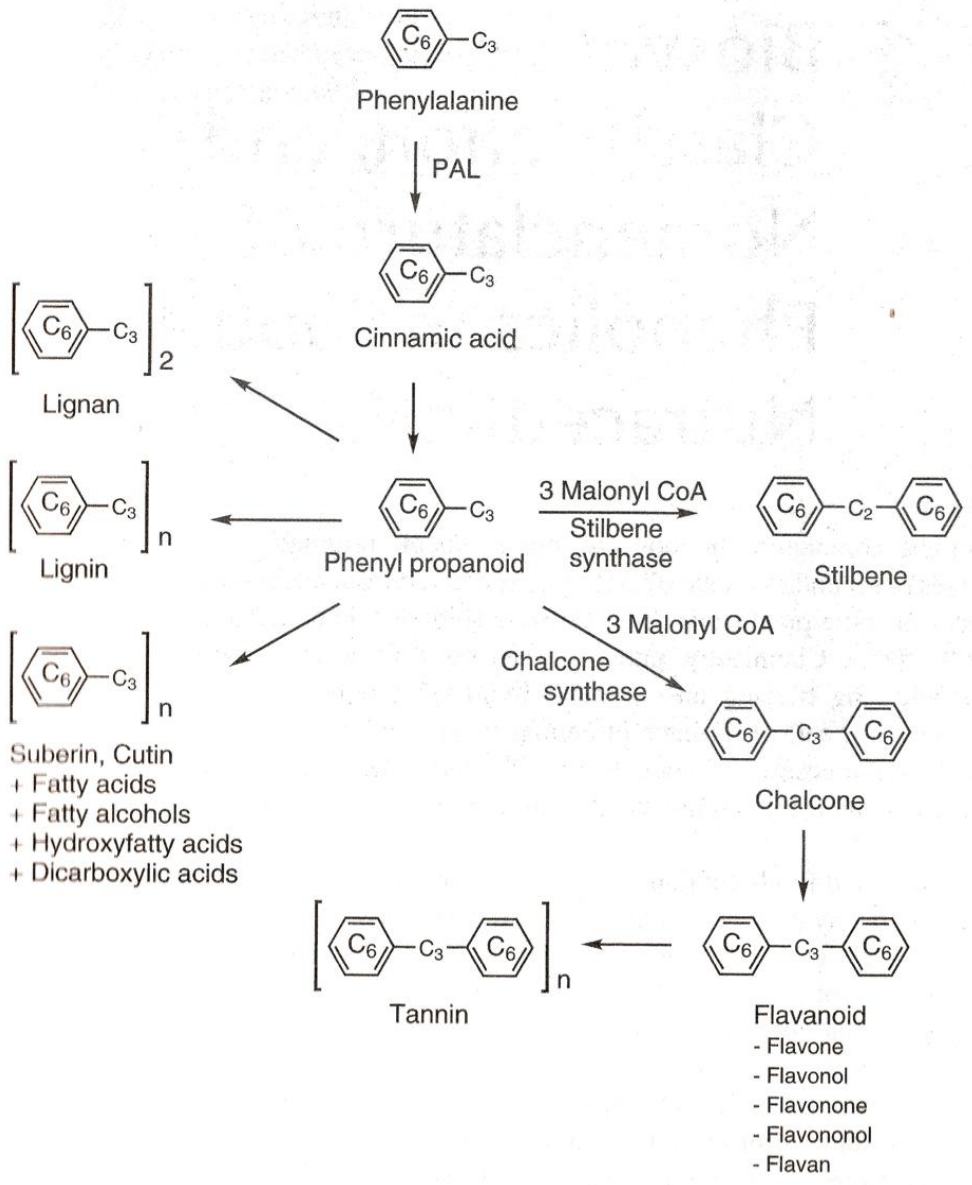

Figure 2. Production of phenylpropanoids, stilbenes, lignans, lignins, suberins, cutins, flavonoids, and tannins from the amino acid phenylalanine (PAL = phenylalanine ammonia lyase). Figure reproduced from Shahidi and Naczk (2004).

Table 4. The major classes of phenolics in plants as defined by their structure and dietary source.

Number of carbon atoms	Basic Skeleton	Class	Examples	Dietary Source
6	C_6	Simple phenols[a]	Catechol, Hydroquinone	Onion
		Benzoquinones[a]	2,6-Dimethoxybenzoquinone	Bamboo
7	C_6-C_1	Hydroxybenzoic acids[b]	*p*-Hydroxybenzoic, Salicylic	Strawberry
8	C_6-C_2	Acetophenones[b]	3-Acetyl-6-methoxybenzaldehyde	Brittle Bush
		Phenylacetic acids[b]	*p*-Hydroxyphenylacetic	Mangosteen
9	C_6-C_3	Hydroxycinnamic acids[a]	Caffeic, Ferulic	Apple
		Phenylpropenes[b]	Myristicin, Eugenol	Nutmeg
		Coumarins[a]	Umbelliferone, Aesculetin	Citrus
		Isocoumarins[b]	Bergenin	Bergenia
		Chromones[b]	Eugenin	Clove
10	C_6-C_4	Naphthoquinones[a]	Juglone, Plumbagin	Walnut
13	C_6-C_1-C_6	Xanthones[a]	Mangiferin	Mango
14	C_6-C_2-C_6	Stilbenes[a]	Resveratrol	Grape
		Anthraquinones[b]	Emodin	Aloe
15	C_6-C_3-C_6	Flavonoids[a]	Quercetin, Cyanidin	Cherry
		Isoflavonoids[a]	Daidzein	Soybean
18	$(C_6$-$C_3)_2$	Lignans[b]	Pinoresinol	Olive
		Neolignans[b]	Eusiderin	Noni
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids[b]	Amentoflavone	Ginko Biloba
n	$(C_6$-$C_3)_n$	Lignins[a]	-	Stone Fruits
	$(C_6)_n$	Catechol melanins[b]	-	-
	$(C_6$-C_3-$C_6)_n$	Condensed tannins[b] (Proanthocyanidins)	-	Grape Seeds

[a] Macheix and others (1990).
[b] Harborne and others (1999).

The structures shown in Figure 4 are composed of a heterocyclic benzopyran ‘C’ ring, the fused aromatic ‘A’ ring, and the phenyl constituent ‘B’ ring. The differences in oxidation level or substitution pattern of the ‘C’ ring subdivides the flavonoids into major subclasses: flavans, flavanones (2-3 bond is saturated), flavones (basic structure), flavonols (hydroxyl group at the 3-position), dihydroflavonols, flavan-3-ols, flavan-4-ols, and flavan-3,4-diols (Aron and Kennedy 2008).

Flavonoids are most commonly found in plants as *O*-glycosides with sugars attached at the C3 position (Hertog and others 1992). The sugars generally occur in the form of hexoses such as glucose, galactose, and rhamnose or pentoses such as arabinose and xylose. The glycosylation increases the water solubility of the molecule and allows for its accumulation in vacuoles of plant cells (Rice-Evans and others 1997). Representing the largest and most ubiquitous class of monomeric flavonoids, the flavan-3-ols comprise the major units of condensed proanthocyanidins, the primary flavonoid in grape seeds (Marais and others 2006; Chung and others 1998).

Tannins are typically divided into two groups. Hydrolysable tannins are esters of phenolic acids and a polyol, usually glucose. The phenolic acids are either gallic acid in gallotannins or other phenolic acids derived from the oxidation of galloyl residues in ellagitannins (Clifford and Scalbert 2000). Proanthocyanidins (condensed tannins), forming the second group of tannins, are polymers made of elementary flavan-3-ol units, and yield anthocyanidins upon heating in acidic media (Santos-Buelga and Scalbert 2000).

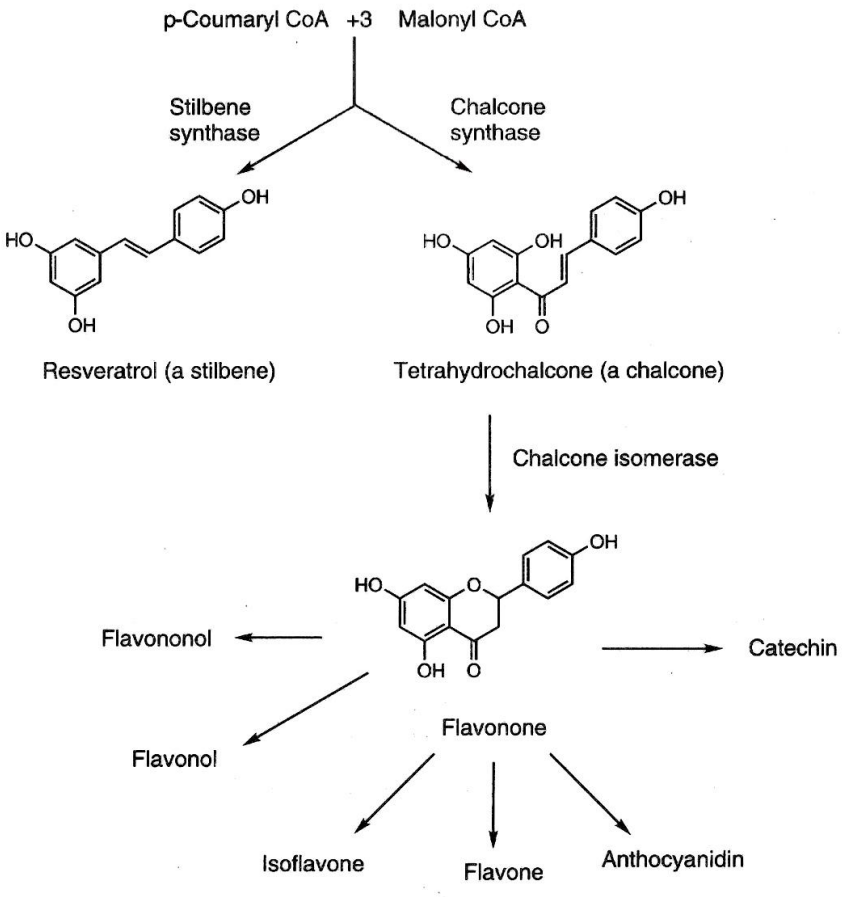

Figure 3. Production of flavonoids and stilbenes from phenylpropanoid and malonyl CoA. Figure adapted from Shahidi and Naczk (2004).

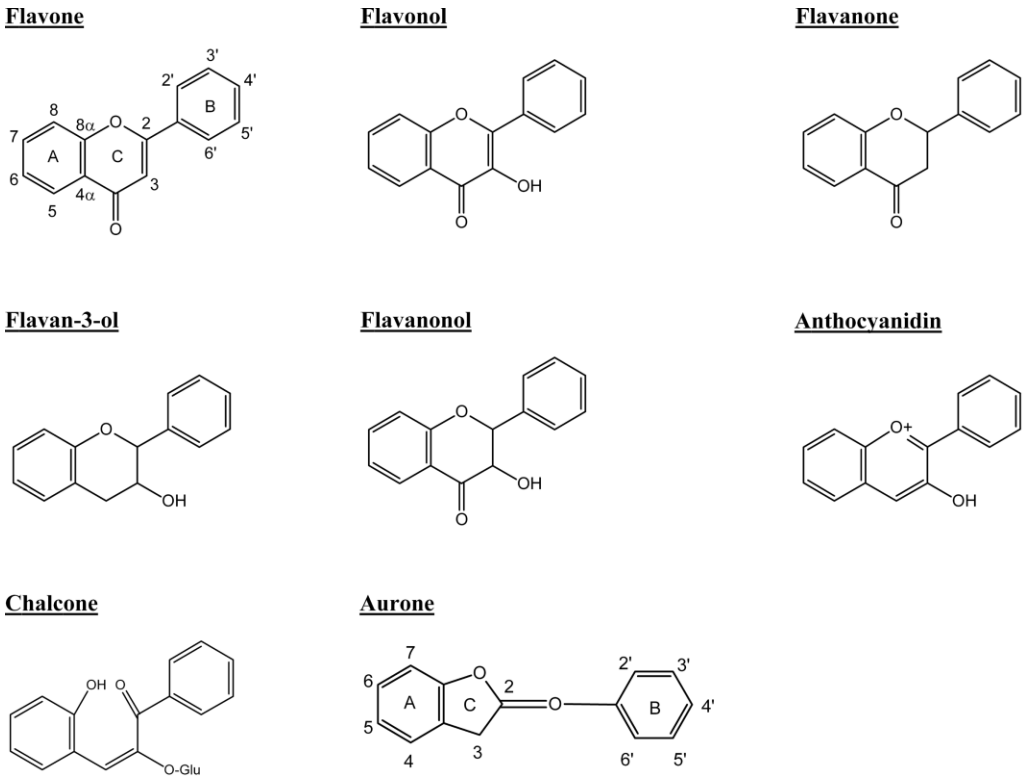

Figure 4. Flavonoid subclasses. Figure adapted from Aron and Kennedy (2008).

Proanthocyanidins are further divided into two categories based on their ‘A’ ring classification: phloroglucinol and resorcinol. The proapigeninidins, propelargonidins, proluteolinidins, procyanidins, prodelphinidins, and protricetinidin all possess a phloroglucinol type ‘A’ ring. Proguibourtinidin, proteracacidin, profisetinidin, promelacacidin and prorobinetinidin all possess a resorcinol type ‘A’ ring (Santos-Buelga and Scalbert 2000).

Within the grape, proanthocyanidins are the major polyphenols in the skins, stems, and seeds. Procyanidins are the predominant proanthocyanidins in grape seeds, while procyanidins and prodelphinidins are dominant in grape skins and stems (Cheynier and Rigaud 1986; Souquet and others 1996).

2. Antioxidant Activities of Plant Phenolics

Growing evidence indicates that oxidative stress, through the production of free radicals, can lead to cell and tissue injury (Figueroa-Romero and others 2008). Free radicals are molecules with an odd (unpaired) number of electrons. The unpaired electrons are increasingly reactive and actively seek to pair with another free electron. Free radicals are generated during oxidative metabolism and normal energy production in the body (Cadenas and Packer 1999). These radicals are involved in many processes in the body including enzyme-catalyzed reactions, electron transport within the mitochondria, signal transduction and gene expression, activation of nuclear transcription factors, oxidative damage to molecules, cells, and tissues, antimicrobial action of macrophages, and aging and disease (Bao and Fenwick 2004; Cadenas and Packer 1999).

Normal human metabolism is dependent on oxygen, a radical, as the terminal electron acceptor. As the two unpaired electrons of oxygen spin in the same direction, oxygen is a bi-radical; however comparatively, it is not a dangerous radical (Halliwell and others 1992). Other

oxygen-derived free radical species, such as superoxide or hydroxyl radicals, formed during metabolism or by ionizing radiation are much stronger oxidants and thus more of a concern (Bao and Fenwick 2004).

In addition to research on the biological effects of these reactive oxygen species, reactive nitrogen species have also been researched (Droge 2002; Wink and Mitchell 1998). NO, or nitrogen monoxide (nitric oxide), is a free radical generated by NO synthase (NOS). This enzyme mediates physiological responses such as vasodilation or brain signal transduction (Wink and Mitchell 1998). However, during inflammation, synthesis of NOS (iNOS) is induced. This iNOS can result in overproduction of NO, causing damage. NO can also react with superoxide to produce the toxic product, peroxynitrite (Wink and Mitchell 1998). Oxidation of lipids, proteins, and DNA can result, thereby increasing the likelihood of tissue injury.

Both reactive oxygen and nitrogen species are involved in normal cell regulation in which oxidation and reduction status are important in signal transduction (Bao and Fenwick 2004). Oxidative stress is increasingly seen as a component in the signaling cascade involved in inflammatory responses, stimulating adhesion molecules, and chemo-attractant production (Halliwell and others 1992). Hydrogen peroxide, which breaks down to produce hydroxyl radicals, can also activate transcription factors involved in stimulating inflammatory responses (Bao and Fenwick 2004). Excess production of these reactive species is toxic and can exert cytostatic effects, membrane damage, and cell death through apoptosis or necrosis (Bao and Fenwick 2004).

Many of the diseases that have been studied to date involve oxidative stress in the form of free radicals. In most cases, free radicals are secondary to the disease process, but in some cases free radicals are causal (Bao and Fenwick 2004). Three different stages of radical-mediated

oxidation of membrane lipids have been proposed: 1) Initiation during which free radicals remove hydrogen from an unsaturated fatty acid to form a lipid radical; 2) Propagation during which the lipid radical plus molecular oxygen forms a lipid peroxy radical, which further breaks down into more radicals; and 3) Termination during which time the new radicals react together or with antioxidants to eliminate free radicals (Cook and Samman 1996).

There is evidence to suggest that the consumption of flavonoid-rich foods, in particular fruits and vegetables, is associated with a lower incidence of cancer, cardiovascular disorders, and other degenerative and chronic diseases caused by oxidative stress (Wildman 2001). The evidence from numerous human studies focused on the consumption of foods containing flavonoids has resulted in six conclusions (Wildman 2001): (1) inverse correlations between flavonoid consumption and the incidence of diseases thought to involve oxidative stress (Cook and Samman 1996); (2) depression of the concentrations of oxidant products such as lipid peroxides; (3) elevation of concentrations of endogenous antioxidants, or prevention of their depletion during oxidant stress; (4) elevated measures of plasma or serum antioxidant capacities determined *ex vivo*; (5) inhibition of exercise-induced muscle tissue breakdown and inflammation; and (6) depression of lipoprotein oxidation rates assessed *ex vivo*.

The antioxidant activities of phenolic compounds mainly depend on their free radical scavenging abilities, determined by their reducing properties as either hydrogen or electron-donating moieties. Flavonoids, such as proanthocyanidins, could act at any stage of the radical-mediated oxidation by blocking initiation via the scavenging of primary radicals such as superoxide, slowing propagation by reacting with peroxy radicals, or accelerating termination by forming antioxidative reaction products. Flavonoids can also prevent the formation of free radicals through several possible mechanisms including direct radical scavenging,

downregulation of radical production, elimination of radical precursors (hydrogen peroxide), metal chelation, inhibition of xanthine oxidase, or elevation of endogenous antioxidants (Wildman 2001).

Two primary conditions exist that characterize a polyphenol as an antioxidant. First, polyphenols delay or inhibit the oxidation of the substrate when present in low concentration compared to the oxidizable substrate. Second, polyphenols are present as stable intermediates, which act as potential terminators of the propagation step by reacting with other free radicals (Rice-Evans and others 1997).

Many *in vitro* studies have demonstrated the high antioxidant activity of polyphenols on synthetic free radicals such as, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric 2,4,6-Tri-pyridyl-S-triazine (TPTZ) and also on the physiologically relevant hydroxyl and superoxide radicals (Proteggente and others 2003). This high antioxidant activity is due to the low redox potential of the polyphenols and their ability to donate several electrons. The role of polyphenols *in vivo* is still unclear as the antioxidant potential of polyphenols is dependent on the absorption and metabolism of these compounds. *In vivo* studies show that flavonoids were present in low levels in human plasma after the ingestion of large amounts of flavonoid rich foods, indicating poor gut barrier absorption (Lotito and Frei 2006). On the other hand, epidemiological as well as human studies suggest that the plasma concentrations may be sufficient to yield potent biological antioxidant activity (Wildman 2001).

3. Health Benefits of Polyphenolics

a. Delay or inhibition of cancer growth.

Diet can play an important role in either promoting or preventing diseases. A large literature base exists describing the successful use of fruits and vegetables in preventing various diseases including cancer. The study performed by Hertog and others (1996) reported a significantly reduced risk of cancer mortality for men with a high fruit intake. A review of over 200 studies found a significant protective effect of fruit and vegetable intake against lung, colon, breast, cervix, esophageal, oral cavity, stomach, bladder, pancreas and ovarian cancer (Block and others 1992). It has also been shown that a diet high in fruits reduced oxidative damage to DNA, which may be one critical step in the onset of some types of cancers (Djuric and others 1998). Numerous studies have suggested that the polyphenolic content and the resulting antioxidant activity of fruits and vegetables contributes to their protective effects against chronic and degenerative diseases (Record and others 2001; Heionen and others 1998).

Animal studies and cell models suggest that the polyphenolics contained in grape seed act as anti-carcinogens by influencing molecular events at the initiation, promotion, and progression stages of cancer (Lin and Weng 2006). Their effect on cellular function is described by several proposed mechanisms. Flavonoids can act as anti-carcinogens by scavenging of free radicals, regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, governance of enzyme activity related to detoxification, oxidation, and reduction, stimulation of the immune system and DNA repair, and the regulation of hormone metabolism (Liu 2002). The most intriguing of these properties is the role of polyphenols in apoptosis, programmed cell death. Apoptosis minimizes leakage of potentially toxic cellular constituents from dying cells. Apoptosis is a key pathway because a

damaged or blocked pathway results in uncontrolled cell division that ultimately leads to tumor formation and propagation. Oxidative stress, cancer, viral infections, and other degenerative diseases are all correlated to improper regulation of apoptosis (Liu 2002).

Several *in vitro* and *in vivo* studies have demonstrated that catechins and other structurally related compounds exhibit anti-proliferative and growth controlling properties in numerous cancerous and normal cell lines. Epigallocatechin-3-gallate, epigallocatechin, and gallocatechin have been shown to exhibit high anti-proliferative action against three cancer lines, MCF-7 breast cancer, UACC-375 melanoma, and HT-29 colon cancer cells (Valcic and others 1996). At 100 μM concentrations, gallocatechin and gallocatechin gallate have been shown to completely inhibit the proliferation of SF-268 cancer cell line from nervous tissue (Seeram and others 2003). *In vivo* studies in mice indicated that pretreatment with 100 mg/kg body weight dietary grape seed proanthocyanidins greatly reduced induced lipid peroxidation and DNA fragmentation in brain and liver tissues (Bagchi and others 2000). Epigallocatechin-3-gallate has shown high toxicity toward AY-27 transitional cancer cells and L1210 mouse leukemia cell lines at concentrations greater than 100 μM (Kemberling and others 2000). Treatment of Caski cervical cancer cells with epigallocatechin gallate showed a dose-dependent inhibition of cell proliferation with a 50% inhibitory concentration of 35 μM and apoptosis at concentrations greater than 100 μM (Ahn and others 2003). The effective concentration of catechins to show *in vitro* antiproliferative activity appears to range between 20–100 μM in different studies depending on the type of catechin and cancer cell line used.

b. Cardiovascular disease

Cardiovascular disease (CVD), and in particular atherosclerosis, remains the leading cause of death in both men and women in the United States (Klatsky 1996). Atherosclerosis is an

inflammatory disease process (Adams and others 2000). Endothelial injury is one of the first events in this process, which is followed by a large number of reactions and molecular responses. All of these events may lead to the formation of atherosclerotic plaques, resulting in constriction of blood vessels and a reduced capacity to dilate. Grape seed proanthocyanidins appear to remedy several of the steps in this complex process. Much epidemiologic evidence indicates that consumption of polyphenolics results in a reduction of CVD risk factors and decreased mortality (Ross 1999). A few of the mechanisms thought to play a key role in the development of atherosclerosis are low-density lipoprotein (LDL) oxidation, platelet aggregation, and nitric oxide (NO) dependent dilation (Pataki and others 2002).

LDLs that transport lipids throughout our bodies are targets for oxidation. The oxidative susceptibility of LDL and the subsequent oxidation of arterial walls are thought to be critical steps in the development of atherosclerosis (Ross 1999). This oxidation occurs through a number of highly reactive oxygen species such as singlet oxygen, O_2, $OH^{\cdot}$, $NO^{\cdot}$, and alkyl peroxyl free radicals. Recent investigations of proanthocyanidins from grape seeds suggest that they are effective at protecting against LDL oxidation via free radical scavenging activities (Arteel and Sies 1999).

Many causal factors exist in the development and progression of atherosclerosis, with platelet hypersensitivity thought to be an important contributor to disease manifestation (Lusis 2000). Platelet aggregation, a result of increased sensitivity to oxidants *in vivo,* is thought to contribute to the initiation and progression of atherosclerosis and to the occurrence of heart disease (Ross 1999; Lusis 2000). Platelet aggregation is associated with an increased release of reactive oxygen species and platelet-vessel wall interactions, resulting in damage to the vascular endothelium (Freedman and Keaney 1999). In addition to their role in the initiation of

atherosclerosis, platelets also contribute to the progression of heart disease by releasing various growth factors that accelerate the proliferation and migration of smooth muscle cells (Ross 1999). Therefore, reducing the activity of platelets would potentially reduce the development and progression of coronary heart disease. Daily intake of platelet inhibitors such as aspirin has been shown to slow the progression of heart disease in animal models (Anderson and others 2001). Similarly, grape products, especially grape seeds, are effective platelet inhibitors, at a minimum concentration of 50 mg/L, and appear to reduce the development of atherosclerosis through this pathway (Vitseva and others 2005).

Procyanidins have recently been shown to possess endothelium-dependent relaxing (EDR) activity in blood vessels (Fitzpatrick and others 2002). The enzyme, nitric oxide synthase (NOS), uses L-arginine and oxygen as substrates to produce NO, which interacts with smooth muscle cells to cause vasorelaxation (Fitzpatrick and others 2002). Furthermore, EDR activity was accompanied by increased levels of cyclic GMP, the vascular smooth muscle cell messenger through which NO acts. It has been recently shown that vasodilating compounds tend to be of the proanthocyanidin type (Fitzpatrick and others 2002).

In blood vessels, NO not only resists vasoconstrictor influences, but also decreases platelet aggregation and adherence of platelets to the endothelium, inhibits oxidation of LDL, and diminishes vascular smooth muscle cell proliferation. In order to determine the respective contributions of grape seed proanthocyanidins on platelet adhesion and intravascular coagulation of blood (thrombosis), male C57BL/6 mice were given either 2 or 20 mg grape seed proanthocyanidin extract/kg body weight (Sano and others 2005). In these mice, injecting them with 20 mg grape seed proanthocyanidin extract/kg body weight increased bleeding time, reduced the thrombus weight and reduced the apparent platelet adhesion to collagen in arterial

walls. No effect was observed with the injection of 2 mg grape seed proanthocyanidin extract/kg body weight (Sano and others 2005). The data collected from animal models suggested that grape seed proanthocyanidins inhibit platelet aggregation when taken orally. Thus, it would appear that consumption of NO-stimulating compounds, such as grape seed proanthocyanidins, in the diet could contribute to the prevention or slowing of atherosclerosis.

Grape seed proanthocyanidins are potent accelerators of NOS activity, quenchers of *in vitro* oxidation of LDL, and reducers of platelet aggregation. A combination of the reduction of these three risk factors suggests the potential for dietary grape seed proanthocyanidins in preventing diseases. Thus, incorporation of grape seed flour into the daily diet through commercial products, such as bread, needs to be researched.

c. Diabetes, glucose, and insulin metabolism

Impaired glucose uptake and insulin resistance are common metabolic alterations which may be a general indicator of age-related disorders and chronic diseases (Preuss and others 2002). Thus, identification of dietary components and natural products that counteract these impairments provides a highly favorable risk to benefit ratio. In one study, an extract of cinnamon, which contained a series of proanthocyanidins was effective in significantly reducing fasting blood glucose in a group of type II diabetic individuals (Khan and others 2003). In addition, these extracts also stimulated glycogen synthesis by activating glycogen synthase and inhibiting glycogen synthase kinase-3β activities, which are commonly known effects of insulin treatment (Jarvill-Taylor and others 2001).

A review of five clinical trials involved nearly 1300 patients in which pycnogenol, a proanthocyanidins rich antioxidant extract from pine bark, was tested for treatment and prevention of retinopathy. The results of this study showed diminished progression of the disease

and partial recovery of visual acuity in subjects ingesting the proanthocyanidin supplement. Pycnogenol treatment effectively improved capillary resistance, reduced blood leakages into the retina, and was as effective as the drug, calcium dobesilate (Schonlau and Rohdewald 2001). Grape seed flour and pycnogenol have a similar polyphenolic make-up, and the inclusion of grape seed flour into novel products geared toward the diabetic community, should be researched.

d. Digestibility and Absorption

Upon ingestion, proanthocyanidins first react with proline-rich proteins in the mouth to yield an astringent sensation. Those compounds which cross the intestinal barrier travel to the liver via the portal vein, where they further degrade into metabolites. Within hours of consumption, these metabolites may reach all tissues as seen in radio-labeling experiments with live rats (Gonthier and others 2003). Confirmation of the presence of low-molecular weight metabolites in the urine and feces of these rats, as well as in chickens and sheep, indicates that polymeric proanthocyanidins may not be absorbed through the intestinal barrier without first being degraded into low-molecular weight metabolites by gut microflora (Scalbert 1991; Gonthier and others 2003). Urine analysis of 69 human subjects after consumption of grape seed extract supplement throughout six weeks (1000 mg/day total polyphenols) supported these results. Results indicated three phenolic acids as breakdown products of proanthocyanidin metabolism: 3-hydroxyphenylpropionic acid, 4-O-methylgallic acid and 3-hydroxyphenylacetic acid (Ward and others 2004).

Of all the classes of flavonoids, proanthocyanidins appear to be the least well absorbed, specifically 10- to 100-fold less than their monomeric constituents (Tsang and others 2005). Polymers with a mean degree of polymerization equal to seven are not as well absorbed by the

human intestine due to their lower permeability through paracellular absorption (movement of ions through intercellular spaces between epithelial cells), and their likely complexation with luminal and mucosa proteins (Manach and Donovan 2004).

4. Methods to Determine Antioxidant Activity

Numerous methods are currently used for the determination of antioxidant activity of botanical extracts. These methods differ from each other in terms of experimental conditions, reaction medium, standards, substrates, and analytical evaluation methods. The comparison of the results and the subsequent interpretation are difficult due to the inherent variability of experimental conditions and differences in the chemical properties of the oxidizable substrates.

The antioxidant capacity methods currently used can be divided into two basic categories due to the chemical reactions involved (Table 5). The first group are hydrogen atom transfer (HAT) reaction-based assays while the second group are the single electron transfer (SET) reaction-based assays. The HAT-based assays use competitive reaction kinetics, with quantitation derived from kinetic curves, while SET-based assays detect the ability of a potential antioxidant to transfer one electron to reduce any compound as an indicator of the endpoint (Huang and others 2005). SET and HAT mechanisms almost always occur together in all samples, with the balance determined by the antioxidants' structure and pH. Both assays are intended to measure the radical scavenging capacity, instead of the preventative antioxidant capacity (Huang and others 2005).

Table 5. *In vitro* antioxidant capacity assays (Huang and others 2005).

Assays involving HAT reactions	**Examples**
	ORAC (Oxygen Radical Absorbance Capacity)
$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$	TRAP (Total Radical Trapping Antioxidant Parameter)
$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet}$	Crocin Bleaching Assay
	IOU (Inhibited Oxygen Uptake)
	Inhibition of Linoleic Acid Oxidation
	Inhibition of LDL Oxidation
Assays involving SET reactions	**Examples**
	TEAC (Trolox Equivalent Antioxidant Capacity)
$M(n) + e \text{ (from AH)} \rightarrow AH^{\bullet} + M(n\text{-}1)$	FRAP (Ferric Ion Reducing Antioxidant Parameter)
	DPPH (Diphenyl-1-Picrylhydrazyl)
	Copper (II) Reduction Capacity
	FCR (Total Phenols Assay by Folin-Ciocalteu Reagent)
Other Assays	**Examples**
	TOSC (Total Oxidant Scavenging Capacity)
	Chemiluminescence
	Electrochemiluminescence
	Inhibition of Briggs-Rauscher Oscillation Reaction

a. HAT-Based Methods

These methods measure the ability of an antioxidant to quench free radicals by hydrogen atom donation. In general, HAT-based assays are composed of a synthetic free radical generator, an oxidizable molecular probe, and an antioxidant (Huang and others 2005). The antioxidant reactivity or capacity measurements are based on competition kinetics between the probe and antioxidant for radicals (Huang and others 2005).

i. Oxygen Radical Absorbance Capacity Assay (ORAC)

One example of a HAT-based method is the ORAC Assay. ORAC measures antioxidant inhibition of peroxyl radical-induced oxidations (Ou and others 2001). In this assay, the peroxyl radical reacts with a fluorescent probe to form a nonfluorescent product, which can then be quantified by fluorescence. Initially this method was developed by (Cao and others 1993), using B-phycoerythrin (B-PE) as a fluorescent probe. However, the use of B-PE has been replaced by fluorescein (FL:3'6'-dihydroxyspiroisobenzofuran-1[3H],9'[9H]-xanthen-3-one) in antioxidant assays because of the shortcomings of B-PE reactivity variability to peroxyl radicals (Cao and Prior 1999), photo-bleaching after exposure to excitation light, and binding of proanthocyanidins to B-PE, resulting in erroneously low ORAC values (MacDonald-Wicks and others 2006).

In the ORAC assay, sample, control, and standards of varying Trolox concentrations are mixed with the FL solution and incubated before the addition of AAPH (2-2'-azobis (2-amidinopropane) dihydrochloride) to initiate the reaction. The probes' reaction with generated peroxyl radicals is followed by the loss of fluorescence over time. The addition of antioxidants causes the scavenging of peroxyl radicals and stops the loss of fluorescent intensity. The ORAC values are obtained by a Trolox standard curve and the net integrated area under the fluorescence decay curve (AUC), defined by $AUC = AUC_{sample} - AUC_{blank}$ (Shahidi and Zhong 2007).

b. SET-Based Methods

These methods involve two components in the reaction mixture, the antioxidants and the probe (oxidant). The degree of color change of the probe is proportional to the antioxidant concentration in the reaction solution. Relative reactivity in SET methods is founded primarily on deprotonation (Lemanska and others 2001) and ionization potential (Wright and others 2001) of the reactive functional groups, making the SET reactions pH dependent. Since there is no oxygen radical present, the correlation of the SET assay results with *in vivo* antioxidant activity is questionable. To make this correlation, it is assumed that antioxidant capacity is equal to the reducing capacity (Benzie and Szeto 1999). SET-based methods are typically utilized to determine antioxidant activity due to their relative speed and ease of use (Huang and others 2005)

i. *Total Phenols Assay by Folin-Ciocalteu Reagent (FCR)*

FCR was initially developed for the analysis of tyrosine, in which oxidation of phenols by a molybdotungstate reagent yields a colored product (Folin 1927). This assay was later extended to the assay of total phenols in wine (Singleton and Rossi 1965), and has since found many applications, most notably total phenolics in plant extracts and beverages. Several studies have found a significant linear relationship between SET-based assays (FRAP, TEAC, DPPH) and the FCR assay, which is not surprising due to the similarity of the chemistry of the assays (Huang and others 2005). FCR assay is based on the reduction of an intensely yellow heteropoly-phosphotubgstate-molybdate anion to a blue colored complex in an alkaline solution in the presence of phenolic compounds (Shahidi and Naczk 2004).

The Folin-Ciocalteu (FC) reagent is nonspecific to phenolic compounds, and can also be reduced by non-phenolic compounds including ascorbic acid, Cu(I), Fe(II), aromatic amines,

sulfur dioxide, sugars, organic acids, and extractable proteins (Prior and others 2005). Phenolic compounds will react with FCR only under basic conditions, where the dissociation of a phenolic proton leads to a phenolate anion, ultimately capable of reducing FCR (McDonald and others 2001).

The total phenols assay by FCR is convenient and reproducible. As a result of these characteristics, a large body of data has been amassed, and FCR has become a routine assay for studying phenolic antioxidants (Huang and others 2005).

ii. ***2,2 diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay***

The DPPH radical is a stable nitrogen radical due to the delocalization of the spare electron throughout the molecule (Prior and others 2005). This delocalization gives rise to a deep purple color, with an absorption maximum at 515 nm. When antioxidants are added to a DPPH solution, absorbance decreases as the odd electron of the nitrogen atom is reduced by receiving a hydrogen atom from the antioxidant (Ozcelik and others 2003). A partial or total loss of the purple color is observed; however, a pale yellow may still remain due to the DPPH picryl group.

While the DPPH assay is a relatively easy and rapid, it does have limitations. DPPH color can be reduced by HAT radical reduction or SET reduction. DPPH bears no similarity to the peroxyl radicals involved in lipid peroxidation, and many antioxidants that react quickly with peroxyl radicals may react slowly or not at all with DPPH due to steric inaccessibility (Prior and others 2005). Also, the reaction kinetics between DPPH and antioxidants are not linear with the DPPH concentrations, and thus EC_{50} (concentration of antioxidant required for a 50% decrease in initial DPPH concentration) determination can become problematic and inappropriate (Brandwilliams and others 1995; Sanchez-Moreno and others 1998).

iii. *Trolox Equivalent Antioxidant Capacity Assay (TEAC)*

The TEAC assay is based on the scavenging ability of antioxidants on the radical anion $ABTS^{\bullet+}$. In this assay, ABTS is oxidized by peroxyl radicals to its radical cation, $ABTS^{\bullet+}$, which is colored. The antioxidant capacity is then determined as the ability of the test compounds to diminish the color by reacting directly with the $ABTS^{\bullet+}$ radical (Figure 5).

In the TEAC assay, $ABTS^{\bullet+}$ must be generated through either chemical reactions [manganese dioxide (Miller and others 1996), ABAP (van den Berg and others 1999), or potassium persulfate (Re and others 1999)] or enzyme reactions [metmyoglobin (Miller and others 1993), hemoglobin or peroxidases (Cano and others 2002)]. $ABTS^{\bullet+}$ reacts rapidly with antioxidants, can be utilized over a wide pH range, and can determine both hydrophilic and lipophilic antioxidant capacities of extracts and body fluids (Prior and others 2005). However, the ABTS radical is not found in biological systems nor does it resemble biological radicals. Because the TEAC assay is simple and has been widely used, many antioxidant values of compounds and food samples have been reported for antioxidant comparison purposes (Gil 2000; Proteggente and others 2002).

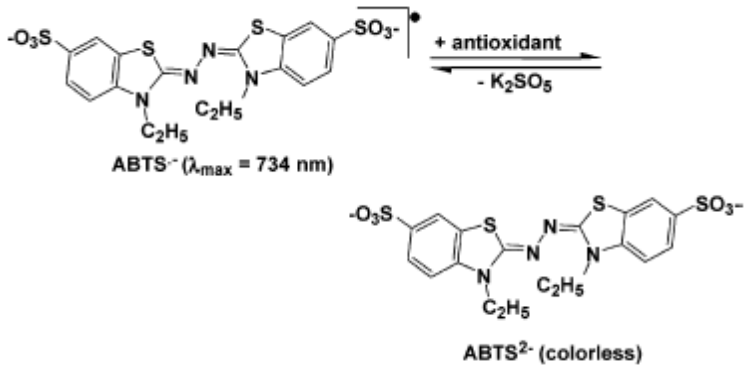

Figure 5. Structure and Reaction Pathway of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (Huang and others 2005).

iv. *Ferric Ion Reducing Antioxidant Power (FRAP)*

The FRAP assay utilizes a ferric salt, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ = 2,4,6-tripyridyl-*s*-triazine) as an oxidant, which is reduced to a blue colored ferrous complex by antioxidants under acidic (pH 3.6) conditions (Figure 6) (Prior and others 2005). The increase in absorbance is measured and compared with that of a Fe(II) standard solution.

The FRAP mechanism is electron transfer rather than mixed SET and HAT as is the case of DPPH. Thus in combination with other methods, it can be useful in determining the mechanisms of different antioxidants. The FRAP assay was developed with the hypothesis that the redox reactions proceed rapidly enough that they are completed within 4 to 6 min; however, sometimes this is not the case. Certain polyphenols such as caffeic acid, tannic acid, ferulic acid, ascorbic acid, and quercetin, slowly increase in absorbance over several hours (Pulido and others 2000). Despite its limitations, FRAP is still a simple, rapid, and inexpensive test of total antioxidant power, with the advantage of determining antioxidant activity directly in whole plasma (Pulido and others 2000).

The effectiveness of antioxidants in foods and biological systems is affected by the overall complexity of the food product. A number of different substrates, system compositions and analytical methods are currently used in testing protocols to evaluate antioxidant capacity. As a result of the numerous methodologies utilized, the results obtained by different researchers are difficult to compare and interpret. Therefore, evaluation of antioxidant activity should be carried out under different experimental conditions, utilizing several different methods to measure the different products of oxidation related to real biological reactions. If numerous

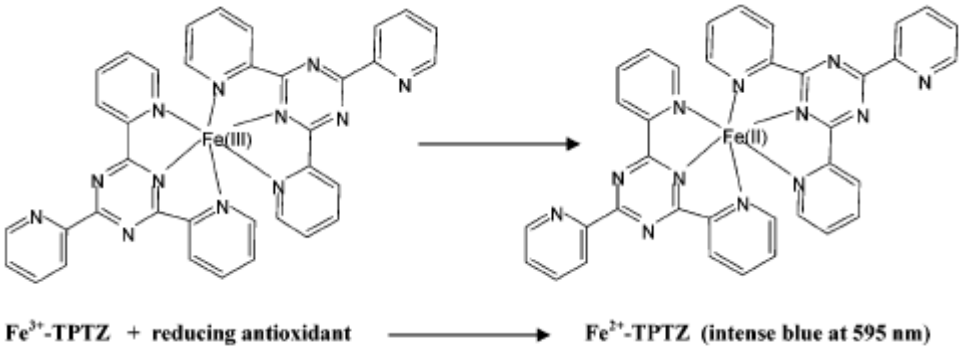

Figure 6. Reaction of the ferric ion reducing antioxidant power (FRAP) assay (Prior and others 2005).

methods are used, such as FRAP, TEAC, and DPPH, researchers can then compare the general trends of the values for individual samples to achieve results with more reliability (Frankel and Meyer 2000).

5. Flour Processing

a. Wheat Flour Processing

Since the formation of gluten is an essential component of bread making and wheat is the contributor of the proteins necessary for its formation, it is logical to presume that a significant proportion of final bread quality arises from the flour milling process. The wheat grain is made up of three components: the inner endosperm, comprising mainly starch and protein; the outer bran, mainly protein and fiber; and the germ, mainly protein, fiber, fat, minerals, and vitamins (Lynn 2000). The level and quality of the gluten-forming proteins (glutenin and gliadin) depends on the wheat variety, agricultural practices, and milling.

Wheat grain can be ground to flour utilizing rudimentary methods, such as mortar and pestle; however, the flours produced would not meet the requirements of the modern day baking industry or the consumer. Instead, flour milling consists of a sequence of conditioning, breaking, grinding and separation operations, which gradually separate the endosperm from the germ and bran (Barnes 1989).

Cleaning, the first stage wheat undergoes before it is milled, consists of separation by size using sieves, separation of stones using specific gravity, air currents to remove minor contaminates, and scouring to remove dirt from the exterior (Barnes 1989). Milling directly after cleaning will produce an inferior product due to contamination by a large quantity of bran. To reduce the amount of bran contained in the flour, wheat is first conditioned. Conditioning

involves the controlled addition of water to make the bran less likely to pulverize during milling (Lynn 2000).

Once the wheat has been correctly conditioned, it is milled. Although there are numerous different milling processes typically, the first stage in wheat milling is break rolling. Break rollers are designed to open up the grains by breaking the grain between a pair of counter-rotating metal rollers (Lynn 2000). After each milling stage, the milled material is separated into fractions. Reduction rolls are then used to reduce large endosperm fractions into flour by counter-rotating rollers. After each break and reduction stage, there is a rotating sieve section which causes the finer, heavier particles to move downwards to the sifting surface while the coarser, lighter particles remain on the top layer of sieves. Different flour streams from the sieving stages are combined in the correct ratio to produce the flour with the required characteristics (Lynn 2000; Barnes 1989).

b. Grape Seed Flour Processing

The grape seed flour processing begins just as winemaking begins with the harvest in the fall. After the winegrapes are weighed and inspected, they enter the stemmer-crusher, which first removes the berries from the stems and then breaks them open to release the juice. The mixture at this point, called the must, is approximately 80% juice, 16% skins, and 4% seeds (Henderson and Rex 2007). In white wines, the must is immediately pressed after destemming-crushing. However, in red wines, the must starts the fermentation process with the skins and seeds present.

From the time the yeast is added, the fermentation typically, takes 1 to 3 weeks, depending on amount and type of yeast added, the nutrients in the must, and the fermentation temperature (Henderson and Rex 2007). In red wine, when fermentation is complete, the wine is separated from the seeds and skins. The majority of wine is drained out by gravity however,

about 10% to 20% is still held within the skins. The skins are removed from the fermentation vessel, loaded into a press and the remaining wine is pressed out of the skins. After the skins and seeds are pressed with as much wine removed as possible, the resulting cake is called pomace.

For the production of grape seed flour, this pomace is collected and tumbled in a trammel screen to separate the larger pieces (primarily grape skins) from the smaller pieces (primarily grape seeds). The seeds are collected and moved to a fluidized bed dryer where the seeds are suspended in high-velocity air heated to 90°C. Most of the water is removed from the seeds utilizing the fluidized bed dryer; however, a final drying to 7% moisture is performed using a shelf dryer.

The dried seeds are then cold-pressed in an expeller press, with temperatures not exceeding 40°C. The expeller press yields two separate products, the oil and the press cake. The oil is collected and sold as a specialty item, while the press cake is collected for subsequent milling. The press cake is ground into baking flour utilizing a micro pulverizer. The product collected from the micro pulverizer is then sieved to produce various particle sizes, for use in different products.

6. Effect of Thermal Processing on Phenolics and Antioxidant Activity

Processing can alter and often damage fruit and vegetable antioxidants, particularly in the case of vitamin C and phenolic antioxidants. Heating, maceration, and separation steps can cause oxidation, thermal degradation, and/or antioxidant leaching, leading to lower levels of antioxidants in processed foods compared to fresh. However, as was found in rice hulls, processing can lead to a dissociation of antioxidants from the plant matrix components, increasing the phenolic content and improving digestive absorption (Kim and others 2006; Jeong and others 2004).

Thermal processing is among the most popular ways of food processing. Through thermal processing, a complex set of chemical reactions takes place that dictate many of the final quality attributes of processed foods. In phenolic compounds, antioxidant activity may experience significant degradation during processing. In a study evaluating the effect of blanching and long term frozen storage, researchers found a 20-30% reduction in vitamin C, β-carotene, and α-carotene, in potatoes, carrots, and spinach due to blanching, while frozen storage only slightly reduced these compounds (Puupponen-Pimia and others 2003). Total phenolic content was positively correlated with antioxidant activity, suggesting that among the compounds tested, phenolic acids contributed to the antioxidant activity. In blueberry juice processing, involving heating, substantial losses of phenolics were observed. Specifically, reduction of anthocyanins, procyanidins, and chlorogenic acid, between 47 and 68%, were observed when comparing a fresh product to the final juice (Kalt 2005). A more recent study of grape seed extracts in bread showed a 30-40% reduction in antioxidant activity, compared to a pure extract, at the completion of processing (Peng and others 2009). The authors attributed the loss in antioxidant activity to either proanthocyanidins complexation with proteins, or the thermal degradation of the proanthocyanidins.

However, one study has demonstrated that processed fruits and vegetables may retain or increase their antioxidant activity throughout heating (Kim and others 2006). These researchers found that the heat treatment of grape seeds liberated phenolic compounds, and subsequently increased the amount of active compounds by up to 46% in the extracts. The authors also suggested that because of the increase in total phenolics, simple heating could be used as a tool to increase antioxidant activity of commercially available grape seed extracts (Kim and others

2006). Thus, it would seem that with respect to the thermal stability of grape seed antioxidants, published studies vary in their conclusions.

7. Phenolic Compounds in Grape Seed Flour

Grape seeds are considered good sources of polyphenolic tannins responsible for the astringent mouthfeel in wine. Grape seeds are concentrated sources of gallic acid, monomeric phenolic compounds, such as (+)-catechin (0.16 mg/mL GSE), (-)-epicatechin (0.66 mg/mL GSE), (-)-epicatechin-3-o-gallate (0.20 mg/mL GSE), and dimeric (0.329 mg/g DW grape seed), trimeric (0.384 mg/g DW grape seed) and polymeric (0.905 mg/g DW grape seed) proanthocyanidins (Saito and others 1998; Kim and others 2006; Khanal and others 2009). (+)-Catechin shows antioxidant activity in human blood plasma by delaying the degradation of α-tocopherol and β-carotene and by inhibiting the oxidation of plasma lipids (Lotito and Fraga 1997). (+)-Catechin has hydroxyl (Moini and others 2002), peroxyl (Scott and others 1993), superoxide (Bors and Michel 1999), and DPPH (Fukumoto and Mazza 2000) radical scavenging activities. (-)-Epicatechin is capable of scavenging hydroxyl radicals (Moini and others 2002), peroxyl radicals (Liu and others 2000), superoxide (Wang and Jiao 2000; Bors and Michel 1999), and DPPH radicals (Fukumoto and Mazza 2000). Gallic acid is a phenolic acid that can scavenge peroxyl radicals (Moldes and others 2003) and DPPH radicals and has also shown activity at stomach pH (Gunckel and others 1998).

Proanthocyanidins are defined as oligomers and polymers of polyhydroxy flavan-3-ol units such as catechin, gallocatechin, and their epimers (Beecher 2004). Proanthocyanidins range in size from dimers through very large polymers and exhibit a wide range of biological activities. Proanthocyanidins are considered to be superior antioxidants compared to their corresponding monomers (catechin or gallocatechin) (Ursini and others 2001).The unique polyhydroxy

phenolic nature of proanthocyanidins and the stability of reduction products (semiquinones and quinines) results in an electron configuration that allows for an easy release of protons and thus substantial antioxidant activity.

Grape seeds have been proven to be rich in phenolic compounds, particularly flavonoids [(+)-catechin and proanthocyanidins], which have demonstrated a wide range of biochemical and pharmacological effects pertaining to their antioxidant activities. The available information seems to suggest that regular consumption of fruits and vegetables should have long-term health benefits, related to their antioxidant activity. Thus, it is reasonable to conclude that consumption of grape seed flour, with potent antioxidant activity, will also result in long-term health benefits. However, for increased availability of health beneficial compounds new food products such as breads rich in these phenolic compounds need to be researched and developed. There is also a need for better understanding of the chemistry of grape seed phenolics not only to determine the antioxidant activities of those compounds, but also to determine grape seed phenolic stability under thermal processing and frozen storage, and the effect of grape seed replacement on bread sensory acceptance and instrumental analyses. Therefore, this study will explore the potential benefit of the use of grape seed flour in a food system such as bread.

CHAPTER THREE

CHANGES IN PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF GRAPE SEED FLOUR USED AS AN INGREDIENT IN BREAD MAKING

INTRODUCTION

Grapes (*Vitis vinifera* L.) belong to a group of the world's largest fruit crops, with the total global production in 2007 exceeding 67 million metric tons (FAOSTAT 2008). The United States is the world's third-largest producer of grapes, and Washington with an annual production of more than 350,000 tons is the second largest producer by state (NASS 2009). There are approximately 57,000 acres of grape (*Vitis* spp.) production in Washington, which is divided approximately equally between wine and juice grapes (NASS 2009). Washington's wine and grape industry contributes $3 billion to the state's economy and over $4.7 billion to the national economy each year.

The production of wine in Washington State has more than doubled over the past decade, making wine grapes the state's fourth largest fruit crop, and its highest value crop. The primary wine grapes grown in Washington (Merlot, Chardonnay, Cabernet Sauvignon, and White Riesling) made up 74% of the total state production. The production of each of the top varieties continues to increase. For instance, Merlot constituted 18% of the state's total production in 2008, a 19% increase in production over 2007 (NASS 2009).

The polyphenols found in the red wine have been linked to numerous health benefits. Red wine's polyphenolic content is correlated to its cardioprotective properties including the oxidation of human LDL (Howard and others 2002), increase in serum HDL levels (Perret and others 2002; Araya and others 2001), modulation of platelet aggregation (Pace-Asciak and others 1996; Pace-Asciak and others 1995), enhanced vasorelaxation, and inhibition of smooth muscle

cell proliferation (Araim and others 2002; Rivard and Andres 2000). The majority of grape polyphenols extracted into the wine are originally present in the skins and seeds, and as a result, the processing of grapes into wine and the length of contact between the wine and the skins and seeds has a significant effect on total phenolic content in the finished wine.

As a result of processing, the skins, stems, and seeds are left behind as waste (pomace). Of the 350,000 tons of grapes grown annually in Washington State, approximately 20% of their total weight is waste in the form of seeds, stems, and skins, with seeds constituting 40-60% of its dry weight (Mazza and Miniati 1993). This translates to between 28,000 and 42,000 tons of grape seed waste for Washington State alone and over 8 million tons worldwide. Removal of this pomace is typically costly, and if it is not treated effectively, it can result in foul odors or ground water contamination (Bonilla and others 1999). Winery waste can have a high chemical oxygen demand (COD) and biological oxygen demand (BOD_5) which originates from its high pollution loads and high content of lipids and other organic substances, such as sugars, polyalcohols, polyphenols, and pectins (Bonilla and others 1999; Schieber and others 2001). Due to the environmental problems related to high COD and BOD_5, it is prudent for wineries and researchers to find novel applications for their grape pomace.

In the past, grape pomace, particularly seeds, was seen only as waste; however, recently alternative applications for this pomace have been explored. The spectrum of other applications has included the production of value-added products such as dietary supplements for disease prevention (Shrikhande 2000), grappa production (Hang and Woodams 2008), laccase production (Moldes and others 2003), and pullulan production (Israilides and others 1998). The oil from grape seeds has also been found to be rich in unsaturated fatty acids, in particular linoleic acid (Schieber and others 2002), and has been recently utilized as a gourmet cooking oil.

For the production of grape seed oil, grape seeds which consist of 7-20% oil, 35% fiber, 29% extractable components including phenolic compounds, 11% proteins, 3% minerals and 7% water, are extracted via an expeller press. The use of an expeller press yields two separate products, the grape seed oil and the press cake. The oil is then collected and sold as cooking oil, while the press cake proceeds on to subsequent milling, creating grape seed flour.

Apart from being a rich source of high-value oil, grape seeds have also been recognized by their high content of phenolic compounds such as gallic acid, monomeric phenolic compounds (catechin and epicatechin), and dimeric, trimeric, and tetrameric proanthocyanidins (Saito and others 1998). Grape seed extracts and proanthocyanidins have been under intense investigation with respect to their potentially beneficial health effects. Recent reports indicate a wide range of biological activities including antioxidant properties and cardioprotective effects (Sato and others 1999), anti-inflammatory effects (Terra and others 2007), anti-ulcer activity (Saito and others 1998), prevention of cataracts (Yamakoshi and others 2002), improvement of insulin sensitivity (Pinent and others 2008), and anti-carcinogenic activity (Hertog and others 1996).

Recently, phenolic antioxidants have been viewed as an important class of food ingredients, which can be added to introduce extra health benefits to various food products. The inclusion of grape seed flour in bakery products such as cookies, cakes, or specialty breads provides a novel method to solve a waste disposal problem while adding extra health benefits. Considering the fact that a heat treatment (baking) is required for numerous bakery products, a pertinent concern is the heat stability of the phenolic compounds and the impact on total phenolic content or antioxidant capacities. It has been reported that the total antioxidant activities of grape seed extracts were enhanced with certain time-temperature combinations of thermal processing

(Kim and others 2006), while the antioxidant activity of blueberry juice was substantially lowered during processing (Kalt 2005).

Thus far, little work has focused on evaluating the relationship between the thermal processing of food and the changes in antioxidant capacities of the phenolic containing additives. There has also been no systematic report on the interaction between grape seed flour and dough matrices, making an investigation into the effect of grape seed flour in the breadmaking process necessary and interesting. The overall objective of this study was to assess changes in the phenolic content and antioxidant activity of grape seed flour used as an ingredient in breadmaking. The specific aims of this research include: 1) the determination of the relationship between the thermal processing of grape seed flour and the antioxidant activity of its extracts; 2) a comparison of the performance of various single electron transfer antioxidant assays on grape seed flour and bread extracts; 3) the assessment of antioxidant activity and stability of polyphenolic compounds in grape seed flour during frozen dough storage and bread production; and 4) the evaluation of the impact of grape seed flour inclusion on sensory quality and antioxidant activity of breads baked with various replacements of wheat flour with Merlot grape seed flour.

MATERIALS AND METHODS

Materials

Polyphenolic and Antioxidant Assay Chemicals

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)], trolox [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], ABAP [2,2'-azobis(2-amidinopropane) dihydrochloride], TPTZ [2,4,6-tri(2-pyridyl)-s-triazine], $FeCl_3 \bullet 6H_2O$ [ferric chloride hexahydrate], DPPH [2,2-diphenyl-1-picrylhydrazyl], Folin-Ciocalteu reagent, vanillin,

procyanidin B2, and tannic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium carbonate, sodium chloride, sodium phosphate, sodium acetate trihydrate, potassium dihydrogen phosphate, hydrochloric acid, glacial acetic acid, and *n*-hexane were purchased from J.T. Baker Inc. (Phillipsburg, NJ). 95% ethanol was purchased from Washington State University Central Stores (WSU, Pullman, WA). All reagents and solvents used were analytical or HPLC grade.

Plant Material

Grape (*Vitis vinifera*, Merlot, Chardonnay, or Cabernet Sauvignon; 2007 and 2008) seed flour (150, 180, 250, or 420 μm) was provided by AprèsVin (Prosser, WA).

Bread

Standardized hard red spring wheat was obtained from the USDA Western Wheat Quality Lab (Pullman, WA). C&H Baker's Special Sugar and SAF Instant Red Label Active Dry Yeast (*Saccharomyces cerevisiae*) was purchased from Spokane Bakery Supply (Spokane, WA). Partially hydrogenated shortening with mono- and di-glycerides (Crisco) and Darigold non-fat dry milk (high temperature process) was purchased from Dissmore's IGA (Pullman, WA). Reagent grade NaCl was purchased from Fisher Scientific (Pittsburgh, PA) and Miller's Strength Malted Barley Flour was obtained from Cargill (Minneapolis, MN).

Methods

Heat treatment

The grape seed flour (2.0 g) was placed in a single layer in an aluminum dish (57 mm diameter) and heated at 120, 150, 180, 210, or 240°C for 10, 20, 30, 40, 50, 60, or 90 min in a gravity convection oven (Precision Model 45EG, Thermo Electron Corp., Waltham, MA).

Following the heat treatment, the flours were cooled to room temperature, sealed in a polyethylene bag and held at -20°C until required for analysis.

Preparation of 70% ethanol extracts of grape seed flour (GSF)

The preparation of 70% ethanolic extracts of bread was modified from the process outlined by Wang and Zhou (2004). Briefly, one gram of heated grape seed flour was weighed and defatted in 30 mL of *n*-hexane at 70°C for 20 min in a shaking water bath (150 rpm). The hexane fraction was then decanted. Each heat-treated grape seed flour (0.1 g) was then extracted for 12 hours with 10 mL of 70% ethanol: water solution in a shaking water bath (150 rpm) at 24°C. The extracts were centrifuged at 1000g for 15 min. The supernatants were filtered through Whatman No. 1 filter paper. Following extraction, the extracts were sealed in plastic scintillation vials, and held at -20°C with the headspace flushed with nitrogen, until required for analysis.

Preparation of bread

Proximate composition of Hard Red Spring Wheat and Merlot grape seed flours

Protein concentration (%) was analyzed using the combustion method (Leco, model FP-428) AACC Method 46-30 (AACC 2000). Flour moisture (%) was tested using 2 g of flour heated in an aluminum dish in a forced-draft oven for 20 min at 130°C, allowed to cool and then weighed following the AACC modified Method (Method 44-16; AACC 2000). The percentage ash calculated from a 4 g sample of flour was ignited and heated for 15 hours at 550°C in a muffle furnace following AACC Method 08-01 (AACC 2000).

Mixing and absorption optimization of wheat and grape seed flour blends

Mixograms were generated using AACC Method 54-40A (AACC 2000; Finney and Shogren 1972), using a 10 g sample of standardized hard red spring wheat flour (HRS-05), obtained from the Western Wheat Quality Lab, or grape seed flour (Merlot 150 μm) blends. The

grape seed flour was added in direct replacement of HRS-05 at levels of 0, 2.5, 5, 7.5, and 10 g per 100 g of hard red spring wheat flour. Briefly, a 10 g sample of each flour blend based on a 14% moisture basis and the appropriate amount of water to give optimum absorption (visually evaluated by the width of the swings of the mixogram curve) were added to the mixing bowl. The mixing for the mixogram was performed for 8 min in a National MFG Co. mixograph mixer (Lincoln, NE) to allow the flour blends to exhibit their mixing time to peak and dough breakdown. The mixograms were then visually evaluated by trained bakers for gluten strength and mixing properties based on previously generated reference curves for low (6-8%), medium (9-11%), or high protein doughs (11-13%) (Figure 7) (Dick and Youngs 1988). Mixogram absorption (MABS), the optimum flour-water absorption, was used to estimate the bread baking absorption (BABS). The mixogram mixing time to peak was used to estimate the optimum bread dough mixing time (MTIME).

Sample Preparation

Bread and dough were prepared using an optimum absorption, optimum mixing, 90 min fermentation straight dough method using 100 g hard red spring wheat flour, 1.8% active dry yeast, 1.5% salt, 6% sugar, 0.3% malt extract, 4% dry milk solids, and 3% partially hydrogenated shortening with mono- and di-glycerides (Crisco) AACC Method 10-10B (AACC 2000; Finney 1945; Finney and Barmore 1945a, 1945b; Finney and Barmore 1943; Finney and others 1976). Grape seed flour was added in direct replacement as described above. Six separate (100 g) batches of dough were prepared for each grape seed flour replacement level. Once mixed, the dough was rested for 10 min at ambient temperature (22°C). After resting, the dough was either pre-frozen in a blast freezer at -20 °C until the core temperature reached -7 °C, then stored at -20 °C for 2 or 6 weeks or immediately proofed in a National MFG. Co. proofing cabinet (Lincoln,

NE, USA) for 90 min at 30°C with 95% relative humidity. Upon completion of frozen storage (either 2 or 6 weeks), the dough was proofed for 180 min. After proofing, both the frozen and unfrozen samples were baked in a National MFG Co. rotary oven (Lincoln, NE, USA) for 21 min at an oven temperature of 218 °C.

Loaf volume

Following baking, the bread loaves were evaluated for mass, height, and volume. The volume of each loaf was measured by a modified rapeseed displacement method (Method 10-05: AACC 2000). The apparatus was set up according to specifications from the rapeseed displacement apparatus at the Western Wheat Quality Laboratory (Pullman, WA).

Instrumental analyses of bread

After one hour of cooling at ambient temperature, the bread was subjected to instrumental analyses. The bread was manually cut into slices of 12.5 mm thickness using a slicing guide. The central two slices were used for color intensity, image analysis, and texture profile analysis (TPA).

Color Intensity

Using a CM-3500 d spectrophotometer (Minolta, Japan), color intensity of the two central slices was measured and expressed as L*a*b* values against a known white background. In color intensity determinations, L* represented whiteness (value 100) or blackness (value 0), a* represented red (+a) or green (-a), and b* represented yellow (+b) or blue (-b).

Image Analysis

Image acquisition

The image acquisition of bread slices took place on the same day of baking. Six loaves of each flour replacement treatment (0, 2.5, 5, 7.5, or 10 g grape seed flour/100 g HRS-05 wheat

flour), storage condition (unfrozen, 2 weeks, or 6 weeks of frozen storage), and non-frozen 0 g GSF/100g wheat flour control were sliced as described above. These combinations yielded a total of 102 loaves. The two central slices had their crust removed and were then scanned in color, with a black background, on both sides using a flatbed scanner (HP Photosmart C4180, Hewlett Packard Co., CA) with 600 dpi of resolution. The images were saved in .jpg format. The images were then opened with Adobe Photoshop Elements 5.0 (Adobe Systems Inc., San Jose, CA) and each image was auto-smart fixed and magnetic lassoed to remove shadows from each slice's edge. The images were saved as .jpg with a resolution of 150 pixels/cm. A set of 408 images (duplicate images of each loaf's two central slices) that presented a wide range of porosity, crumb, coarseness and crumb heterogeneity was evaluated.

Image thresholding

The bread crumb images were analyzed utilizing an image analysis program built within IMAQ Vision Builder 6.1 (National Instruments, Austin, TX) (Pitts 2009). Using an automated program, the blue color plane was extracted from the images. The threshold levels of each image were manually adjusted, the border objects were removed, and the images were then passed through a particle filter to remove cells smaller than 5 pixels. The remaining cells were then properly closed with a three row by three column structuring element of ones and separated with a three row by three column structuring element of ones. The image was again passed through a particle filter to remove cells fewer than 5 pixels. The following crumb features were obtained from particle analysis of each slice of bread: (1) mean cell diameter (mm), which is the average of the longest chord for each cell present in the crumb image; (2) minimum cell diameter (mm); (3) maximum cell diameter (mm); and (4) the number of cells/mm^2 present in the cut surface of the bread crumb.

Texture Profile Analysis (TPA)

The two central slices of each loaf were taken for texture measurement by a TA-xT2i texture analyzer (Stable Micro System, UK) with a 36 mm diameter aluminum plunger. The tests were conducted under the following conditions (Esteller and others 2006): compression force, 0.5 *g*; compression speed, 2 mm/s; and distance, 40% compression of two stacked 12.5 mm slices. Measurements were carried out at ambient temperature (22°C) and data were automatically processed by the Texture Exponent 32 Program (v. 2) software supplied with the instrument. The following equation was used to determine firmness (Bourne 2006):

Firmness = Peak force of the first product compression

Experimental design of sensory evaluation

Sensory evaluation by consumer panel

Sensory evaluation of bread was conducted using a consumer acceptance test with some modification (Meilgaard and others 1999). Ten attributes of bread broken down into four categories including appearance (crumb darkness, crust darkness, porosity), taste (sweetness, bitterness, astringency, sourness), oral texture (hardness, adhesiveness), and overall acceptance. These attributes were selected according to a list of standardized lexicon terms for bread evaluation (Meilgaard and others 1999). Consumers were provided with a list of definitions of each sensory attribute (Table 6), and evaluations were made using a 7-point scale anchored with dislike very much (1) and like very much (7) hedonic ratings of each attribute. Filtered deionized water was provided for rinsing the palate. The deionized water was filtered over a Milli-Q Reagent Water System (Millipore, Bedford, MA, USA) containing carbon, deionizing, and trace organic scavenger filters. For the interstimulus protocol during bread evaluations, panelists were instructed to rinse with deionized water and wait at least 30 seconds between samples. All

sensory sessions were carried out in separate booths equipped with a computerized system and sensory software (Compusense *five plus*, release 4.6, Compusense, Inc., Guelph, ON) where sensory data were recorded directly. Samples were assigned 3-digit codes and their serving orders were randomized by the software.

Three separate consumer acceptance panels were performed by panelists. Consumers were recruited from the staff and students of WSU. The first sensory panel was composed of 87 participants, all between the ages of 18 and 70, with a composition of 35 males and 52 females. Each consumer received each fresh bread treatment (0, 2.5, 5, 7.5, and 10% GSF replacement), a total of five samples. The second sensory panel was composed of 97 participants, all between the ages of 18 and 70, with a composition of 43 males and 54 females. Each consumer received a total of six samples consisting of (unfrozen 0 (control), and 6 week frozen 0, 2.5, 5, 7.5, and 10%), a total of six samples. The final sensory panel was composed of 87 participants, all between the ages of 18 and 70, with a composition of 39 males and 48 females. Each consumer received a total of six samples consisting of (unfrozen 0 (control), and 2 week frozen 0, 2.5, 5, 7.5, and 10%).

A completely randomized design was used for sample presentation. Specifically, the bread pieces were presented in a random serving order of five or six flights of one sample each (either 0, 2.5, 5, 7.5, or 10% GSF replacement) for a total of five or six samples. Each sensory panel was performed the morning after baking (samples were stored overnight at room temperature in closed Ziploc polyethylene bags (S.C. Johnson & Son, Inc., Racine, WI), and consumers were given 12.5 x 12.5 x 12.5 cm cubes of each sample containing both crust and crumb sections.

Preparation of 70% ethanol extracts of bread

The preparation of 70% ethanolic extracts of bread was modified from the process outlined by Wang and Zhou (2004). Briefly, one gram of lyophilized and ground bread dough or crumb was accurately weighed and defatted in 30 mL of *n*-hexane at 70°C for 20 min in a shaking water bath (150 rpm). The hexane fraction was then decanted and dried under a stream of nitrogen. Defatted sample (0.1 g) was extracted for 12 hours with 10 mL of 70% ethanol: water solution in a shaking water bath (150 rpm) at 24°C. The extracts were then centrifuged at 1000g for 15 min. The supernatants were then filtered through filter paper (Whatman No. 1). Following extraction, the extracts were sealed in plastic scintillation vials, and stored at -20°C with the headspace flushed with nitrogen until required for analysis.

Photometric determination of phenolic compounds and antioxidant activity (GSF and Bread)

Total phenolic content (TPC): Folin-Ciocalteu assay

Total phenols were determined using Folin-Ciocalteu reagent according to the method by Singleton and Rossi (1965), as modified by Gutfinger (1981), with some minor changes. Briefly, the 70% ethanolic grape seed or bread extracts (0.5 mL) were mixed with 0.1 mL of 50% Folin-Ciocalteu reagent and 0.5 mL of 2% Na_2CO_3, and centrifuged at 12000*g* in a VWR Galaxy 14D Micro-centrifuge (West Chester, PA) for 5 min. The absorbance was measured with a Genesys 10-S spectrophotometer (Thermo Electron Corp., Waltham, MA) at 750 nm after 30 min of incubation, covered and in the dark, at ambient temperature. TPC was expressed as tannic acid equivalents (mg TAE/100g dry GSF, and mg TAE/100g defatted bread, respectively). The standard curve was generated with 2.5, 5, 10, 20, 30, 40, 50, 100, 200, and 500 mg/L of tannic acid.

Flavan Determination: Vanillin-HCl assay

Total flavanols were determined using the vanillin-HCl method according to Butler (1982) with slight modifications. Fifteen microliters of sample extract was taken to dryness at 22°C by a roto-vap (Buchi rotovapor R-200, Switzerland) and redissolved in 2.5 mL of a solution containing 4% HCl and 0.5% vanillin in glacial acetic acid, prepared immediately before use. The absorbance was measured with a Genesys 10-S spectrophotometer at 510 nm after 5 min of incubation, covered and in the dark, at ambient temperature. A standard curve was established with 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 µM/L procyanidin B2. Total flavanol results were expressed as mg Procyanidin B2/g dry weight grape seed flour.

Antioxidant activity: DPPH

The antioxidant activity of the extracts of both GSF and bread was measured using a relative DPPH scavenging capacity (RDSC) method described by Cheng and others (2006) using the free radical 2,2 diphenyl-1-picrylhydrazl (DPPH). A microplate scanning spectrophotometer PowerWave X-I with KC4 v.3.0 PowerReports^{tm} Software (Bio-Tek Instruments, Inc., Winooski, VT) was used to determine the concentration of DPPH. Two hundred microliters of 70% ethanol solvent was added to blank wells, 100 µL of 70% ethanol to control wells, and 100 µL of either 70% ethanolic extract of GSF or bread, or 100 µL of trolox solutions in 70% ethanol were added to appropriate wells in a 96-well clear, flat-bottom, microplate (BD Falcon 353915, Fisher Scientific, Pittsburgh, PA) with an eight-channel pipetter. Prior to analysis the 70% ethanolic extracts were diluted 50-fold with 70% ethanol. One hundred microliters of 0.208 mM DPPH was added to control, standard, and sample wells and the microplates were covered with microplate sealing tape (Nunc®, Sigman-Aldrich, Milwaukee, WI) (Cheng and others 2006). The plate was shaken on low (level 1) for 5 seconds before each reading. The decrease in

absorbance was determined at 515 nm every min for 2 h. The DPPH (quenched) was calculated as follows:

$$\%DPPH_{(quenched)} = [1-((A_{sample} - A_{blank})/(A_{control} - A_{blank}))] \times 100$$

where A represents the absorbance at 515 nm.

To determine antioxidant activity, the area under the curve (AUC) of %DPPH versus antioxidant-DPPH reaction time for each sample was calculated. The AUC was utilized because it considers both the kinetic and thermodynamic measurements of the radical-antioxidant reactions (Huang and others 2005). An example of the DPPH AUC is expressed mathematically as follows:

$$A = \int b \rightarrow 0 \; f(x)\, dx$$

where A represents area and *x* represents time in min.

The integral for the GSF, bread, and trolox standards at concentrations of 50 - 1000 µM, was expressed numerically using the trapezoidal rule for calculation (Yu 2008):

$$AUC = 0.5\, X_0 + (X_1 + X_2 + X_3 + X_4 + X_5 + \ldots\ldots\ldots\ldots + X_{y-1}) + 0.5_{Xy}$$

where:

X_0: %DPPH quenched at time 0.

X_1, X_2, X_3, etc.: % DPPH quenched at each minute to steady state

X_y: %DPPH quenched when the steady time was reached.

The standard curve was generated by plotting AUC values for trolox at different concentrations. Antioxidant activity was calculated using the standard curve prepared and expressed as µmol Trolox Equivalent (TE) per g sample dry matter using the following equation:

$$\mu moles\ TE/g = (\mu moles/L) \times DF \times (L_{solvent}/g_{sample})$$

Where:

DF: Dilution factor for sample extract

$L_{solvent}$: Volume of solvent used for extraction of the sample

g_{sample}: Amount of sample used for extraction

Antioxidant activity: TEAC

This assay is based on the decolorization of the radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) $ABTS^{\circ+}$ after reduction to ABTS. Spectrophotometric methods were performed as described by Schilling and others (2007). A phosphate buffer was prepared by mixing 818 mL of a Na_2HPO_4 solution (66 mmol/L) with 182 mL of a KH_2PO_4 solution (66 mmol/L) and 150 mmol sodium chloride. For the daily preparation of the radical solution, 0.5 mL of an ABTS solution (20 mmol/L) in the phosphate buffer was mixed with 100 mL of an ABAP solution (2.5 mmol/L) in the phosphate buffer and heated for 15 min in a 60°C water bath. The reaction was initiated by adding 1.96 mL of the $ABTS^{\circ+}$ solution to 40 µL of the sample or trolox standard solutions or 40 µL of 70% ethanol as a control (van den Berg and others 1999; van den Berg and others 2000). The mixture was allowed to stand for 6 min at ambient temperature before the absorption was measured at 734 nm (Genesys 10-S, Thermo Electron Corp., Waltham, MA, USA). Seventy percent ethanolic solutions of trolox in a range of 50-1000 µmol/L were used for calibration of the TEAC and FRAP assays. TEAC radical scavenging results were expressed as µmol Trolox equivalents/ g dry weight sample.

Antioxidant activity: FRAP

This method is based on the increase in absorption at 593 nm due to the formation of tri-pyridyl-*S*-triazine complexes with Fe^{2+} [TPTZ-Fe(II)] in the presence of a reductive agent (Benzie and Strain 1996; Benzie and Szeto 1999). The FRAP reagent was prepared from 2.5 mL

of a TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and 2.5 mL of a $FeCl_3$ solution (20 mmol/L) mixed with 25 mL of an acetate buffer (0.3 mol/L, pH 3.6). For the determination of the antioxidant capacity, the FRAP reagent (1.5 mL) was mixed with 100 µL of water and 100 µL of the sample, standard, or control. The mixture was allowed to stand for 4 min at ambient temperature before the absorption at 593 nm was measured. The calibration curve was performed with trolox as described above.

Statistical analysis

All baking tests and chemical determinations were performed in triplicate, with the results reported as the means of those determinations with the standard deviations. The total phenolic, antioxidant and total flavanol results, along with the instrumental analysis data for both the dough and the bread were analyzed for significant differences using two-way analysis of variances (ANOVA) (PROC GLM using LSMEANS) and mean separations via Tukey's HSD multiple comparisons (SAS Inst., Inc., version 9.1; Cary, NC) at the $p \leq 0.05$ confidence level. The independent variables evaluated during heating of grape seed flour experiments included, heating temperature, length of heating time, and harvest year while the dependent variable was the resulting phenolic content or antioxidant activity. The independent variables for the bread baking treatments were frozen storage length, GSF replacement level, and the type of sample (bread or dough) while the dependent variable was the resulting phenolic content or antioxidant activity. Evaluation of relationships between variables was carried out by computing the relevant linear regression coefficient (r^2).

For the sensory evaluation results, consumer panel evaluations were analyzed for significant differences by two-factor (panelist and GSF-replacement) ANOVA and Tukey's HSD multiple comparisons using XLSTAT (version 2009.2; Addinsoft, Paris, France). The ANOVA

performed used a mixed effects model, with panelists held as a random effect and the bread as a fixed effect. Principal components analysis (PCA) with no rotation was also performed using XLSTAT.

RESULTS AND DISCUSSION

Grape Seed Flour

Various solvents such as 70% methanol/water (Yilmaz and Toledo 2004), ethyl acetate (Guendez and others 2005), 70% acetone/water (Baker and others 1995), 70% ethanol/water (Kim and others 2006), methanol, ethanol, isopropanol, and 50% ethanol/water (Lafka and others 2007) have been used in the extraction of phenolic compounds from grape seeds. Lafka and others (2007) investigated the effect of different solvents on the extraction of phenolic compounds from grape seeds and the subsequent effect on antioxidant activity as measured by Folin-Ciocalteu (TPC) and DPPH assays. These researchers reported that a mixture of 50% ethanol/water resulted in the maximum phenol extraction compared to methanol, ethanol, acetone, ethyl acetate, and isopropanol; however, the corresponding antioxidant activity was not as high as extracts from the same solvents. In response, Lafka and others (2007) proposed that the optimal solvent for polyphenolic extraction and the subsequent antioxidant activity was a 70% ethanol/water mixture. Considering these studies, it was determined that a 70% ethanol/water mixture would be used for the extraction of the polyphenolic compounds from the GSF.

Effects of heating conditions on the antioxidant activities of grape seed press residue extracts

Total Phenolic Content (TPC; Folin-Ciocalteu assay)

The TPC of 150 µm Merlot seed flour extracts from two separate harvest years (2007 and 2008) are presented in Table 7. TPC in 2007 Merlot seed flour was not significantly impacted by

heating temperatures at 120 or 150°C, except at 120°C for 20 min; however, heating at or above 180°C caused significant decreases in TPC in both 2007 and 2008 harvest years ($P<0.05$). TPC in 2007 Merlot seed flour increased, albeit not significantly, from 79.90 mg TAE / g DW GSF in the unheated control to 86.70 mg tannic acid equivalents (TAE) / g DW GSF after 10 min at 120°C, and to 84.04 mg TAE / g DW GSF after 30 min of heating at 150°C. TPC in 2008 Merlot seed flour significantly increased with heat treatment, from 134.20 mg TAE / g DW GSF in the control to 141.40 mg TAE / g DW GSF after 10 min at 150°C ($P<0.05$).

Total phenolics in grape seeds are composed of a complex group of substances including, phenolic acids, flavanols, flavan-3-ols, and flavanonols, these compounds are closely associated with the color, taste, and nutritional quality of plant foods, their antioxidant activity occupying their most important biological property (Macheix and others 1990). Therefore, it would be ideal to avoid the change in total phenolics that occurs at high heating temperatures. Three possible mechanisms have been proposed to explain the changes of phenolic content of samples exposed to high temperature (Maillard and Berset 1995). These three mechanisms include: 1) the release of bound phenolic compounds, 2) partial degradation of lignin leading to the release of phenolic acid derivatives, and 3) the beginning of thermal degradation of the phenolic compounds. In the present study, the observation of significant reduction in TPC activity at heating at or above 180°C suggests that thermal degradation is the main mechanism involved. The increase in TPC when the 2008 Merlot seed flour was heated at 150°C for 10 min may be explained by the liberation of phenolic compounds. Recent studies on rice hulls have shown that simple heat treatment (100°C for 30 min), similar to the heat treatments used in the present study, can increase the TPC by converting insoluble phenolic compounds to soluble phenolics or phenolic derivatives (Jeong and others 2004).

A previous study also showed that in red grape pomace peels (skins), drying via an air circulating oven at 60°C did not have a detrimental effect on the phenolic concentration or their antioxidant activity (Larrauri and others 1997). At 100 and 140°C, a significant reduction in the extractability of total phenolics and proanthocyanidins was noted. These researchers also noted that the loss of condensed tannins was significantly lower than the loss in total extractable phenolics. They attributed this difference to the higher molecular weight of the condensed tannins which are commonly bound to either fiber or protein. This more complex chemical structure may have explained the condensed tannins resistance to thermal degradation.

In the present study, a significant reduction in TPC was not realized until temperatures were at least 80°C higher than those reported by Larrauri and others (1997) at 180°C. This finding agrees with the results from Kim and others (2006) who noted that a significant reduction in the total phenolics of Campbell early grape seeds occurred only at or above 200°C. The increased temperature required to cause a significant reduction in total phenolics of grape seeds could be due to the greater content of condensed tannins in the seeds compared to the skins included in red grape pomace. The higher content of condensed tannins could add to the resistance against thermal degradation.

Differences were also noticed between the TPC for GSF from the 2007 and 2008 harvest years. The TPC for the 2007 unheated control GSF was 79.9 mg tannic acid/g DW GSF compared to 134.2 mg tannic acid/g DW in the 2008 unheated GSF, a 68% increase. Despite the differences in initial TPC values, the trends in TPC values across heating times and temperatures between the two years are very similar. The differences in initial concentrations between the two years could be explained by differences in viticultural and enological practices prior to our receipt of the GSF. One of the most important determinants of the phenolic content of grape

seeds is the climatic conditions of that year. Total phenolic content of grapes is heavily dependent upon the extent to which berry temperature is elevated as a result of sunlight exposure (Bergqvist and others 2001). Winemaking practices could also result in differences between TPCs of GSF. Since the GSF used in this study was Merlot flour, the seeds were exposed to a maceration period. The length of the maceration period varies from winery to winery, causing variable amounts of phenolic extraction from the seeds into the wines. Thus, if climatic conditions changed year over year or enological practices varied from year to year, the contents of phenolics, flavanols, and the resultant antioxidant activities contained within the GSF would also vary. As we were not privy to the history of the grape pomace prior to drying, we can only speculate as to why differences were observed.

Total Flavanol Content (TFC; Vanillin-HCl)

Flavanol content estimated by Vanillin-HCl assay showed a similar trend as the TPC in GSF extract. TFC in the 2007 GSF showed no significant change when heated at 120°C for up to 90 min (Table 8a). However, significant decreases were noticed when GSF was heated at 150°C for more than 10 min, or at or above 180°C for any length of time. In 2008, significant TFC increases versus the unheated control GSF were observed when heated at 120°C for 10, 20, 40, 50, or 60 min (Table 8b). The highest TFC was 115.67 mg Procyanidin B2/g DW at a heating temperature of 120°C for 20 min compared to the unheated control at 95.23 mg Procyanidin B2/g DW, a 21.5% increase. However, significant decreases in 2008 GSF TFC were seen when heated at 150°C for any length of time. The significant decreases in TFC when GSF was heated at 150°C suggests that the flavanols measured by Vanillin-HCl were more susceptible to thermal degradation than the TPC measured by Folin-Ciocalteu. A recent study on the effects of heat processing on green tea agrees with this observation (Wang and others 2000). The researchers

found that after roasting tea leaves at 160°C for 10 min, the TPC measured by Folin-Ciocalteu was reduced by 11.46% while the total flavanol content measured by HPLC was reduced by 57.5%. This could be explained by the fact that the Folin-Ciocalteu method lacks specificity as it is based on the reducing power of phenolic hydroxyl groups, and detects all phenols and breakdown products with varying sensitivity. On the other hand, the Vanillin assay is specific for a narrow range of flavanols (monomers and polymers) and dihydroxychalcones that have a single bond at the 2,3-position and free meta-hydroxy groups on the flavanol B-ring (Sarkar and Howarth 1976). Thus, the specificity of the Vanillin assay excludes the flavanol breakdown products, while the Folin-Ciocalteu reagent may react with these products.

Antioxidant activity of 70% ethanolic extracts from grape seed flour originating from grape seed oil production: DPPH, TEAC, and FRAP.

DPPH assay

The stable free radical DPPH has been used extensively for the determination of antioxidant activity, specifically for the free radical scavenging activities of pure antioxidant compounds that have been extracted from fruits, plants, or food materials. During the assay, the color of the DPPH solution changes from purple to yellow, due to the formation of diphenylpicryl-hydrazyl, with a reduction reaction produced by either a hydrogen radical or electron donation process (Ozcelik and others 2003).

The DPPH free radical scavenging activity of 2007 and 2008 GSF extracts are shown in Tables 9. The 2007 GSF DPPH radical scavenging activity significantly increased from 256.45 µmol Trolox/g DW to 280.59 µmol Trolox/g DW after treating at 120°C for 40 min, a 9.4% increase. However, heating of the 2007 GSF at or above 180°C caused significant decreases compared to the control. The 2008 GSF DPPH radical scavenging activity was unaffected when heated at or below 150°C, however; heating at or above 180°C caused significant decreases in

radical scavenging activity. The length of time exposed to the heating treatment also caused significant effects in the DPPH radical scavenging activity of 70% ethanolic grape seed flour extracts. For both harvest years, the general trend showed that as the heating time increased, the antioxidant activity decreased with greater decreases observed at higher heating temperatures. At the lowest heating temperature, 120°C, the DPPH radical scavenging activity increased up to 40 min of heating and then decreased.

The area under the curve (AUC) was used to compare heating time/temperature combinations because this method considers both inhibition time and reaction kinetics. As a result, the data gathered utilizing the AUC method provided more robust results than methods which simply use a fixed time or inhibition degree (Huang and others 2002). The steady state for the DPPH reactions was reached by 43 min. This time was used to compare the samples of interest using the AUC with the AUC of known amounts of μmol Trolox in a cycle of 43 min.

Yemis and others (2008) found utilizing conventional colorimetric analysis of DPPH that Muscat seed extract contained 4.63 mmol Trolox/mg extract. This amount was substantially higher than the DPPH radical scavenging activity found in this study for either 2007 or 2008 Merlot seed flour. The difference could be due to the fact that the Merlot seed flour used here is an oil press residue while the Muscat seed used by Yemis and others (2008) was fresh and had not undergone wine processing or oil pressing. The composition of the phenolic compounds contained in Samtrot grape seed extract compared to grape seed oil press residue greatly varies as well (Maier and others 2009). While the Samtrot grape seeds were characterized by an evenly distributed proportion of procyanidin B1 (14%), catechin (31.5%), procyanidin B2 (18.5), epicatechin (22.4%), and epicatechin gallate (13.4%), the seed oil press residue was dominated by catechin (47%) and showed a very low content of epicatechin gallate (1.9%). The grape seed

oil press residue also showed a significantly lower radical scavenging activity compared to unprocessed grape seeds (Maier and others 2009). The reduced antioxidant activity in the press residue led Maier and others (2009) to conclude that a balanced mixture of various phenolic compounds resulted in an enhanced antioxidant activity of 'Samtrot' grape seeds.

TEAC assay

The results of the TEAC assay for the 2007 and 2008 harvest years are shown in Table 10. The activity of the GSF extracts were expressed as µmol Trolox/g DW GSF. High TEAC values indicate that the mechanism of antioxidant action of the extracts was hydrogen donation which could terminate the oxidation process by converting once free radicals to stable forms (Huang and others 2005). The 2007 GSF TEAC activity significantly increased from 1108.0 µmol Trolox/g DW GSF in the unheated control to 1508.0 µmol Trolox/g DW GSF when heated at 150°C for 10 min; however, the TEAC activity significantly decreased compared to the control at all other time-temperature combinations. The 2008 GSF TEAC activities also significantly decreased compared to the control at all time temperature combinations.

Influence of the solvent in which the reaction takes place is an important factor that could affect the mechanism of the overall reaction (Perez-Jimenez and others 2008). Therefore, in order to compare results obtained from this study with others, special attention had to be paid to the solvents and methodology utilized. In a recent study, comparing the effectiveness of different solvents on the extraction of Cabernet Sauvignon grape seed powder, Li and others (2008) found that in 70% ethanol extracts of the powder, a TEAC value of 673.5 µmol Trolox/g DW GSF resulted. These results are similar to the results in the present study for the 2007 (1108 µmol Trolox/g DW GSF) and 2008 (1873 µmol Trolox/g DW GSF) unheated controls.

FRAP Assay

The ability of GSF extracts to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain (1996). An antioxidant which is capable of donating a single electron to the ferric-TPTZ (Fe(III)-TPTZ) complex reduces this complex into a blue ferrous-TPTZ (Fe(II)-TPTZ).

In general, the trend for ferric ion reducing activity of the GSF did not vary markedly from the DPPH or TEAC free radical scavenging activities. The 2007 GSF FRAP reducing activity was significantly increased when heated at 120°C for up to 90 min or at 150°C for 10, 20, or 30 min (Table 11a). The highest 2007 FRAP reducing activity observed was 265.33 µmol Trolox/g DW GSF at 120°C for 10 min compared to the control which was 192.32 µmol Trolox/g DW GSF, a 38% increase. Heating at or above 180°C caused significant reductions in the FRAP reducing activity, following the same trend as the TPC, DPPH, and TEAC assays.

The 2008 GSF FRAP reducing activity was significantly increased compared to the unheated flour when heated at 120°C for 60 min and 150°C for 10 or 20 min (Table 11b). The 2008 GSF FRAP reducing activity followed the trend which was observed using the TPC, TEAC, and DPPH assays in that at 180°C or above, a significant reduction in reducing activity occurred.

Guo and others (2003) found grape seed extracts had the highest FRAP reducing activity compared to the pulp, skin, and seeds of 27 other antioxidant rich fruits. The FRAP reducing activity in their grape seed extracts was 555.4 µmol Trolox/g DW (Guo and others 2003). In the present study, the unheated GSF extracts were measured at either 192.32 µmol Trolox/g DW (2007) or 373.42 µmol Trolox/g DW (2008). These values were lower than those reported by Guo and others (2003) but the grape seeds used in that study were unprocessed. Even though the

Merlot GSF used in this study has gone through both wine processing and oil pressing, it still retained substantial FRAP reducing activity. In fact, of the 27 antioxidant rich fruits studied by Guo and others (2003), only white pomegranate skin had a higher FRAP reducing activity than the 2008 Merlot GSF.

Effect of flour particle size and grape seed variety on TPC and Antioxidant Activity

Gallic acid, catechin, and epicatechin have previously been reported to be in high concentrations in grape seeds (Santos-Buelga and Scalbert 2000). In the present study, differences were found between the concentrations of total phenolics in GSF produced from Cabernet Sauvignon and Chardonnay grapes. Cabernet Sauvignon GSF had a significantly lower TPC compared to Chardonnay GSF at the 425 and 250 μm particle size (Table 12a). However, at the 180 μm particle size, the TPC of Cabernet Sauvignon and Chardonnay GSF were not significantly different.

Particle size and wine grape variety had a significant effect on the FRAP reducing capacity of the corresponding seed flours (Table 12b). For the Cabernet Sauvignon grape seed variety, as the particle size decreased, the FRAP reducing capacity increased from 208.9 (425μm) to 609.8 (180μm), a 192% increase. The highest FRAP reducing capacity in the Chardonnay flour was the 250 μm which was significantly higher than either the 425 μm or 180 μm flours. The FRAP reducing capacity of Chardonnay flour was significantly higher than the Cabernet Sauvignon flour at particle sizes of 425 and 250 μm; however, at 180 μm the Cabernet Sauvignon flour was significantly higher.

Particle size and variety both exhibited significant effects on TEAC radical scavenging activity (Table 12c). The TEAC activity of Chardonnay GSF was consistently higher than the TEAC activity of Cabernet Sauvignon GSF. TEAC scavenging activity for Cabernet Sauvignon

GSF followed a general trend of increasing as the particle size decreased. However, the TEAC activity of Chardonnay flour was significantly higher in the larger particle sizes. This indicates that the phenolic compounds in Chardonnay GSF that exhibit activity against the ABTS radical are associated with larger flour particles.

The DPPH radical scavenging activity of Cabernet Sauvignon and Chardonnay GSF was significantly impacted by particle size and variety (Table 12d). Significant differences between the 425 μm and 250 μm flours were found in both the Cabernet Sauvignon and Chardonnay GSF. However, further particle size reduction from 250 to 180 μm did not significantly affect the DPPH radical scavenging activity of the flours. At the 425 μm particle size, the Chardonnay GSF showed a higher DPPH radical scavenging activity compared to the Cabernet Sauvignon ($P<0.05$).

A reduction in TPC and antioxidant activity in Cabernet Sauvignon GSF was expected for all particle sizes compared to Chardonnay seeds as during winemaking, more flavonol leaches out of the Cabernet Sauvignon seeds due to maceration. However, Yilmaz and Toledo (2004) found that the flavanol content of Chardonnay and Merlot seeds were similar despite the Merlot flavonoids having time to leach out in the wine. This result agrees with the results found for the 180 μm GSF.

Phenolic extraction efficiency is highly dependent on sample preparation including, drying, milling, and particle size reduction of flour carried out prior to the extraction (Nagy and Simandi 2008). Results for Cabernet Sauvignon showed that as the flour particle size decreased from 450 to 180μm, the TPC or antioxidant activity increased. Chardonnay flour TPC and antioxidant activity was highest at the 250μm particle size. The Cabernet Sauvignon TPC increased 214% as the particle size decreased from 450 to 180μm. The Chardonnay TPC

increased 60% as the particle size decreased from 450 to 250 μm, but decreased 20% as the particle size decreased from 250 to 180μm. Typically, higher extraction efficiencies can be achieved when utilizing smaller particle sizes because of an increase in the mass transfer surface, allowing more total area to be exposed to the extracting solvent (Nagy and Simandi 2008). While studying the supercritical CO_2 extraction of paprika, Nagy and Simandi (2008), discovered that at a 1.5 mm particle size, low extraction efficiency was observed, (~45%), but when the particles were smaller (~0.2mm), the efficiency was significantly higher (~90%).

Correlation of antioxidant activity, TFC, and TPC values

Phenolic compounds have been reported to act as antioxidants not only because they are hydrogen or electron donors but because they stabilize radical intermediates, thus preventing oxidation of various food ingredients (Ricardo-Da-Silva and others 1991). To evaluate antioxidant properties of GSF extracts based on their phenolic contents, coefficients of linear correlations between TPC (Folin-Ciocalteu), total flavanol contents (Vanillin-HCl), TEAC, FRAP, and DPPH were determined in 2007 (Table 13a) and 2008 (Table 13b) Merlot GSF.

The DPPH and FRAP assays were highly correlated with results obtained by the Folin-Ciocalteu method ($r^2 = 0.922$ and $r^2 = 0.919$, respectively). The lowest correlation was found between the TEAC assay and Folin-Ciocalteu method ($r^2 = 0.741$). The 2008 harvest year results obtained by the FRAP assay were strongly correlated between the results obtained with the Folin-Ciocalteu method ($r^2 = 0.966$). The lowest correlation was found between the 2008 TPC and DPPH assays ($r^2 = 0.811$). The strong correlations found between DPPH and FRAP (2007) and FRAP and TPC (2008) suggest a relationship between phenolic compound concentration in the Merlot GSF and their free radical scavenging and ferric reducing capacities. Therefore, the presence of phenolic compounds in Merlot seed extracts contributes significantly to their

antioxidant potential. These results are in agreement with earlier research which reports that ferric reducing potential can be related to phenolic content (Katalinic and others 2006).

In addition to being correlated with total phenolics, the antioxidant assays were also correlated to total flavanol content (TFC) determined by the Vanillin-HCl method. In 2008 flour, the correlation between TFC and FRAP was $r^2 = 0.901$. TFC and TPC were strongly correlated in both 2007 ($r^2 = 0.869$) and 2008 ($r^2 = 0.936$). These results indicate a relationship between the mean values for the total content of flavanols and phenolics in Merlot GSF.

The strong correlation between the DPPH and FRAP assays of GSF extracts deserves further discussion. Antioxidants are reducing agents and thus are capable of donating a single electron or hydrogen atom for reduction (Huang and others 2005). In this study, the strong correlation between DPPH and FRAP indicated that compounds present in the GSF extracts are capable of reducing DPPH radicals and ferric ions. In Greek wines, Arnous and others (2000) published strong correlations between DPPH radical scavenging ability and ferric ion reducing ability. In general, the ferric ion reducing ability of antioxidants correlated with the results from other methods which were used to estimate antioxidant capacity (Pulido and others 2000). In the present study, the strong correlations between FRAP and DPPH, TPC, and TFC suggest that the ferric ion reducing ability of the polyphenols is a critically important factor in defining the free radical scavenging capacity of these compounds.

Bread Containing Grape Seed Flour

Dough and Bread properties

The moisture, ash, and protein content of both the hard red spring wheat flour and 150 µm Merlot seed flour used in dough preparation are shown in Table 14. The protein and ash contents of the wheat flour alone were 13.02% and 3.97% respectively, while the protein and ash

contents of the GSF were 17.38% and 4.85% respectively. These findings suggest that the replacement of wheat flour with GSF in bread at 2.5, 5, 7.5 or 10g/100g flour increases the ash content and protein content of the flour used in the bread making. In breads, an increase in wheat protein content is typically associated with an increase in the percentage of large glutenin polymers and loaf volume (Aamodt and others 2005). However, the increase in protein content associated with the inclusion of GSF in the bread decreases the gluten content. Flours with a decreased gluten content produce loaves with smaller loaf volume and smaller slice area (Aamodt and others 2005).

The temperature changes in the bread crumb and bread crust are shown in Figure 8. The temperatures within the bread crumb did not exceed 101.3°C, and reached a steady temperature of ~100°C at approximately 9 min, remaining at that temperature for the remainder of baking (12 min). The crust temperatures, taken just below the crust, did not exceed 102.5°C. The crust reached a steady temperature of ~100°C at approximately 4 min, and remained at that temperature for the remainder of baking (17 min). In an industrialized process the bread is passed through four baking zones 115, 130, 156, and 176°C for a total time baking time of 27.4 min (Therdthai and others 2002). Therdthai and others (2002) found that the crumb temperature did not exceed 99°C, similar to the internal temperature reached in this experiment.

Total Phenolic Content (TPC) of Bread with GSF replacement.

The TPC in both frozen and unfrozen bread and dough made with GSF significantly increased with increasing concentrations of GSF (Table 15). For example, compared to the wheat flour control frozen dough, the TPC of unfrozen dough increased 241%, 651%, 1113%, or 1779% at 2.5, 5, 7.5, or 10 g GSF/100g flour, respectively. In the unfrozen bread, the TPC increased 1614%, 3019%, 4603%, or 6545% compared to the wheat flour control bread at 2.5, 5,

7.5, or 10 g GSF/100g flour, respectively. Frozen storage of dough containing GSF caused significant reductions of up to 14% in TPC at 2 weeks storage or longer. However, the control dough did not see a significant reduction in TPC after 6 weeks of frozen storage (Table 15).

The TPC of bread crumb containing GSF is consistently higher than doughs across all replacement and frozen storage variants. However, a higher TPC in the control dough versus the bread illustrates a reversal of this trend (Table 15). As was previously mentioned, the phenolic content of GSF was not significantly reduced until it was exposed to temperatures at or exceeding 180°C and as the crumb temperature did not exceed 101°C, no reduction in phenolic content was expected. Lee and others (2003) explained that upon heating insoluble phenolic compounds can be converted to soluble phenolic compounds, and in the present study, phenolic compounds once bound to the gluten matrix could be solubilized. It is likely that the combination of the thermal stability and the solubilization of phenolic compounds contributed to the increase in the TPC of the bread.

The recovery of TPC in unfrozen dough and bread compared to the TPC in the control bread (0 % GSF replacement) revealed a significantly reduced TPC recovery rate at 24% ± 3.3% for the dough and 31% ± 1.4% for the bread across all GSF replacement levels (Figure 9). This reduction in recovery was attributed to interactions between the GSF and wheat proteins during dough preparation, in which the gluten network was being developed via a cross-linking structure by disulfide bonds through the interchange reaction of the thiol group (Wang and Zhou 2004). In this process, the free radical GS● (thiyl) is initiated by the broken disulfide bonds during dough mixing and these free radicals are quickly quenched by the addition of the antioxidants BHA and BHT (Dong and Hoseney 1995). Since GSF phenolic compounds are potent free radical scavengers, they might show activity that is similar to BHA or BHT within

the dough matrix. However, the catechins in GSF may also initiate their own corresponding semiquinone free radicals at neutral pH, as demonstrated in green tea catechins (Zhu and others 1997). Thus, the combined effect of GSF being both a potent free radical scavenger and a possible initiator shows that the GSF phenolic compounds could interact with GS● and be involved in the thiol interchange reaction. The involvement of GSF in this reaction may cause binding by the GSF phenolic compounds thus reducing their extractability and affecting dough rheological properties. The lower extraction rate found in the dough compared to the finished bread can be explained by the conversion of insoluble phenolic compounds to soluble phenolic compounds through the simple heating imposed on the system during the baking process, as was shown by Lee and others (2003).

Total Flavanol Content of Bread with GSF Replacement

Total flavanol content (TFC) determined by the Vanillin-HCl assay showed a similar trend as TPC. Significant increases in TFC were observed with increasing GSF replacement levels (Table 16). For example, compared to the control, the TFC increased 0%, 100%, 150%, or 337% in unfrozen dough, while TFC in unfrozen bread increased 44%, 189%, 245%, or 401% at 2.5, 5, 7.5, or 10 g GSF/100g flour, respectively. However, unlike TPC, the TFC was not significantly increased in the bread compared to the dough, except in the unfrozen dough with GSF replacement at or greater than 5 g/100g flour. Also unlike TPC, the TFC was not consistently significantly affected by frozen storage at six weeks (Table 16). The TPC recovery rates are similar to the reduced recovery rates seen for TFC in Figure 10. Proanthocyanidins, the main antioxidant contained in grape seeds (Shahidi and Naczk 2004), are condensed tannins which are known to interact with proteins (Poncet-Legrand and others 2007). Thus, the reduced TFC recovery rate in both the bread and dough can be explained by proanthocyanidin binding

with proteins in the bread, particularly gluten, forming insoluble complexes likely through similar mechanisms as mentioned under the TPC section. This indicates that the flavanols measured by TFC may form more stable complexes with the bread protein, which in turn are not solubilized through heating below 101°C. Poncet-Legrand and others (2007) found that grape seed flavanols bind to proteins which are rich in proline via hydrophobic interactions and hydrogen bonding, with hydrogen bonding being the main mechanism. The strength and number of hydrogen bonds depends on the degree of polymerization and galloylation of the grape proanthocyanidins. Increasing either of these parameters greatly increases the interactions between the proanthocyanidins and the proteins.

Antioxidant Activity of GSF Containing Bread Crumb and Dough: TEAC, FRAP, and DPPH.

DPPH Radical Scavenging Assay

Heat treatment is among the most popular forms of food processing (Randhir and others 2008). During heating, an array of complex chemical reactions takes place and determines the sensory characteristics, nutritional value, and safety of the final processed products. While some compounds are destroyed during thermal processing, new compounds can be generated. Some of these compounds contribute to the color and flavor of foods, but some compounds with various biological activities can also form as a result of heat-induced reaction. Some of these compounds formed through heating contain substantial antioxidative and/or chemopreventative properties.

Frozen dough has been increasingly exploited by the baking industry due to preservation problems associated with fresh breads and a growing bread market demand. The quality of bread made from frozen dough is influenced by the original formulation as well as the mixing time, freezing rate, storage duration, and thawing rate (Inoue and Bushuk 1991; Inoue and Bushuk 1992). These factors can act independently or together to reduce the quality of the bread by

reducing loaf volume through reduced yeast activity or damaged gluten matrices (Lucas and others 2005). Despite the reduction in quality caused by frozen storage, there is a growing market demand within the bread industry for frozen bread dough (Selomulyo and Zhou 2007).

In the present study, the antioxidant activity of both defatted bread and dough extract significantly increased with the replacement of 2.5, 5, 7.5, or 10 g GSF/100g wheat flour (Table 17). From Table 17, a replacement of Merlot GSF up to 2.5 g/100g resulted in a significantly higher DPPH radical scavenging activity of the bread and dough. However, as the replacement rate of GSF increased from 2.5 to 10 g/100g flour in the bread and dough, the radical scavenging activity consistently decreased by 14.8%. Also, when the dough and bread extracts were compared to the unheated GSF extract (2008) and calculated for equivalent concentrations, the DPPH radical scavenging activity of the unfrozen dough and bread was reduced by 62% and 57%, respectively. These data suggest that thermal degradation is not the main cause of radical scavenging loss. This phenomenon could be brought about during the mixing of the dough through the reactions of GSF phenolics (particularly proanthocyanidins) with gluten proteins or bread starches to yield large complexes that were not well extracted by the solvents utilized in this experiment. GSF extracts have a higher proanthocyanidin concentration compared to wheat flour and proanthocyanidins have been reported to complex with carbohydrate and protein fractions (McCallum and Walker 1990), making them less extractable. GSF included within a bread system can also be modified by active oxidative enzymes such as polyphenol oxidase in bread (Quinde and others 2006) or oxidized by available O_2. Also, during carmelization and breakdown of sugars in wheat during baking, the furfural derivatives formed may go through condensation with proanthocyanidins (McCallum and Walker 1990). These alternatives to

thermal degradation may explain the decrease in TPC and DPPH radical scavenging activity observed in both the bread and the dough after mixing and baking.

The DPPH radical scavenging activity of bread containing GSF is consistently higher than the dough at replacement levels of 2.5 or 5 g/100g flour, but no significant differences were observed at replacement levels of 7.5 or 10 g/100g flour. However, the dough containing 0 g GSF/100g flour shows a reversal of this trend with a significantly higher radical scavenging activity in the dough versus the bread (Table 17). These data suggest that the phenolics extracted from the wheat flour (0 g GSF/100g flour) are more susceptible to thermal degradation than the GSF phenolics, and thus show a significantly reduced DPPH radical scavenging activity. During frozen storage, the GSF containing bread and dough did not show significant changes in the DPPH radical scavenging activity. These data suggest that the phenolics lost due to frozen storage were likely not the phenolics which were active against the DPPH free radical.

TEAC assay

The TEAC assay was performed to evaluate the ability of the test samples to quench $ABTS^{+}$ cations. Higher antioxidant capacity is usually related to a decrease in absorbance (van den Berg and others 1999). The TEAC results followed a similar trend as the DPPH assay (Table 18). A higher replacement of Merlot GSF resulted in a significantly higher TEAC scavenging activity of the corresponding bread and dough. However, unlike the DPPH assay, as the replacement rate of GSF increased from 2.5 to 10 g/100g flour in the bread and dough, the scavenging activity only increased approximately 20%. When the dough and bread extracts were compared with the 2008 unheated GSF extract and calculated for equivalent concentrations, the TEAC scavenging activity of the unfrozen dough and bread was reduced by ~75%. This reduction in scavenging activity was higher than observed in the DPPH assay and indicated that

the phenolics which interacted with the proteins and starches in the bread and dough matrix were more reactive with the ABTS radical than with the DPPH radical.

The TEAC scavenging activity of dough containing GSF was higher than the bread at replacement levels of 2.5 or 5 g/100g flour when stored at -20°C for 6 weeks. At replacement levels of 7.5 or 10 g/100g flour, the scavenging activity of the bread either did not significantly change or significantly increased compared to the dough. However, in dough containing 0 g GSF/100g flour, a significantly higher ABTS scavenging activity was observed in the dough compared to the bread. These data suggest that the phenolics extracted from the wheat flour (0 g GSF/100g flour) are more susceptible to thermal degradation than the GSF phenolics, thus showing a significantly reduced TEAC scavenging activity.

Frozen dough storage showed significant changes in the TEAC scavenging activity of both GSF containing breads and dough. The TPC of GSF-containing breads and doughs had a 14% decrease in activity after two weeks of frozen storage. However, the TEAC data for GSF containing doughs showed an increase in activity when stored at -20°C for two weeks or longer. The TEAC data for 2.5 and 5 g GSF/100g flour containing bread significantly increased when the dough was stored frozen for 6 weeks. However, at higher replacement levels, the increased frozen storage time caused significant decreases in TEAC activity. These data show a synergistic effect between frozen storage and the TEAC radical scavenging activity at GSF replacement levels of ≥7.5 g/100g flour. For instance, the replacement of 7.5 g GSF/100g flour in breads when stored for six weeks versus two weeks decreased by 9.6%, while at 10 g GSF/100g flour the TEAC scavenging decreased by 16.4%. Thus, it would appear that the longer the frozen storage time and the higher the GSF replacement the larger the decrease in bread TEAC activity.

The bread dough was stored in polyethylene bags at freezing temperatures. Polyethylene allows some oxygen transmission with longer frozen storage time allowing greater exposure to oxygen. The larger reduction in TEAC activity based on frozen storage time might be due to an exposure to oxygen over a longer period of time (Murcia and others 2009). The phenolics contained in GSF are potent oxygen scavengers and readily quench oxygen, thus resulting in a reduction in the antioxidant potential measured by the TEAC assay.

FRAP Assay

The results of ferric reducing capacities of GSF containing bread and dough extracts are presented in Table 19. The trend for the ferric ion reducing activities of the extracts was similar as observed with DPPH and TEAC assays. Similar to the DPPH radical scavenging assay, as the replacement rate of GSF increased from 2.5 to 10 g/100g flour in both the bread and dough, the ferric ion reducing capacity consistently increased by 11%. Also, when the dough and bread extracts were compared with the 2008 unheated GSF extract and calculated for equivalent concentrations, the ferric iron reducing activity of the unfrozen dough and bread was reduced by 80% and 69%, respectively.

In contrast to the DPPH and TEAC radical scavenging activities, the FRAP reducing capacity showed no significant difference between the control bread and dough. This indicates that thermal treatment did not significantly reduce the ferric ion reducing potential of wheat phenolics.

Similar to the TEAC assay and in contrast with the DPPH assay, frozen dough storage showed significant changes in the TEAC scavenging activity of GSF containing breads and dough. The ferric ion reducing capacity of the dough and bread was unaffected by frozen storage up to 7.5 g GSF/100g flour. However, at 10g GSF/100g flour, FRAP reducing capacity was

reduced when stored frozen for two weeks or longer ($P<0.05$). Again, a relationship was seen between the replacement of 10 g GSF/100g flour and frozen storage for two weeks or longer. The chemical mechanisms behind this synergism are not yet fully understood and should be the focus of further research.

Correlation of antioxidant activity, TFC, and TPC values for frozen and unfrozen dough and bread extracts.

Correlations between the antioxidant activity of 70% ethanolic extracts of dough and bread from all assays Folin-Ciocalteu, Vanillin-HCl, DPPH, TEAC, and FRAP were all above r^2=0.796 (Table 20a and b). Most assays, including DPPH, TEAC, and FRAP showed a high correlation with TPC. In plums, nectarines and peaches, high correlations (greater than r^2 =0.9) were also reported between antioxidant activities as determined by DPPH or FRAP and TPC (Gil and others 2002). The strong correlations found between bread crumb and dough FRAP with TPC indicates a strong positive relationship between the phenolic compound concentration in the Merlot GSF and their ferric reducing capacities. Therefore, the presence of phenolic compounds in Merlot seed extracts contributes significantly to their antioxidant potential.

The antioxidant activities were also correlated with total flavanol content (TFC) determined by the Vanillin-HCl method. Specifically, FRAP and TEAC assays had correlations of r^2 = 0.975 and 0.912, respectively, to TFC. Moderate correlations were observed between bread dough and crumb TFC and the other antioxidant assays. The TFC of bread dough (r^2 = 0.871) and crumb (r^2 = 0.980) were strongly correlated to TPC. These results suggest that the bread crumb phenolics, which were determined by the Folin-Ciocalteu method, were largely flavanols. In the dough, the slightly weaker linear correlation shows that there may be phenolics others than flavanols present in the system.

The strong correlation between FRAP and TEAC in both the bread dough and crumb deserves further attention. Antioxidants are compounds which are capable of donating a single electron or hydrogen atom for reduction; however, not all reducing agents are antioxidants. In this study, the strong correlation indicates that the compounds present in the 70% ethanolic extracts of GSF containing bread dough and crumb are capable of reducing ABTS radicals along with ferric ions. Thaipong and others (2006) reported that generally, the ferric ion reducing ability of antioxidants correlated with the results from ABTS, DPPH, and ORAC assays. This phenomenon was also found in this study with the FRAP assay being strongly correlated to all other antioxidant and phenolic assays.

GSF-fortified bread is a functional food product with a relatively high antioxidant activity. When the ferric ion reducing potential of GSF-fortified bread at varying concentrations (2.5 to 10 g/100g flour) was compared to antioxidant rich fruits, it was shown that the bread even after thermal processing was equivalent on a per gram basis to the fruits (Figure 11). As illustrated in Figure 11, the ferric ion reducing potential of 5 g/100 g flour GSF-fortified bread (4.93 µmol trolox/g DW) is slightly higher than the reducing potential of Tatli pomegranate (4.69 µmol trolox/g fresh fruit), while the 10 g/100 g flour GSF fortified bread was only slightly lower than the antioxidant rich Bluecrop blueberry. Blueberries are a rich source of phytochemicals and have been heralded for their potential health benefits (Russell and others 2007).

Effect of GSF replacement on loaf volume

Unfrozen Dough

GSF had a negative effect on the volume of bread (Table 21) and as the concentration of GSF increased, the loaf volume decreased. In the unfrozen dough, the replacement of GSF

significantly decreased the loaf volume by 7.0%, 12.8%, 18.4%, or 26.1% at replacement levels of 2.5, 5, 7.5, or 10 g GSF/100 g flour, respectively. This indicates a direct effect on loaf volume associated with the replacement of wheat flour with GSF in bread.

Frozen Dough

A significant reduction in bread volume was found after 2 or 6 weeks of frozen storage at or above 5g GSF/100g flour (Table 21). The frozen storage decreased the bread volume by 4.8% to 33.4% in 2 weeks of frozen storage and by 4.1% to 33.0% at the end of 6 weeks of frozen storage. These results generally agree with those previously reported by other researchers on the effects of frozen storage on white wheat dough (Bhattacharya and others 2003; Selomulyo and Zhou 2007).

The effect of freezing on the volume of bread loaves with added GSF is also shown in Table 21. The average volumes of GSF containing bread were compared to those of the control (no GSF added) at 6 weeks of frozen storage. Control bread or bread containing GSF showed no significant reduction in volume after the second week of frozen storage. However, after the sixth week of frozen storage, breads containing 5 g GSF/100 g flour or more had a significantly decreased loaf volume compared to the two week frozen storage loaves. Frozen storage exhibited a more pronounced effect on bread volume after two weeks of frozen storage, but the replacement of GSF remained the main cause of loaf volume reduction.

The volume reduction associated with bread made from dough that has been stored frozen longer than 2 weeks could be due to a loss in dough strength, dough structure, or yeast survival and gassing power (Selomulyo and Zhou 2007). The loss in dough strength related to frozen storage has been found to be from either the release of reducing substances from the yeast (Hsu and others 1979) or the reduction of gluten cross-linking caused by ice crystallization

during the frozen storage period (Varriano-Marston and others 1980), leading to poor gas retention. The reduction in loaf volume due to a loss of dough structure may be due to either the depolymerization of glutenin subunits which make up the part of the gluten matrix (Ribotta and others 2001) or the formation of ice crystals and the subsequent separation of starch granules from the gluten matrix (Varriano-Marston and others 1980). Either of these two mechanisms can cause a discontinuous gluten matrix, subsequently weakening the gluten network, causing poor gas retention and a reduction in bread volume or an excessive proofing time. Yeast survival and gassing power have also been found to be lost during frozen storage. Inoue and others (1994) showed that the gassing power of frozen dough remained at a level similar to that of unfrozen dough after 2 weeks of frozen storage but the gassing power significantly decreased after 6 or 10 weeks of frozen storage. It is reasonable to suspect that the loss in loaf volume is due to the combined effects of gluten matrix damage and the reduced gassing power of yeast in the frozen dough system. The combination of these two mechanisms would result in a reduced gas production and retention and a lower quality final product.

Impact of GSF replacement on bread color

The GSF used in the present study was a fine mesh flour (150µm), which was a dark purple to brown color in appearance. Therefore, it was expected that bread containing GSF would result in a different color than the control. Comparison of bread color between the control bread and bread with five levels of GSF replacement across three frozen storage times is described in Table 22. Spectrophotometric measurement showed a consistent trend in bread color. For bread with more GSF replacement, L^* value significantly decreased but the a^* value significantly increased. This indicated that as GSF replaced the wheat flour in the bread, brightness decreased and it became a more intense red color. The b^* value did not show a similar

trend as the *L** or the *a** values. Instead, the replacement of GSF caused a decreased *b** value, an indication of more blue color that remained constant across all replacement levels. Despite the significant decrease in *L** and *a** values compared to the control, the results of the acceptance panel performed by either 87 (0 or 2 weeks frozen storage) or 97 (6 weeks frozen storage), consumers showed no significant difference in color acceptance of crumb across most frozen storage times and most GSF concentrations. A significant difference in color acceptance was observed at 10 g/100 g flour stored frozen for six weeks where the acceptance rating decreased from a mean of 5.1 in the control to 4.5 at 10g/100g flour. It would seem that the decrease in brightness and the color change (increased red and blue intensity) associated with replacement values up to 7.5 g GSF/100g flour did not significantly affect the acceptance of the color of the bread. However, once the concentration of GSF exceeded 7.5 g/100g flour, the acceptance significantly decreased.

Peng and others (2009) found that in the visual evaluation of the color changes in bread fortified with 1 g grape seed extract (GSE)/500g flour, 70% of the panelists liked the color of the fortified bread better than the control bread. However, only a 13% reduction in the L* value of the bread was noticed, from 71.35 in the control to 62.13 in the bread containing 1 g GSE/500g flour (Peng and others 2009). In the present study, a significant difference in the visual acceptance of crumb darkness was not observed until the L* value was reduced by 45%, from a mean of 83.77 in the control to 46.35 for the 10 g GSF/100g flour bread stored for 6 weeks. This shows that consumers in this study still accepted breads with a significant decrease in brightness, up to a 45% reduction from the control.

Impact of GSF on bread porosity

Porosity refers to the extent of perforation of the bread crumb, including holes and cracks that allow the permeation of air. The results on the acceptance of porosity from the consumers did not show any significant difference ($P>0.05$) between the GSF replacement levels or across the storage levels (Table 23). However, as the concentration of GSF increased, so did the consumer acceptance of porosity. This suggested that consumers prefer a denser crumb structure with more cells/mm^2, a smaller mean pore diameter or more tightly packed cells.

Results of the image analysis of the bread crumb revealed that as the GSF increased, the number of cells/mm^2 also increased but the mean diameter of the cells decreased (Figure 12, 13 and 14). For instance, the number of cells/mm^2 increased from 0.55 in the control to 1.21 with 10 g GSF/100g flour, in the unfrozen dough, a 220% increase, while the mean cell diameter decreased from 0.49 mm in the control to 0.39 mm with 10 g GSF/100g flour. There were also significant decreases in the mean cell diameter across frozen storage times at concentrations of GSF at or above 7.5 g/100g flour.

Bread porosity is significantly affected by the mixing, proofing, and freezing processes (Baardseth and others 2000). Air is incorporated into the dough during mixing, and forms gas cells which in turn act as nucleation sites for CO_2 gas generation by yeast during proofing (Campbell 2003). The oxygen which has been incorporated aids in the oxidation of ascorbic acid to dehydroascorbic acid, helping in the formation of the gluten matrix (Campbell 2003). Bread made from frozen dough is often subject to a weakened gluten network either by depolymerization or ice crystallization which affects gluten cross-linking (Selomulyo and Zhou 2007). The image analysis results of porosity indicate that the formation of cells in the dough was significantly affected by the replacement of GSF during mixing and frozen storage.

However, the effect of the replacement of GSF seemed to be more directly related to the differences in mean cell diameter and the number of cells/mm^2.

Changes in the texture profile: firmness in bread with added GSF

Firmness is commonly used as an indicator to determine bread quality as a change in hardness is frequently accompanied by a loss of quality during storage (Spices 1990). Thus firmness was selected as one of the quality indices in both sensory evaluation and instrumental analysis.

In this study, as evaluated by texture profile analysis (TPC) the firmness of bread increased with increased GSF replacement (Table 24). The increments of increase in firmness of the unfrozen bread, two week frozen storage bread, and six weeks frozen storage bread were comparable across the different GSF replacement levels. Compared to the control, the increase in firmness of the unfrozen bread ranged from 8% with 2.5 g GSF/100g flour to 106% with 10 g GSF/100g flour. For the two weeks frozen storage bread, increases in firmness ranged from 25% with 0 g GSF/100g flour to 167% with 10g GSF/100g flour. After six weeks of frozen storage, the change in firmness ranged from 0.5% with 0 g GSF/100g flour to 192% with 10g GSF/100g flour. However, after six weeks of frozen storage, only the breads containing 7.5g GSF/100g flour or greater exhibited a significant increase in firmness over their respective unfrozen breads.

Interestingly, the breads made from dough which was frozen for two weeks were consistently more firm than breads made from dough which had been frozen for six weeks. These results do not agree with that found by other researchers (Kenny and others 1999). These researchers found that as the length of frozen storage time increased, bread firmness as measured by TPA also increased. The reason for the increased firmness exhibited by the two week frozen storage dough may be explained by varying degrees of weakened gluten strength and reduced

yeast activity caused by larger ice crystal formation. Dough strength diminishes with a slower freezing rate, longer storage time and higher storage temperatures (Yi and Kerr 2009). Yi and Kerr (2009) found that freezing to an internal dough temperature of -30°C within 27 min caused the least amount of damage to the gluten structure. In this study, an internal temperature of -7°C was reached within 60 min for the 6 week frozen storage dough and 70 min for the 2 week frozen dough, thus more damage to the gluten matrix may have occurred in the 2 week frozen dough due to larger ice crystals. This increased damage caused by a lower freezing rate could have caused the increased firmness.

Despite the increase in the firmness of bread with increasing GSF concentration, no significant differences in the consumer acceptance of hardness between GSF concentrations or length of frozen storage were observed in the sensory data. The lack of a relationship between the sensory acceptance and TPA measurement of hardness may indicate that either the change in firmness, measured by TPA, is not perceived or that increasing firmness is not related to a decrease in acceptance.

Changes in GSF Bread Taste Profile: Sweetness, Bitterness, and Astringency

One of the most important taste characteristics in food acceptance is sweetness (Meilgaard and others 1999). Bitterness and astringency were also important characteristics of taste and mouthfeel to evaluate due to the bitter and astringent nature of grape seed flavan-3-ols contained in concentrated levels in the GSF. The highest replacement level of GSF (10 g/100g flour) in the unfrozen and six week frozen storage breads resulted in a significant reduction in consumer acceptance of both sweetness and astringency compared to the replacement of 5 g GSF/100g wheat flour (Table 25).

The taste of bitterness and the tactile sensation of astringency are elicited primarily by the flavan-3-ols in GSF including; (+)-catechin, (-)-epicatechin, and especially proanthocyanidins (Kielhorn and Thorngate 1999). Taste receptor cells that confer bitterness are primarily associated with papillae on the tongue, while transduction mechanisms for their perception are compound specific (Lesschaeve and Noble 2005). A replacement level of 10 g GSF/100g flour resulted in a significant decrease in acceptance after frozen storage for 6 weeks. However, all other replacement levels and frozen storage times did not result in a significant change in the sensory acceptance of bitterness.

Astringency is a chemically induced complex set of tactile sensations which stems from the precipitation of proline-rich salivary proteins in the mouth causing a loss of lubrication (Kallithraka and others 2001). Numerous researchers have shown that flavan-3-ols including proanthocyanidins, the main antioxidant in GSF, can precipitate proline-rich salivary proteins (Poncet-Legrand and others 2007). A replacement level of 10g GSF/100g flour resulted in a significant decrease in the acceptance of astringency and sweetness of the bread. This suggested that as the concentration of GSF increased so did the corresponding flavanol content, resulting in decreased astringency acceptability. This increase in the content of flavanols could result an increase in the intensity of bitterness and astringency which both have been shown to suppress the sense of sweetness in model solutions of grape seed tannin (Smith and others 1996). The suppression of sweetness could also cause a reduced acceptance of bitterness, astringency, and sweetness at the highest GSF replacement level. Large variations in the astringency acceptance ratings were noticed and could be due to the different salivary flow characteristics of each individual panelist. Panelists who generate a greater amount of saliva are generally expected to have a reduced sensitivity to astringency (Drobna and others 2004).

Changes in the Overall Acceptance of Bread with added GSF

The overall consumer acceptance of GSF-containing bread was not significantly impacted by GSF concentration or length of frozen storage time, except at 5 g GSF/100g flour frozen for 6 weeks (Table 26). The 5 g GSF/100g flour replacement level was either equal to or higher than the mean acceptance ratings for the unfrozen controls at all frozen storage times. This suggests that the replacement of wheat flour with GSF in breads at concentrations up to 5 g/100g flour does not significantly impact the overall acceptance of bread and could potentially increase the acceptability of the bread. The incorporation of yam flour into white breads also increased acceptability of the breads up to a 5% replacement rate of wheat flour, at which point, the overall acceptability decreased (Hsu and others 2004).

Principal component analysis (PCA) was performed to understand how sensory attributes including; hardness, porosity, crumb darkness, bitterness, sweetness, and astringency contributed to the acceptance of the breads. Samples which are further away on the biplots are perceptually more different than samples which are found closer together (Meilgaard and others 1999). Similarly, attributes plotted close to samples on the biplot are more similar than attributes which are farther away in the graphical space.

Figure 15, illustrating the unfrozen bread consumer acceptance panel, shows that the 2.5% GSF bread (2.5 g GSF/100g flour) and the 5% GSF bread were similar, and their acceptance ratings were associated with increased sensory acceptance for crumb darkness, overall acceptance, hardness, and astringency. The 0% GSF bread acceptance was associated with an increased acceptance of crust darkness, while the 7.5% and 10% GSF breads were defined by a reduction in the acceptance of sweetness, bitterness, astringency, crumb darkness, and overall acceptance. In the 2 week frozen storage bread sensory panel the 2.5% GSF bread

and the unfrozen control bread (0 g GSF/100g flour) were similar, and were characterized by an increase in the sensory acceptance for crust darkness, and a decrease in the acceptance for porosity, hardness, and astringency (Figure 16). The 7.5% GSF bread acceptance was defined by an increase in the acceptance of astringency and sweetness, while the 10% GSF bread was defined by an increased acceptance of hardness, and a decrease in the acceptance of crumb darkness, overall acceptance, and bitterness. Two week frozen storage 5% GSF-containing bread acceptance ratings were defined by an increase in the acceptance of bitterness, sweetness, overall acceptance, and crumb darkness. At 6 weeks of frozen storage, the consumer acceptance panel found that 0% and 2.5% GSF breads were similar, and were characterized by an increase in the acceptance ratings of crumb darkness, sweetness, hardness, and overall acceptance (Figure 17). The 5% and 7.5% GSF-containing breads were also similar, in the same quadrant, and were defined by a reduction in the acceptance of sweetness, hardness, and crumb darkness. The unfrozen control dough was defined by increases in acceptance of bitterness, astringency, and crumb darkness, while the 10% GSF-containing bread was characterized by a decrease in the acceptance of overall acceptance, bitterness, astringency, porosity, and crumb darkness.

An examination of the PCA biplots showed that breads containing ≥5% GSF were perceptually different from the unfrozen control breads. In addition to becoming more different than the control, breads containing ≥7.5% GSF were characterized by a reduction in overall acceptance and the acceptance of key bread sensory attributes including, crumb darkness, bitterness, astringency, and sweetness. While the replacement of up to 5% GSF created bread that was significantly perceptually different from the control breads, it still exhibited acceptable sensory properties.

CONCLUSIONS

The TPC, TFC, DPPH radical scavenging activity, TEAC radical scavenging activity, and FRAP reducing potential all demonstrated that the press residues of grape seed oil production are polyphenolic rich waste products with high antioxidant activity against various free radicals. The heating of Merlot GSF caused significant decreases TPC and antioxidant activity when heated at ≥180°C but remained significantly unchanged at lower temperatures. A strong correlation between antioxidant properties and TPC was found, indicating that phenolic compounds are the major contributor to the antioxidant properties measured by TEAC, DPPH, and FRAP assays. The strong correlations between the mean values of FRAP and all other assays suggest that the ferric ion reducing ability of polyphenols is an important factor dictating their free radical scavenging ability. The high correlation between FRAP and DPPH assays along with TPC (Folin-Cioclateu) also shows that the combination of the DPPH and FRAP assays best illustrate antioxidant activity

The replacement of wheat flour with grape seed in bread making resulted in a dose dependent increase in the TPC and radical scavenging activity of the breads. However, recovery of phenolics was low and could have been caused by a small amount of thermal degradation or more likely through phenolic complexation with proteins during the flour mixing. Thermal degradation was a small factor as significant increases in baked bread crumb total phenolic and antioxidant activity compared to the dough was noted.

The replacement of GSF coupled with frozen storage had effects on bread quality. The instrumental analysis results showed significant impacts of GSF replacement (at all levels) on bread brightness, porosity, hardness, and loaf volume. A synergistic effect between the GSF and frozen storage at higher replacement levels (7.5 and 10 g GSF/100g flour) was noticed. The

exact mechanism responsible for the effects of GSF and frozen storage on the bread and dough matrix is unknown. However, it is possibly due to the combination of the interaction between grape seed phenolics and gluten proteins during dough mixing and the reduced activity of yeast after frozen storage. Despite the effects of the replacement of GSF on the bread and the resulting instrumental values, the overall consumer acceptance of the breads containing GSF did not reveal significant differences from the control. In general, a higher GSF replacement (>5g/100g flour) led to a more dense loaf, reduced overall acceptance, and a decrease in the acceptance of astringency and sweetness. Thus, the replacement of no more than 5 g GSF/100g flour is recommended for GSF-fortified bread that contains increased TPC and antioxidant activity while still maintaining acceptable instrumental and sensory properties.

SUGGESTIONS FOR FUTURE STUDIES

In order to assess the effects of processing on individual phenolic compounds within the GSF, high performance liquid chromatography – mass spectrometry analysis should be performed. The determination of individual phenolics in the grape seeds before and after the grape seeds are pressed into oil would provide insight into the extractability of grape seed phenolics into the oil and any degradation or isomerization of phenolic compounds prior to grinding into flour. The GSF could then be included into a bread baking regime and again assessed for the changes in phenolic compounds. Following this procedure would yield a complete picture of what happens throughout grape seed processing and the reductions caused by each of the processing steps.

Another intriguing study would be to take electron micrographs of different varieties of GSF which have been ground to different particle sizes. The flours could then be included into a bread baking model, and the structures of the flours could be correlated with their impact on bread quality. The idea behind this is that the structures of the GSF are rough and instead of the phenolic compounds binding to and disrupting the gluten matrix, the GSF may actually be physically tearing it apart. Thus, the interactions between GSF and protein containing systems would be further understood.

In order to better view the relationships between the time, temperature, and harvest years of grape seed flour on total phenolics and antioxidant activity, response surface modeling should be performed. Once the response surfaces have been created, mathematical prediction and optimization equations can be formulated. Response surface modeling could also be performed on the bread baking regime to determine the optimum bake time, proof time, and temperature needed to optimize phenolic stability.

An *in vivo* intervention study to determine whether the daily consumption grape seed bread has any physiological impact should be undertaken. Proanthocyanidins have been shown to be poorly absorbed by the body. However, monomeric phenolics such as catechin and epicatechin which are both contained in GSF are fairly readily absorbed. The participants' blood could be taken before the study and then again after the study to determine the increase in antioxidant potential in the blood.

Further product development could also be performed. In this study, significant losses in loaf volume related to the inclusion of GSF were shown. To combat this issue, reducing or oxidizing dough enhancers could be used. Another way to approach the loaf volume problem may be to add back in the amount of gluten lost due to the replacement of wheat flour with GSF. Encapsulation of GSF or grape seed extract could also be used in future bread baking studies to prevent the interaction of grape seed phenolics with gluten matrices.

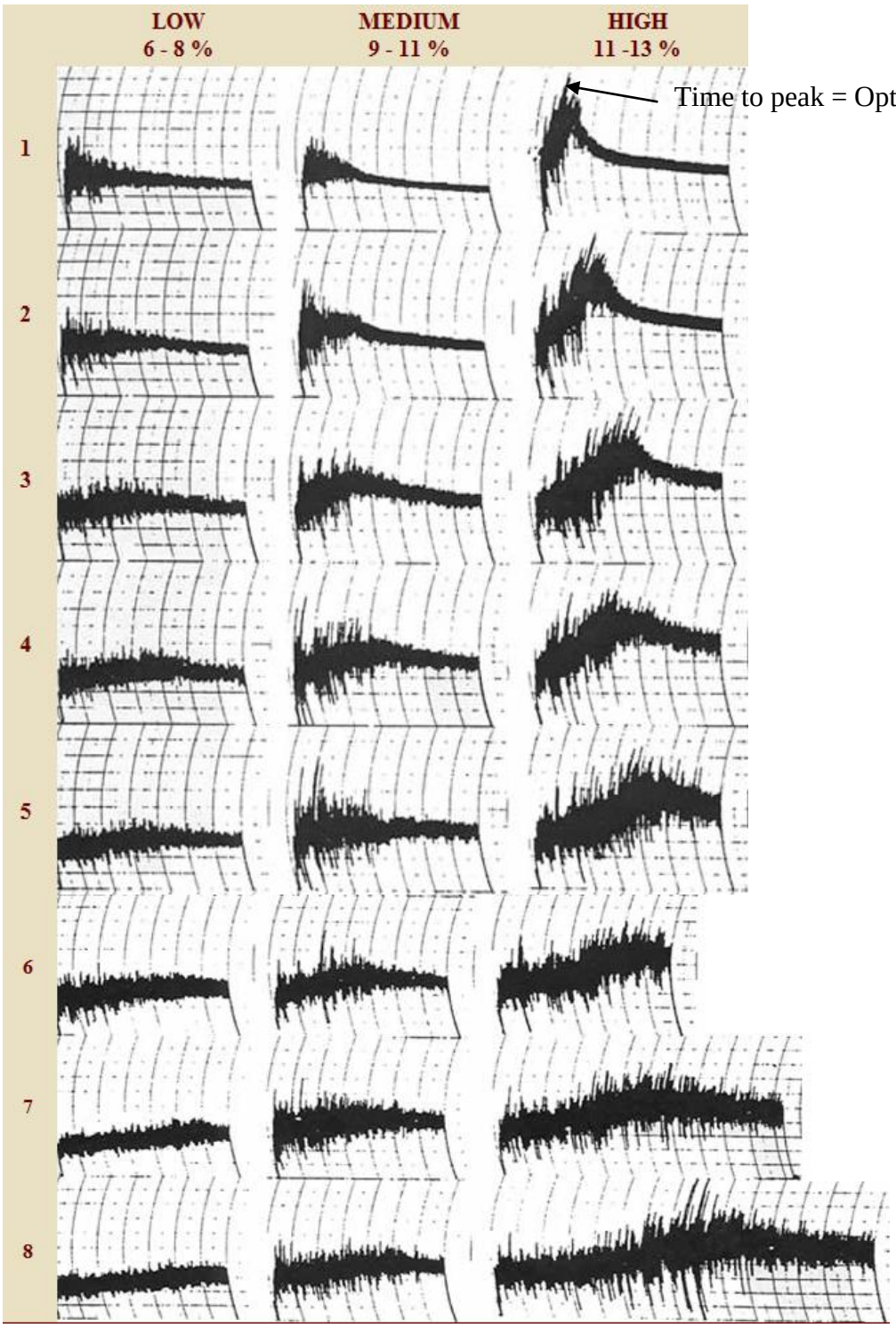

Figure 7. Mixogram Reference Chart used by experienced bakers at the Western Wheat Quality Laboratory to determine, optimum water absorption and mixing time (Dick and Youngs 1988). Low (6-8%), medium (9-11%) and high (11-13%) refers to the protein content (%) of the flour with classification numbers 1 through 8 used to describe the mixing tolerance of the flour (1=low tolerance and 8=high tolerance).

Table 6. Sensory appearance, taste, and oral texture attributes and corresponding descriptions used in-booth by the consumer panelists for the evaluation of Merlot grape seed flour breads.

Attribute	Description
Crust Darkness	Degree of color darkness of the crust, ranging from light brown to dark brown.
Crumb Darkness	Degree of darkness in the bread interior, ranging from white to dark brown.
Porosity	The extent of perforation of the bread crumb, encompassing holes and cracks, which allow the permeation of air.
Sweetness	Fundamental taste commonly associated with sucrose.
Bitterness	Having a harsh disagreeably sharp taste like quinine.
Astringency	The drying or puckering sensation commonly associated with tannins.
Sourness	Fundamental taste sensation elicited by acids.
Hardness	Force required to bite completely through sample placed between molars.
Adhesiveness	Force required to remove the sample completely from the palate, using the tongue during consumption.

Table 7. Effect of heating time and temperature on the total phenolic content (mg tannic acid/g dry weight grape seed flour) of 70% ethanol extracts from (a) 2007 and (b) 2008 150 µm Merlot Seed Flour.

a.	**Heating Time (min)**							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	79.90 ± 2.48^{abcdv}	86.70 ± 6.46^{av}	68.46 ± 1.45^{ew}	75.31 ± 2.15^{dw}	79.51 ± 4.93^{cdv}	78.85 ± 4.23^{bcdv}	82.66 ± 9.43^{abcv}	85.65 ± 7.96^{abv}
150	79.90 ± 2.48^{abv}	77.64 ± 0.76^{abw}	82.11 ± 5.18^{abv}	84.04 ± 8.12^{av}	78.68 ± 6.22^{abv}	75.54 ± 0.69^{bcv}	69.51 ± 2.44^{cdw}	64.10 ± 4.84^{dw}
180	79.90 ± 2.48^{av}	71.28 ± 3.60^{bw}	59.90 ± 5.82^{cx}	56.36 ± 3.98^{cx}	48.96 ± 1.99^{dw}	37.08 ± 4.23^{efw}	43.55 ± 3.70^{dex}	32.61 ± 3.97^{fx}
210	79.90 ± 2.48^{av}	45.26 ± 5.37^{bx}	31.83 ± 3.86^{cy}	21.01 ± 5.59^{dy}	12.88 ± 3.34^{ex}	10.18 ± 2.51^{ex}	11.56 ± 1.24^{ey}	10.78 ± 3.04^{ey}
240	79.90 ± 2.48^{av}	7.03 ± 0.88^{by}	4.38 ± 0.50^{bcz}	-4.68 ± 2.13^{dz}	-1.48 ± 2.69^{cdy}	0.45 ± 5.08^{bcdy}	-2.03 ± 3.58^{cdz}	4.76 ± 3.02^{bcy}

$^{a-f}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b.	**Heating Time (min)**							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	134.20 ± 2.93^{abv}	139.74 ± 9.12^{av}	134.82 ± 6.94^{abv}	138.08 ± 6.01^{av}	130.05 ± 1.98^{bv}	130.05 ± 0.39^{bv}	116.68 ± 6.86^{cv}	131.04 ± 5.71^{bv}
150	134.20 ± 2.93^{bv}	141.40 ± 2.52^{av}	140.36 ± 6.48^{abv}	129.02 ± 4.85^{bw}	112.44 ± 2.67^{cw}	112.59 ± 4.90^{cw}	99.53 ± 2.25^{dw}	92.69 ± 7.61^{ew}
180	134.20 ± 2.93^{av}	75.13 ± 4.22^{bw}	59.84 ± 2.70^{cw}	53.26 ± 1.71^{dx}	48.70 ± 3.65^{dex}	42.69 ± 2.91^{ex}	35.13 ± 2.16^{fx}	19.38 ± 1.03^{gx}
210	134.20 ± 2.93^{av}	16.79 ± 1.64^{bx}	8.45 ± 4.12^{cx}	7.88 ± 3.03^{cdy}	5.96 ± 0.86^{cdy}	1.81 ± 0.36^{dy}	8.13 ± 3.16^{cdy}	4.51 ± 1.33^{cdy}
240	134.20 ± 2.93^{av}	5.13 ± 1.33^{by}	-6.48 ± 1.68^{cy}	-6.99 ± 0.54^{cz}	-5.91 ± 2.65^{cz}	-4.04 ± 1.09^{cy}	-4.46 ± 2.07^{cz}	-1.30 ± 1.56^{cy}

$^{a-g}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 8. Effect of heating time and temperature on the total flavanol content (mg Procyanidin B2/g dry weight grape seed flour (GSF)) of 70% ethanol extracts from (a) 2007 and (b) 2008 150µm Merlot seed flour.

a.	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	60.32 ± 0.89^{abv}	65.14 ± 1.74^{abv}	64.18 ± 6.71^{abv}	61.67 ± 2.31^{abv}	59.55 ± 1.77^{abv}	62.44 ± 4.42^{abv}	65.92 ± 10.95^{av}	58.59 ± 5.50^{bv}
150	60.32 ± 0.89^{av}	57.81 ± 2.91^{abw}	51.26 ± 4.74^{bcw}	44.51 ± 0.67^{cdw}	40.07 ± 2.34^{dew}	47.21 ± 0.579^{cw}	45.47 ± 5.70^{cdw}	33.13 ± 1.46^{ew}
180	60.32 ± 0.89^{av}	40.65 ± 0.89^{bx}	30.82 ± 0.34^{cx}	30.24 ± 2.03^{cx}	21.37 ± 0.89^{dx}	16.55 ± 0.00^{dex}	15.20 ± 1.20^{dex}	11.73 ± 0.34^{ex}
210	60.32 ± 0.89^{av}	20.02 ± 1.16^{by}	7.68 ± 0.34^{cy}	4.40 ± 0.00^{cy}	3.05 ± 0.34^{cy}	3.05 ± 0.34^{cy}	2.27 ± 1.20^{cy}	1.50 ± 0.00^{cy}
240	60.32 ± 0.89^{av}	1.50 ± 0.00^{bz}	5.94 ± 0.34^{by}	1.12 ± 0.34^{by}	0.93 ± 0.00^{by}	0.93 ± 0.00^{by}	1.70 ± 0.34^{by}	1.89 ± 0.34^{by}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b.	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	95.23 ± 1.00^{efv}	106.80 ± 17.18^{cdv}	115.67 ± 13.25^{av}	100.24 ± 4.85^{dev}	114.13 ± 2.74^{abv}	103.34 ± 17.53^{cdv}	108.53 ± 10.99^{bcv}	90.99 ± 2.61^{fv}
150	95.23 ± 1.00^{av}	85.78 ± 4.85^{bw}	85.58 ± 1.34^{bw}	77.10 ± 6.65^{cdw}	83.46 ± 2.91^{bcw}	73.63 ± 5.47^{cdw}	76.13 ± 3.52^{dw}	61.09 ± 3.06^{ew}
180	95.23 ± 1.00^{av}	58.59 ± 4.10^{bx}	38.14 ± 2.03^{cx}	33.23 ± 0.579^{cdx}	30.43 ± 0.579^{dex}	25.80 ± 0.00^{efx}	22.52 ± 1.46^{fx}	14.23 ± 0.579^{gx}
210	95.23 ± 1.00^{av}	12.69 ± 0.34^{by}	7.87 ± 0.00^{bcy}	6.90 ± 0.34^{bcy}	6.13 ± 0.00^{bcy}	6.13 ± 0.00^{bcy}	5.55 ± 0.00^{cy}	4.98 ± 0.00^{cy}
240	95.23 ± 1.00^{av}	6.13 ± 1.16^{by}	6.90 ± 0.34^{by}	5.74 ± 0.34^{by}	4.98 ± 0.00^{by}	7.87 ± 1.00^{by}	4.98 ± 0.00^{by}	5.55 ± 0.579^{by}

$^{a-g}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-y}$ Different letters within a column are significantly different ($P < 0.05$), n =3.

Table 9. Effect of heating time and temperature on the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (µmol Trolox/g dry weight grape seed flour (GSF)) of 70% ethanol extracts from (a) 2007 and (b) 2008 150µm Merlot seed flour.

a.	**Heating Time (min)**							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	256.45 ± 17.5^{bcv}	264.80 ± 18.7^{abv}	257.57 ± 9.95^{abcv}	258.07 ± 13.6^{abcv}	280.59 ± 27.5^{av}	276.62 ± 5.22^{abv}	267.64 ± 7.92^{abv}	235.49 ± 9.45^{cv}
150	256.45 ± 17.5^{av}	254.24 ± 3.68^{av}	239.07 ± 16.7^{abv}	230.18 ± 10.8^{bw}	239.80 ± 6.69^{abw}	223.98 ± 20.3^{bcw}	242.81 ± 29.7^{abw}	201.76 ± 43.2^{cw}
180	256.45 ± 17.5^{av}	209.79 ± 9.68^{bw}	193.77 ± 18.2^{bw}	193.71 ± 9.30^{bx}	165.61 ± 17.4^{cx}	119.81 ± 4.60^{dx}	122.70 ± 12.2^{dx}	139.44 ± 41.1^{dx}
210	256.45 ± 17.5^{av}	165.08 ± 16.1^{bx}	87.81 ± 7.17^{cx}	74.67 ± 31.8^{cy}	65.07 ± 22.9^{cdy}	46.52 ± 17.8^{dy}	15.20 ± 7.79^{ey}	5.99 ± 3.75^{ey}
240	256.45 ± 17.5^{av}	-62.24 ± 9.71^{bcdy}	-80.05 ± 12.1^{cdy}	-61.98 ± 2.83^{bcdz}	-66.40 ± 3.00^{bcdz}	-83.90 ± 11.3^{dz}	-60.29 ± 10.7^{bcz}	-46.64 ± 4.44^{bz}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.
$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b.	**Heating Time (min)**							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	269.26 ± 11.2^{av}	283.43 ± 4.17^{av}	277.88 ± 8.83^{av}	275.62 ± 0.71^{av}	280.73 ± 6.33^{av}	279.85 ± 6.61^{av}	289.06 ± 5.89^{av}	275.66 ± 6.82^{av}
150	269.26 ± 11.2^{av}	286.57 ± 6.61^{av}	288.98 ± 2.39^{av}	281.60 ± 0.73^{av}	280.51 ± 13.00^{av}	285.11 ± 2.09^{av}	273.10 ± 2.51^{av}	272.76 ± 4.36^{av}
180	269.26 ± 11.2^{av}	253.91 ± 14.55^{abw}	240.63 ± 3.04^{bcw}	222.00 ± 16.3^{cw}	230.44 ± 12.4^{dcw}	194.46 ± 8.92^{dw}	188.39 ± 16.5^{dw}	160.58 ± 32.3^{ew}
210	269.26 ± 11.2^{av}	138.50 ± 24.1^{bx}	101.10 ± 13.7^{cx}	83.99 ± 5.78^{cdx}	75.71 ± 27.1^{dex}	57.35 ± 8.88^{ex}	64.25 ± 7.92^{dex}	26.16 ± 4.22^{fx}
240	269.26 ± 11.2^{av}	-9.30 ± 24.0^{by}	-12.81 ± 9.39^{by}	-14.09 ± 2.54^{by}	-11.63 ± 5.86^{by}	-10.90 ± 30.3^{by}	-15.03 ± 6.67^{by}	-2.48 ± 5.70^{by}

$^{a-f}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 10. Effect of heating time and temperature on the Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox/g dry weight grape seed flour (GSF)) of 70% ethanol extracts from (a) 2007 and (b) 2008 150µm Merlot seed flour.

a.	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	1108.0 ± 96.0^{av}	1116.0 ± 63.5^{aw}	630.7 ± 68.0^{dv}	617.3 ± 48.2^{dw}	518.7 ± 104.1^{ev}	1113.3 ± 20.1^{av}	918.7 ± 46.2^{bv}	777.3 ± 20.1^{cv}
150	1108.0 ± 96.0^{bv}	1508.0 ± 115.4^{av}	647.7 ± 28.1^{dev}	1094.7 ± 33.3^{bv}	588.0 ± 48.7^{efv}	558.7 ± 16.7^{fx}	743.7 ± 12.2^{cw}	689.3 ± 53.3^{cdw}
180	1108.0 ± 96.0^{av}	553.3 ± 53.3^{cx}	521.3 ± 52.1^{cw}	561.3 ± 33.3^{cw}	569.3 ± 28.1^{cv}	670.7 ± 28.1^{bw}	318.7 ± 12.2^{ex}	401.3 ± 32.3^{dx}
210	1108.0 ± 96.0^{av}	468.0 ± 13.9^{by}	244.0 ± 8.00^{cdx}	260.0 ± 16.0^{cx}	209.3 ± 9.24^{cdew}	193.3 ± 20.1^{cdey}	174.7 ± 16.7^{dey}	153.3 ± 4.62^{ey}
240	1108.0 ± 96.0^{av}	49.00 ± 14.0^{bz}	26.33 ± 2.31^{by}	21.67 ± 3.06^{by}	22.33 ± 1.15^{bx}	25.67 ± 6.11^{bz}	29.00 ± 3.46^{bz}	41.00 ± 3.46^{bz}

$^{a-f}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3

b.	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	1873.3 ± 46.2^{av}	1177.3 ± 123.0^{cw}	1148.0 ± 28.8^{cdv}	1089.3 ± 53.3^{dw}	1470.7 ± 23.1^{bv}	1393.3 ± 4.62^{bv}	1188.0 ± 8.00^{cv}	1073.0 ± 0.00^{dv}
150	1873.3 ± 46.2^{av}	1260.0 ± 8.00^{bv}	1113.3 ± 88.1^{cv}	1281.3 ± 53.3^{bv}	1084.0 ± 21.2^{cdw}	1033.3 ± 154.8^{dw}	937.3 ± 37.0^{ew}	892.0 ± 50.0^{ew}
180	1873.3 ± 46.2^{av}	604.0 ± 97.0^{cx}	737.3 ± 59.0^{bw}	716.0 ± 104.9^{bx}	585.3 ± 46.2^{cdx}	350.7 ± 52.1^{ex}	521.3 ± 54.5^{dx}	385.3 ± 25.7^{ex}
210	1873.3 ± 46.2^{av}	500.0 ± 21.7^{by}	342.7 ± 48.2^{cdx}	324.0 ± 27.7^{cdy}	356.0 ± 56.0^{cy}	284.0 ± 21.7^{cdx}	241.3 ± 12.2^{ey}	270.7 ± 62.1^{dey}
240	1873.3 ± 46.2^{av}	225.0 ± 7.21^{bz}	23.00 ± 2.00^{cy}	23.7 ± 4.16^{cz}	22.33 ± 4.16^{cz}	30.33 ± 3.06^{cy}	29.00 ± 7.21^{cz}	22.33 ± 1.15^{cz}

$^{a-g}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3

Table 11. Effect of heating time and temperature on the ferric ion reducing antioxidant power (FRAP) (µmol Trolox/g dry weight grape seed flour (GSF)) of 70% ethanol extracts from (a) 2007 and (b) 2008 150µm Merlot seed flour.

a. 2007	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	192.3 ± 21.2^{cv}	265.3 ± 23.5^{av}	255.3 ± 5.77^{abv}	262.6 ± 21.3^{abv}	241.5 ± 7.84^{bv}	240.9 ± 6.89^{bv}	245.0 ± 23.3^{abv}	240.1 ± 27.8^{bv}
150	192.3 ± 21.2^{cdv}	218.6 ± 7.48^{bw}	247.7 ± 12.3^{av}	213.1 ± 27.3^{bcw}	175.7 ± 10.6^{dw}	175.2 ± 17.2^{dw}	194.9 ± 3.72^{cdw}	190.1 ± 18.3^{dw}
180	192.3 ± 21.2^{abv}	200.9 ± 5.09^{aw}	176.4 ± 25.4^{bcw}	168.3 ± 8.39^{cdx}	147.1 ± 3.44^{dex}	115.8 ± 6.55^{fgx}	136.8 ± 27.4^{efx}	109.1 ± 6.75^{gx}
210	192.3 ± 21.2^{av}	134.6 ± 1.20^{bx}	97.97 ± 7.56^{cx}	78.72 ± 5.16^{cdy}	68.27 ± 7.51^{dey}	63.12 ± 2.89^{dey}	52.52 ± 7.05^{efy}	38.73 ± 1.20^{fy}
240	192.3 ± 21.2^{av}	9.787 ± 1.05^{by}	4.788 ± 0.26^{by}	3.727 ± 0.00^{bz}	3.273 ± 0.00^{bz}	3.424 ± 0.26^{bz}	4.939 ± 0.26^{bz}	8.273 ± 0.00^{bz}

$^{a-g}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. 2008	Heating Time (min)							
Temperature (°C)	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**90**
120	373.4 ± 18.4^{bv}	347.4 ± 35.1^{cw}	372.2 ± 6.39^{bw}	376.0 ± 23.4^{bv}	329.5 ± 6.58^{cv}	382.4 ± 12.2^{abv}	399.0 ± 10.1^{av}	349.0 ± 24.3^{cv}
150	373.4 ± 18.4^{bv}	408.0 ± 23.2^{av}	410.1 ± 29.7^{av}	349.9 ± 5.46^{cw}	299.9 ± 30.7^{dw}	339.3 ± 4.60^{cw}	292.8 ± 24.1^{dw}	292.2 ± 21.6^{dw}
180	373.4 ± 18.4^{av}	257.5 ± 18.9^{bx}	193.4 ± 23.5^{cx}	185.2 ± 6.19^{cx}	194.5 ± 2.92^{cx}	152.1 ± 2.15^{dx}	154.6 ± 9.08^{dx}	117.5 ± 6.05^{ex}
210	373.4 ± 18.4^{av}	100.1 ± 7.57^{by}	71.00 ± 1.57^{cy}	61.46 ± 2.76^{cy}	57.67 ± 1.89^{cy}	50.39 ± 6.75^{cdy}	53.27 ± 1.64^{cdy}	34.18 ± 2.76^{dy}
240	373.4 ± 18.4^{av}	5.394 ± 0.53^{bz}	9.485 ± 0.53^{bz}	5.849 ± 1.39^{bz}	4.636 ± 0.00^{bz}	4.939 ± 0.52^{bz}	6.000 ± 0.00^{bz}	8.273 ± 0.00^{bz}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{v-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 12. The effects of flour particle size and grape variety on (a) total phenolic content (TPC) (mg tannic acid/g dry weight), (b) ferric ion reducing antioxidant power (FRAP) (µmol Trolox/g DW), (c) Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox/g DW), and (d) 2,2 diphenyl-1-picrylhydrazyl (DPPH) (µmol Trolox/g DW) assays of 70% ethanolic extracts of 2008 Cabernet Sauvignon and Chardonnay grape seed flour (GSF).

a. TPC (mg tannic acid/g dry weight)	**Sieve Opening (µm)**		
Flour type	**425**	**250**	**180**
Cabernet Sauvignon	62.30 ± 2.96^{cz}	168.4 ± 8.34^{bz}	195.8 ± 10.1^{ay}
Chardonnay	149.6 ± 1.96^{cy}	239.4 ± 4.96^{ay}	189.6 ± 10.1^{by}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{y-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. FRAP (µmol Trolox/g DW)	**Sieve Opening (µm)**		
Flour type	**425**	**250**	**180**
Cabernet Sauvignon	208.9 ± 8.86^{cz}	543.0 ± 15.3^{bz}	609.8 ± 25.3^{ay}
Chardonnay	416.3 ± 13.5^{cy}	602.1 ± 29.2^{ay}	488.1 ± 21.3^{bz}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{y-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

c. TEAC (µmol Trolox/g DW)	**Sieve Opening (µm)**		
Flour type	**425**	**250**	**180**
Cabernet Sauvignon	742.7 ± 48.2^{cz}	1492 ± 34.9^{bz}	1615 ± 76.0^{ay}
Chardonnay	1772 ± 56.0^{ay}	1825 ± 61.1^{ay}	1658 ± 33.3^{by}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{y-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

d. DPPH (µmol Trolox/g DW)	**Sieve Opening (µm)**		
Flour type	**425**	**250**	**180**
Cabernet Sauvignon	248.9 ± 12.6^{bz}	287.8 ± 9.11^{ay}	280.9 ± 14.1^{ay}
Chardonnay	275.2 ± 12.6^{by}	297.0 ± 6.14^{ay}	283.3 ± 14.7^{aby}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{y-z}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 13. The coefficients of linear correlation (r^2) between total phenolic content via Folin-Ciocalteu (TPC), total flavanol content via Vanillin-HCl (TFC), Trolox equivalent antioxidant capacity (TEAC), 2,2 diphenyl-1-picrylhydrazyl radical scavenging (DPPH), and ferric ion reducing antioxidant power (FRAP) assays of 70% ethanolic extracts of (a) 2007 and (b) 2008 Merlot grape seed flour (GSF) (150µm).

a. 2007 Merlot Flour				
	TFC	**TPC**	**DPPH**	**TEAC**
TPC	0.869		0.922	0.741
DPPH	0.809	0.922		0.718
TEAC	0.716	0.741	0.718	
FRAP	0.876	0.919	0.925	0.700

b. 2008 Merlot Flour				
	TFC	**TPC**	**DPPH**	**TEAC**
TPC	0.936		0.811	0.893
DPPH	0.75	0.811		0.791
TEAC	0.846	0.893	0.791	
FRAP	0.901	0.966	0.893	0.880

Table 14. Protein, moisture, and ash content of hard red spring wheat flour and 2008 Merlot grape seed flour (GSF) (150 µm).

	Hard Red Spring Wheat (HRS-05)	2008 Merlot Grape Seed Flour (150 µm)
Protein Content (%)	13.0 ± 0.10	17.4 ± 0.28
Moisture Content (%)	14.8 ± 0.02	7.38 ± 0.03
Ash Content (%)	3.97 ± 0.02	4.85 ± 0.01

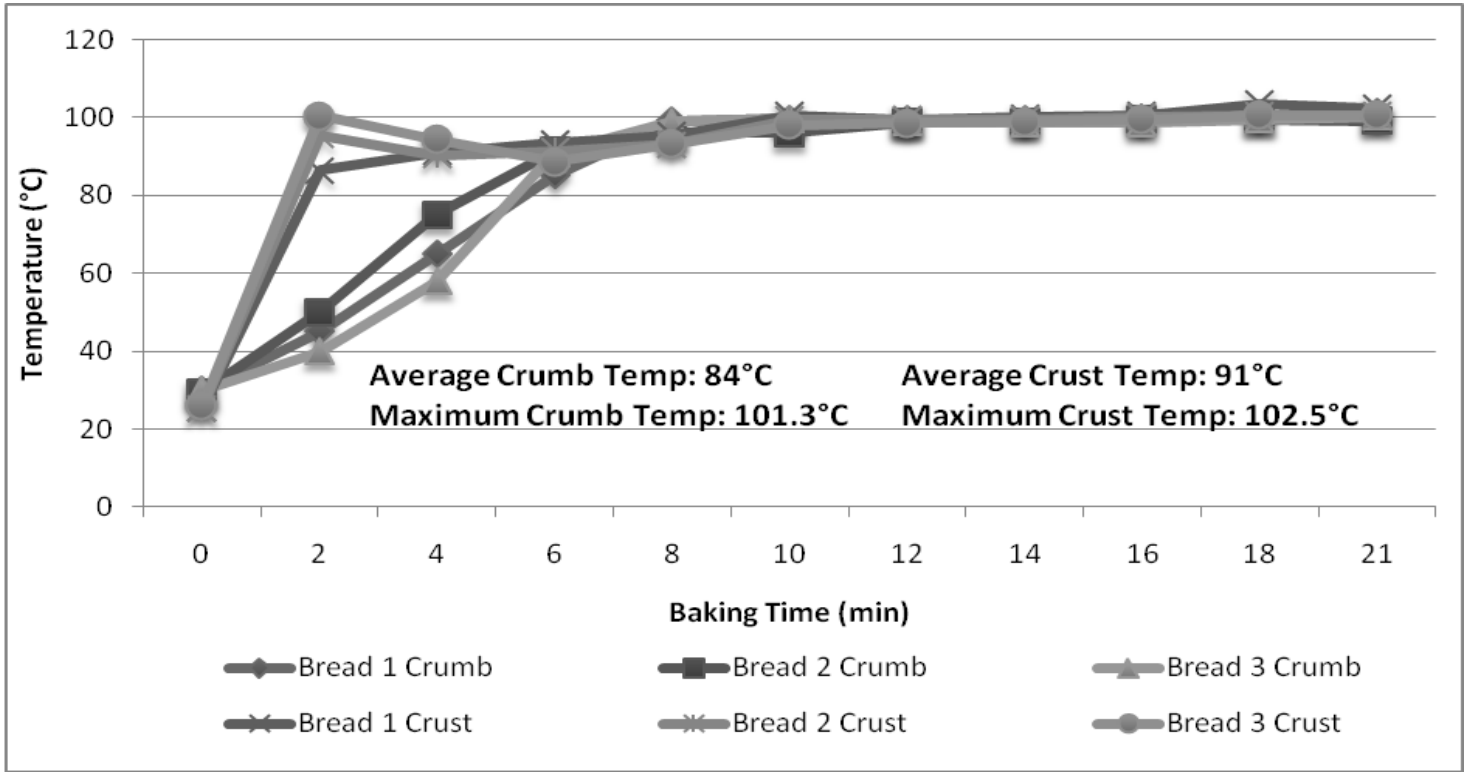

Figure 8. Central crumb and crust temperatures of bread made with 0% Merlot grape seed flour (GSF) during baking. Bread 1, 2 and 3 refer to triplicate loaves that were baked at 218°C for 21 min.

Table 15. Total phenolic content (TPC) (mg Tannic Acid / g (DW) in Merlot grape seed flour (GSF) or non-GSF containing dough or bread crumb compared across GSF replacement levels and frozen storage times of (a) 0 weeks, (b) 2 weeks, or (c) 6 weeks. Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour.

	150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)					
a. 0 weeks frozen storage	**0**	**2.5**	**5**	**7.5**	**10**	
0 weeks - Dough	0.204 ± 0.037^{ex}	0.696 ± 0.055^{dy}	1.532 ± 0.025^{cy}	2.474 ± 0.131^{by}	3.833 ± 0.113^{ay}	
0 weeks - Crumb	0.064 ± 0.029^{ey}	1.097 ± 0.082^{dx}	1.996 ± 0.062^{cx}	3.010 ± 0.116^{bx}	4.253 ± 0.064^{ax}	
b. 2 weeks frozen storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
2 weeks - Dough	0.310 ± 0.116^{ex}	0.179 ± 0.033^{fx}	0.687 ± 0.026^{dy}	1.472 ± 0.064^{cy}	2.398 ± 0.082^{by}	3.174 ± 0.059^{ay}
2 weeks - Crumb	0.062 ± 0.021^{ey}	0.062 ± 0.056^{ey}	0.941 ± 0.019^{dx}	1.778 ± 0.040^{cx}	2.628 ± 0.045^{bx}	3.655 ± 0.062^{ax}
c. 6 weeks frozen storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
6 weeks - Dough	0.146 ± 0.070^{ex}	0.144 ± 0.042^{ex}	0.649 ± 0.038^{dy}	1.455 ± 0.025^{cy}	2.327 ± 0.060^{by}	3.234 ± 0.019^{ay}
6 weeks - Crumb	0.198 ± 0.107^{ex}	0.190 ± 0.110^{ex}	1.012 ± 0.021^{dx}	1.931 ± 0.039^{cx}	3.010 ± 0.097^{bx}	3.977 ± 0.105^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column (not broken by a line) are significantly different ($P < 0.05$), n = 3.

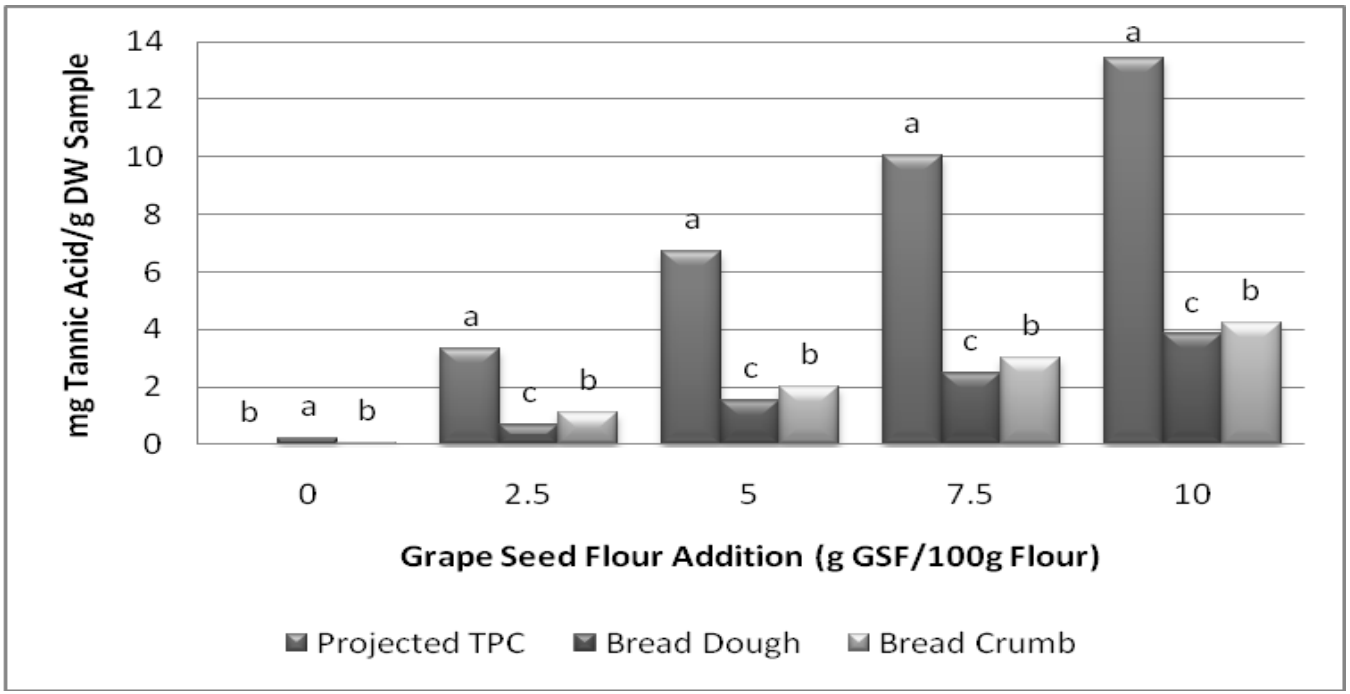

Figure 9. Recovery of total phenolics in 70% ethanolic extracts of unfrozen bread or dough systems compared to the theoretical recovery calculated based on 2008 unheated Merlot grape seed flour (GSF) TPC at the appropriate GSF replacement levels (Projected TPC). Different letters within replacement levels (a-c) indicate significant differences ($P<0.05$).

Table 16. Effect of baking at 218°C for 21 min on the total flavanol content (TFC) (mg Procyanidin B2/g DW GSF) of Merlot grape seed flour (GSF) bread made from dough which was stored frozen (-20°C) for 0 weeks (a), 2 weeks (b), or 6 weeks (c). Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour.

a. 0 weeks frozen storage	**150µm Merlot Seed Flour Replacement (g GSF/100g Flour)**				
Frozen Storage	**0**	**2.5**	**5**	**7.5**	**10**
0 weeks - Dough	0.514 ± 0.111^{cx}	0.514 ± 0.111^{cx}	1.029 ± 0.294^{by}	1.285 ± 0.111^{by}	2.250 ± 0.589^{ay}
0 weeks - Crumb	0.578 ± 0.193^{cx}	0.835 ± 0.111^{cx}	1.671 ± 0.401^{bx}	1.992 ± 0.111^{bx}	2.893 ± 0.841^{ax}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. 2 weeks frozen storage	**150µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
2 weeks - Dough	0.579 ± 0.000^{cx}	0.643 ± 0.485^{cx}	0.579 ± 0.000^{cx}	1.285 ± 0.294^{bx}	1.607 ± 0.111^{bx}	2.314 ± 0.510^{ax}
2 weeks - Crumb	0.450 ± 0.111^{dx}	0.579 ± 0.334^{dx}	0.964 ± 0.193^{cdx}	1.478 ± 0.401^{bcx}	1.928 ± 0.510^{abx}	2.250 ± 0.294^{ax}

$^{a-d}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

c. 6 weeks frozen storage	**150µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
6 weeks - Dough	0.642 ± 0.111^{cx}	0.579 ± 0.000^{cx}	0.964 ± 0.193^{bcx}	1.414 ± 0.485^{bx}	2.250 ± 0.401^{ax}	2.121 ± 0.193^{ax}
6 weeks - Crumb	0.579 ± 0.000^{dx}	0.643 ± 0.111^{dx}	1.092 ± 0.111^{cdx}	1.542 ± 0.193^{bcx}	1.928 ± 0.193^{bx}	2.507 ± 0.192^{ax}

$^{a-d}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

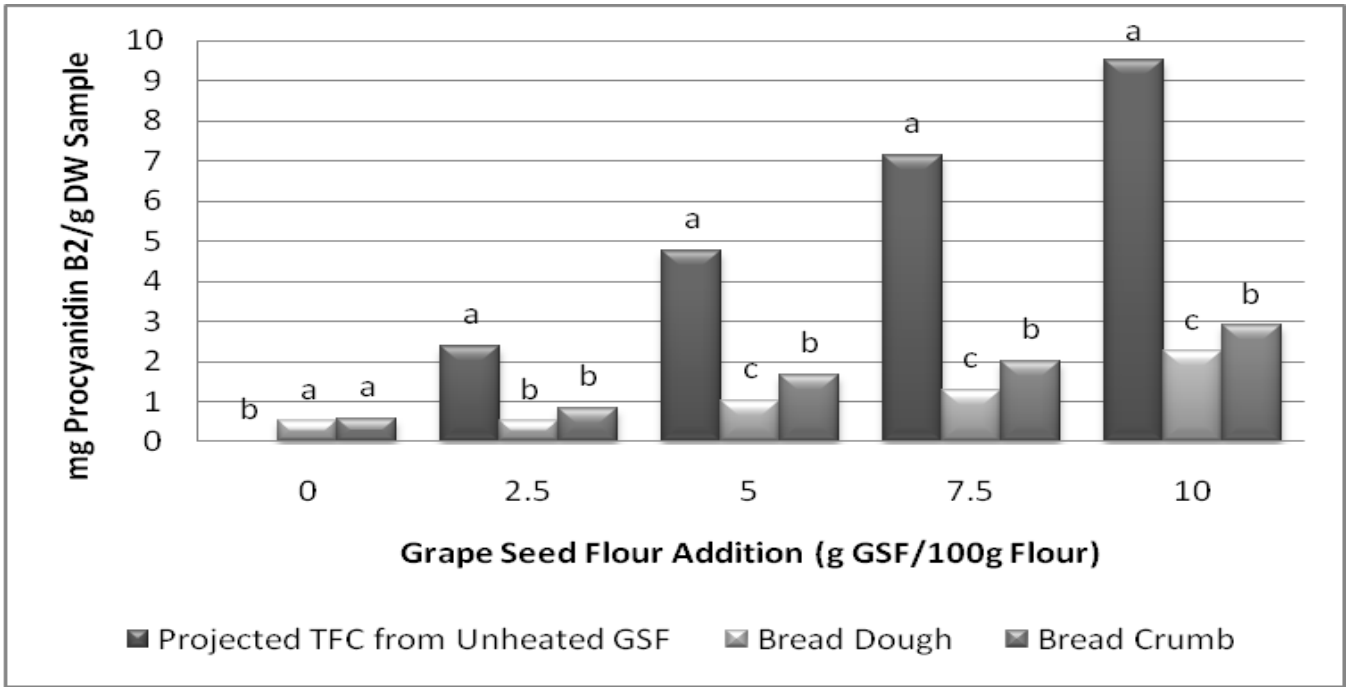

Figure 10. Recovery of total flavanols in 70% ethanolic extracts of unfrozen bread or dough systems compared to the recovery calculated based on 2008 unheated Merlot grape seed flour (GSF) TPC at the appropriate GSF replacement levels. Different letters within replacement levels (a-c) indicate significant differences ($P<0.05$).

Table 17. Effect of baking on the 2,2 diphenyl-1-picrylhydrazyl DPPH radical scavenging activity (µmol Trolox/g DW GSF) of Merlot grape seed flour (GSF) bread made from dough which was stored frozen (-20°C) for 0 weeks (a), 2 weeks (b), or 6 weeks (c). Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour.

a. 0 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**				
Frozen Storage	**0**	**2.5**	**5**	**7.5**	**10**
0 weeks - Dough	1.52 ± 0.10^{dx}	3.24 ± 0.24^{cy}	5.23 ± 0.45^{by}	6.97 ± 0.28^{ax}	7.64 ± 0.08^{ax}
0 weeks - Crumb	0.00 ± 0.00^{ey}	3.87 ± 0.14^{dx}	6.50 ± 0.26^{cx}	7.07 ± 0.05^{bx}	7.56 ± 0.04^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. 2 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
2 weeks - Dough	0.44 ± 0.67^{ex}	1.47 ± 0.04^{dx}	2.94 ± 0.38^{cx}	5.63 ± 0.68^{bx}	6.25 ± 0.55^{by}	7.52 ± 0.13^{ax}
2 weeks - Crumb	0.62 ± 0.54^{ex}	0.00 ± 0.00^{fy}	2.99 ± 0.09^{dx}	6.16 ± 0.41^{cx}	6.87 ± 0.41^{bx}	7.44 ± 0.13^{ax}

$^{a-f}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

c. 6 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
6 weeks - Dough	0.94 ± 0.91^{dx}	1.56 ± 0.17^{dx}	3.07 ± 0.20^{cx}	5.62 ± 0.36^{by}	6.97 ± 0.17^{ax}	7.37 ± 0.24^{ax}
6 weeks - Crumb	0.60 ± 0.54^{dx}	0.02 ± 0.04^{ey}	3.19 ± 0.33^{cx}	6.67 ± 0.14^{bx}	7.10 ± 0.18^{abx}	7.20 ± 0.44^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 18. Effect of baking on the Trolox equivalent antioxidant capacity (TEAC) (µmol Trolox/g DW GSF) of Merlot grape seed flour (GSF) bread made from dough which was stored frozen for 0 weeks (a), 2 weeks (b), or 6 weeks (c). Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour.

a. 0 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**				
Frozen Storage	**0**	**2.5**	**5**	**7.5**	**10**
0 weeks - Dough	14.4 ± 0.20^{ex}	17.8 ± 0.69^{dx}	21.7 ± 0.64^{cx}	29.3 ± 1.47^{bx}	35.5 ± 1.33^{ay}
0 weeks - Crumb	7.66 ± 0.83^{ey}	14.5 ± 0.50^{dy}	19.7 ± 1.17^{cx}	27.1 ± 3.64^{bx}	41.8 ± 0.53^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. 2 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
2 weeks - Dough	12.5 ± 0.64^{ex}	11.8 ± 1.56^{ex}	16.3 ± 0.70^{dx}	21.9 ± 1.96^{cx}	28.8 ± 1.06^{by}	31.9 ± 0.61^{ay}
2 weeks - Crumb	7.73 ± 0.99^{ey}	7.73 ± 0.46^{ey}	14.5 ± 0.70^{dx}	20.9 ± 0.61^{cx}	35.3 ± 1.89^{bx}	44.6 ± 2.27^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

c. 6 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
6 weeks - Dough	17.9 ± 0.42^{dx}	18.5 ± 3.19^{dx}	23.1 ± 0.70^{cx}	29.7 ± 1.50^{bx}	31.0 ± 0.72^{bx}	38.2 ± 0.80^{ax}
6 weeks - Crumb	8.80 ± 0.40^{ey}	10.3 ± 0.70^{ey}	17.4 ± 0.69^{dy}	24.9 ± 1.21^{cy}	31.9 ± 1.21^{bx}	37.3 ± 1.70^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 19. Effect of baking on the ferric ion reducing antioxidant power (FRAP) (µmol Trolox/g DW GSF) of Merlot grape seed flour (GSF) bread made from dough which was stored frozen for 0 weeks (a), 2 weeks (b), or 6 weeks (c). Merlot bread was made at wheat flour replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g.

a. 0 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**				
Frozen Storage	**0**	**2.5**	**5**	**7.5**	**10**
0 weeks - Dough	0.719 ± 0.021^{ex}	1.930 ± 0.134^{dy}	3.226 ± 0.064^{cy}	5.251 ± 0.043^{by}	7.572 ± 0.396^{ay}
0 weeks - Crumb	0.844 ± 0.021^{ex}	3.115 ± 0.037^{dx}	4.930 ± 0.161^{cx}	6.695 ± 0.238^{bx}	10.497 ± 0.262^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

b. 2 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	Unfrozen-0	0	2.5	5	7.5	10
2 weeks - Dough	0.881 ± 0.077^{ex}	0.819 ± 0.000^{ex}	1.794 ± 0.057^{dy}	3.140 ± 0.183^{cy}	5.263 ± 0.259^{by}	6.818 ± 0.098^{ay}
2 weeks - Crumb	0.943 ± 0.021^{ex}	0.943 ± 0.021^{ex}	2.967 ± 0.134^{dx}	4.868 ± 0.057^{cx}	7.214 ± 0.336^{bx}	8.991 ± 0.183^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

c. 6 weeks frozen storage	**150 µm Merlot Seed Flour Replacement (g GSF/100g Flour)**					
Frozen Storage	**Unfrozen-0**	**0**	**2.5**	**5**	**7.5**	**10**
6 weeks - Dough	0.930 ± 0.000^{ex}	0.942 ± 0.021^{ex}	2.103 ± 0.130^{dy}	3.881 ± 0.130^{cy}	5.238 ± 0.057^{by}	7.251 ± 0.057^{ay}
6 weeks - Crumb	0.967 ± 0.064^{ex}	0.979 ± 0.043^{ex}	2.956 ± 0.140^{dx}	4.979 ± 0.223^{cx}	6.645 ± 0.130^{bx}	8.189 ± 0.064^{ax}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 3.

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 3.

Table 20. The coefficients of linear correlation (r^2) between total phenolic content via Folin-Ciocalteu (TPC), total flavanol content via Vanillin-HCl (TFC), Trolox equivalent antioxidant capacity (TEAC), 2,2 diphenyl-1-picrylhydrazyl (DPPH), and ferric ion reducing antioxidant power (FRAP) assays of 70% ethanolic extracts of Merlot grape seed flour (GSF) containing (a) bread dough and (b) bread crumb.

a. GSF Containing Bread Dough				
	TFC	**TPC**	**DPPH**	**TEAC**
TPC	0.871		0.897	0.858
DPPH	0.815	0.897		0.846
TEAC	0.851	0.858	0.846	
FRAP	0.897	0.989	0.907	0.912

b. GSF Containing Bread Crumb				
	TFC	**TPC**	**DPPH**	**TEAC**
TPC	0.980		0.859	0.939
DPPH	0.841	0.859		0.796
TEAC	0.912	0.939	0.796	
FRAP	0.975	0.980	0.860	0.961

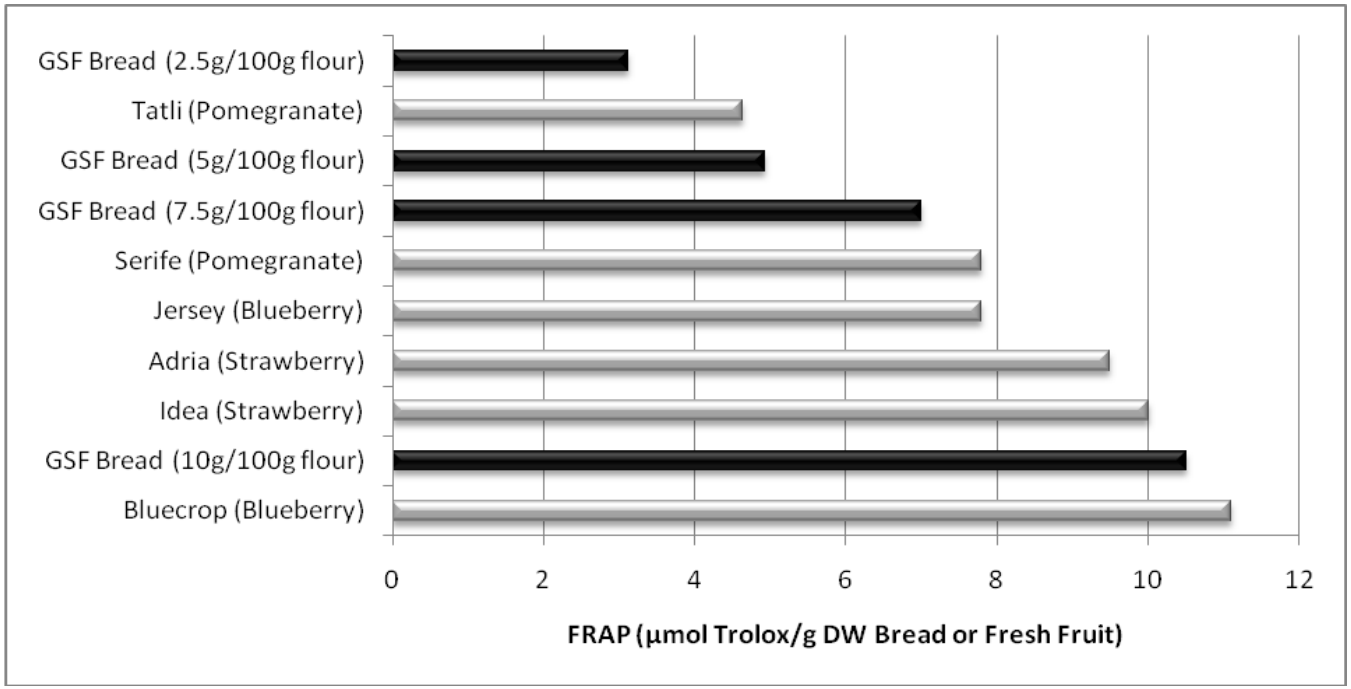

Figure 11. Comparison of antioxidant activity of Merlot grape seed flour (GSF)-fortified breads with different varieties of blueberry (Connor and others 2002), strawberry (Guo and others 2003), and pomegranate (Gil 2000). Results expressed as ferric ion reducing antioxidant power (FRAP) equivalents (µmol Trolox/g dry weight bread or fresh fruit).

Table 21. Volume measured by rapeseed displacement (expressed as cc) of Merlot grape seed flour (GSF) bread made from the frozen and unfrozen dough processes, with or without the replacement of GSF. Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Data in parentheses are percentages of the reduction in volume of bread versus the corresponding sample made by the unfrozen dough process.

	Bread with GSF replacement of wheat flour (cc)					
Frozen Storage	**Unfrozen - 0 g GSF/100 g Flour**	**0 g GSF/100 g Flour**	**2.5 g GSF/100 g Flour**	**5 g GSF/100 g Flour**	**7.5 g GSF/100 g Flour**	**10 g GSF/100 g Flour**
0 weeks	-	1007.5 ± 24.03^{ax}	936.7 ± 20.66 (7.0%)^{bx}	878.3 ± 45.35 (12.8%)^{cxy}	821.7 ± 34.45 (18.4%)^{dx}	745.0 ± 19.24 (26.1%)^{ex}
2 weeks	1067.5 ± 26.41^{ax}	1016.7 ± 62.82 (4.8%)^{bx}	915.0 ± 51.72 (14.3%)^{cx}	899.2 ± 59.95 (15.8%)^{cx}	820.0 ± 24.29 (23.2%)^{dx}	710.8 ± 37.74 (33.4%)^{exy}
6 weeks	1024.2 ± 21.54^{ax}	982.5 ± 54.20 (4.1%)^{ax}	908.3 ± 36.83 (11.3%)^{bx}	842.5 ± 44.13 (17.7%)^{cy}	750.0 ± 36.47 (26.8%)^{dy}	685.8 ± 16.56 (33.0%)^{ey}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 6.
$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$), n = 6.

Table 22. Comparison of Merlot grape seed flour (GSF) bread color intensity values as measured by L*, a*, and b* between sensory acceptance (expressed along a 7-point scale, where 1 = dislike extremely and 7=like extremely) and spectrophotometric measurement. Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Results are expressed as the mean determination followed by the standard deviation. For consumer evaluation of each bread at each storage time, n=87 for 0 and 2 weeks storage, and n=97 for 6 weeks of storage. For spectromphotometric measurements, n=3.

			Bread with GSF Replacement				
	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
Crumb darkness acceptance by consumers	0	-	5.1 ± 0.5^{ax}	5.3 ± 1.4^{ax}	5.2 ± 1.3^{ax}	4.9 ± 1.6^{ax}	4.8 ± 1.6^{ax}
	2	5.1 ± 1.6^{ax}	5.1 ± 1.5^{ax}	5.3 ± 1.1^{ax}	5.4 ± 1.3^{ax}	5.1 ± 1.3^{ax}	5.0 ± 1.5^{ax}
	6	5.2 ± 1.6^{ax}	5.1 ± 1.5^{ax}	5.2 ± 1.3^{ax}	4.9 ± 1.5^{abx}	4.9 ± 1.6^{abx}	4.5 ± 1.6^{bx}
Color values measured by spectrophotometer	Frozen Storage (weeks)						
*L**	0	-	82.75 ± 0.44^{ay}	62.33 ± 0.77^{by}	54.96 ± 0.84^{cx}	49.73 ± 0.97^{dx}	45.42 ± 0.51^{ey}
	2	84.27 ± 0.73^{ax}	82.15 ± 0.46^{by}	62.72 ± 0.99^{cxy}	55.75 ± 0.62^{dx}	50.58 ± 0.91^{ex}	46.35 ± 0.56^{fx}
	6	83.77 ± 1.14^{ax}	83.86 ± 1.11^{ax}	63.23 ± 0.99^{bx}	55.62 ± 0.88^{cx}	49.80 ± 0.83^{dx}	46.35 ± 0.56^{fx}
*a**	0	-	-1.73 ± 0.21^{ey}	7.12 ± 0.26^{dx}	10.21 ± 0.31^{cx}	11.65 ± 10.13^{bx}	12.76 ± 0.07^{ax}
	2	-2.09 ± 0.21^{fx}	-1.05 ± 0.29^{ez}	6.84 ± 0.14^{dy}	9.50 ± 0.19^{cz}	11.13 ± 0.19^{by}	12.09 ± 0.16^{ay}
	6	-2.21 ± 0.22^{ex}	-2.05 ± 0.17^{ex}	7.06 ± 0.17^{dx}	9.78 ± 0.10^{cy}	11.56 ± 0.21^{bx}	12.68 ± 0.22^{ax}
*b**	0	-	17.68 ± 0.59^{ay}	15.49 ± 0.24^{cx}	16.48 ± 0.31^{bx}	16.28 ± 0.21^{bx}	16.13 ± 0.09^{bx}
	2	18.30 ± 0.39^{ax}	17.79 ± 0.45^{bxy}	14.80 ± 0.17^{dy}	15.75 ± 0.14^{cz}	15.90 ± 0.20^{cy}	15.71 ± 0.22^{cy}
	6	18.00 ± 0.51^{ay}	18.02 ± 0.53^{ax}	15.36 ± 0.23^{cx}	16.13 ± 0.14^{by}	16.37 ± 0.35^{bx}	16.13 ± 0.33^{bx}

$^{a-f}$ Different letters within a row are significantly different ($P < 0.05$), n = 12.

$^{x-z}$ Different letters within a column are significantly different ($P < 0.05$).

Table 23. Consumer acceptance of Merlot grape seed flour (GSF) bread's appearance (expressed along a 7-point scale, where 1 = dislike extremely and 7=like extremely) and image analysis of crumb porosity including the number of cells per mm^2, mean cell diameter (mm), maximum and minimum cell diameter (mm). Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Results are expressed as the mean determination followed by the standard deviation. For consumer evaluation of each bread at each storage time, n=87 for 0 and 2 weeks storage, and n=97 for 6 weeks of storage. For cell measurements, n=3.

			Bread with GSF replacement of wheat flour				
	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
A: Results of sensory evaluation Porosity acceptance	0	-	5.3 ± 1.4^{ax}	5.4 ± 1.3^{ax}	5.3 ± 1.2^{ax}	5.2 ± 1.2^{ax}	5.4 ± 1.2^{ax}
	2	5.2 ± 1.4^{ax}	5.2 ± 1.3^{ax}	5.2 ± 1.3^{ax}	5.4 ± 1.3^{ax}	5.4 ± 1.2^{ax}	5.4 ± 1.2^{ax}
	6	5.2 ± 1.3^{ax}	5.1 ± 1.6^{ax}	5.3 ± 1.1^{ax}	5.0 ± 1.4^{ax}	5.3 ± 1.3^{ax}	5.0 ± 1.3^{ax}
B: Results of image analysis	Frozen Storage (weeks)						
Number of cells per mm^2	0	-	0.55 ± 0.09^{ey}	0.80 ± 0.11^{dy}	0.97 ± 0.09^{cz}	1.12 ± 0.08^{by}	1.21 ± 0.06^{ay}
	2	0.66 ± 0.06^{dx}	0.73 ± 0.26^{dx}	0.92 ± 0.12^{cx}	1.21 ± 0.11^{bx}	1.31 ± 0.04^{ax}	1.36 ± 0.05^{ax}
	6	0.63 ± 0.06^{dx}	0.68 ± 0.04^{dx}	0.87 ± 0.13^{cx}	1.14 ± 0.07^{by}	1.29 ± 0.05^{ax}	1.32 ± 0.09^{ax}
Mean cell diameter, mm	0	-	0.49 ± 0.09^{ay}	0.38 ± 0.02^{dx}	0.42 ± 0.02^{bx}	0.41 ± 0.02^{bcx}	0.39 ± 0.03^{cdx}
	2	0.52 ± 0.02^{bx}	0.54 ± 0.08^{ax}	0.39 ± 0.02^{cx}	0.39 ± 0.02^{cy}	0.36 ± 0.02^{dy}	0.34 ± 0.01^{ey}
	6	0.46 ± 0.04^{ey}	0.47 ± 0.03^{az}	0.37 ± 0.02^{cx}	0.42 ± 0.02^{bx}	0.38 ± 0.02^{cy}	0.35 ± 0.02^{dy}
Min cell diameter, mm	0	-	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}
	2	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}
	6	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}	0.01 ± 0.00^{ax}
Max cell diameter, mm	0	-	6.49 ± 3.38^{ay}	3.43 ± 0.81^{cy}	5.13 ± 1.99^{bx}	4.35 ± 1.33^{bcx}	3.48 ± 0.77^{cx}
	2	7.27 ± 1.73^{ax}	8.23 ± 2.08^{ax}	4.42 ± 1.06^{cdxy}	5.70 ± 2.32^{bx}	4.56 ± 1.21^{bcx}	3.24 ± 1.20^{dx}
	6	7.66 ± 3.02^{ax}	5.93 ± 0.74^{by}	5.03 ± 1.32^{bcx}	5.29 ± 2.02^{bcx}	4.23 ± 1.67^{cdx}	3.49 ± 0.99^{dx}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 12.
$^{x-z}$ Different letters within a column are significantly different ($P < 0.05$).

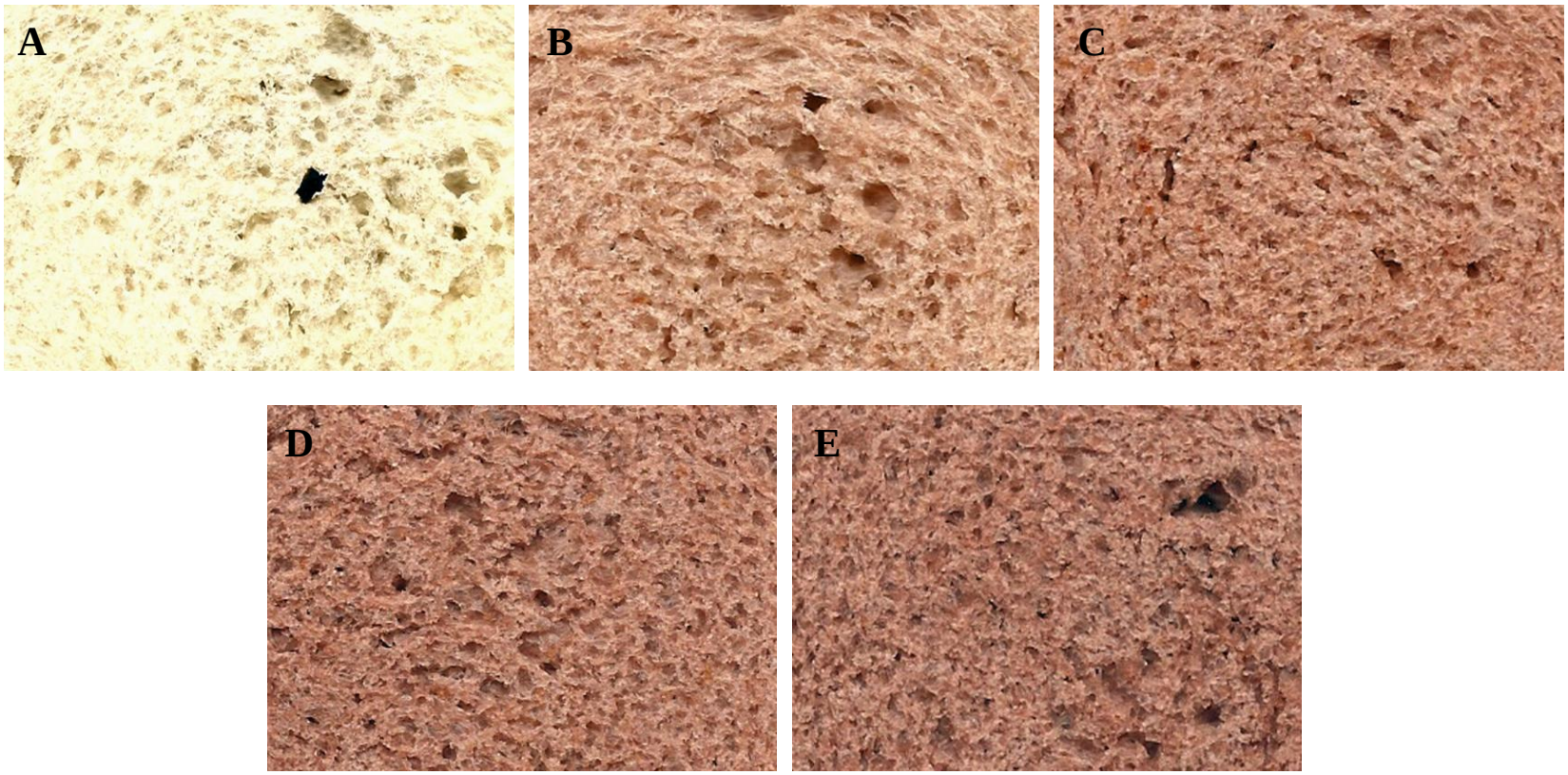

Figure 12. Crumb images of the center slices of unfrozen breads made using Merlot grape seed flour (GSF). The GSF bread was using increasing replacement of wheat flour with GSF: (a) Control bread (0 g GSF/100 g flour), (b) bread with 2.5 g GSF/100 g flour, (c) bread with 5 g GSF/100 g flour, (d) bread with 7.5 g GSF/100 g flour, and (e) bread with 10 g GSF/100g flour.

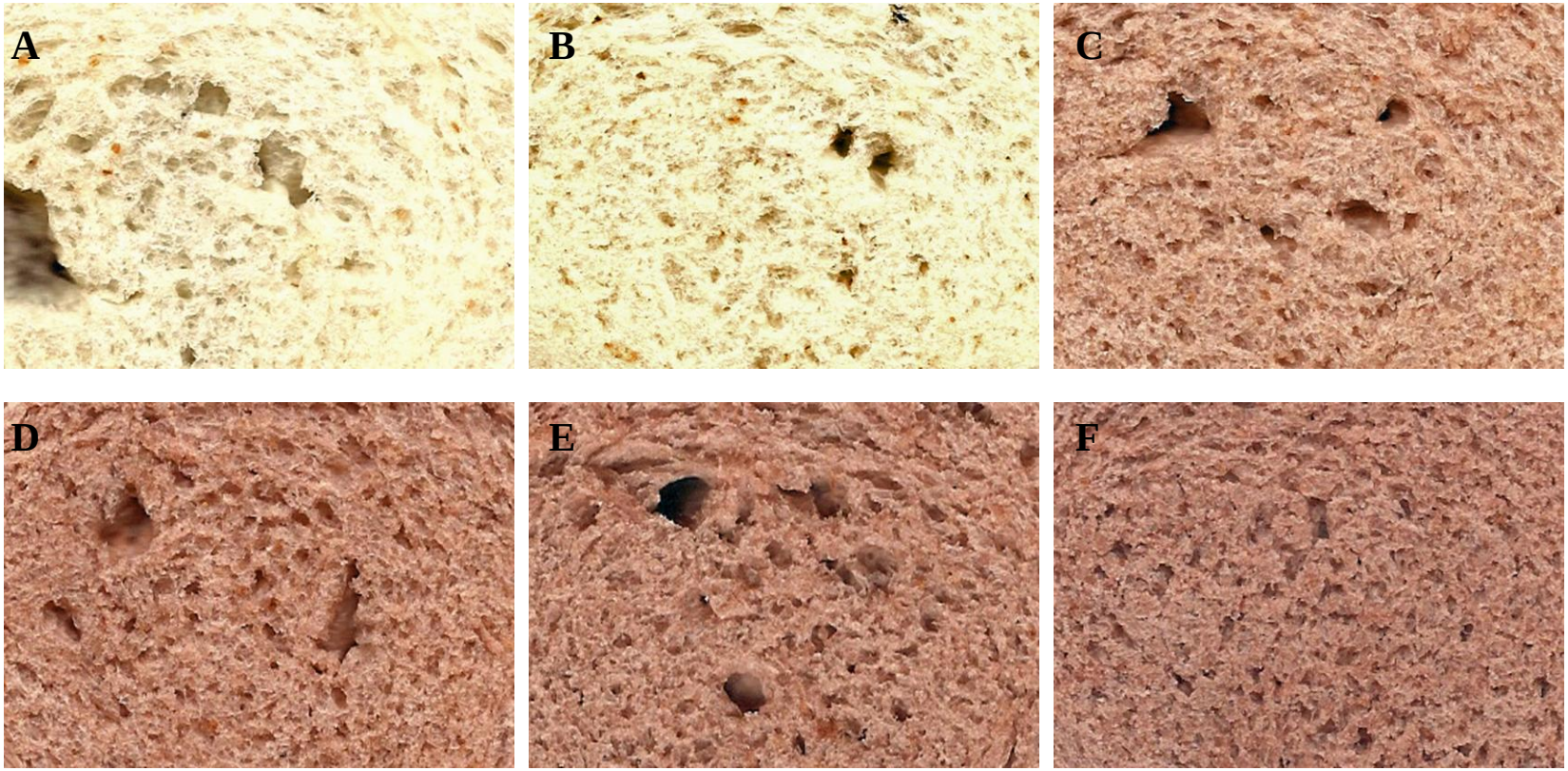

Figure 13. Crumb images of the center slices of breads made using Merlot grape seed flour (GSF) stored frozen for two weeks. The GSF bread was using increasing replacement of wheat flour with GSF: (a) Control bread (0 g GSF/100 g flour), (b) bread with 0 g GSF/100g flour, (c) bread with 2.5 g GSF/100 g flour, (d) bread with 5 g GSF/100 g flour, (e) bread with 7.5 g GSF/100 g flour, and (f) bread with 10 g GSF/100g flour.

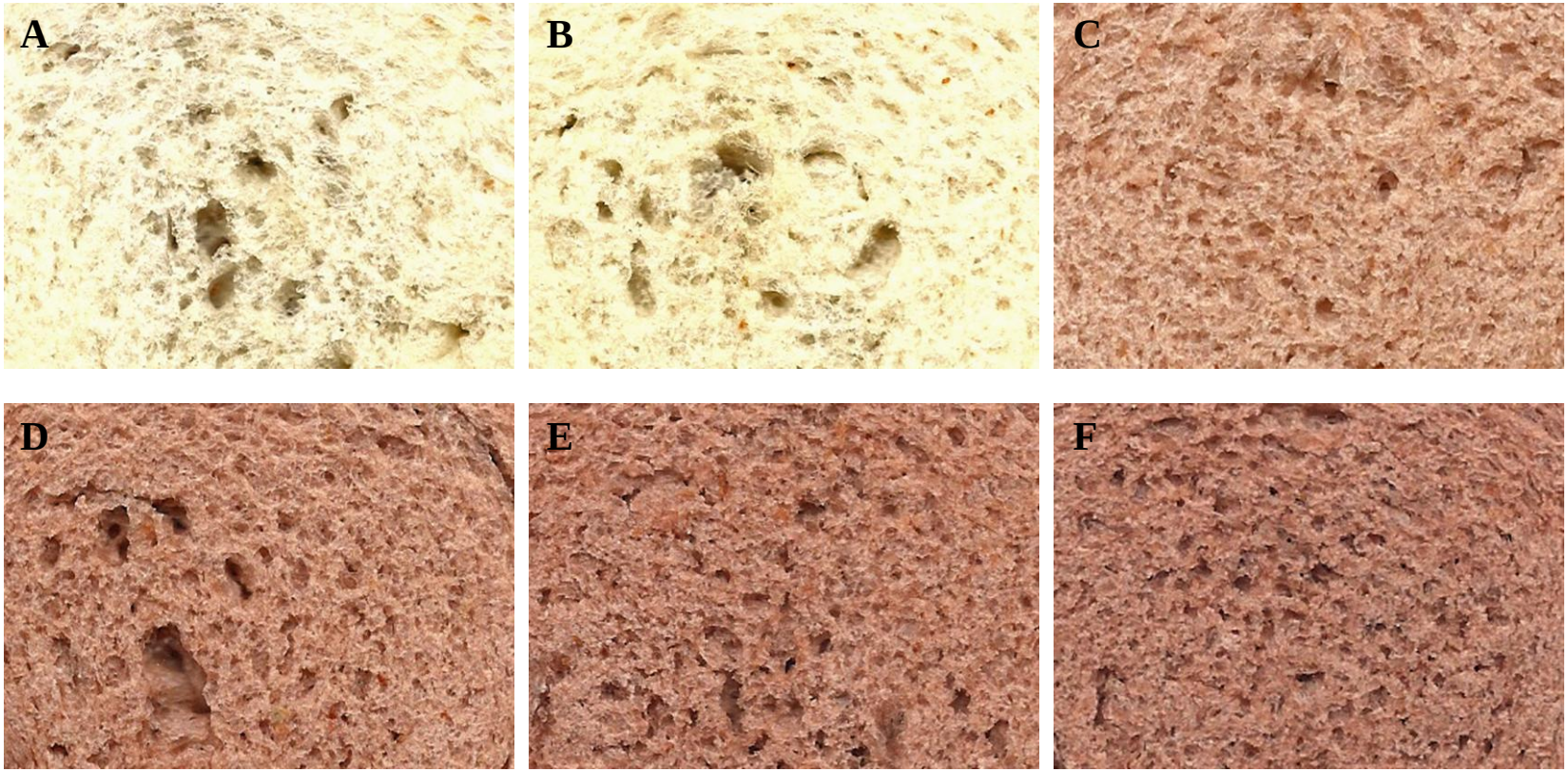

Figure 14. Crumb images of the center slices of breads made using Merlot grape seed flour (GSF) stored frozen for six weeks. The GSF bread was using increasing replacement of wheat flour with GSF: (a) Control bread (0 g GSF/100 g flour), (b) bread with 0 g GSF/100g flour, (c) bread with 2.5 g GSF/100 g flour, (d) bread with 5 g GSF/100 g flour, (e) bread with 7.5 g GSF/100 g flour, and (f) bread with 10 g GSF/100g flour.

Table 24. Consumer acceptance of the grape seed flour (GSF) bread's hardness (expressed along a 7-point scale, where 1 = dislike extremely and 7=like extremely) and texture profile analysis (TPA) of firmness (expressed in g). Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Results are expressed as the mean determination followed by the standard deviation. For consumer evaluation of each bread at each storage time, n=87 for 0 and 2 weeks storage, and n=97 for 6 weeks of storage. For firmness evaluations, n=3 measurements.

			Bread with GSF replacement of wheat flour				
	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
A: Results of sensory evaluation Hardness acceptance	0	-	5.0 ± 1.3^{ax}	5.3 ± 1.4^{ax}	5.2 ± 1.3^{ax}	5.1 ± 1.3^{ax}	5.1 ± 1.5^{ax}
	2	5.0 ± 1.6^{ax}	5.2 ± 1.5^{ax}	5.3 ± 1.4^{ax}	5.2 ± 1.4^{ax}	5.1 ± 1.4^{ax}	5.1 ± 1.3^{ax}
	6	5.1 ± 1.5^{ax}	5.2 ± 1.4^{ax}	5.2 ± 1.5^{ax}	4.9 ± 1.5^{ax}	5.0 ± 1.3^{ax}	5.0 ± 1.4^{ax}
	Frozen Storage (weeks)						
B: Results of TPA Firmness (*g*)	0	-	212.90 ± 27.5^{cy}	230.34 ± 24.1^{cy}	248.06 ± 31.67^{cy}	326.56 ± 64.6^{bz}	437.88 ± 57.64^{az}
	2	247.77 ± 36.0^{ex}	310.63 ± 23.0^{dx}	338.76 ± 24.9^{dx}	420.20 ± 39.1^{cx}	515.20 ± 29.4^{bx}	661.03 ± 46.4^{ax}
	6	198.92 ± 14.2^{dx}	199.89 ± 18.9^{dy}	273.56 ± 30.9^{cy}	293.50 ± 56.6^{cy}	407.53 ± 90.7^{by}	579.98 ± 36.9^{ay}

$^{a-e}$ Different letters within a row are significantly different ($P < 0.05$), n = 12.
$^{x-z}$ Different letters within a column are significantly different ($P < 0.05$).

Table 25. Consumer acceptance of grape seed flour (GSF) bread's sweetness (a), astringency (b), and bitterness (c). Acceptance was evaluated along a 7-pt hedonic scale where 1 = dislike extremely and 7=like extremely. Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Results are expressed as the mean determination followed by the standard deviation. For consumer evaluation of each bread at each storage time, n=87 for 0 and 2 weeks storage, and n=97 for 6 weeks of storage.

a. Sweetness	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	Bread with GSF replacement of wheat flour				
			0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
Sweetness Acceptance by Consumers	0	-	5.2 ± 1.5^{abx}	5.3 ± 1.2^{ax}	5.3 ± 1.2^{ax}	5.2 ± 1.3^{abx}	4.9 ± 1.4^{bx}
	2	5.1 ± 1.4^{ax}	5.2 ± 1.5^{ax}	5.1 ± 1.3^{ax}	5.3 ± 1.1^{ax}	5.3 ± 1.2^{ax}	5.2 ± 1.3^{ax}
	6	5.1 ± 1.2^{abcx}	5.4 ± 1.3^{abx}	5.5 ± 1.2^{ax}	5.3 ± 1.1^{abcx}	5.0 ± 1.2^{bcx}	4.9 ± 1.3^{cx}
b. Astringency	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	Bread with GSF replacement of wheat flour				
			0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
Astringency Acceptance by Consumers	0	-	4.9 ± 1.3^{abcx}	5.0 ± 1.2^{ax}	4.9 ± 1.1^{abx}	4.5 ± 1.3^{bcx}	4.5 ± 1.3^{cxy}
	2	4.6 ± 1.3^{ax}	4.7 ± 1.4^{ax}	4.6 ± 1.4^{ax}	4.9 ± 1.1^{ax}	4.7 ± 1.2^{ax}	4.8 ± 1.3^{ax}
	6	4.9 ± 1.3^{ax}	4.6 ± 1.5^{abcx}	4.8 ± 1.2^{abx}	4.6 ± 1.1^{abcx}	4.5 ± 1.3^{bcx}	4.3 ± 1.4^{cy}
c. Bitterness	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	Bread with GSF replacement of wheat flour				
			0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
Bitterness Acceptance by Consumers	0	-	4.8 ± 1.4^{ax}	4.9 ± 1.2^{ax}	4.8 ± 1.2^{ax}	4.8 ± 1.3^{ax}	4.5 ± 1.3^{ax}
	2	4.8 ± 1.3^{ax}	4.6 ± 1.4^{ax}	4.6 ± 1.4^{ax}	4.8 ± 1.2^{ax}	4.7 ± 1.2^{ax}	4.6 ± 1.4^{ax}
	6	4.7 ± 1.3^{ax}	4.7 ± 1.5^{abx}	4.8 ± 1.2^{ax}	4.7 ± 1.3^{abx}	4.5 ± 1.3^{abx}	4.3 ± 1.4^{bx}

$^{a-c}$ Different letters within a row are significantly different ($P < 0.05$).

$^{x-z}$ Different letters within a column are significantly different ($P < 0.05$).

Table 26. Overall consumer acceptance of the grape seed flour (GSF) bread made from frozen and unfrozen dough. Acceptance was evaluated along a 7-pt hedonic scale where 1 = dislike extremely and 7=like extremely. Merlot bread was made at replacement levels of 0, 2.5, 5, 7.5 and 10 g GSF/100 g wheat flour. Results are expressed as the mean determination followed by the standard deviation. For consumer evaluation of each bread at each storage time, n=87 for 0 and 2 weeks storage, and n=97 for 6 weeks of storage.

	Frozen Storage (weeks)	Unfrozen 0 g GSF/100 g Flour	Bread with GSF replacement of wheat flour				
			0 g/100 g flour	2.5 g/100 g flour	5 g/100 g flour	7.5 g/100g flour	10 g/100 g flour
Overall Acceptance by consumers	0	-	5.1 ± 1.5^{abx}	5.3 ± 1.2^{abx}	5.4 ± 1.1^{ax}	5.1 ± 1.3^{abx}	4.8 ± 1.4^{bx}
	2	5.0 ± 1.5^{ax}	5.1 ± 1.5^{ax}	5.0 ± 1.4^{ax}	5.2 ± 1.2^{axy}	5.2 ± 1.3^{ax}	4.8 ± 1.4^{ax}
	6	4.9 ± 1.5^{ax}	5.0 ± 1.5^{ax}	5.1 ± 1.4^{ax}	4.9 ± 1.3^{ay}	4.9 ± 1.4^{ax}	4.7 ± 1.4^{ax}

$^{a-b}$ Different letters within a row are significantly different ($P < 0.05$).

$^{x-y}$ Different letters within a column are significantly different ($P < 0.05$).

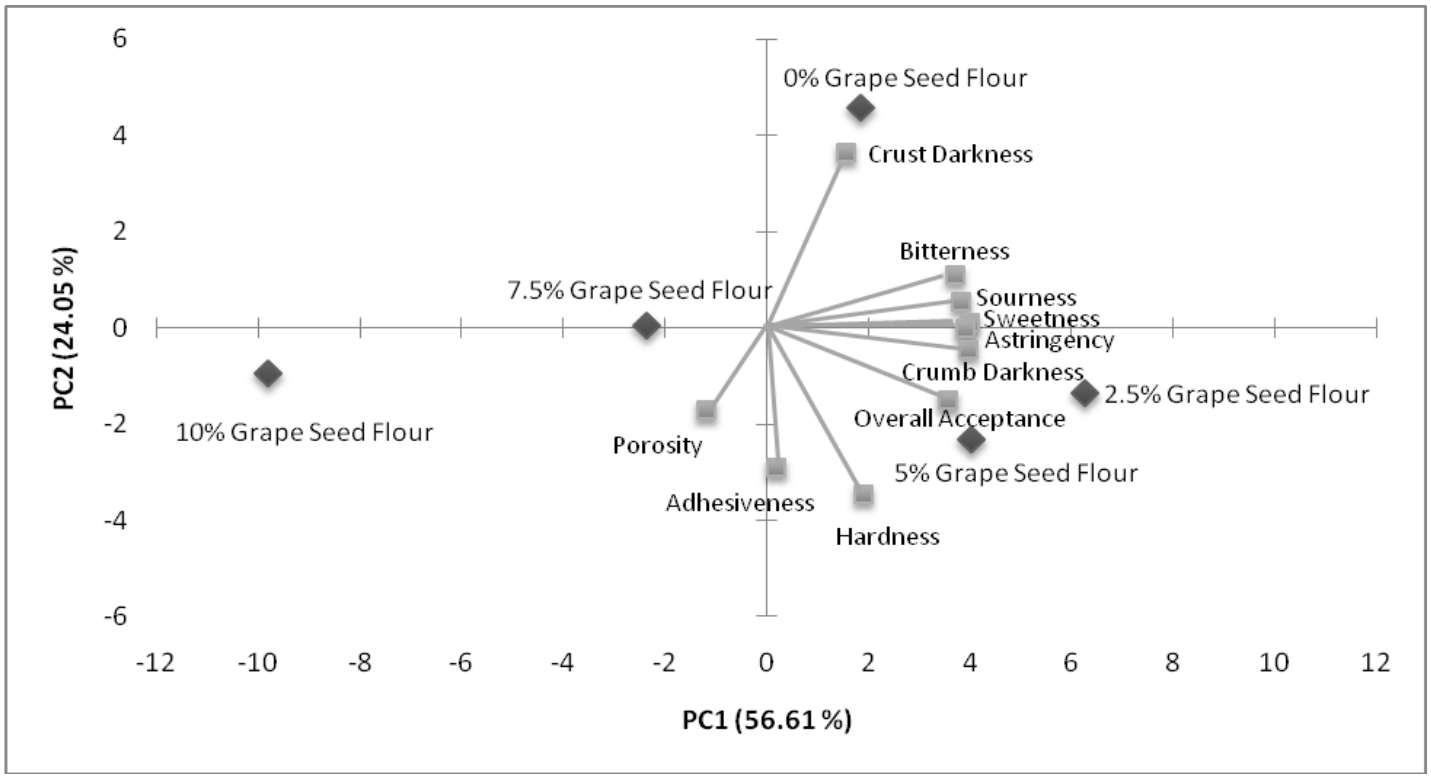

Figure 15. Biplot of the principal component analysis of unfrozen grape seed flour (GSF) bread prepared using different replacement of white wheat flour with GSF (0, 2.5, 5, 7.5 and 10%). The attributes evaluated by the consumers included acceptance of appearance (crust darkness, crumb darkness), texture (hardness, adhesiveness, porosity), taste and flavor (sweetness, sourness, astringency) and overall acceptance. PC1 accounted for 56.6% of the variation in the data while PC2 acounted for 24.1% of the variation in the data.

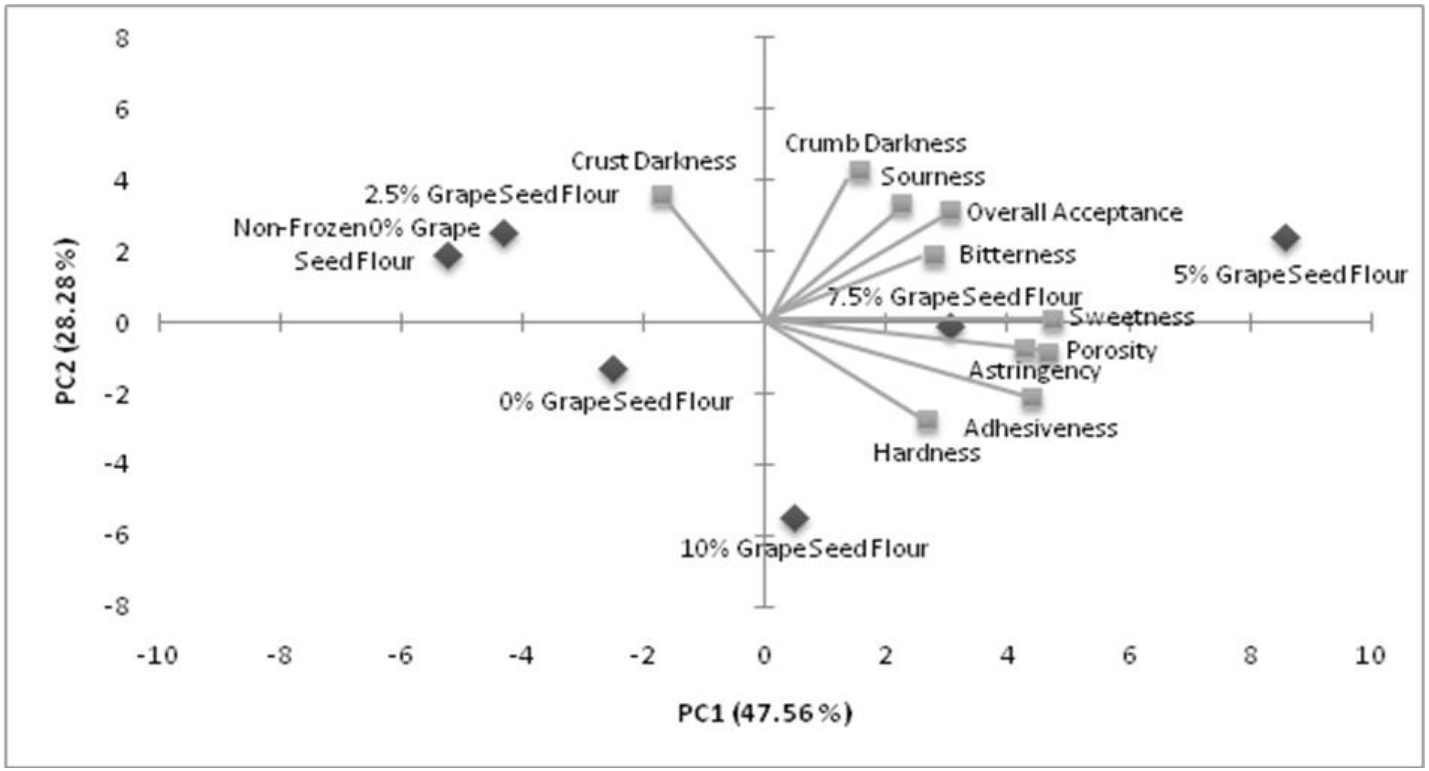

Figure 16. Biplot of the principal component analysis of 2 week frozen storage grape seed flour (GSF) bread prepared using different replacement of white wheat flour with GSF (0, 2.5, 5, 7.5 and 10%). The attributes evaluated by the consumers included acceptance of appearance (crust darkness, crumb darkness), texture (hardness, adhesiveness, porosity), taste and flavor (sweetness, sourness, astringency) and overall acceptance. PC1 accounted for 47.6% of the variation in the data while PC2 acounted for 28.3% of the variation in the data.

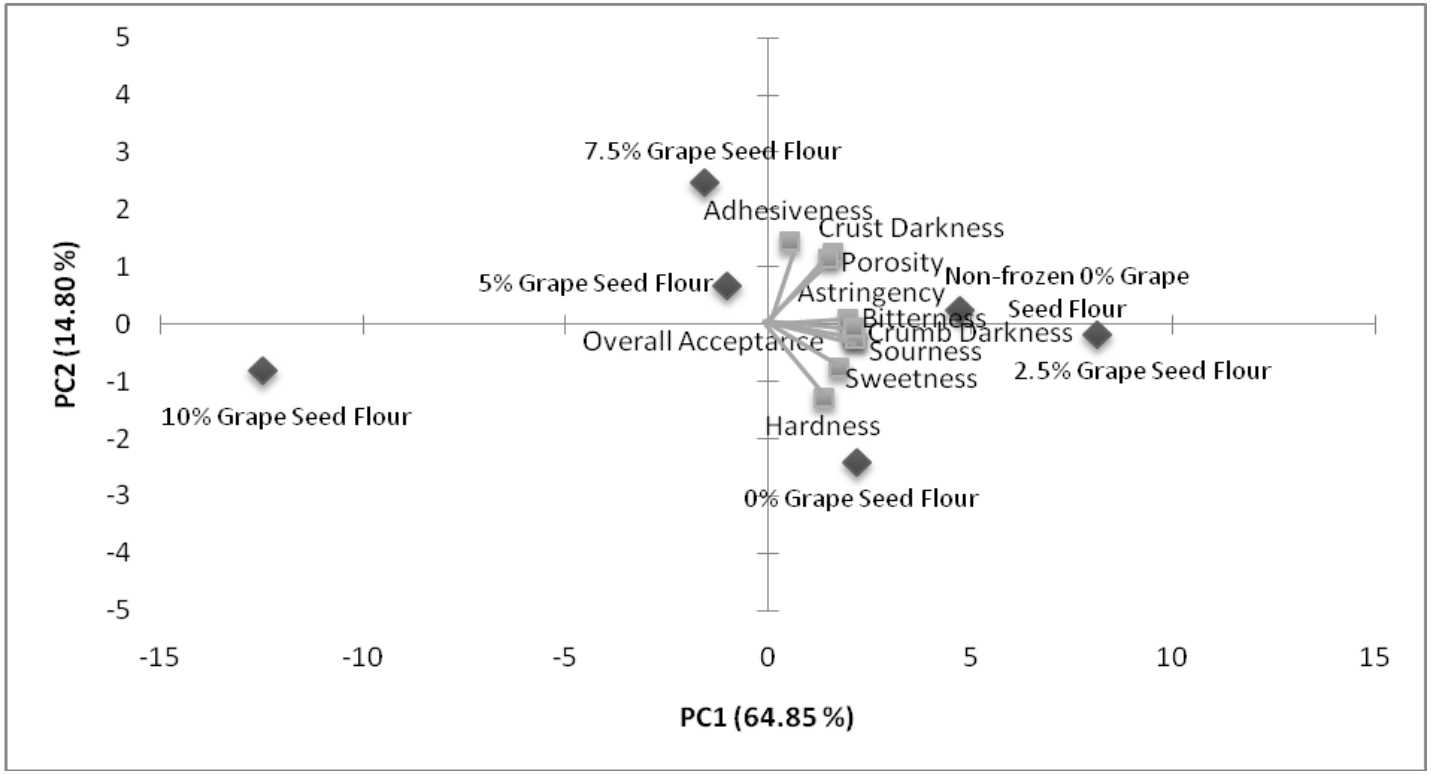

Figure 17. Biplot of the principal component analysis of 6 week frozen storage grape seed flour (GSF) bread prepared using different replacement of white wheat flour with GSF (0, 2.5, 5, 7.5 and 10%). The attributes evaluated by the consumers included acceptance of appearance (crust darkness, crumb darkness), texture (hardness, adhesiveness, porosity), taste and flavor (sweetness, sourness, astringency) and overall acceptance. PC1 accounted for 64.9% of the variation in the data while PC2 acounted for 14.8% of the variation in the data.

LIST OF REFERENCES

AACC. 2000. Approved Methods of the AACC,10th ed. St. Paul, MN: The Association.

Aamodt A, Magnus EM, Faergestad EM. 2005. Hearth bread characteristics: Effect of protein quality, protein content, whole meal flour, DATEM, proving time, and their interactions. Cereal Chem. 82(3):290-301.

Adams MR, Kinlay S, Blake GJ, Orford JL, Ganz P, Selwyn AP. 2000. Atherogenic lipids and endothelial dysfunction: Mechanisms in the genesis of ischemic syndromes. Ann. Rev. Med. 51:149-167.

Ahn WS, Yoo J, Huh SW. 2003. Protective effects of green tea extracts (polyphenol EGC and EGCG) on human cervical lesions. Eur. J. Cancer Prev. 12:383-390.

Anderson HV, McNatt J, Clubb FJ, Herman M, Maffrand JP, DeClerk F, Ahn C, Buja LM, Willerson JT. 2001. Platelet inhibition reduces cyclic flow variations and neointimal proliferation in normal and hypercholesterolemic-atherosclerotic canine coronary arteries. Circulation 104:2331-2337.

Araim O, Ballantyne J, Waterhouse AL, Sumpio BE. 2002. Inhibition of vascular smooth muscle cell proliferation with red wine and red wine polyphenols. J. Vasc. Surg. 35:1226-1232.

Araya J, Rodrigo R, Orellana M, Rivera G. 2001. Red wine raises plasma HDL and preserves long-chain polyunsaturated fatty acids in rat kidney and erythrocytes. Br. J. Nutr. 86:189-195.

Aron PM, Kennedy JA. 2008. Flavan-3-ols: Nature, occurrence and biological activity. Mol. Nutr. Food Res. 52(1):79-104.

Arteel GE, Sies H. 1999. Protection against peroxynitrite by cocoa polyphenol oligomers. Febs Lett. 462(1-2):167-170.

Baardseth P, Kvaal K, Lea P, Ellekjaer MR, Faergestad EM. 2000. The effects of bread making process and wheat quality on French baguettes. J. Cereal Sci. 32(1):73-87.

Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG. 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. p 187-197.

Baker J, Bridle P, Garciaviguera C, Kallithraka S. 1995. Survey of solvents for the extraction of grape seed phenolics. Phytochem. Anal. 6:265-267.

Bao Y, Fenwick R. 2004. Phytochemicals in Health and Disease. New York, NY: Marcel Dekker, Inc. 346 p.

Barnes PJ. 1989. Wheat in Milling and Baking. In: Palmer GH, editor. Cereal Science and Technology. Aberdeen, Great Britain: Aberdeen University Press. p 367-412.

Beecher GR. 2004. Proanthocyanidins: Biological activities associated with human health. Pharm. Biol. 42:2-20.

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. 239(1):70-76.

Benzie IFF, Szeto YT. 1999. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J. Agric. Food Chem. 47(2):633-636.

Bergqvist J, Dokoozlian N, Ebisuda N. 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin Valley of California. Am. J. Enol. Vitic. 52:1-7.

Bhattacharya M, Langstaff TM, Berzonsky WA. 2003. Effect of frozen storage and freeze-thaw cycles on the rheological and baking properties of frozen doughs. Food Res. Int. 36(4):365-372.

Bianco A, Uccella N. 2000. Biophenolic components of olives. Food Res. Int. 33(6):475-485.

Bjornholm S. 2007. Varieties. Seattle, WA.

Block G, Patterson B, Subar A. 1992. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer. 18:1-29.

Bonilla F, Mayen M, Merida J, Medina M. 1999. Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. Food Chem. 66(2):209-215.

Bors W, Michel C. 1999. Antioxidant capacity of flavanols and gallate esters: Pulse radiolysis studies. Free Radic. Biol. Med. 27(11-12):1413-1426.

Bourne M. 2006. Texture Profile Analysis. Hamilton, MA: Texture Technologies.

Brandwilliams W, Cuvelier ME, Berset C. 1995. Use of a Free-Radical Method to Evaluate Antioxidant Activity. Food Sci. Technol.-Lebensm.-Wiss. Technol. 28(1):25-30.

Bravo L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:317-333.

Cadenas E, Packer L. 1999. Understanding the process of aging. New York, NY: CRC Press. 366 p.

Campbell GM. 2003. Bread Aeration. Cornwall: CRC Press. 374 p.

Cano A, Alcaraz O, Acosta M, Arnao MB. 2002. On-line antioxidant activity determination: comparison of hydrophilic and lipophilic antioxidant activity using the ABTS+ assay. Redox Report. 7:103-109.

Cao G, Alessio HM, Cutler RG. 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic. Biol. Med. 14(303-311).

Cao G, Prior RL. 1999. Measurement of oxygen radical absorbance capacity in biological samples. Methods Enzymol. 299:50-62.

Cheng ZH, Moore J, Yu LL. 2006. High-throughput relative DPPH radical scavenging capacity assay. J. Agric. Food Chem. 54(20):7429-7436.

Cheynier V, Rigaud J. 1986. HPLC separation and characterization of flavonols in the skin of *Vitis vinifera* var. Cinsault. Am. J. Enol. Vitic. 37:248-252.

Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. 1998. Tannins and human health: a review. Crit. Rev. Food Sci. Nutr. 38:421-464.

Clifford MN, Scalbert A. 2000. Ellagitannins - nature, occurrence and dietary burden. J. Food Sci. Agric. 80:1118-1125.

Connor AM, Luby JJ, Hancock JF, Berkheimer S, Hanson EJ. 2002. Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage. J. Agric. Food Chem. 50(4):893-898.

Cook N, Samman S. 1996. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem. 7:66-76.

Dick JW, Youngs VL. 1988. Evaluation of durum wheat, semolina, and pasta in the United States. In: Fabriani G, Lintas C editors. Durum Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemistry. p 237-248.

Djuric Z, Depper JB, Uhley V. 1998. Oxidative DNA damage levels in blood from women at high risk for breast cancer are associated with dietary intakes of meats, vegetables, and fruits. J. Am. Diet Assoc. 98:524-528.

Dong W, Hoseney RC. 1995. Effect of certain breadmaking oxidants and reducing agents on dough rheological properties. Cereal Chem. 72:58-64.

Drobna Z, Wismer WV, Goonewardene LA. 2004. Selection of an astringency reference standard for the sensory evaluation of black tea. J. Sens. Stud. 19:119-132.

Droge W. 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82(1):47-95.

Esteller MS, Zancanaro O, Palmeira CNS, da Silva Lannes SC. 2006. The effect of kefir addition on microstructure parameters and physical properties of porous white bread. Eur. Food Res. Technol. 222(1-2):26-31.

FAOSTAT. 2008. FAO Statistical Database.

Figueroa-Romero C, Sadidi M, Feldman EL. 2008. Mechanisms of disease: The oxidative stress theory of diabetic neuropathy. Reviews in Endocrine & Metabolic Disorders 9(4):301-314.

Finney KF. 1945. Methods of estimating and the effect of variety and protein level on the baking absorption of flour. Cereal Chem. 22:149-158.

Finney KF, Barmore MA. 1943. Yeast variability in wheat variety test baking. Cereal Chem. 20:194-200.

Finney KF, Barmore MA. 1945a. Optimum vs. fixed mixing time at various potassium bromate levels in experimental bread baking. Cereal Chem. 22:244-254.

Finney KF, Barmore MA. 1945b. Varietal responses to certain baking ingredients essential in evaluating the protein quality of hard winter wheats. Cereal Chem. 22:225-243.

Finney KF, Shogren MD. 1972. A Ten-Gram mixograph for determining and predicting functional properties of wheat flours. Bakers Digest. 46(2):32.

Finney PL, Magoffin CD, Hoseney RC, Finney KF. 1976. Short-time baking systems. I. Interdependence of yeast concentration, fermentation, time and oxidation requirement. Cereal Chem. 53:126-134.

Fitzpatrick DF, Bing B, Maggi DA, Fleming RC, O'Malley RM. 2002. Vasodilating procyanidins derived from grape seeds. Ann. NY Acad. Sci. 957:78-89.

Folin O. 1927. Tyrosine and tryptophan determinations in proteins. J. Biol. Chem. 73:649-672.

Frankel EN, Meyer AS. 2000. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. Sci. Food Agric. 80(13):1925-1941.

Freedman JE, Keaney JF. 1999. Nirtic oxide and superoxide detection in human platelets. Methods Enzymol. 301:61-70.

Fukumoto LR, Mazza G. 2000. Assessing antioxidant and prooxidant activities of phenolic compounds. J. Agric. Food Chem. 48(8):3597-3604.

Gale. 2009. Retail Bakeries. In: Group G, editor. Encyclopedia of American Industries. Online Edition. Farmington Hills, Mich: Business and Company Resource Center.

Galet P. 2002. Identifying . . . the grape bunches. In: Montalbetti C, editor. Hachette Wine Libraries Grape Varieties. London: Cassell Illustrated. p 32-35.

Gil MI. 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 48:4581-4589.

Gil MI, Tomas-Barberan FA, Hess-Pierce B, Kader AA. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. J. Agric. Food Chem. 50(17):4976-4982.

Gonthier MP, Donovan JL, Texier O, Felgines C. 2003. Metabolism of dietary procyanidins in rats. Free Radic. Biol. Med. 35:837-844.

Goode J. 2005. The Biology of the Grape Vine. In: Lumsden H, editor. The Science of Wine: From Vine to Glass. London: Octopus Publishing Group. p 22-23.

Guendez R, Kallithraka S, Makris DP, Kefalas P. 2005. Determination of low molecular weight polyphenolic constituents in grape (Vitis vinifera sp.) seed extracts: Correlation with antiradical activity. Food Chem. 89(1):1-9.

Gunckel S, Santander P, Cordano G, Ferreira J, Munoz S, Nunez-Vergara LJ, Squella JA. 1998. Antioxidant activity of gallates: an electrochemical study in aqueous media. Chem. Biol. Interact. 114:45-59.

Guo CJ, Yang JJ, Wei JY, Li YF, Xu J, Jiang YG. 2003. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr. Res. 23(12):1719-1726.

Halliwell B, Gutteridge JMC, Cross CE. 1992. Free radicals, antioxidants, and human disease. Nutr. Today. 37:103-109.

Hang YD, Woodams EE. 2008. Methanol content of grappa made from New York grape pomace. Bioresour. Technol. 99(9):3923-3925.

Harborne JB, Baxter H, Moss GP. 1999. Phytochemical dictionary: Handbook of bioactive compounds from plants. 2nd ed. London: Taylor & Francis. 976 p.

Heionen IM, Meyer AS, Frankel EN. 1998. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. J. Agric. Food Chem. 46:4107-4112.

Henderson JP, Rex D. 2007. What is Wine? In: Serenka R, editor. About Wine. Clifton Park, NY: Thomson Delmar Learning. p. 562.

Hertog MG, Bueno-de-Mesquita HB, Fehily AM. 1996. Fruit and vegetable consumption and cancer mortality in the Caerphilly study. Cancer Epidemiol. Biomarkers Prev. 5:673-677.

Hertog MG, Hollman PCH, Katan MB. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commobnly consumed in the Netherlands. J. Agric. Food Chem. 40:2379-2383.

Hollman PCH, Arts ICW. 2000. Flavonols, flavones and flavanols - nature, occurrence and dietary burden. J. Sci. Food Agric. 80(7):1081-1093.

Howard A, Chopra M, Thurnham D. 2002. Red wine consumption and inhibition of LDL oxidation: what are the important components? Med Hypotheses. 59:101-104.

Hsu CL, Hurang SL, Chen WL, Weng YM, Tseng CY. 2004. Qualities and antioxidant properties of bread as affected by the incorporation of yam flour in the formulation. Int. J. Food Sci. Technol. 39(2):231-238.

Hsu K, Hoseney R, Seib S. 1979. Frozen dough I. Factors affecting the stability of yeasted doughs. Cereal Chem. 56:419-424.

Huang DJ, Ou BX, Hampsch-Woodill M, Flanagan JA, Prior RL. 2002. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate flourescence reader in 96-well format. J. Agric. Food Chem. 50(16):4437-4444.

Huang DJ, Ou BX, Prior RL. 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53(6):1841-1856.

Inbar Y, Chen Y, Hadar Y, Verdonck O. 1988. Composting of Agricultural Wastes for Their Use as Container Media - Simulation of the Composting Process. Biological Wastes. 26(4):247-259.

Inoue Y, Bushuk W. 1991. Studies on Frozen Doughs. 1. Effects of Frozen Storage and Freeze-Thaw Cycles on Baking and Rheological Properties. Cereal Chem. 68(6):627-631.

Inoue Y, Bushuk W. 1992. Studies on Frozen Doughs. 2. Flour Quality Requirements for Bread Production from Frozen Dough. Cereal Chem. 69(4):423-428.

Inoue Y, Sapirstein HD, Takayanagi S, Bushuk W. 1994. Studies on Frozen Doughs. 3. Some Factors Involved in Dough Weaking During Frozen Storage and Thaw-Freeze Cycles. Cereal Chem. 71(2):118-121.

Israilides C, Smith A, Harthill J, Barnett C, Bambalov G, Scanlon B. 1998. Pullulan content of the ethanol precipitate from fermented agro-industrial wastes. Appl. Microbiol. Biotechnol. 49:613-617.

IWSR. 2009. U.S. Poised to Become World's Largest Wine-Consuming Market. VINEXPO. New York, NY.

Jarvill-Taylor KJ, Anderson RA, Graves DJ. 2001. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. J. Am. Coll. Nutr. 20:327-336.

Jeong SM, Kim SY, Kim DR, Jo SC, Nam KC, Ahn DU. 2004. Effect of heat treatment on antioxidant activity of citrus peels. J. Agric. Food Chem. 52:3389-3393.

Kallithraka S, Bakker J, Clifford MN, Vallis L. 2001. Correlations between saliva protein composition and some T-1 parameters of astringency. Food Qual. Preference. 12:145-152.

Kalt W. 2005. Effects of production and processing factors on major fruit and vegetable antioxidants. J. Food Sci. 70(1):R11-R19.

Katalinic V, Milos M, Kulisic T, Jukic M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94:550-557.

Keller G. 2009. World wine consumption falls for the first time in years. The Huffington Post.

Kemberling JK, Yamamoto T, Hara A. 2000. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer Lett. 157:15-21.

Kenny S, Wehrle K, Denehy T, Arendt EK. 1999. Correlation between empiracle and fundamental rheology measurements and baking performance of frozen bread dough. Cereal Chem. 76:421-425.

Khan A, Safdar M, Khan A, Khattak KN, Anderson RA. 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 26:3215-3218.

Khanal RC, Howard LR, Prior RL. 2009. Procyanidin Content of Grape Seed and Pomace and Total Anthocyanin Content of Grape Pomace as Affected by Extrusino Processing. J. Food Sci. 74:174-183.

Kielhorn S, Thorngate JH. 1999. Oral sensations associated with the flavan-3-ols (+)-catechin and (-)-epicatechin. Food Qual. Preference. 10(2):109-116.

Kim SY, Jeong SM, Park WP, Nam KC, Ahn DU, Lee SC. 2006. Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts. Food Chem. 97(3):472-479.

Klatsky AL. 1996. Alcohol, coronary disease, and hypertension. Annu. Rev. Med. 47:149-160.

Lafka TI, Sinanoglou V, Lazos ES. 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chem. 104(3):1206-1214.

Larrauri JA, Ruperez P, SauraCalixto F. 1997. Effect of drying temperature on the stability of polyphenols and antioxidant activity of red grape pomace peels. J. Agric. Food Chem. 45(4):1390-1393.

Lee SC, Kim JH, Jeong SM, Kim DR, Ha JU, Nam KC. 2003. Effect of far-infrared radiation on the antioxidant activity of rice hulls. J. Agric. Food Chem. 51:4400-4403.

Lemanska K, Szymusiak H, Tyrakowska B, Zielinski R, Soffer AEM, Rietjens IMCM. 2001. The influence of pH on the antioxidant properties and the mechanisms of antioxidant action of hydroxyflavones. Free Radic. Biol. Med. 31:869-881.

Lempert P. 2008. 'Healthy' gains in breads. Facts, Figures, and the Future. Food Marketing Institute. p 1.

Lesschaeve I, Noble AC. 2005. Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. Am. J. Clin. Nutr. 81:330-335.

Lin JK, Weng MS. 2006. In: Grotewold E, editor. The Science of Flavonoids. New York: Springer Science and Business Media. p 273.

Liu RH. 2002. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. J. Nutr. 134:3479S-3485S.

Liu ZQ, Ma LP, Zhou B, Yang L, Liu ZL. 2000. Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low-density lipoprotein. Chem. Phys. Lipids 106:53-63.

Lotito SB, Fraga CG. 1997. (+)-Catechin prevent human plasma oxidation. Free Radic. Biol. Med. 24(3):435-441.

Lotito SB, Frei B. 2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence or epiphenomenon? Free Radic. Biol. Med. 41:1727-1746.

Lucas T, Grenier A, Quellec S, Le Bail A, Davenel A. 2005. MRI quantification of ice gradients in dough during freezing or thawing process. J. Food Eng. 71:98-108.

Lusis AJ. 2000. Atherosclerosis. Nature (Lond.) 407:233-241.

Lynn A. 2000. Current practice in milling and baking. In: Morris PC, Bryce JH, editors. Cereal Biotechnology. Boca Raton, FL: CRC Press LLC and Woodhead Publishing Limited. p 243.

MacDonald-Wicks LK, Wood LG, Garg ML. 2006. Methodology for the determination of biological antioxidant capacity in vitro: a review. J. Sci. Food Agric. 86(13):2046-2056.

Macheix JJ, Fleuriet A, Billot J. 1990. Fruit Phenolics. Boca Raton, FL: CRC Press. 378 p.

Maier T, Schieber A, Kammerer D, Carle R. 2009. Residues of grape (*Vitis vinifera* L.) seed oil production as a valuable source of phenolic antioxidants. Food Chem. 112:551-559.

Maillard MN, Berset C. 1995. Evolution of antioxidant activity during kinling: Role of insoluble bound phenolic acids of barley and malt. J. Agric. Food Chem. 43:1789-1793.

Manach C, Donovan JL. 2004. Pharmacokinetics and metabolism of dietary flavonoids in humans. Free Rad. Res. 38:771-785.

Marais JP, Deavours B, Dixon RA, Ferrira D. 2006. In: Grotewold E, editor. The Science of Flavonoids. Columbus, Ohio: Springer Science and Business Media Inc. p 1-46.

Mazza G, Miniati E. 1993. Grapes. Anthocyanins in fruits, vegetables, and grains Boca Raton, Ann Harbor, London, Tokyo: CRC Press. p 149-199.

McCallum JA, Walker JL. 1990. Proanthocyanidins in wheat bran. Cereal Chem. 67(3):282-285.

McDonald S, Prenzler PD, Antolovich M, Robards K. 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem. 73(1):73-84.

Meilgaard M, Civille GV, Carr BT. 1999. Sensory evaluation techniques, 3rd ed. New York: CRC Press. 488 p.

Miller NJ, Diplock AT, Rice-Evans CA, Davies MJ, Gopinathan V, Milner A. 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sci. 84:407-412.

Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. 1996. Antioxidant activities of carotenes and xanthophylls. FEBS Lett. 384:240-242.

Moini H, Guo QO, Packer L. 2002. Xanthine oxidase and xanthine dehydrogenase inhibition by the procyanidin-rich french maritime pine bark extract, Pycnogenol((R)): A protein binding effect. In: Buslig, BSMJA, editor. Kluwer Academic/Plenum Publ. p 141-149.

Moldes D, Gallego P, Rodriguez-Couto S, Sanroman A. 2003. Grape seeds: the best lignocellulosic waste to produce laccase by solid state cultures of *Trametes hirsuta*. Biotechnol. Lett. 25:491-495.

Mullins MG, Bouquet A, Williams LE. 1992a. The grapevine and its relatives. Biology of the Grapevine. Cambridge, NY: Cambridge University Press. p 17-36.

Mullins MG, Bouquet A, Williams LE. 1992b. Grapevine Structure. Biology of the Grapevine. Cambridge, NY: Cambridge University Press. p 37-79.

Murcia MA, Jimenez AM, Martinez-Tome M. 2009. Vegetables antioxidant losses during industrial processing and refrigerated storage. Food Res. Int. 42:1046-1052.

Nagy B, Simandi B. 2008. Effects of particle size distribution, moisture content, and initial oil content on the supercritical fluid extraction of paprika. p 293-298.

NASS. 2009. Grape Release. In: Agriculture USD, editor. Olympia, WA.

Nelson B. 2009. Grape Facts 2010. In: America WG, editor. Washington D.C.

Ou B, Hampsch-Woodill M, Prior RL. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J. Agric. Food Chem. 49:4619-4626.

Ozcelik B, Lee JH, Min DB. 2003. Effects of light, oxygen, and pH on the absorbance of 2,2-diphenyl-1-picrylhydrazyl. J. Food Sci. 68(2):487-490.

Pace-Asciak CR, Hahn S, Diamandis EP. 1995. The red wine phenolics trans-resveratrol and quercitin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. Clin. Chim. Acta. 236:207-219.

Pace-Asciak CR, Hahn S, Rounova O. 1996. Wines and grape juices as modulators of platelet aggregation in healthy human subjects. Clin. Chim. Acta. 246:163-182.

Pataki G, Barta J, Varsanyi I. 1997. Decomposition of anticarcinogen factors of the beetroot during juice and nectar production. Cancer Lett. 114:105-106.

Pataki T, Bak I, Kovacs P, Bagchi D, Das DK, Tosaki A. 2002. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. Am. J. Clin. Nutr. 75(5):894-899.

Peng X, Ma J, Cheng K-W, Jiang Y, Chen F, Wang M. 2009. The effects of grape seed extract fortification on the antioxidant activity and quality attributes of bread. Food Chem. 119:49-53.

Perez-Jimenez J, Arranz S, Tabernero M, Diaz-Rubio ME, Serrano J, Goni I, Saura-Calixto F. 2008. Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of. Food Res. Int. 41(3):274-285.

Perret B, Ruidavets JB, Vieu C. 2002. Alcohol consumption is associated with enrichment of high-density lipoprotein particles in polyunsaturated lipids and increased cholesterol esterification rate. Alcohol Clin. Exp. Res. 26:1134-1140.

Pinent M, Castell A, Baiges I, Montagut G, Arola L, Ardevol A. 2008. Bioactivity of flavonoids on insulin-secreting cells. Compr. Rev. Food. Sci. Food Saf. 7(4):299-308.

Pitts M. 2009. Image Processing. Pullman, WA.

Poncet-Legrand C, Gautier C, Cheynier V, Imberty A. 2007. Interactions between flavan-3-ols and poly(L-proline) studied by isothermal titration calorimetry: Effect of the tannin structure. J. Agric. Food Chem. 55(22):9235-9240.

Preuss HG, Bagchi D, Bagchi M. 2002. Protective effects of a novel niacin-bound chromium complex and a grape seed proanthocyanidin extract on advancing age and various aspects of syndrome X. Ann. NY Acad. Sci. 957:250-259.

Prior RL, Wu XL, Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53(10):4290-4302.

Proteggente A, Pannala A, Paganga G, Van Buren L, Wagner E, Wiseman S, Van de Put FHMM, Dacombe C, Rice-Evans CA. 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamic C composition. Free Rad. Res. 36:217-233.

Proteggente AR, Wiseman S, van de Put FHMM, Rice-Evans CA. 2003. The relationship between the phenolic composition and the antioxidant activity of fruits and vegetables. In: Rice-Evans CA, Packer L, editors. Flavonoids in health and disease. New York, NY: Marcel Dekker, Inc. p 71-95.

Pulido R, Bravo L, Saura-Calixto F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J. Agric. Food Chem. 48:3396-3402.

Puupponen-Pimia R, Hakkinen ST, Aarni M, Suortti T, Lampi AM, Eurola M, Piironen V, Nuutila AM, Oksman-Caldentey KM. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. J. Sci. Food Agric. 83(14):1389-1402.

Quinde Z, Ullrich SE, Baik BK. 2006. Genotypic variation in color and discoloration potential of barley-based food products. Cereal Chem. 81(6):752-758.

Randhir R, Kwon YI, Shetty K. 2008. Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innovative Food Science & Emerging Technologies 9(3):355-364.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231-1237.

Record IR, Dreosti IE, McInerney JK. 2001. Changes in plasma antioxidant status following consumption of diets high of low in fruit and vegetables or following dietary supplememtation with an antioxidant mixture. Br. J. Nutr. 85:459-464.

Ribotta PD, Leon AE, Anon MC. 2001. Effects of freezing and frozen storage of doughs on bread quality. J. Agric. Food Chem. 49:913-919.

Ricardo-Da-Silva JM, Rigaud J, Cheynier V, Cheminat A, Moutounet M. 1991. PROCYANIDIN DIMERS AND TRIMERS FROM GRAPE SEEDS. Phytochem. 30(4):1259-1264.

Rice-Evans CA, Miller NJ, Paganga G. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2:152-159.

Rivard A, Andres V. 2000. Vascular smooth muscle cell proliferation in the pathogenesis of atherosclerotic cardiovascular diseases. Histol. Histopathol. 15:557-571.

Robinson J. 2006. The Oxford companion to wine, 3rd ed. Oxford, NY: Oxford University Press. 813 p.

Ross R. 1999. Atherosclerosis- An inflamatory disease. N. Engl. J. Med. 340:115-126.

Russell WR, Labat A, Scobbie L, Duncan SH. 2007. Availability of blueberry phenolics for microbial metabolism in the colon and the potential inflammatory implications. Mol. Nutr. Food Res. 51(6):726-731.

Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N. 1998. Antiulcer activity of grape seed extract and procyanidins. J. Agric. Food Chem. 46(4):1460-1464.

Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. 1998. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 76(2):270-276.

Sano T, Oda E, Yamashita T, Naemura A, Ijiri Y, Yamakoshi J, Yamamoto J. 2005. Anti-thrombotic effect of proanthocyanidin, a purified ingredient of grape seed. Thrombosis Res. 115(1-2):115-121.

Santos-Buelga C, Scalbert A. 2000. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. J. Sci. Food Agric. 80(7):1094-1117.

Sarkar SK, Howarth RE. 1976. Specificity of the vanillin test for flavanols. J. Agric. Food Chem. 24:317-320.

Sato M, Maulik G, Ray PS, Bagchi D, Das DK. 1999. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. J. Mol. Cell. Cardiol. 31(6):1289-1297.

Scalbert A. 1991. Antimicrobial properties of tannins. Phytochem. 30:3875-3883.

Schieber A, Muller D, Rohrig G, Carle R. 2002. Effects of grape cultivar and processing on the quality of cold-pressed grape seed oils. Mitteilungen Klosterneuberg 52:29-33.

Schieber A, Stintzing FC, Carle R. 2001. By-products of plant food processing as a source of functional compounds - recent developments. Trend Food Sci. Technol. 12(11):401.

Schonlau F, Rohdewald P. 2001. Pycnogenol for diabetic retinopathy. A review. Int. Ophthalmol. 24:161-171.

Scott BC, Butler J, Halliwell B, Aruoma OI. 1993. EVALUATION OF THE ANTIOXIDANT ACTIONS OF FERULIC ACID AND CATECHINS. Free Radic. Res. Commun. 19(4):241-253.

Seeram NP, Zhang Y, Nair MG. 2003. Inhibition of proliferation of human cancer cells and cyclooxygenase enzymes by anthocyanidins and catechins. Nutr. Cancer. 46:101-106.

Selomulyo VO, Zhou WB. 2007. Frozen bread dough: Effects of freezing storage and dough improvers. J. Cereal Sci. 45(1):1-17.

Shahidi F, Naczk M. 2004. Phenolics in food and nutraceuticals. Boca Raton: CRC Press. 576 p.

Shahidi F, Zhong Y. 2007. Measurement of antioxidant activity in food and biological systems. In: Shahidi F, Ho CT, editors. Antioxidant Measurement and Application.Washington D.C.: ACS Symposium Series 956.

Shrikhande AJ. 2000. Wine by-products with health benefits. Food Res. Int. 33(6):469-474.

Singleton VL, Rossi JA. 1965. A colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Smith AK, June H, Noble AC. 1996. Effects of viscosity on the bitterness and astringency of grape seed tannin. Food Qual. Pref. 7:161-166.

Souquet JM, Cheynier V, Brossaud F, Moutounet M. 1996. Polymeric proanthocyanidins from grape skins. Phytochem. 43:509-512.

Spices R. 1990. Application of rheology in the bread industry. In: Faridi H, Faubion JM, editors. Dough Rheology and Baked Product Texture. New York: Van Nostrand Reinhold. p 343-361.

Terra X, Valls J, Vitrac X, Merrillon JM, Ardevol L, Arola L, Arola A, Blade C, Fernandez-Larrea J, Pujadas G, Salvado J, Blay M. 2007. Grape-seed procyanidins act as antiinflammatory agents in endotoxin-stimulated RAW 264.7

macrophages by inhibiting NFkB signaling pathway. J. Agric. Food Chem. 55:4357-4365.

Thaipong K, Boonprokob U, Crosby K, Cisneros-Zevallos L, Byrne DH. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Comp. Anal. 19:669-675.

Therdthai N, Zhou WB, Adamczak T. 2002. Optimization of the temperature profile in bread baking. J. Food Eng. 55(1):41-48.

Tsang C, Auger C, Mullen W, Bornet A. 2005. The absorption, metabolism and excretion of flavan-3-ols and procyanidins followintg the ingestion of grape seed extract by rats. Brit. J. Nutr. 94:170-181.

Ursini F, Rapuzzi I, Toniolo R, Tubaro F, Bontempelli G. 2001. Characterization of antioxidant effect of procyanidins. Methods Enzymol. 335:338-350.

Valcic S, Timmermann BN, Alberts DS. 1996. Inhibitory effect of six green tea catechins and caffeine on the growth of four selected human tumor cell lines. Anticancer Drugs. 7:461-468.

van den Berg R, Haenen G, van den Berg H, Bast A. 1999. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem. 66(4):511-517.

van den Berg R, Haenen G, van den Berg H, van der Vijgh W, Bast A. 2000. The predictive value of the antioxidant capacity of structurally related flavonoids using the Trolox equivalent antioxidant capacity (TEAC) assay. Food Chem. 70(3):391-395.

Varriano-Marston E, Hsu KM, Mahdi J. 1980. Rheological and structural changes in frozen dough. Bakers Digest. 54:32-34.

Vitseva O, Varghese S, Chakrabarti S, Folts JD, Freedman JE. 2005. Grape seed and skin extracts inhibit platelet function and release of reactive oxygen intermediates. J. Cardiovasc. Pharmacol. 46(4):445-451.

Wang LF, Kim DM, Lee CY. 2000. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. J. Agric. Food Chem. 48(9):4227-4232.

Wang R, Zhou WB. 2004. Stability of tea catechins in the breadmaking process. J. Agric. Food Chem. 52(26):8224-8229.

Wang R, Zhou WB, Isabelle M. 2007. Comparison study of the effect of green tea extract (GTE) on the quality of bread by instrumental analysis and sensory evaluation. Food Res. Int. 40(4):470-479.

Wang SY, Jiao HJ. 2000. Scavenging capacity of berry crops in superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J. Agric. Food Chem. 48(11):5677-5684.

Ward NC, Croft KD, Puddey IB, Hodgson JM. 2004. Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic acid, an important metabolite of proanthocyanidins in humans. J. Agric. Food Chem. 52:5545-5549.

Wildman REC. 2001. Handbook of Nutraceuticals and Functional Foods. New York, NY: CRC Press. 568 p.

Wink DA, Mitchell JB. 1998. Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic. Biol. Med. 25(4-5):434-456.

Wright JS, Johnson ER, DiLabio GA. 2001. Predicting the activity of phenolic antioxidants: Theoretical method, analysis of substituent effects, and application to major families of antioxidants. J. Am. Chem. Soc. 123:1173-1183.

Yamakoshi J, Saito M, Kataoka S, Tokutake S. 2002. Procyanidin-rich extract from grape seeds prevents cataract formation in hereditary cataractous (ICR/f) rats. J. Agric. Food Chem. 50:4983-4988.

Yemis O, Bakkalbasi E, Artik N. 2008. Antioxidative activities of grape (Vitis vinifera) seed extracts obtained from different varieties grown in Turkey. Int. J. Food Sci. Technol. 43(1):154-159.

Yi JH, Kerr WL. 2009. Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. Food Sci. Technol. 42(9):1474-1483.

Yilmaz Y, Toledo RT. 2004. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. J. Agric. Food Chem. 52(2):255-260.

Yu L. 2008. Wheat antioxidants. Hoboken, NJ: John Wiley & Sons. 292 p.

Zhu QY, Zhang A, Tsang D, Huang Y, Chen ZY. 1997. Stability of Green Tea Catechins. J. Agric. Food Chem. 45:4624-4628.