ABSTRACT

Title of Thesis:	ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF TOMATO SEED WASTES AND POTENTIAL USE AS A FUNCTIONAL FOOD
	Elena Bailoni, Master of Science 2020
Thesis Directed By:	Dr. Liangli Yu, Department of Nutrition and Food Science

Tomato seeds are a major waste product of the tomato processing industry. To find a use for tomato seeds, two products made from the seeds, tomato seed flour and tomato seed oil, were investigated for their health beneficial properties. Tomato seed flour showed total phenolic and radical scavenging assay values similar to other healthful foods thought to be beneficial for human health. It also contained specific chemical compounds that are known to be beneficial for reducing the risk of chronic diseases. Tomato seed flour was added to ketchup to determine how it performed in food system. Tomato seed flour did not significantly affect most of the physical properties of ketchup and may be an effective functional food additive. The tomato seed flour did show potential as a thickener. The use of tomato seed flour in food systems may be beneficial to businesses, the environment, and for human health.

ANTIOXDIANT AND FREE RADICAL SCAVENGING PROPERTIES OF TOMATO SEED WASTES AND POTENTIAL USE AS A FUNCTIONAL FOOD

by

Elena Bailoni

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Advisory Committee:
Professor, Dr. Liangli Yu, Chair
Dr. Hee-Jung Song
Dr. Monica Whent

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List of Abbreviations

AAA – Aromatic amino acids

AAPH – 2,2'-Azobis(2-amidinopropane) dihydrochloride

ABTS – 2,2'azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

ANOVA – Analysis of variance

DPPH – 2,2-diphenyl-1-picrylhydrazyl

EC – Effective concentration

ET – Electron transfer

FC – Folin-Ciocalteu

FDA – Food and Drug Administration

FL – Fluorescein

FR – Free radicals

GAE – Gallic acid equivalent

HPLC – High Performance Liquid Chromatography

IC – Inhibitory concentration

ORAC – Oxygen radical absorbance capacity

PUFA – Poly-unsaturated fatty acids

ROS – Reactive oxygen species

SAA – S-containing amino acids

SFA – Saturated fatty acids

TE – Trolox equivalent

TEAC – Trolox equivalent antioxidant capacity (another name for ABTS assay)

TPC – Total phenolic content

Trolox – 6-hydroxy-2,5,7,8-tetramethylchoman-2-carboxylic acid

UFA – Unsaturated fatty acids

UHPLC – Ultra high performance liquid chromatography

USDA – United States Department of Agriculture

Chapter 1: Tomato Seed Oil and Flour Studies

Tomato Seed Oil and Flour Production

Tomato Seed Waste

In 2017, the United States produced over 22 billion pounds of processed tomato products (USDA, 2018a). These products include sauces, pastes, and juices (Al-Wandawi, Rahman, and Al-Shaikhly, 1985). These processes produce waste, including dry waste consisting of unused tomato seeds and skins (Gould, 2013). Rossini et al (2013) suggests one flow diagram outlining where seeds are wasted in some tomato processing facilities; after tomatoes are received, washed, ground, and sorted, the juice from the tomatoes is extracted. It is during this extraction that residues, including the peels and seeds, are removed from the product and considered a waste or byproduct. After extraction, the product may go on to be pulped, concentrated, and may have other ingredients added before pasteurization. This process represents the possible production of tomato purees, ketchups, and pastes. The tomatoes may go through a possible preheating treatment before juice extraction (Hayes, Smith, and Morris, 1998). By the time the waste is removed, the mixture may have already been slightly cooked. Through dewatering, sorting, and further processing, the seeds can be separated from the peels and used as an oil or meal/flour, depending on the particle size of the dry waste (Hayes, Smith, and Morris, 1998).

Common disposal techniques for dry tomato wastes include returning the waste to fields, transporting them to landfills (Jesse, Schultz, and Bomben, 1975), or feeding

the waste to animals (Gould, 2013). In the canned tomato industry, from farm to retail, 59% of the weight of tomatoes harvested is listed as losses (USDA, 2018b). It's estimated that pulped tomatoes create 1.13% dry waste by mass, and approximately 45% of this waste consists of tomato seeds (Rabak, 1917; Rossini et al, 2013). If this estimate holds for the 22 billion pounds of processed tomato products, approximately 114 million pounds of tomato seeds were discarded as waste in the US in 2017.

Reducing waste in the food chain is becoming an increasingly important issue. In 2009, Hall Guo, Dore, and Chow (Hall et al., 2009) outlined the massive amounts of fresh water and fossil fuel energy used on wasted food. It is worthwhile to find a beneficial use for tomato seeds instead of using our current limited resources to simply discard of them.

Potential Use of Tomato Seeds

Transforming tomato seeds into oil or flour products is a physical change that enhances the usability and value of the seeds, making it a value-added product by USDA definition (Agriculture Marketing, 2019). And though the FDA does not define functional foods, it is a term commonly used in the market to describe foods that function to improve health from their nutraceutical components or value (Ross, 2000).

The growing market for these types of products makes them worthwhile for both researchers and companies; the market is predicted to be worth 578.23 billion USD by 2025, experiencing an 8.8% growth worldwide every year (Grand View Research,

2017). Additionally, botanical supplements are expected to be in increasing demand over this period as consumers turn to natural supplements to improve their health (Grand View Research, 2017).

Investigations into the use of tomato seeds as a functional food are worthwhile. Banerjee et al (2017) concluded that processing wastes may have higher concentrations of secondary metabolites, such as polyphenols, when compared to the pulp of plants. Rudra et al (2015) also stated that the seeds of tomatoes are a richer source of polyphenols when compared to tomato pulp.

Tomato seeds have been studied for potential use as an oil ingredient as early as 1914, with studies looking into the components of tomato seed oils in 1919 (Jamieson and Bailey, 1919). Tomato seed oil studies have shown potential benefits for use in the nutraceutical, pharmaceutical, cosmetic (Zuorro, Lavecchia, and Medici, 2012), and fuel (Giannelos, Sxizas, and Lois, 2005) industries. Rossini et al (2013) stated that the best use for tomato seed wastes is to turn them into vegetable oil. Defatted tomato seed flour was studied for its beneficial properties in 1985 (Al-Wandawi, Rahman, and Al-Shaikhly, 1985). Limited studies have been conducted on tomato seed flour uses, including its integration into wheat bread (Carlson, Knorr, and Watkins, 1981).

Previously, tomato processing wastes have been studied by several groups for their potential use as a nutraceutical. Antioxidants were identified in microwave-assisted extraction of tomato processing wastes (Pinela et al., 2017), as well as high levels of

lycopene from supercritical extraction of the oil fractions of tomato seed wastes (Kehili et al., 2016) and from microemulsion extraction of tomato paste production waste using saponin (Amiri-Rigi, Abbasi, and Scanlon, 2016). Tomato pomace, the skin and seed waste that results from tomato processing, has been identified as a good source of antioxidants, phenolic compounds, lycopene, and color components (Silva et al., 2018).

Tomato Seed Oil and Flour Processing Techniques

To begin the oil making process, seeds are removed/separated from all other parts of the fruit and cleaned. The oil from the seeds can then be extracted using a variety of different methods. For seeds with high oil content, they are simply heated then pressed by an expeller to extract the edible oil (National Edible Oil, 2019). More commonly, a solvent extraction needs to be performed in combination with a heating and pressing pretreatment. Hexane is a commonly used solvent for oil extraction (PennState Extension, 2013). The preprocessed cake is flaked and mixed with decreasing concentrations of the chosen solvent. The residual oil content of the flakes may be as low as 0.5-0.7% after solvent extraction (SRS International, 2013). The mixture is heated during or after solvent extraction, the purpose of which is to evaporate off the solvent so only the oil remains. The oil and solvent mixture may be further distilled until all of the solvent is removed. The solvent is recycled and used for additional extractions (PennState Extension, 2013). The resulting defatted flakes become what are referred to as the seed meal/flour.

Often, seed oils are further refined to remove undesirable materials or to make the oil more appealing to the consumer (Gunstone, 2002). The exact refining process depends on the type of oil, but often oils are neutralized to remove free fatty acids, bleached to remove impurities and possible constituents that can induce color changes, and deodorized to remove undesirable smells and tastes (National Edible Oil, 2019). The refining process also removes some desirable components such as antioxidants and vitamins (Gunstone, 2002), specifically during bleaching (PennState Extension, 2013). Bleaching allows oils to retain their characteristic color for a longer time, as it removes components that induce lipid oxidation and result in these color changes. When the oil is bleached, it's mixed with bleaching clay and heated to high temperatures (90-110°C). The undesirable components attach to the clay particles, as do some desirable components (PennState Extension, 2013). A balance between refining enough to achieve an oil up to industry and consumer standards and not refining so much as to remove all desirable components must be achieved. Oils are called crude if they are unrefined and refined if they undergo any of this further processing (National Edible Oil, 2019). However, commercially refined oils don't list the ways or methods by which they were refined.

Tomato Seed Oil Composition

Key Studies Table Summaries

Limited studies have attempted to characterize and quantify the components of tomato seed oil and the resulting tomato seed flour. Most if not all of these studies only analyze one of these products. Fourteen studies conducted from 1919 to 2014

were identified as having sufficient and valuable information regarding the properties and components of tomato seed oil. Some of this information includes the fatty acid profile of tomato seed oil, sterol content, physicochemical properties of the oil, ideal storage conditions, oxidative stability, color, antioxidant activity, and antibacterial activity.

When relevant, crude and refined oil samples are noted and listed separately. Unless specifically stated, all laboratory extracted oil samples are assumed to be crude. All commercial oil samples are assumed to be refined.

Table 1. Tomato Seed Oil Extractions. Table shows the solvent, extraction method, and source of tomato seeds of the literature used to analyze tomato seed oil.

Extraction Solvent	Extraction Method	Tomato Seed Source	Source
Petroleum Ether	Soxhlet/Rotary Evaporator	Tomatoes grown in Italy	Giuffre and Capocasale, 2016
	Soxhlet/Rotary Evaporator/Degummed	Waste from Greek tomato plant	Lazos et al, 1998
	Reflux with redistilled solvent	Waste from Greek tomato plant	Tsatsaronis and Baskov, 1972
	Continuous volatile solvent method	Various sources from United States	Jamieson and Bailey, 1919
Hexane	Stirring/Centrifugation/Rotary Evaporator	Waste from California tomato plant	Shao et al, 2014
	Soxhlet/Rotary Evaporator	Unknown, seeds separated from pulp. Study in Greece	Giannelos et al, 2004
	Soxhlet	Waste from Brazil tomato plant, hot break	Cantarelli et al, 1993
	Soxhlet	Waste from Brazil tomato plant, cold break	Cantarelli et al, 1993
	Dionex accelerated solvent extractor, dried under nitrogen	Frozen from California company	Eller et al, 2010

	In lab conditions (no source)	Wastes from Egypt	Hassanien et al, 2014
	Soxhlet	Pomace from Iran	Fahimdanesh and Bahrami, 2013
	Soxhlet	Wastes from Greek tomato plant	Kiosseoglou and Boskou, 1989
	Soxhlet	Tomatoes grown in Greece	Kiosseoglou and Boskou, 1989
Acetone	Supercritical extraction in autoclave	Waste from Turkish tomato plant	Demirbas, 2009
	Soxhlet	Waste from Turkish tomato plant	Demirbas, 2009
Supercritical Carbon Dioxide	Pilot Plant Extractor	Frozen from California company	Eller et al, 2010
Absolute Ethanol	Dionex accelerated solvent extractor, dried under nitrogen	Frozen from California company	Eller et al, 2010
Ether and/or carbon tetrachloride	Continuous volatile solvent method*	Various sources from United States	Jamieson and Bailey, 1919
None	Expeller/press	Various sources from United States	Jamieson and Bailey, 1919
Chloroform-methanol	"Dried, comminuted and extracted threefold"	Waste from Poland	Malecka, 2002

*laboratory refined or mentions of laboratory refined

Table 1.1. Unrefined Tomato Seed Oil Fatty Acid Profile. Fatty acids identified in tomato seed oil using unrefined oil. Range shows the highest and lowest values, average shows the percent average of all studies that identified the fatty acid.

Fatty Acid	Common Name	Range (%)	Average (%)	Source(s)
C12:0	Lauric	0.01-0.3	0.12	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Cantarelli et al, 1993
C13:0	Trideclic	0.001	0.001	Tsatsaronis and Baskov, 1972
C14:0	Myristic	0.08-2.4	0.535	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016
C15:0	Pentadeclic	0.02	0.02	Tsatsaronis and Baskov, 1972
C16:0	Palmitic	12.26-23.4	15.12285714	Tsatsaronis and Baskov, 1972; Kiosseoglou and Boskou, 1989; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016

C17:0	Heptadecanoic	0.08-0.4	0.23375	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Giannelos et al, 2004; Giuffre and Capocasale, 2016
C18:0	Stearic	4-9.4	5.611428571	Tsatsaronis and Baskov, 1972; Kiosseoglou and Boskou, 1989; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016
C19:0	Nonadecylic	0.01	0.01	Tsatsaronis and Baskov, 1972
C20:0	Arachidic	0.27-1.3	0.560009091	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016
C22:0	Behenic	0.08-0.7	0.191428571	Tsatsaronis and Baskov, 1972; Fahimdanesh and Bahrami, 2013;

				Cantarelli et al, 1993; Giannelos et al, 2004; Giuffre and Capocasale, 2016
C23:0	Tricosylic	0.02	0.02	Tsatsaronis and Baskov, 1972
C24:0	Lignoceric	0.08-0.17	0.125	Tsatsaronis and Baskov, 1972; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Giannelos et al, 2004
C25:0	Pentacosylic	0.02	0.02	Tsatsaronis and Baskov, 1972
C26:0	Cerotic	0.1	0.1	Tsatsaronis and Baskov, 1972
C27:0	Heptacosylic	0.005	0.005	Tsatsaronis and Baskov, 1972
C28:0	Montanic	0.005	0.005	Tsatsaronis and Baskov, 1972
C14:1	Myristoleic	0.02-0.25	0.11	Hassanien et al, 2014; Demirbas, 2009; Giuffre and Capocasale, 2016
C16:1	Palmitoleic	0.08-6.8	1.470909091	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016

C17:1	Heptadecenoic	0.04-0.38	0.117142857	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004; Giuffre and Capocasale, 2016
C18:1	Oleic	17.16-29.7	23.27714286	Tsatsaronis and Baskov, 1972; Kiosseoglou and Boskou, 1989; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016
C18:1 t	Eladic	0.03-0.08	0.048	Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004; Giuffre and Capocasale, 2016
C18:2	Linoleic	37.6-61	51.57857143	Tsatsaronis and Baskov, 1972; Kiosseoglou and Boskou, 1989; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas,

				2009; Giuffre and Capocasale, 2016
C18:2 t		0.08-0.1	0.086666667	Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Giannelos et al, 2004
C18:3	Linolenic	0.4-3.5	1.757692308	Tsatsaronis and Baskov, 1972; Kiosseoglou and Boskou, 1989; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Lazos et al, 1998; Cantarelli et al, 1993; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016
C18:3 t		0.04	0.04	Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004
C20:1	Ecosenoic/Gadoleic	0.08-0.6	0.16	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004; Demirbas, 2009; Giuffre and Capocasale, 2016

Unsaturated (all)	68.6-81.72	77.61684211	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Cantarelli et al, 1993; Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004; Demirbas, 2009; Lazos et al, 1998; Kiosseoglou and Boskou, 1989; Giuffre and Capocasale, 2016
Saturated (all)	15-31.3	19.77615789	Tsatsaronis and Baskov, 1972; Hassanien et al, 2014; Cantarelli et al, 1993; Fahimdanesh and Bahrami, 2013; Giannelos et al, 2004; Demirbas, 2009; Lazos et al, 1998; Kiosseoglou and Boskou, 1989; Giuffre and Capocasale, 2016

Common names retrieved from: AES Laboratories Pvt. Ltd. (2015).

Table 1.3. Refined Tomato Seed Oil Fatty Acid Profile. Fatty acids identified in tomato seed oil using refined oil. Range shows the highest and lowest values, average shows the percent average of all studies that identified the fatty acid.

Fatty Acid	Common Name	Range (%)	Average (%)	Source(s)
C14:0	Myristic	0.1	0.1	Lazos et al, 1998
C16:0	Palmitic	13.6	13.6	Lazos et al, 1998
C17:0	Heptadecanoic	0.1	0.1	Lazos et al, 1998
C18:0	Stearic	6	6	Lazos et al, 1998
C20:0	Arachidic/Eicosanoic	0.2	0.2	Lazos et al, 1998
C22:0	Behinic	0.1	0.1	Lazos et al, 1998
C24:0	Lignoceric	0.1	0.1	Lazos et al, 1998
C16:1	Palmitoleic	0.6	0.6	Lazos et al, 1998
C18:1	Oleic	22.1	22.1	Lazos et al, 1998
C18:2	Linoleic	54	54	Lazos et al, 1998
C18:2 t		0.8	0.8	Lazos et al, 1998
C18:3	Linolenic	2.1	2.1	Lazos et al, 1998
Unsaturated (all)		79-80.6	79.8	Jamieson and Bailey, 2019; Lazos et al, 1998
Saturated (all)		14.7-20.2	16.9666667	Jamieson and Bailey, 2019; Lazos et al, 1998

Table 1.4. Tomato Seed Oil Sterol Contents. Sterols identified in tomato seed oil separated by crude (unrefined) and refined oils. Range shows the highest and lowest values, average shows the percent average of all studies that identified the sterol.

Sterol	Crude Range (%)	Crude Average (%)	Source(s)	Refined Range (%)	Refined Average (%)	Source(s)
Cholesterol	5.20-15.09	9.282	Lazos et al, 1998; Jamieson and Bailey, 1919; Eller et al, 2010	16	16	Lazos et al, 1998
Brasicasterol	0.298-9.8	2.5006	Lazos et al, 1998; Eller et al, 2010; Malecka, 2002	1.1	1.1	Lazos et al, 1998
24-Methylenecholesterol	1.2	1.2	Lazos et al, 1998	0.9	0.9	Lazos et al, 1998
Campesterol	4.79-29.5	9.505	Lazos et al, 1998; Jamieson and Bailey, 1919; Eller et al, 2010; Malecka, 2002	6.1	6.1	Lazos et al, 1998
Stigmaterol	4.5-14.4	10.232	Lazos et al, 1998; Eller et al, 2010; Malecka, 2002	14.6	14.6	Lazos et al, 1998
Delta$_7$-Campesterol	0.3	0.3	Lazos et al, 1998	trace	trace	Lazos et al, 1998
Clerosterol	Trace	Trace	Lazos et al, 1998			
beta-Stitosterol	43.2-54.38	49.86	Lazos et al, 1998; Jamieson and Bailey, 1919; Malecka, 2002	53	53	Lazos et al, 1998

Delta$_{5}$-Avenasterol	3.32-10.8	6.94	Lazos et al, 1998; Jamieson and Bailey, 1919; Malecka, 2002	7	7	Lazos et al, 1998
Delta$_{7,24}$-Stigmastadienol	0.5	0.5	Lazos et al, 1998	0.3	0.3	Lazos et al, 1998
Delta$_{7}$-Stigmastenol	0.4	0.4	Lazos et al, 1998	0.2	0.2	Lazos et al, 1998
Delta$_{7}$-Avenasterol	0.1	0.1	Lazos et al, 1998	trace	trace	
Erythrodiol	0.1	0.1	Lazos et al, 1998			
Campestanol	0.93	0.93	Jamieson and Bailey, 1919			
Delta$_{5}$-Stigmasterol	8.29	8.29	Jamieson and Bailey, 1919			
Sitostanol	0.49	0.49	Jamieson and Bailey, 1919			
Delta$_{7}$-Stigmasterol	10.85	10.85	Jamieson and Bailey, 1919			
Citrostandienol	0.94-1.77	1.5125	Jamieson and Bailey, 1919; Eller et al, 2010			
Dihydrolanosterol	3.95-4.46	4.213333333	Eller et al, 2010			
Cycloartenol	10.07-11.11	10.69	Eller et al, 2010			
Cholestanol	0.709-1.11	0.848666667	Eller et al, 2010			
Lathosterol	1.05-1.07	1.06	Eller et al, 2010			
Dihydrospinasterol	3.6-3.79	3.726666667	Eller et al, 2010			
Sitosterol	32.52-33.85	33.22666667	Eller et al, 2010			
Beta-Amyrin	0.893-1.41	1.073	Eller et al, 2010			

Table 1.5. Physicochemical Properties of Tomato Seed Oil. Studies were separated by solvent and extraction type. Range shows lowest and highest values, average shows average value for all studies in the same column.

	Petroleum Ether, Soxhlet (Giuffre and Capocasale, 2016; Lazos et al, 1998)		**Petroleum Ether, Soxhlet, Purified (Lazos et al, 1998)**		**Hexane, centrifuge (Shao et al, 2014)**		**Hexane, Soxhlet (Giannelos et al, 2004; Cantarelli et al, 1993; Fahimdanesh and Bahrami, 2013)**		**Hexane, no source for extraction (Hassanien et al, 2014)**	**Acetone, Soxhlet (Demirbas, 2009)**		**Acetone, Supercritical in autoclave (Demirbas, 2009)**	
	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Oil Content (DW%)	19.84 - 23.44	21.9967											
Refractive Index (n_{20})	1.4581- 1.4662	1.46195	1.461	1.461	1.471	1.471	1.4720- 1.4733	1.47265		1.4733	1.4733	1.4714	1.4714
Density (kg/m_3)	0.916	0.916	0.9156	0.9156	0.901	0.901	0.9151	0.9151					
Specific gravity (g/mL)							0.9181- 0.9186	0.91835		0.9177	0.9177	0.9185	0.9185
Kinematic viscosity at 40°C (mm_2/s)							28	28					
Viscosity at 21°C (mPa.s)	75	75	74	74									

Iodine value (g 1/100 g oil)	105	105	104	104	126	126	124-126.4	125.125	105.4	126.8	126.8	109.7	109.7
Saponification value (mg KOH/g oil)	184	184	186	186	177	177	156-195	177.7	184.5	190.2	190.2	183.6	183.6
Peroxide value (meq/kg oil)	9.3	9.3	9.1	9.1	0.63	0.63							
Acid Value (mg KOH/g oil)					0.22	0.22				0.241	0.241	0.006	0.006
Free acidity (g%)	1.01-7.77	3.41	0.05	0.05									
OSI - 120C (h)	2.01-4.72	3.54333333											
p-Anisidine Value	1.21-3.93	2.38333333											
L* (1 cm)	26.95-28.5	27.94			39.26	39.26							
a* (1 cm)	1.98-22	7.435	10	10	30.56	30.56							
b* (1 cm)	5.04-10	7.5525	2	2	50.4	50.4							
L* (2 cm)	24.32-24.87	24.58											
a* (2 cm)	1.34-1.63	1.52											
b* (2 cm)	0.58-1.65	1.26											
Cloud Point (°C)							-8.9	-8.9					

Pour Point (°C)							-16.1	-16.1
Flash Point (°C)							189	189
Smoke Point (°C)	176	176	208	208				

Table 1.6. Tomato Seed Oil Tocopherol Content. Studies were separated and extraction solvents and methods noted, as this can affect results.

Antioxidant	**Supercritical CO2 (Eller et al, 2010)**	**Hexane, Dionex (Eller et al, 2010)**	**Hexane (Hassanien et al, 2014)**	**Absolute Ethanol, Dionex (Eller et al, 2010)**	**Purified commercial Oil (Muller et al, 2012)**
Alpha-tocopherol (mg/g)	0.05	0.04	0.0371	0.03	0.2456
Beta-tocopherol (mg/g)					0.0347
Gamma-tocopherol (mg/g)	1.05	1.03	1.0788	0.9	1.095
Delta-tocopherol (mg/g)	0.01	0.01	0.0023	0.01	
Lutein (mg/g)					0.0048
Beta-carotene (µg/g)	0.93	4.53		4.06	
Lycopene cis 1 (µg/g)	0	1.34		0.58	
Lycopene cis 2 (µg/g)	0	3.02		1.06	
Lycopene all trans (µg/g)	0	12.07		7.5	

Lycopene cis 3 (µg/g)	0	9.43	7.85	
Total lycopene (µg/g)		25.86	16.99	95.6

Table 1.7. Tomato Seed Oil Antioxidant Assay Data. Studies were isolated to compare the four that completed antioxidant assays. Demirbas (2009) had range and average results as different samples were tested. Eller et al (2010) used three different extraction methods, which are noted for the columns under the study name.

Antioxidant Activity Assay	**Demirbas, 2009**		**Ma et al, 2013**	**Eller et al, 2010**			**Muller et al, 2012**
	range	**average**		**SC-CO2**	**Hexane Dionex**	**Absoulte Ethanol Dionex**	
Total Phenolic Substances - Nitric Oxide Method (%)	2.48-4.27	3.5					
Antioxidative Activity - Ferric Thiocanate Method (absorbance at 480 nm vs incubation period)	>0.2->0.8 over 4 days						
DPPH (IC50 µg/mL)			1590.1				
HRST/FRAP Method (EC50 µg/mL)			2.76				
ORAC (µmol Trolox equiv/g)				0.96	1.47	1.17	
FRAP (mmol alpha-TE/kg)							3.2
Alpha TEAC (mmol alpha-TE/kg)							6 (exact number not reported)
DPPH (mmol alpha-TE/kg)							4 (exact number not reported)
LPSC (mmol alpha-TE/kg)							15.1

Further Analysis

In addition to the laboratory extracted oils listed in **Table 1**, one laboratory obtained commercial tomato seed oil from China (Ma et al, 2013) and another from Italy (Muller et al, 2012). Ma et al (2013) analyzed the antibacterial activity of tomato seed

oil by a disc diffusion test. The concentration of tomato seed oil ranged from 2 µg/disc to 100 µg/disc. Seven model bacteria were used and the zone of inhibition was reported as millimeters. Acetone was used as a negative control and roxithromycin and chloramphenicol were used as positive controls. The researchers suggest that the results showed that tomato seed oil has moderate antibacterial activity. The oil specifically showed medium activity against *S. aureus, E. coli, S. flexneri,* and *P. mirabilis*. The tomato seed oil showed overall larger zones of inhibition against *P. mirabilis* when compared to the two positive controls.

The group also determined the chemical composition of the tomato seed oil using GC-MS. The analysis could not be included in specific tables since the amount of each compound was based on the total weight, and the complete profile was not listed. 87 unique compounds were identified. The five major compounds found in the oil by percent composition were cycloeucalenol (25.67%), oleic acid (16.70%), linoleic acid (7.85%), 5 alpha-cholest-8-en-3 beta-ol, 14-methyl-, acetate (6.20%), and palmitic acid (6.08%). Of the other compounds identified, many included phenols, alcohols, phytochemicals, and fatty acids (Ma et al, 2013).

Muller et al (2012) analyzed the redox properties of tomato seed oil cultured macrophages. Using cell cultures, they were able to measure and analyze the effect of tomato seed oil on ROS and ROS sensitive proteins. The research group determined that tomato seed oil was able to reduce ROS species in cells and inhibit some ROS

mediated cell signaling pathways by controlling some of the proteins involved, including MAPKs and NF-kB.

Some of the work conducted on tomato oil samples specifically analyzed the effect of tomato cultivar on its physicochemical properties (Giuffre and Capocasale, 2016). This may cause the variation of some tomato seed oil properties, both physicochemical and otherwise. The fourteen studies included tomato seeds from tomatoes grown in Italy, California, Turkey, Greece, Brazil, Iran, and Egypt. Some of the seeds were from tomato plants that had the seeds specifically separated for analysis and others came from commercial waste sources, i.e. tomato pomace generated from a hot or cold break process. Commercial oils that were gifted to labs were from companies based in China (Ma et al, 2013) and Italy (Muller et al, 2012). Since it is not a popular product, there is currently no standard for tomato seed oil properties, though it is assumed that refined tomato seed oils would all yield similar results. The extraction method and solvent may also affect the properties and components of the oils.

Tables 1.2 and **1.3** show that on average, tomato seed oils consist of mostly unsaturated fatty acids, with oleic making up about 23% of the UFA and linoleic making up about 51% of the UFA in crude oils. Refined oils differ slightly from these numbers. The other fatty acid that makes up the majority of the tomato seed oil profile is palmitic acid, a saturated fatty acid. UFA and especially PUFA are considered beneficial for human health when compared to SFA. Oleic acid is an

omega-9 fatty acid and linoleic an essential omega-6 fatty acid. Linoleic acid cannot be synthesized in the body and is necessary to maintain normal body functions (Simopoulos 1999).

The FAO Codex Standards for Fats and Oils from Vegetable Sources can be used to compare the average profile of tomato seed oil with the average profile of the 15 listed common commercial vegetable oils. The majority of the data comes from unrefined tomato seed oils, that are what will be considered for comparison, however, most of the standards presented by FAO are for refined oils. The most notable characteristics of tomato seed oil is its fatty acid profile, mostly linoleic and oleic acids. Many vegetable oils also contain high linoleic and oleic acids, with maize (corn), soybean, and sunflower seed oils having similar proportions to tomato seed oil (approximately one-fifth of the composition being oleic and one half of the composition between linoleic). Based on the literature, tomato seed oil is comprised of 37.6-61% linoleic acid. This range is greater than the range given for coconut oil (1-2.5%), palm oil (9-12%), and virgin olive oils (3.5-21%). All found refractive indexes, densities, iodine values, and saponification numbers fall within the range of reported numbers for the various vegetable oils. Gamma-tocopherol had the largest value per gram in all tomato seed oils, ranging from 0.9-1.095 mg per gram (Food and Agriculture Organization, 2001). The only vegetable oils listed that also fall within this range, and also represent the highest reported amounts of gamma-tocopherol, are maize (corn) oil, sesame seed oil, and soybean oil. While only a brief overview, the physical and chemical profile of tomato seed oil obtained from the

literature indicates that the profile of the oil is similar to other commercially available oils (Food and Agriculture Organization, 2001).

Chemical properties, individual antioxidant content, and current antioxidant assay data were presented according to the extraction method, as the values and actual reported data varied greatly depending on the extraction. Notably, when looking at the performed antioxidant assays, only one lab group chose to perform more than two assays. It is generally accepted that at least two assays must be performed to have an understanding of antioxidant activity but using more than two should be done when looking to draw conclusions (Huang, Ou, and Prior, 2005). Further, none of the current antioxidant assay results used the most accepted standard for their corresponding assays. Some only presented the data qualitatively and another used inhibition concentration, generally not used for food components. The research group who conducted four antioxidant assays used the FRAP, a version of ABTS, DPPH, and LPSC (Muller et al, 2012). The results were expressed in mmols of alpha-TE per kg of oil. These assays are more commonly expressed in Trolox equivalents, a water-soluble derivative of TE instead of directly comparing it to TE. Using similar standards in antioxidant assays is important for replicability and comparability between labs. While all assays have their advantages and disadvantages, there are specific disadvantages of the FRAP and LPSC assays used in this study. Not all reductants that reduce Fe(III), the reaction that occurs in the FRAP assay, are antioxidants. The LPSC assay, a chemiluminescence-based peroxyl radical assay,

involves detection of CL, of which the mechanism of generation is unknown and can cause confusion when interpreting results (Karadag et al, 2009).

Tomato Seed Flour Composition

Table Summaries of Key Studies

As discussed, tomato seed flour or meal is the waste product of tomato seed oil production. It mainly contains the hydrophilic components of the tomato seed, as most hydrophobic components remain in the oil when extracted. Tomato seed meal can also be seen as the "de-oiled" fibrous part of the tomato seed. As such, most extractions that aim to create tomato seed meal are very similar to extractions for tomato seed oil; the portion that is retained for the study is what's different.

Eight studies with data regarding the composition of tomato seed meal were reviewed and summarized. They include details regarding extractions, contents of the meal, contents of protein concentrates, amino acid contents, in vivo protein tests, functional properties, and antioxidant assays.

Table 1.8. Tomato Seed Flour Extraction Methods and Sources. Extraction solvent, method, and sources are listed for the literature used to analyze tomato seed flour properties.

Extraction Solvent	Extraction Method	Tomato seed source	Source
Petroleum Ether	Soxhlet	3 cultivars grown in Italy	Giuffre and Capocasale, 2016
Hexane	Not stated	Waste from processing plant in India	Sogi et al, 2004
		Waste from processing plant in India	Sogi et al, 2002
	Soxhlet	Waste from processing plant in Greece	Liadakis et al, 1995
		Tomatoes from India	Sarkar and Kaul, 2014
Gasoline	Not stated	Study in Uzbekistan	Turakhozhaev, 1979
Purchased Seeds/Cake		UK	Sarkar et al, 2016
		India	Rao 1990

Table 1.9. Tomato Seed Flour Contents. Content analysis done on tomato seed flours were separated out by extracted tomato seed flour and purchased tomato seed flour. Range shows the lowest and highest values, and average shows the average value for all studies that identified the same content or compound.

	Extracted		**Source(s)**	**Purchased**		**Source(s)**
	range	average		range	average	
Nitrogen (%)	5.6-6.55	6.08666667	Giuffre and Capocasale, 2016			
Crude Protein (%)	28.66-40.94	34.824	Giuffre and Capocasale, 2016; Liadakis et al, 1995; Sarkar and Kaul, 2014	40.1		Rao 1990
Moisture (%)	8.1		Liadakis et al, 1995	4		Rao 1990
Crude Fat (%)	1.3		Liadakis et al, 1995	6.1		Rao 1990
Ash (%)	4.6		Liadakis et al, 1995	9.4		Rao 1990
Total sugars (%)	3.2		Liadakis et al, 1995			
Total dietary fiber (%)	54.1		Liadakis et al, 1995	21		Rao 1990
K (mg/100 g)	1046		Liadakis et al, 1995			
Na (mg/100 g)	70		Liadakis et al, 1995			
Ca (mg/100 g)	294		Liadakis et al, 1995	265		Rao 1990
Mg (mg/100 g)	491		Liadakis et al, 1995	536		Rao 1990
P (mg/100 g)	903		Liadakis et al, 1995	982		Rao 1990
Fe (mg/100 g)	10		Liadakis et al, 1995			
Mn (mg/100 g)	6		Liadakis et al, 1995			

Cu (mg/100 g)	2	Liadakis et al, 1995	3.8	Rao 1990
Zn (mg/100 g)	4	Liadakis et al, 1995	7.3	Rao 1990
Chromium (mg/100g)			0.3	Rao 1990
Nicotinic acid (mg/100 g)			2.9	Rao 1990
Riboflavin (mg/100 g)			**0.45**	**Rao 1990**

Table 1.10. Tomato Seed Flour Protein Isolate Studies. Studies in which tomato seed flour was isolated to a protein isolate were analyzed. All samples were from extracted sources. The components are listed as the range, lowest and highest values, and average values. Where there was only one study, the range number shows the exact number reported.

Extracted			**Source(s)**
	Range	Average	
Crude Protein (%)	71.3-99.57	87.51	Liadakis et al, 1995; Sarkar and Kaul, 2014; Turakhozhaev, 1979
Ash (%)	3.4-3.8		Liadakis et al, 1995; Turakhozhaev, 1979
Total sugars (%)	0.8		Liadakis et al, 1995
Total dietary fiber (%)	16.1		Liadakis et al, 1995
K (mg/100 g)	193		Liadakis et al, 1995
Na (mg/100 g)	596		Liadakis et al, 1995
Ca (mg/100 g)	525		Liadakis et al, 1995
Mg (mg/100 g)	102		Liadakis et al, 1995
P (mg/100 g)	570		Liadakis et al, 1995
Fe (mg/100 g)	15		Liadakis et al, 1995

Mn (mg/100 g)	2	Liadakis et al, 1995
Cu (mg/100 g)	2	Liadakis et al, 1995
Zn (mg/100 g)	3	Liadakis et al, 1995

Table 1.11. Tomato Seed Flour Amino Acid Profile. Studies done on tomato seed flour's amino acid profile were analyzed. Studies were separated out based on purchased tomato seed flour or tomato seed flour protein concentrate. Average reflects the average value of the purchased and protein concentrate values.

Amino Acid (g/ 16g N)	**Purchased**	**Source**	**Protein Concentrate**	**Average**	**Source(s)**
Lysine	5.2	Rao 1990	3.7-5.963	4.8315	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Histidine	2.9	Rao 1990	1.9-2.5	2.2	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Arginine	10.4	Rao 1990	5.55	5.55	Turakhozhaev, 1979
Aspartic acid	10.8	Rao 1990	11	11	Turakhozhaev, 1979
Theronine	4.1	Rao 1990	3.649-7.2	5.4245	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Serine	5.3	Rao 1990	5.75	5.75	Turakhozhaev, 1979

Glutamic acid	14.9	Rao 1990		24.7	24.7	Turakhozhaev, 1979
Proline	4.4	Rao 1990		7.3	7.3	Turakhozhaev, 1979
Glycine	4.9	Rao 1990		6.35	6.35	Turakhozhaev, 1979
Alanine	4	Rao 1990		6	6	Turakhozhaev, 1979
Cystine	-	Rao 1990	-	-		Turakhozhaev, 1979
Methionine	1	Rao 1990	1.95-5.519		3.7345	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Valine	3.1	Rao 1990		7.9	7.9	Turakhozhaev, 1979
Isoleucine	3.3	Rao 1990	4.75-4.93		4.84	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Leucine	6.2	Rao 1990	4.75-7.79		6.27	Sarkar and Kaul, 2014; Turakhozhaev, 1979
Tryosine	3.7	Rao 1990		2.55	2.55	Turakhozhaev, 1979
Phenylanaline	4.6	Rao 1990		4.28	4.28	Turakhozhaev, 1979
Tryptophan	0.8	Rao 1990		1.236		Sarkar and Kaul, 2014
SAA				3.058		Sarkar and Kaul, 2014
AAA				8.732		Sarkar and Kaul, 2014

Table 1.12: Tomato Seed Flour Protein Analysis Studies. Studies in which protein efficiency and retention analysis were done on tomato seed flour. Studies were separated based on analysis of either the whole flour or the protein concentrate portion. Range reflects the lowest and highest value within a column, average is the average value of all studies.

	Flour		**Source(s)**	**Protein Concentrate**		**Source(s)**
	range	average				
Protein Efficiency ratio	0.81-1.41	1.11	Sogi et al, 2004; Rao 1990	1.45-2.66	2.055	Sogi et al, 2004; Sarkar and Kaul, 2014
Corrected protein efficiency ratio	1.93		Sogi et al, 2004	1.99		Sogi et al, 2004
Protein Utilization	0.22-0.73	0.475	Sogi et al, 2004; Rao 1990	0.69		Sogi et al, 2004
Net protein retention	2.52		Sogi et al, 2004	2.51		Sogi et al, 2004

Further Analysis

Extractions of the tomato seed flours were similar to those of the tomato seed oil. Hexane was the most popular solvent in the studies and Soxhlet extraction protocol was commonly used. Two studies purchased a de-oiled meal.

Tomato seed flour contains an average of 34% protein (Giuffre and Capocasale, 2016; Liadakis et al, 1995; Sarkar and Kaul, 2014). One study that extracted the flour showed it has 54.1% fiber (Liadakis et al, 1995), while another that purchased the tomato seed flour showed it has 21% fiber (Rao 1990). Both extracted and purchased

tomato seed flours contain high amounts in mg/100 g of calcium, magnesium, and phosphorus relative to other minerals. The extracted tomato seed flour also showed a high amount of potassium.

Three studies further extracted a concentrated protein isolate from the tomato seed meal. Liadakis et al (1995) isolated proteins from tomato seed meal by extracting with deionized water and keeping the pH constant with 0.5N NaOH. The temperature was kept between 30-50 °C. After the mixture was centrifuged, the supernatant was adjusted to a pH of 3.9 using 0.5N HCl. The precipitate was centrifuged and dried. Sarkar and Kaul (2014) extracted protein isolate with 1 M NaCl solution at 50 °C. The pH was kept at 8 with 0.1 N NaOH. The mixture was centrifuged and the supernatant collected. It was then adjusted to pH 4 with 0.1 N HCl, centrifuged and dried. Turakhozhaev et al (1979) extracted protein with 0.2% NaOH and vacuum filtered the mixture. The protein was precipitated with a 5% HCl solution at pH 5.5. The protein was separated in a centrifuge and dried. All three protein extractions identified different isoelectric points: 3.9, 4, and 5.5. The resulting 3.9 and 4 values are very close to each other. The study that identified the isoelectric point of 5.5 did so, based on the alkali-soluble fractions of the protein, which may account for the difference.

Sarkar et al (2015) examined the protein composition of tomato seed flour using SDS-PAGE. The main proteins identified in an extracted protein isolate had

molecular weights of 48, 33, 20, 19, and 10 kDa. The researchers hypothesize that the 48-19 kDa proteins coincide with albumin and globulin fractions of the protein identified by previous researchers, while the 10 kDa fraction was identified for the first time. This study identified some functional properties of the protein isolate, concluding that tomato seed protein emulsions flocculated and creamed around their isoelectric point, pH 2-4. However, emulsions at pH 6-8 with high levels of salt were stable, as well as emulsions that underwent heat treatment. The emulsions were also stable over seven days of storage.

Sogi et al (2002) also analyzed functional properties of tomato seed protein concentrate, as well as tomato seed meal. The group concluded that tomato seed does not have good foaming properties, however, the meal has a good emulsion capacity and stability. The cream layer was larger and held better over a storage period of 50 hours when compared to the protein isolate. The meal also had higher water absorption and fat absorption rates, supporting these findings. Even so, the group suggests the protein isolate may be a better choice for some food systems due to the lack of cellular matter.

Sarkar and Kaul (2014) found that tomato seed meal had a 21% radical inhibition per gram of sample against the DPPH radical. They also found the total phenolic content of the meal was 20.1 mg tannic acid equivalent per 100 g, suggesting it is a moderate source of phenolics. This study was able to positively correlate phenolics and

antioxidant activity, though only one assay against a radical was used and the standard used for TPC is not what is suggested in most literature.

This group also analyzed some antinutritional factors of tomato seed bran, meal, and protein isolate. Trypsin and phytate both reduce the bioavailability of protein (Gilani et al, 2005). Phytate concentration in µg/g was reduced from 26.16 in the bran to 5.29 in the meal to 3.48 in the protein isolate. Trypsin inhibitory activity was also reduced as the tomato seed was broken down from the bran to meal to protein isolate. In TIU/mg, the values were 12.5 for the bran, 6.58 for the meal, and 2.65 for the protein isolate.

Antioxidants and Their Testing Methods

Free Radicals, Antioxidant Compounds, and the Relation to Human Health

Chronic diseases are amongst the leading causes of death globally. Cardiovascular disease is the leading cause of death in the developed countries (Reuland, McCord, and Hamilton, 2013). Hyperglycemia (diabetes) and obesity rates are also raising worldwide (Zhang et al., 2015). Sixty percent of US adults have at least one chronic condition (Buttorff, Ruder, and Bauman, 2017). They are not only responsible for death and disability, but also come with a high economic cost. The CDC estimates that adults with chronic health conditions account for 90% of total healthcare spending (Buttorff, Ruder, and Bauman, 2017). The initiation or progression of at least 70 disorders, including cancer, diabetes, atherosclerosis, anemia, and

neurodegenerative disorders, has been linked to reactive oxygen species (ROS) (Ferrari and Torres, 2003).

Free radicals (FR) are chemical species that have unpaired electrons (Ferrari and Torres, 2003). If the radicals can receive electrons, they are an oxidant; if they can give electrons, they are a reducer. Oxidants in the body, also known as reactive oxygen species (ROS), can be generated in a variety of ways. Mitochondrial respiration, reperfusion, inflammation, and metabolism of foreign compounds all may generate ROS (Khan *et al.*, 2008). Some common ROS generated in the human body include superoxide, hydrogen peroxide, and hydroxyl radicals. Reactive non-radicals such as hydrogen peroxide and singlet oxygen are also generated and may lead to oxidative damage through secondary reactions (Khan *et al.*, 2008; Ferrari and Torres, 2003).

A balance of reactive oxygen species and antioxidants in the body is important. An excess of ROS causes oxidative damage, which further leads to DNA breaks, modifications, and cross-links (Khan et al., 2008). The result of these reactions can be desirable. Some amount of damage to a cell can be beneficial when it prevents the proliferation of aged or unhealthy cells. Furthermore, evidence shows that maintaining some level of ROS in the body may increase lifespan and prevent chronic diseases through currently unknown mechanisms (Ristow, 2014). However, without a balance of pro- and anti-oxidants, excess ROS leads to harmful levels of oxidative

damage (Sun et al., 2002) linked to changes in transcription or transduction pathways, replication errors, genomic instability, and epigenetic changes (Lopez-Lazaro, 2007). Additionally, ROS can oxidize lipids and proteins in the body. The oxidative damage of polyunsaturated fatty acids, known as lipid peroxidation, is especially harmful because it creates a chain reaction that leads to harmful secondary products. Lipid peroxidation creates fatty acid peroxyl radicals that can further oxidize other fats and molecules in the body (Nimse and Pal, 2015). The resulting products of lipid peroxidation include aldehydes, which are active compounds that can lead to cell damage (Pryor and Porter, 1990). Reactive oxygen species also oxidize essential proteins and enzymes in the body, possibly leaving them nonfunctional (Ferrari and Torres, 2003).

When the body maintains normal levels of stress and functionality, it produces its own antioxidant compounds to quench/clear free radicals. The enzymes superoxide dismutase, glutathione peroxidase, and catalase work to achieve a natural balance of ROS. In the cytosol, mitochondria, or peroxisomes, they remove or reduce commonly produced ROS (Klaunig and Kamedulis, 2004). When overexpressed, antioxidant enzymes have been shown to reduce or reverse malignant features of cancer cells (Zhang *et al.,* 2002), demonstrating their importance.

A problem arises when the load of oxidants is too much for the body's natural antioxidants to process and maintain balance. Though the production of ROS can be

natural, some lifestyle choices contribute to excess oxidative stress. Exposure to free radicals in the environment is possible from pollutants and cigarette smoke. Physical and psychological stress, including but not limited to physical activity, calorie restriction (Ristow, 2014), and traumatic events (Schiavone *et al.,* 2013) have all been linked to an increase in the production of ROS. Additionally, the expression of our body's antioxidant enzymes may decrease with age. The activities of these enzymes appear to decrease in aged mammals compared to young ones; this has been linked to increases in oxidative damage in older animals compared to younger animals (Tian *et al.,* 1998). A study conducted to determine the effect of oxidative damage to the kidney over the life cycle of rats found that as rats age, the production of oxidants began to exceed the production and capabilities of antioxidant enzymes (Jena *et al.*, 2017).

While synthetic antioxidant compounds are an option for the prevention of oxidative damage, there is concern that they may be associated with potential health risks (Safer and Al-Nughamish, 1999). Phytochemicals in plants such as phenolics including polyphenols and other antioxidant compounds may be a potential alternative source of natural antioxidants. Many studies have linked an increase in plant consumption to positive health benefits (Willett, 2002), and it's believed antioxidant phytochemicals are the source of their health benefits. Studies have also shown that polyphenols in foods can prevent pathways that lead to cardiovascular disease, obesity-related inflammatory responses, and diabetes (Zhang et al., 2015). Previously, fruit wastes,

including cherry tomato wastes, were found to contain high amounts of antioxidants (Deng et al., 2012). These wastes included the peels and seeds.

Polyphenols are metabolites of plant species (Beckman, 2000). They are known to contribute to the flavor, color, and smell of plant products (Pandey and Rizvi, 2009) and may be found in plant tissue, cell walls, or in cell vacuoles (Wink, 1997). They contain phenol rings and occur in a conjugated form with at least one sugar residue linked to hydroxyl groups (Pandey and Rizvi, 2009).

Polyphenols are classified into four different groups based on their chemical structure (Spencer *et al.*, 2008). Lignans are diphenolic and contain 2,3-dibenzylbutane (Pandey and Rizvi, 2009). Stilbenes are another group of polyphenols and contain a two-carbon methylene bridge that connects two phenyls (Pandey and Rizvi, 2009).

The remaining two classes of polyphenols are the more widely studied of the four, mainly due to their abundance. Phenolic acids are derivatives of benzoic acid or cinnamic acid (Pandey and Rizvi, 2009). This class accounts for one-third of polyphenols in the human diet and are associated with acidic foods such as fruits (Pandey and Rizvi, 2009). The final class of polyphenols is flavonoids. They are the most common polyphenolic compound in the human diet. They contain two aromatic rings bound by three carbon atoms (Pandey and Rizvi, 2009). Over 4,000 kinds of flavonoids have been identified and are further classified into six sub-classes based on

their heterocycle (Pandey and Rizvi, 2009). The six sub-classes are flavonols, flavanones, flavanols, flavones, anthocyanins, and isoflavones (Pandey and Rizvi, 2009).

Another antioxidant phytochemical group found in plant species are carotenoids. They are pigments found in most colored plant species (Eggersdorfer and Wyss, 2018). Over 650 have been identified in nature (Khoo *et al.*, 2011), 100 in the human food chain (Milani *et al.*, 2017), and 40 in the human bloodstream (Zimmer and Hammond, 2007). Since human body cannot synthesize carotenoids, any found in the body must come from plant sources (Zimmer and Hammond, 2007). Some common carotenoids include alpha and beta-carotene, lutein, lycopene, and zeaxanthin (Eggersdorfer and Wyss, 2018). Beta-carotene specifically has been studied for its potential anti-cancer properties (Burton and Ingold, 1984).

Some vitamins found in diets can also act as antioxidant species. Vitamins C, A, and E are such antioxidant vitamins. One study found that while healthy centenarians had decreased natural antioxidant enzymes in their blood, they did have increased levels of vitamins A and E, possibly linking them to longevity (Mecocci et al., 2000). They are also essential for bodily functions and cannot be synthesized (Kasote *et al.*, 2015). Vitamin C, also known as ascorbic acid, is a water-soluble antioxidant vitamin, said to be the most important water-soluble antioxidant (Klimczak and Gliszcynska-Swiglo, 2015). In the body, vitamin C/ascorbic acid can donate an electron to other

radicals to stop oxidation, forming the ascorbate radical that is then able to be regenerated as ascorbate (Nimse and Pal, 2015).

Phenolic compounds, carotenoids, and vitamins have all been linked to antioxidant activities through a positive correlation of phenolic and free radical scavenging assays (Pandey and Rizvi, 2009; Sung and Lee, 2010). A higher total phenolic content is associated with a stronger antioxidant activity (Sun, Chu, Wu, and Liu, 2002). Additionally, many of these compounds have been studied for their beneficial health properties for various chronic diseases. It's believed that these active compounds work as functional foods due to their antioxidant, antibacterial, and antiviral activities, influence over detoxifying enzymes, control of hormones and endocrines, and their abilities to decrease platelet aggregation, blood pressure, and alter cholesterol metabolism (Lampe, 1999). Generally, increased consumption of plant products has long been linked to a decrease in the risk of developing chronic diseases (World Health Organization, 2003).

Due to the established link between oxidative damage and chronic diseases and the antioxidant effect polyphenols have on these pathways, it is worthwhile to study food components that possess polyphenols. Tomato seed wastes may be one such component. Investigating total phenolic content and using different antioxidant assay methods can help further link phytochemicals to their antioxidant properties.

Total Phenolic, Antioxidant, and Radical Scavenging Assays and Quantification Methods

There are two mechanisms by which an antioxidant may quench a free radical. An antioxidant can donate a hydrogen atom or transfer a single electron to reduce an oxidant (Prior, Wu, and Schaich, 2005). The assays used to detect the antioxidant capacity of a sample measure one or both of these mechanisms.

In 2005, Huang, Ou, and Prior stated that applying multiple ET-based assays (such as TPC, DPPH, and ABTS) can lead to good correlations between results, showing a positive relationship between total phenolic contents and antioxidant activity (Huang, Ou, and Prior, 2005). The group also stated that the ORAC assay should be the assay of choice when evaluating peroxyl radical scavenging capacity (Huang, Ou, and Prior, 2005). When evaluating foods for beneficial components, oftentimes multiple antioxidant capacity assays and the total phenolic assay are used (Deng et al., 2012; Fu et al., 2011), and Prior, Wu, and Schaich (2005) stated that "no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed or complex system." Therefore, it is beneficial to perform multiple assays on samples to ensure correlation between total phenolics, scavenging capacity, and antioxidant activity.

The Folin-Ciocalteu assay, or the total phenolic content (TPC) assay, has been developed and used over many years to determine the total phenolics in samples. The method was first developed and used in 1927 (Folin and Ciocalteu, 1927) and

improved upon in 1965 (Singleton and Rossi, 1965). The assay measures the reducing capacity of a sample as phenolic compounds reduce the FC reagent, causing it to turn from a yellow to blue color. The reaction is nonspecific for all phenolic compounds. While the reaction that occurs isn't completely understood, it's believed that FC reagent is a phosphomolybdate-phosphotungstate complex, in which yellow Mo(VI) is reduced to blue Mo(V). The color change is measured in a spectrophotometer at an absorbance of 765 nm. A gallic acid standard is used to compare samples, in which the total phenolic contents are reported as milligrams of gallic acid equivalent per gram of sample (Huang, Ou, and Prior, 2005).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is commonly used in laboratories to assess the reducing ability of antioxidants in a sample (Prior, Wu, and Schaich, 2005). DPPH•, a free radical, is reduced to DPPH in the presence of antioxidants. The assay conditions cause a decolorization reaction, where purple DPPH• changes its color when reduced to DPPH. A spectrophotometer continuously reads the absorbance of the samples at 515 nm to determine the degree of decolorization (Pisoschi, Cheregi, and Danet, 2009). Trolox is used as a standard and the assay results are reported as micromoles of Trolox equivalents per gram of original sample.

2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is the salt that forms the $ABTS^{\bullet+}$ radical when oxidized by potassium persulphate (Liu, 2010) or manganese dioxide (Su, Yin, Charles, Zhou, Moore, and Yu, 2007). This radical is a blue-green

color that absorbs at 743 nm. When antioxidants are present, the nitrogen atom present in $ABTS^{\bullet+}$ quenches the hydrogen atom in the antioxidant, leading to decolorization (Pisoschi and Negulescu, 2011). Trolox is used as a standard to compare the sample against, in which the reaction is measured at a fixed time point (Prior, Wu, and Schaich, 2005). The results are reported as micromoles of Trolox equivalent per gram of original sample. The ABTS assay is sometimes referred to as the Trolox equivalent antioxidant capacity (TEAC) assay in literature (Prior, Wu, and Schaich, 2005). Multiple methods can be used to generate $ABTS^{\bullet+}$, with some methods choosing to call it the ABTS or TEAC assay, but all are based on the same reaction mechanism (Prior, Wu, and Schaich, 2005).

The DPPH and ABTS based assays typically measure single electron transfer capabilities in samples. However, these radicals can be reduced by hydrogen transfer, so these assays could be classified as measuring both the hydrogen and single electron transfer capabilities in samples (Jimenez et al, 2004).

The Oxygen Radical Absorbance Capacity (ORAC) assay was first reported by Cao and Prior (1999) as the first assay to run all reactive species reaction to completion with the capability to quantify antioxidant capacity by combining inhibition time and inhibition percentage. The quantification of the assay is based on an area under the curve calculation. The original ORAC assay detected damage to B- or R-phycoerythrin based on its decreased fluorescence emission. The antioxidant capacity

is based on the net area under the curve that quantified a decreased rate and amount of product over time (Prior, Wu, and Schaich, 2005). Later, Ou, Hampsch-Woodill, and Prior (2001) updated the method to use fluorescein as the fluorescent probe. They validated this method and determined that using the FL probe in an ORAC assay accurately measures the hydrophilic antioxidant activity against peroxyl radicals generated from AAPH. Further development of the assay was completed to validate its ability to be completed with a multichannel liquid handling system with a microplate fluorescence reader in a 96-well format (Huang et al, 2002). This allowed the assay to be carried out without the use of specialized equipment, making it more easily reproducible in a variety of lab spaces. Trolox is used as the standard for the ORAC assay at different concentrations and it is carried out at a controlled, constant temperature (37 °C) (Karadag, Ozcelik, and Saner, 2009).

Other antioxidant assays and methods of detection can be used to measure the antioxidant capacity of food samples. These include but are not limited to the ferric reducing antioxidant power (FRAP) assay, the hydroxyl radical averting capacity (HORAC) assay, the total peroxyl radical trapping antioxidant parameter (TRAP) assay, the lipid peroxidation assay, the potassium ferricyanide reducing power (PFRAP) assay, the cupric reducing antioxidant power (CUPRAC) assay, the total oxidant scavenging (TOSC) assay, chemiluminescence assays, photochemiluminescence assays, electrochemical-based methods, and biosensor-

based methods (Ou, Hampsch-Woodill, and Prior, 2001; Pisoschi and Negulescu, 2011).

No one assay can capture the full antioxidant capacity of a sample. They all use different mechanisms and quantification systems that may detect certain types of antioxidants more accurately or less accurately, leading to the need to perform multiple assays to draw conclusions. Each assay has advantages and disadvantages that must be considered.

The TPC assay using the Folin-Ciocalteu reagent is advantageous because it is simple, requires no special equipment, and is easily reproducible when the correct reference standard is used (Prior, Wu, and Schaich, 2005; Huang, Ou, and Prior, 2005). When performed properly, it is precise in its ability to detect phenolics using gallic acid as a reference (Prior, Wu, and Schaich, 2005). However, the reagent can react with several organic and nonphenolic substances such as sugars and acids, as well as inorganic substances such as iron, potassium, and sodium based compounds (Prior, Wu, and Schaich, 2005; Peterson, 1979). And the TPC assay is known by its ability to detect phenolics, it is truly based on a reduction reaction. It is also carried out in an aqueous phase, making it less relevant for hydrophobic substances (Karadag, Ozcelik, and Saner, 2009). Still, the TPC assay is useful due to its known ability to detect polyphenols. A positive relationship between the TPC assay and antioxidant assays have long been established, and as it is the only assay that is

assumed to be based on the phenol content, making it useful to perform in conjunction with other assays (Prior, Wu, and Schaich, 2005).

The DPPH assay also has drawbacks. It is easily interfered by carotenoids (Noruma, Kikuchi, and Kawakami, 1997). It's also believed that the color loss could be due to hydrogen or electron transfer (Prior, Wu, and Schaich, 2005). The radical is also not similar to peroxyl radicals, and radicals that may easily and quickly react with peroxyl radicals may react slowly or not at all with DPPH. Electron transfer tends to happen quicker, and hydrogen transfer slower, so the detected antioxidant capacity may only reflect part of the true value (Karadag, Ozcelik, and Saner, 2009). Some groups chose to report the results of a DPPH assay as EC_{50}, or the concentration of the sample needed to react with 50% of the reagent, DPPH. However, it has been found that DPPH concentration is not linear to the reaction kinetics between DPPH and antioxidants (Karadag, Ozcelik, and Saner, 2009), making this an inaccurate way of expressing antioxidant capacity using the DPPH assay. Though it has drawbacks and does not accurately express all antioxidants in a sample, the DPPH assay is simple, quick, and requires no special equipment (Prior, Wu, and Schaich, 2005). Due to this, it has seen widespread use and is a common assay used when considering antioxidant content. When expressed using micromoles of Trolox equivalent per gram of sample, it is comparable between labs. It is one of the more commonly used assays when considering the past work completed on tomato seed antioxidant capacity (Sarkar and Kaul, 2014; Ma et al, 2013; Muller et al, 2012).

The ABTS assay has limitations; the assay measures the ability of a substance to react with the ABTS radical rather than its true oxidative capabilities. Additionally, some compounds may take a long time to react with the radical and are not detected when by some fixed time points used, such as four to six minutes (Karadag, Ozcelik, and Saner, 2009). The radical is also not biologically relavent, so the ability to react with this radical may not reflect the ability of a substance to react with biological radicals (Prior, Wu, and Schaich, 2005). Van den Berg et al (1999) concluded that the assay is more useful as a ranking order or comparison between samples and less as a quantitate measurement of antioxidant capacity. Just as in the DPPH assay, some groups choose to report the capacity using IC_{50} or $EC_{50,}$ but ABTS concentration is also not linear to the reaction kinetics between ABTS and antioxidants, making it an inaccurate way of expressing antioxidant capacity (Karadag, Ozcelik, and Saner, 2009). However, the ABTS assay also has many advantages. Since it is referred to as the TEAC assay in most literature, the assumption is that most groups may use a Trolox equivalent in reporting, making it easily comparable. Additionally, it can be used to measure both hydrophilic and hydrophobic antioxidant compounds and can be performed over a wide range of pH values (Arnao 2000; Karadag, Ozcelik, and Saner, 2009). Due to its simple and quick nature, the ABTS assay has been widely used for a variety of substances (Prior, Wu, and Schaich, 2005). Since it has been stated that this assay should be used as a comparison, its common use in antioxidant capacity testing makes it worthwhile.

The ORAC assay has been identified as detecting antioxidants using the hydrogen transfer mechanism. It is limited to hydrophilic chain-breaking antioxidants that work against peroxyl radicals (Karadag, Ozcelik, and Saner, 2009). The assay is also criticized for its requirements of a specific and constant assay temperature, long assay time, and requirement of a fluorometer (Prior, Wu, and Schaich, 2005). However, it is one of the most highly regarded antioxidant assays because of its ability to detect antioxidants that do and do not have a lag phase. Since other antioxidant assays are often completed in a few minutes, where the ORAC assay typically runs for an hour, antioxidants that may react are given sufficient time to be detected (Karadag, Ozcelik, and Saner, 2009). The updated fluorescent probe that is used in detection is also inexpensive, and when an automated plate reader is used, the assay itself and calculations can be automated (Karadag, Ozcelik, and Saner, 2009). Since the assay calculates the area under the fluorescence decay curve compared to a sample, the quantification accounts for the lag time, initial rate, and total inhibition in one single value (Prior, Wu, and Schaich, 2005). It is considered to have desirable up to highly desirable characteristics in terms of simplicity, instrumentation, and biological relevance; the only assay to have only positive associated values for these characteristics when compared to eight other commonly performed antioxidant assays (Prior, Wu, and Schaich, 2005).

Other commonly reported antioxidant assays include the FRAP, TRAP, TOSC, and LDL oxidation methods. While worthwhile to perform in some instances, the scope of the measured antioxidant capacity and logistics of these assays made them less valuable for this research. These methods were not chosen over the aforementioned assays due to their specific drawbacks.

The FRAP assay measures a sample's ability to reduce Fe (III) to Fe (II). However, there are substances that are not antioxidants that can reduce Fe (III). For instance? Additionally, some antioxidants can't reduce Fe (III), making it hard to determine if this method over or underestimates true antioxidant capacity (Karadag, Ozcelik, and Saner, 2009). The results of this assay are also known to vary widely depending on the endpoint used, and the ability of a substance to reduce Fe (III) isn't biological relevant (Prior, Wu, and Schaich, 2005).

The TRAP assay is based on a sample's ability to interfere with peroxyl radicals. This method has its limitations in that the values obtained completely depend on the endpoint used; this varies so much between labs that comparisons are extremely difficult (Karadag, Ozcelik, and Saner, 2009). The TRAP assay also requires specialized equipment and a high level of expertise, as it's intended for in vivo measurements in serum or plasma (Prior, Wu, and Schaich, 2005).

The TOSC assay can measure hydroxyl radicals, peroxyl radicals, and peroxynitrite (Prior, Wu, and Schaich, 2005). However, this method is not suitable for antioxidant capacity measurement and comparison between labs and other food samples. It requires a very long reaction time and requires manual injections into a gas chromatograph (Prior, Wu, and Schaich, 2005).

The LDL oxidation assay is highly biologically relevant, as it takes isolated low-density lipoproteins from blood samples, oxidizes them, and measures the peroxidation (Handelman et al, 1999). However, it requires constant isolation of LDL from different blood samples as needed, and using biological samples makes it difficult to produce consistent results (Prior, Wu, and Schaich, 2005).

Since there are various methods to determine the antioxidant content of food samples, it is important that assays are standardized to follow accepted procedures so results can be compared between labs. However, many assays are relatively new and undergo updating in their procedures, leading to a lack of consistent data available for some food products. It is the suggestion of Prior, Wu, and Schaich (2005) that the ORAC, TEAC/ABTS, and F-C/TPC methods be standardized for the use of determining antioxidant capacity. These three methods were selected based on the range of antioxidants and antioxidant mechanisms they can detect, along with their varying ease of reproducibility and automation. As such, these three methods are used to determine the antioxidant capacity of the food samples, along with the DPPH

assay, as most of the available literature on the antioxidant capacity of tomato seed samples used the DPPH assay.

Successful assays also depend on the successful extraction of the desirable components from the compound being studied. Sultana, Anwar, and Ashraf (2009) tested the effectiveness of four extraction solutions and two extraction methods on the antioxidant activity of plant materials. In general, they found that using aqueous methanol and ethanol (80%) have higher extraction yields for plant material samples. Cacace and Mazza (2003) studied various concentrations of aqueous ethanol and found that using more than a 60% ethanol/water solution to extract phenolics resulted in a decrease in total extracted phenolics in black currants. Generally, aqueous methanol solutions have been successfully used to extract phenolic compounds and result in high antioxidant capacity outcomes from hydrophobic (oil) samples (Dehpour et al, 2009; Gulluce et al, 2005; Lutterodt et al, 2011). Aqueous ethanol solutions have shown similar success in extracting and yielding in high phenolic and antioxidant capacity results for hydrophilic samples, specifically flours (Bonoli et al, 2004; Inglett et al, 2010; Lutterodt et al, 2011). Ngo et al (2017) also compared different solvents for use in antioxidant assays and determined that 50% acetone should be recommended for phenolic and antioxidant assays.

For specific antioxidant compounds, other methods can be used for exact quantification. A spectrophotometric method can be used to quantify beta-carotene

contents. Beta-carotene belongs to the carotenoids class of compounds as a known pigment that aids in photosynthesis and photoprotection in plants (Mayne, 1996). Since it's a pigment, using a UV-vis spectrophotometer can be useful in its detection.

There are many methods to quantify beta-carotene, including high performance liquid chromatography (HPLC), gas chromotography (GC), and electrophoresis. Using a spectrophotometric method is advantageous because it is inexpensive, quick, and accurate. It's been found that beta-carotene contents are easily detectible in fruit and vegetable samples at about 461 nm, with a limit of detection of about 0.04% and a relative standard deviation of less than 6.4% (Zahra et al, 2016).

While there is a possibility for interfering components in the spectrophotometric method, it's been established as a simple and rapid procedure for measuring beta-carotene content (Lime et al,1957). Thus, determining beta-carotene content with a similarly prepared standard using a spectrophotometer is often reported research (Karnjanawipagul et al, 2010; Sanusi and Adebiyi, 2009) and has been cited as a precise method when analyzing its ability to accurately detect beta-carotene (Biswas, Sahoo, and Chatli, 2011).

HPLC systems can be used to identify and quantify many different non-volatile components depending on the techniques used. It can quantify the amount of vitamin C in a sample and identify exact chemical components in samples.

Chromatography is used to separate components with a mobile and stationary phase. Different types of chromatography exist, including thin layer, gas, and liquid chromatography. HPLC is advantageous to other types of chromatography because of the many different separation techniques available, the possibility to manipulate the mobile phase, and because there is no need to derivatize the sample (Fallon, Booth, and Bell, 1987). In HPLC systems, a sample is injected through the system and portioned within the phases; those with more attraction to the stationary phase stay in the system longer, while those with more attraction to the mobile phase move quickly. The stationary phase is typically a packed column made of steel, silica, or glass, while the mobile phase is a liquid, gas, or supercritical fluid. In the case of an HPLC system, the mobile phase will always be liquid.

An HPLC system contains the mobile phase reservoir, a pump, injector, column, and detector with data recording unit. These parts allow the system to deliver specific volumes of a sample into specific gradients of the mobile phase. As the sample and mobile phase move through the system, the compounds in the sample will be separated. Compounds with a stronger attraction for the mobile phase, as opposed to the column, move quickly through the system and onto the detector. Compounds with a stronger attraction to the stationary phase take longer to move through the system. Knowing what kind of components is more or less attracted to the different phases can help in their identification and quantification. The detector typically used

in HPLC system is a UV-Vis detector. Since it's concentration dependent, knowing the wavelength at which a component shows absorption can be used to determine the concentration of that component in the sample (Waters, 2019a). Another possible detector for an HPLC system is a mass spectrometer. LC-MS (liquid chromatography-mass spectrometry) is used to detect ions in a sample, thus detecting mass differences of various components in a sample. It can be used to identify exact components in a sample. An MS resource library can be used to identify components (ThermoFisher, n.d.).

Since there are many possibilities for different phase types, chromatography can be used to separate a wide range of very similar components (Fornstedt, Forssen, and Westerlund, 2015). There are different modes of separation used in HPLC systems. They are all based on size, charge, or polarity. Separations based on size involve a stationary phase with a particular pore size. Molecules too big to fit in these pores will come out of the column first, while smaller molecules will spend more time in the stationary phase going through the pores, thus separating molecules based on size. Separations based on charge utilize an anion exchanger or cation exchanger stationary phase particles. In the case of charges, opposites attract, so molecules with a charge opposite to that of the stationary phase stay in the system longer. These separations typically rely on a pH gradient over time in the mobile phase. Separations based on polarity rely on the fact that molecules with similar polarities are attracted to each other. In an HPLC system, molecules are more attracted to whichever phase more

closely matches its polarity. In normal phase chromatography, the stationary phase is polar and the mobile phase is nonpolar. In reverse phase chromatography, the stationary phase is nonpolar and the mobile phase is polar (Waters, 2019b). Reverse phase HPLC is the most popular, with more than half of all separations using reverse phase techniques (Fallon, Booth, and Bell, 1987). Reversed phase HPLC has been used to quantify the amount of vitamin C in food samples (Fontannaz, Kilinc, and Heudi, 2006); even specifically in tomato samples (Abushita et al, 1997). LC-MS techniques on the basis of mass separation and compound detection have been successfully used to detect specific components in other seed flours (Choe et al, 2018). Additionally, a database for the metabolomes of tomatoes detected by LC-MS has been developed by one lab group (Moco et al, 2006), and the flavonols of different parts of the tomato identified by another (Stewart et al, 2000).

Since polyphenols may also contribute to color, measuring the color of samples can be useful. Additionally, it's a useful comparative tool for detecting differences in samples; oil quality is often correlated to color (Smouse, 1995). Many factors can influence color, including the presence of polyphenols, the presence of contaminants, and any lipid oxidation/rancidity (Smouse, 1995). Color changes may be related to storage conditions, where changes in color indicate quicker spoiling.

Color can be accurately quantified and measured with a colorimeter. Using the CIELAB color scale, the measurements L, a, and b are taken on a colorimeter. The

“L” measures how black (L= 0) or white (L= 100) a sample is. The “a” and “b” measurements are on axis and are less direct measurements. The “a” coordinate is measured on a horizontal axis, where more positive numbers correlate with a red color and more negative numbers correlate with a green color. The “b” coordinate is measured on a vertical axis, where positive numbers indicate a yellow color and negative indicate a blue color. At the center of these axis would be gray, where “a” and “b” are zero (McGuire, 1992). Although the “a” and “b” coordinates cannot be read in isolation, since they represent the full color spectrum, statistical analysis can be completed using colorimeter results to show how much color differs between samples.

Comparable Data

As discussed, it can be difficult to obtain comparable data for antioxidant capacity assays. Many labs use different standards that cannot be compared and run limited assays that are not possible to perform by all labs. A brief review of the antioxidant capacity of comparable products to tomato seed oil and flour are listed below. Data was selected to be used if it contained an assay used in this research with the same standard (or convertible standard), which are as follows: The TPC assay using F-C reagent expressed as mg of gallic acid equivalents per gram of sample, the ABTS/TEAC assay expressed as micromoles of Trolox equivalent per gram of sample, the DPPH assay expressed as micromoles of Trolox equivalents per gram of sample, and the ORAC assay (as conducted for hydrophilic antioxidants) expressed as micromoles of Trolox per gram of sample.

The phenolic contents and antioxidant capacities of commonly used commercial oils and flours were investigated. However, due to different standard solutions and reported equivalents, it is somewhat difficult to find comparable data. Xuan et al (2018) reported the total phenolic content of some common oils using the same standard as the collected data (mg GAE/g oil). The group reported the total phenolic content using this standard of the following oils: sunflower 4.39, safflower 1.76, canola 3.0, cotton seed 8.22, grape seed 15.56, flaxseed 39.16, avocado 11.31, chia seed 4.86 and, sesame seed 10.46. Ninfali et al (2005) investigated the antioxidant capacity of some common oils using an ORAC assay expressed as μmol TE/100 g, which is a comparable standard for the assays performed. Converting the results to μmol TE/g of sample, the group reported the following results: refined peanut oil and extra virgin olive oil contained 1.06 and 11.5 μmol TE/g sample, respectively.

Cereal grains were also investigated for their antioxidant activity. The total phenolic contents of some grains and flours with comparable standards were found. Xuan et al (2018) reported rice bran as having 19.59 mg GAE/g sample. Ragaee, Abdel-Aal, and Noaman reported the total phenolic content of six common cereal grains in μg GAE per gram of sample. These numbers were converted to mg GAE per gram of sample and were as follows: Hard wheat 0.562, soft wheat 0.501, barley 0.879, millet 1.387, rye 1.026, and sorghum 4.128.

A recently published study by Silva et al (2018) analyzed the phenolic content and radical scavenging capacity of tomato pomace using the DPPH, TEAC (ABTS), FRAP, and TPC assays. Not all of the data are comparable, as the DPPH assay was expressed as the percent inhibition and the FRAP assay was not conducted in the present study. However, the ABTS assay is easily comparable and a gallic acid standard was used in the TPC assay, making it comparable to the present study. The study performed the assays on six different batches of commercially produced tomato pomace/waste, leading to significantly different results for the assays. The results for the $ABTS^{\bullet+}$ scavenging capacity as µmol TE/g sample were as follows: 2.1485, 1.9472, 1.23804. 2.2129, 2.5073 and 2.2924. The results for the TPC assay as mg GAE/g sample were as follows: 0.1232, 0.2275, 0.1896, 0.1755, 0.1008, and 0.1294. While the assays were performed on tomato seed pomace, a combination of the waste skin and seeds, the data is useful to understand the range of values that should be obtained from tomato seed oil and flour.

Chapter 2: Tomato Seed Flour

Introduction

Abstract

Many commercial tomato products produce tomato seeds as a waste. Tomato seeds can have their oils extracted and the hydrophilic meal is the tomato seed flour. In order to study their potential health beneficial properties, tomato seed flour samples were extracted with 50% acetone and evaluated for their phenolic contents, radical savaging capacities, vitamin C content, and chemical composition. The two tested flours contained an average of 1.8398 and 1.7540 mg gallic acid equivalents/g in total phenolics, which were significantly different from each other, likely due to the effect of tomato cultivar. These values were higher than those previously reported for tomato wastes and higher than some commonly used flours, such as wheat and rye. The flours were not significantly different for any of the tested free radical scavenging capacities. The values obtained from the ABTS, DPPH, and ORAC assays were comparable to some common vegetables. The ORAC values of the flour extracts were higher than those reported for whole tomato seeds, possibly showing that the beneficial components of tomato seeds concentrate in this portion. The flours had an average vitamin C content of 8.317 micrograms/g, much lower than that reported for whole tomato seeds. UHPLC-HRMS was used to identify chemical compounds, including quercetin and kaempferol isomers, flavonoids known for their health beneficial properties. The results of this study show that tomato seed flour may

be beneficial to health when compared to other commonly used flours and could be utilized as a value-added product.

Introduction

Tomato seeds are a waste product of many tomato processes, including the making of tomato paste, sauce, and ketchup. The seeds can be made into tomato seed oil, and this process produces tomato seed flour. Tomato seed flour is currently seen as a waste product of tomato seed oil production. Finding a use for tomato seed flours can reduce environmental waste and increase profits for food companies. Many groups have studied the properties of tomato seed oil, but few have investigated tomato seed flour.

Whole tomatoes are known to contain carotenoids, specifically lycopene, and antioxidant compounds such as vitamin C. Beneficial compounds in tomatoes have been associated with a decreased risk of cancer and cardiovascular disease; the health benefits have been attributed to antioxidants with free radical scavenging capacity (Canene-Adams et al, 2005). Additionally, polyphenols that act as antioxidants may reduce inflammation and aid in the prevention of other chronic diseases, such as obesity and diabetes (Zhang et al, 2015). If some of these compounds or properties can be identified in tomato seed flour, it could also be considered a food that is beneficial for human health.

Previously, other vegetable seed flours have been investigated for their health beneficial properties. Broccoli, carrot, and cucumber seed flours were found to contain phenolic compounds and measurable free radical scavenging capacity (Choe et al, 2018). Tomato seed flour may also contain components beneficial to human health, making it a potential functional food ingredient. Tomato seed flour has been investigated for its potential use as a protein, as its amino acid profile in its protein isolate form has been studied (Rao, 1990; Sarkar and Kaul, 2014). Besides its potential use as a protein, Sarkar and Kaul (2014) also investigated some radical scavenging and phenolic properties of tomato seed flour. Tomato seed meal (flour) reportedly had a 21% radical inhibition per gram of sample against the DPPH radical and 20.1 mg tannic acid equivalent per 100 grams in terms of total phenolic content, suggesting it is a moderate source of phenolics. This study was able to positively correlate phenolics and antioxidant activity in tomato seed flour, and identify that it does contain beneficial health components.

Some research has been competed regarding the use of tomato seed as a functional food ingredient. Most include the entire tomato pomace, where all tomato waste components (skin, seeds, and pulp) have been combined. Whole tomato seeds have been found to increase phenolic contents and free radical scavenging capacity of tarhana, a traditional Turkish soup (Isik and Yapar, 2017). Tomato seed flour on its own could serve as a potential functional food ingredient, and its actual functionality compared to dried seeds or a combination of skin, seeds, and pulp would most likely

be improved. Defatted flour would likely be more consistent in food products, making tomato seed flour worthwhile to study.

The current study aims to identify specific health beneficial compounds in the defatted tomato seed flour, as well as quantify the phenolic contents and free radical scavenging capacity of the flour using different assay types. This is the first time that tomato seed flour has been studied for chemical composition. The results of this study may indicate the usefulness of tomato seed flour as functional food and as a potential agricultural product that can reduce waste and increase profits for food companies.

Materials and Methods

Materials

Tomato seed flour samples were gifted from Botanic innovations (Spooner, WI, USA). Methanol, 3,4,5-trihydroxybenzoic acid (Gallic acid), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin and Ciocalteu's phenol reagent (FC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), manganese oxide, 2,2′-Azinobis (2-amidinopropane) dihydrochloride (AAPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and Fluorescein (FL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetone, 30 percent ACS-grade hydrogen peroxide, DEPC-Treated water and nuclease-free water were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was prepared by an ELGA Purelab ultra Genetic polishing system with >5 ppb TOC and resistivity of 18.2 mΩ

(Lowell, MA, USA). Ethanol was purchased from Pharmco-Aaper (Brookfield, CT, USA). Two different samples of tomato seed flour were used for the study. The samples were referred to as Tomato Seed Flour "1" or "2." The tomato seed flours were both brown in color. Tomato Seed Flour 1 and Tomato Seed Flour 2 contained both large chunks and small pieces of the tomato seed.

Methods

Seed Flour Extraction. For total phenolic content, radical scavenging capacity assays, and vitamin C quantification, approximately ten grams of seed flour was accurately weighed and combined with 50 mL of the 50% acetone and extracted for 24 hours at ambient temperature. The two flour samples, 1 and 2, were extracted in triplicate. 50% acetone extracts failed to yield identifiable compounds in UHPLC (Ultra-High Performance Liquid Chromatography) analysis. Instead, ten grams of one of the tomato seed flours was accurately weighed. 50 mL of ethanol was added to the bottom of a Soxhlet apparatus, and 50 additional mL was poured over prior to extraction. After 24 hrs, the solvent was evaporated, and the residue was re-dissolved in methanol. This solution was centrifuged at 1000 rpm for 5 min and the supernatant collected. The solution was again evaporated, and the remaining particles dissolved in 50 mL ethanol. All samples were stored in the fridge until testing.

Total Phenolic Contents. The TPC assay was completed according to laboratory procedures (Moore et al, 2005). A 20% Na_2CO_3 (w/v) solution was prepared. Gallic

acid stock solution and working solutions for the standard curve were prepared in solvent. For the assay, 3 mL of ultrapure water, 50 µL of the sample, standard, or blank (solvent), and 250 µL Folin-Ciocalteu reagent were added to a test tube. The mixture was vortexed for five seconds and allowed to stand for at least a minute. 750 µL 20% Na_2CO_3 was added. The tops of the tubes were covered and allowed to sit in the dark for two hours at room temperature. After this time, a spectrophotometer was blanked at 765 nm using the solvent blank. The absorbance of all standards and samples were measured. A standard curve was developed using the standards. Results were reported as milligrams of Gallic Acid equivalent per gram of sample (mg GAE/g).

$ABTS^{\bullet+}$ Scavenging Capacity. The ABTS scavenging capacity assay was completed according to laboratory procedures (Moore et al, 2005). ABTS working solution was prepared by reacting ABTS with manganese oxide and diluting the mixture to an absorbance of 0.700 ± 0.005 at 734 nm. 2 mL of ABTS and 160 µL of the sample to be measured was added to a test tube. After centrifuging for 30s, absorbance was read at 734 nm after a total of 90s. Trolox was used as the standard and results were reported as µmol Trolox Equivalent per gram of sample (µmol TE/g).

Relative DPPH Radical Scavenging Capacity. The Relative 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay was completed according to laboratory procedure (Cheng, Moore, and Yu, 2006) adapted for a microplate reader.

DPPH solution absorbance was read at 515 nm and diluted so that the absorbance read between 0.9 and 1.0. Trolox working standards were prepared. The samples were diluted in a 1:15 ratio. A 96 well plate reader was used to complete the assay. 200 µL of solvent was added to the blank wells. 100 µL of solvent, standard, and sample was added in triplicate to its respective well. 100 µL of DPPH was added to the solvent, standard, and sample wells. The assay was carried out with the lab protocol on a Victor multilabel plate reader (Perkin-Elmer, Turku, Finland) for 1.5 hours. The area under the curve was generated from the program and a standard curve developed from the Trolox standards. Results were reported as µmol TE per gram of sample (µmol TE/g).

Oxygen Radical Absorbing Capacity (ORAC). The ORAC assay was completed according to the lab's established protocol (Moore et al, 2005). AAPH solution, working fluorescein solution, and trolox standards were prepared. Samples were diluted using a dilution factor from previous preliminary lab data. Flour samples were diluted in a 1:500 ratio. In addition to the triplicate samples tested, each of those triplicate samples was added to the plate in triplicate. A Victor multilabel plate reader (Perkin-Elmer, Turku, Finland) was preheated to 37°C. The final reaction mixture consisted of 225 µL of 8.16×10^{-8} M FL, 30 µL of sample or solvent blank or standard, and 25 µL of 0.36 M AAPH. The fluorescence of the mixture was recorded every 2 min over 2 h at 37 °C. Excitation and emission wavelengths were 485 and 520 nm, respectively. A standard curve was developed from the AUC data from the

Trolox standards. Data from the samples were used to obtain μmol of Trolox per gram of original sample (μmol TE/g).

High-Performance Liquid Chromatography with Ultra Violet Detector. Vitamin C contents were measured using a laboratory HPLC-UV procedure and using ascorbic acid as a standard. The UV spectrum was measured at 278 nm. A luna C18 column, 4.6 mm × 250 mm and 5 μm particle size, was used. HPLC-grade water with 0.1% formic acid (v/v) was used as solvent A, and acetonitrile with 0.1% formic acid (v/v) was used as solvent B. The elution was carried out with 5% of solvent B at the beginning, increasing via a linear gradient to 13% B at 5 min; increasing to 20% B at 10 min; increasing to 27% B at 25 min; increasing to 33% B at 40 min; increasing to 50% B at 45 min; increasing to 90% B at 46 min; keeping 90% until 51 min; and the post run time for re-equilibration was 10 min. The injection volume was 5 μL, with a flow rate of 1 mL/min and an oven temperature of 40 °C. The final results were expressed in micrograms of Vitamin C per gram of sample.

Ultra High-Performance Liquid Chromatography Photo Diode Array High-Resolution Multi-Stage Mass Spectrometry (UHPLC-PDA-ESI/HRMS). Previous laboratory procedures were carried out for the UHPLC-HRMS analysis (Choe et al, 2018). Analysis was performed on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, USA) with an Agilent 1290 Infinity liquid chromatography coupled with a DAD detector. The UV-vis spectrum scanning was from 190 to 600

nm. A luna C 18 column, 4.6 mm × 250 mm and 5 μm particle size, was used. HPLC grade water with 0.1% formic acid (v/v) was used as solvent A, and acetonitrile with 0.1% formic acid (v/v) was used as solvent B. The elution was carried out with 5% of solvent B at the beginning, increasing via a linear gradient to 13% B at 5 min; increasing to 20% B at 10 min; increasing to 27% B at 25 min; increasing to 33% B at 40 min; increasing to 50% B at 45 min; increasing to 90% B at 46 min; keeping 90% until 51 min; and the post-run time for re-equilibration was 10 min. The injection volume was 5 μL, with a flow rate of 1 mL/min and an oven temperature of 40 °C. The HRMS was conducted in a negative ionization mode with the optimized parameters as follows: spray voltage at 4.5 kV, capillary temperature at 325 °C, capillary voltage at -50 V, and tube lens offset voltage at -120 V. The mass range was m/z 100-1000 with a resolution of 30,000. Data was post-processed using QualBrowser part of Thermo Scientific Xcalibur 2.2 software.

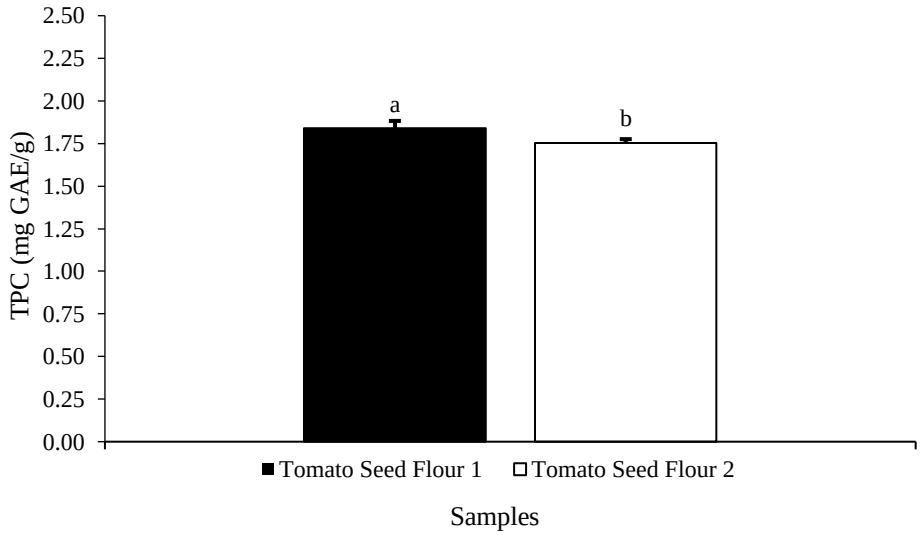
Statistical Analysis. Statistical analysis was performed using SAS programming. Means plus the standard deviation (n = 3 unless otherwise noted) were used for all data points. For comparison, a t test was completed to compare the two samples. Significant difference is declared at $P < 0.05$.

Results and Discussion

Phenolics, Radical Scavenging, and Vitamin Tests

Total Phenolic Contents - The Tomato Seed Flour 1 sample had a total phenolic content (TPC) of 1.8398 GAE/g, and the TPC for Tomato Seed Flour 2 was 1.7540 GAE/g. The total phenolic contents of the two seed flour samples were significantly different ($P < 0.05$) as shown in **Figure 2.1**. This may be due to the effect of different tomato lots being used to process the seeds.

Figure 2.1. Total phenolic Contents (TPC) of Tomato Seed Flours. GAE is gallic acid equivalent. Error bars represent standard deviation (n = 3). Different letters indicate significant difference at $P < 0.05$.



The results in the present study show more phenolic contents in the seed fractions than another group found in six tested combined seed and skin samples, ranging

between 0.1008 and 0.2275 mg GAE/g (Silva et al, 2018). The 2018 study did acknowledge the low TPC value obtained from this assay, possibly due to the processing of the samples; the skin and seed combination used was obtained as pomace waste from tomato processors. The present values are also higher than an average hydrophilic total phenolic content of tomato seeds of three different cultivars reported of 0.220 mg GAE/g (Toor and Savage, 2004) and higher than reported values for five seeds coming from different types of tomatoes, ranging from 0.673 – 1.218 mg GAE/g (Valdez-Morales et al, 2014). The discrepancies between all groups may be caused by the natural differences of different tomatoes grown in different locations, or by slight differences in method including the time the components were given to react. Additionally, the Silva et al (2018) and Valdez-Morales et al (2014) studies extracted the seeds with methanol, which may not fully capture the hydrophilic contents of the tomato seeds. Toor and Savage (2004) extracted with acetone. The larger values for the seed flour could also be caused by a concentration of hydrophilic components in the seed flour due to the nature of its production. The present total phenolic contents of tomato seed flour are higher than available reported values for tomato seeds. When comparing the present values to available data on the TPC of other flours, tomato seed flour contains approximately three times as much phenolic contents as hard and soft wheat, two times as much as barely, about 25% more than millet, and about 75% more than rye, while rice bran and sorghum contained more phenolics per gram (Xuan et al, 2018; Ragaee, Abdel-Aal, and Noaman, 2006).

Radical Scavenging Capacity Assays - The radical scavenging capacity values are listed in **Table 2.1**. The two flour samples were not significantly different from each other in any of the tests. The data shows that tomato seed flour may be a promising free radical scavenger, especially given the high values achieved in the ORAC assay; potentially since to the ORAC assay has been identified as detecting antioxidants using the hydrogen transfer mechanism and is limited to hydrophilic chain-breaking antioxidants that work against peroxyl radicals (Karadag, Ozcelik, and Saner, 2009).

Table 2.1. Free Radical Scavenging Capacities of Tomato Seed Flours. TE is Trolox equivalent. Scavenging capacities reported as mean ± SD (n = 3). Letters in a column indicate significant difference at $P < 0.05$. No significant difference was detected between samples for identical assays.

	DPPH (μmol TE/g)	ORAC (μmol TE/g)	ABTS (μmol TE/g)
Tomato Seed Flour 1	$3.57_a \pm 0.05$	$141.29_a \pm 19.68$	$0.86_a \pm 0.13$
Tomato Seed Flour 2	$3.81_a \pm 0.12$	$124.60_a \pm 10.03$	$0.77_a \pm 0.04$

The ABTS radical scavenging capacity for the tomato seed flours showed they contained 0.8608 and 0.7652 μmol of Trolox per gram, averaging to 0.8130 μmol TE/g. These values are lower than those reported by Silva et al (2018) for six

different tomato pomaces, which ranged from 1.9472 to 2.5073 µmol TE/g. It's also lower than the reported value by Toor and Savage (2004) for the hydrophilic fraction of tomato seeds at 1.140 µmol TE/g. However, the tomato seed values do fall into the range reported by Valdez-Morales et al (2014) for the seeds of five different kinds of tomato, which were reported to range between 0.351-1.396 µmol TE/g; tomato seed flours tested were closest to the results of the ABTS assay from the whole seed of cherry tomatoes at 0.850 µmol TE/g. These results may indicate that the discrepancies in reported values are due to the lipophilic portion of the seed, removed when tomato seed flour is processed.

Deng et al (2012) reported the antioxidant capacity of 56 common vegetables in µmol TE/g using the ABTS assay. The research group measured both the lipophilic and hydrophilic fractions of the vegetables and added them together for the total antioxidant capacity. When looking at the total antioxidant capacities, tomato seed flour was much lower than all other tested vegetables. All reported vegetables had a range of 6.93-33.63 µmol Trolox per gram on a dry weight basis. However, when considering just the hydrophilic fraction, the tomato seed flour falls more within the presented range. Since tomato seed flour represents mostly the hydrophilic fraction of the tomato seed, this may be a more worthwhile comparison. The range of hydrophilic fraction reported was 0.07-19.01 µmol TE/g, with many of the values falling within the 0.2-1.5 µmol TE/g range. This shows that tomato seed flour has comparable antioxidant activity to the hydrophilic fraction of some common

vegetables, with the mean value achieved in the present study very similar to the reported value for broccoli (0.87 µmol TE/g), onion (0.90 µmol TE/g), and snap beans (0.70 µmol TE/g).

The DPPH radical scavenging capacity assay for the tomato seed flours showed that they contained 3.5659 and 3.8065 µmol of Trolox per gram, averaging to 3.6862 µmol TE/g. Valdez-Morales et al (2014) also reported the DPPH scavenging capacities of different tomato seeds as µmol TE/g; all values were less than those obtained for tomato seed flour, ranging from 0.830 – 1.562 µmol TE/g. This may indicate that the concentrated hydrophilic components in tomato seed flours are better at scavenging the DPPH radicals than in the total tomato seed. The DPPH assay is also easily interfered by carotenoids (Noruma, Kikuchi, and Kawakami, 1997), which are known to be found in tomato fractions.

The obtained antioxidant capacities can be compared to a study done on common fruits and vegetables which reported the DPPH radical scavenging capacity as µmol Trolox per 100 grams. According to the DPPH assay, tomato seed flour has comparable antioxidant activity to red potatoes, found to contain about 350 µmol Trolox per 100 grams (Miller, 2000), whereas tomato seed flour would contain about 360 µmol Trolox per 100 grams. Tomato seed flour has a higher measured value than celery, cucumber, head lettuce, whole tomatoes, yellow onions, green beans, carrots, and cauliflower. Peas were reported to have 300 µmol Trolox per 100 grams, and

white potatoes reported to have 400 µmol Trolox per gram, so tomato seed flour may also be comparable to these products given variation and standard deviations (Miller, 2000).

The tomato seed flours showed ORAC values of 141.29 and 124.60 µmol Trolox per gram, averaging to 132.945 µmol TE/g. These values are up to ten times higher than those reported for whole tomato seeds by Valdez-Morales et al (2014), who reported that tomato seeds contained a range of 10.283-18.250 µmol TE/g. Again, this increase could be due to the concentrated hydrophilic components in tomato seed flour and possible different seeds, as the ORAC assay is known for detecting hydrophilic chain-breaking antioxidants (Karadag, Ozcelik, and Saner, 2009).

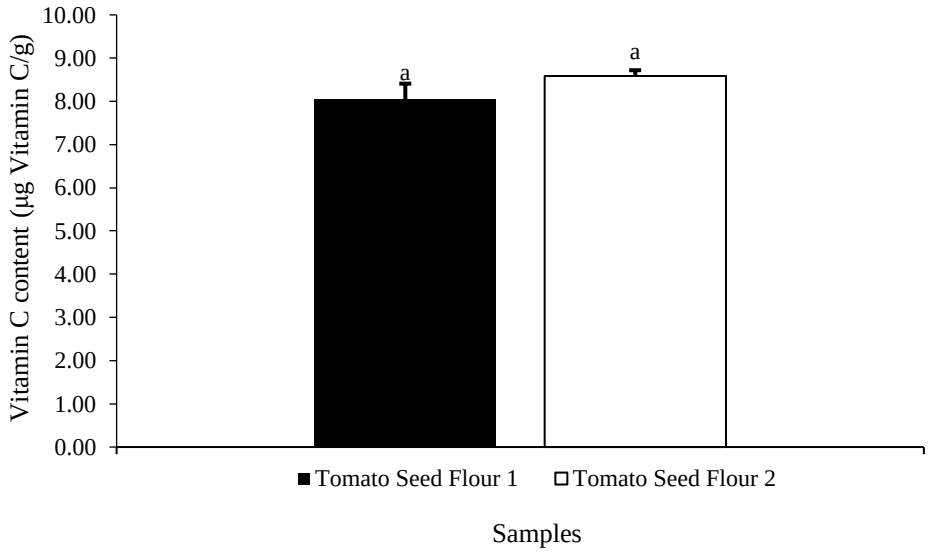
Wu et al (2004) reported ORAC assay results separated by lipophilic and hydrophilic portions. Since tomato seed flour contains mostly the hydrophilic fraction of the tomato seed, these values can be compared to the present study. The only vegetable with hydrophilic ORAC values reported in the hundreds by Wu et al (2004) were dry pinto, red kidney, and small red beans, with values of 119.37, 144.04, and 145.39 µmol TE/g, respectively. All other vegetables had values in the tens or lower. No fruits or grain based foods had values in the hundreds. The tomato seed flour value is comparable to the reported value of walnuts at 130.57 µmol TE/g.

Compared to a previous laboratory study (Choe et al, 2018), tomato seed flour has higher radical scavenging capacity against the DPPH radical and in the ORAC assay when compared to cucumber seed flour (2.64 and 28.63 µmol TE/g, respectively). Against the ABTS radical, cucumber flour extract resulted in a higher value (6.81 µmol TE/g); higher values were obtained for all three assays for carrot and broccoli seed flours. The result of the carrot seed flour ORAC assay showed that it contained 143.91 µmol TE/g, close to the value obtained for one of the tomato seed flours in the current study. Altogether, comparing these values allows for the vegetable seed flours to be ranked and compared in their radical scavenging capacity.

Vitamin C Contents - The tomato seed flour samples did not differ from each other in vitamin C concentration as shown in **Figure 2.2**. The average micrograms of vitamin C per gram of Tomato Seed Flour 1 was 8.04454 and the average micrograms of vitamin C per gram of sample for Tomato Seed Flour 2 was 8.58993. This averages to 8.317 micrograms vitamin C/g or 0.008317 mg vitamin C/g; milligrams of vitamin C per gram is a more commonly used value for expressing vitamin C is was used for comparison. Toor and Savage (2004) reported the ascorbic acid content of tomato seeds, the same as the vitamin C content, as 0.016 mg ascorbic acid/g. This value is double of what was found in the tomato seed flour, possibly indicating that some vitamin C is removed in the flour making process. Another study found that whole seed byproducts contained 14.4 µg vitamin C/g (Knoblich et al, 2005), which shows

that some vitamin C is lost in the flour-making process or is retained in the tomato seed oil.

Figure 2.2. HPLC Analysis of Vitamin C Content of Tomato Seed Flours. Error bars represent standard deviation (n = 3). No significant difference between samples was detected at $P < 0.05$.



Vitamin C contents of some common fruits and vegetables were investigated. HPLC has been used to quantify tomato juice, which was found to have 3.2 mg of vitamin C per 100 grams (George et al, 2005). Canned beets and peaches contain 2 and 3 mg of vitamin C per 100 grams, respectively (Vanderslice and Higgs, 1984), which represent some of the lowest reported values. While the tomato seed flour did contain some vitamin C, it occurs at a very low level.

Chemical Composition

UHPLC-HRMS was first performed with the 50% acetone extracts. There were no detectable compounds using the acetone extracts, so one of the tomato seed flour samples (sample "2") was extracted using a Soxhlet apparatus in ethanol. The purpose of the UHPLC-HRMS was to identify and not quantify components, so one sample was sufficient. The chemical composition of tomato seed flour has not been investigated except in the case of protein composition. **Table 2.2** listed the identified compounds.

Table 2.2. Characterization of Compounds Present in Tomato Seed Flour. Theor. [M-H]- and Exptl. [M-H]- were theoretical and experimental m/z of molecular ions, respectively. Peak figures that correspond to the Peak ID can be found in Appendix A.

Peak ID	tR (min)	Theor. [M-H]-	Exptl. [M-H]-	Chemical Formula	Tentative Identification
1	1.68	133.0375	133.0135	$C_4H_7NO_4$	Aspartate
2	2.15	128.17	128.0346	$C_7H_{12}O_2$	Hexahydro-benzoic acid
3	18.44	137.0233	137.0252	$C_7H_6O_3$	Salicylic acid
4	19.51	625.00	625.1384	$C_{27}H_{30}O_{17}$	Quercetin-di-O-glucoside isomer
5	20.18	609.00	609.1425	$C_{27}H_{30}O_{16}$	Kaempferol-di-O-glucoside isomer

6	25.01	228.25	228.1017	$C_{14}H_{12}O_3$	Resveratrol
7	35.16	462.40	461.2653	$C_{21}H_{18}O_{12}$	Kaempferol 3-O-glucuronide

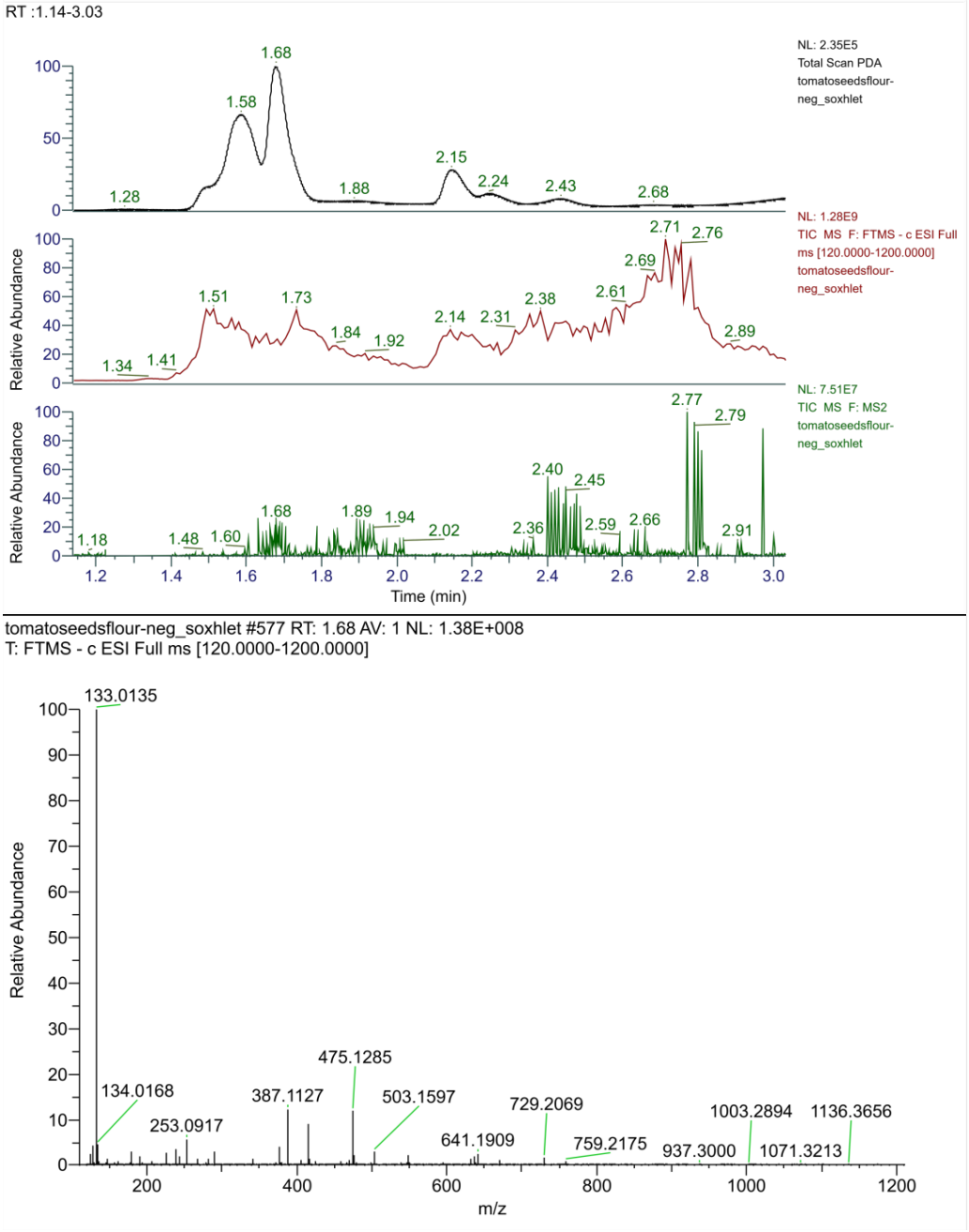
Seven peaks were identified and matched with a tentative compound. Approximate theoretical m/z values were given for isomers. Mass spectrum databases and previous studies identifying compounds in whole tomatoes or whole tomato seeds were used to confirm the proposed tentative identifications. For example, MassBank of North America was effectively used (Horai et al, 2010) to search for experimental m/z values with matching retention times. In the case of aspartate, the experimental m/z value was input into the search and the database was searched for closely matching m/z and retention times. **Figure 2.3** shows this representative spectrum. Spectrum data for other identified peaks can be found in Appendix A. A submitted spectrum for aspartate (Rasche et al, 2012) was found with nearly identical m/z peak and a retention time of 1.73 min. Journals for tomato and tomato seed chemical compounds were then searched to confirm if it had previously been identified in other research. Turakhozhaev (1979), Rao (1990), and Le Gall et al (2003) confirmed the presence of aspartate in tomatoes and in tomato seed flour's amino acid profile.

Aspartate, kaempferol di-O-glucoside isomers, and quercetin-di-O-glucoside isomers were previously identified in a whole tomato metabolite profile (Le Gall et al, 2003). Aspartic acid was also previously identified as a significant portion of tomato seed

flour's amino acid profile (Turakhozhaev, 1979; Rao, 1990). Kaempferol 3-O-glucuronide is linked to flavonoid glucoside isomers, so these may also explain the presence of this specific flavonoid. Salicylic acid (Moco et al, 2006) and resveratrol (Nicoletti et al, 2007) have also been previously identified in tomato fruits. Salicylic acid has been widely studied in tomato seeds for its ability to induce stress tolerance, possible by enhancing the plant's antioxidant activity (Senaratna et al, 2000). Previously, 4-hydroxybenzoic acid was identified in tomato processing byproducts (Kalogeropoulos et al, 2012), which may explain the hexahydro-benzoic acid found in the present study.

The tentative identification of these seven compounds is important in understanding the components of both tomatoes and tomato seeds. The majority of the compounds are thought to make up a large portion of the tomato fruit's phenolic content but might actually be partially contained in the tomato seed. The identification of the flavonoid compounds, specifically the kaempferol isomers, quercetin isomers, and resveratrol reflect why the tomato seed flour showed significant phenolic and radical scavenging properties.

Figure 2.3. UHPLC-HRMS Data for Retention Time 1.68 Minutes. Total PDA (photo diode array), MS (mass spectrum), and MS2 (tandem MS spectrum) with full MS spectrum at 1.68 minutes retention time. This total spectrum data was used to identify aspartate in tomato seed flour.



The identified compounds may have specific and important health beneficial properties. Quercetin and its isomers are associated with significant antioxidant and anti-inflammatory activities. Some studies suggest that it may protect against oxidant related chronic diseases, such as cardiovascular disease, diabetes, and cancer (Jan et al, 2010). Kaempferol and its isomers have nearly identical activities and properties (Kashyap et al, 2017). The similar properties are due to their almost identical chemical structure. The two flavonoids have the typical flavonoids structure of two phenyl rings connected to a heterocyclic ring; both contain a 3-hydroxy flavone backbone and only differ in that quercetin has an additional hydroxyl group on its heterocyclic ring (Dabeek and Marra, 2019).

Benzoic acid and its derivatives, which include salicylic acid, are phenolic acids. They are known to have some antioxidant activity and are effective hydroxy radical scavengers (Rice-Evans, Miller, and Paganga, 1996). Resveratrol has many health beneficial properties both in the prevention and treatment of oxidative damage related chronic diseases. It is specifically toted for its cardiovascular health benefits (Marques, Markus, and Morris, 2009).

The identified chemical compounds point to a source of tomato seed flour's phenolic contents and antioxidant and free radical scavenging properties. They also indicate some potential health benefits that tomato seed flour may have, specifically in the area of cardiovascular health as some of the specific polyphenols found are known to have protective cardiovascular effects.

Discussion

Analyzing the data compared to the study completed by Silva et al (2018) in which tomato skin and seeds were analyzing together, the data shows that the ABTS scavenging capacities form this study for the flour were less than that of the tomato seed and skin combination. This makes sense, as removing the skins from the sample would probably remove some components that would scavenge free radicals.

Standard deviations may be the reason why all other tests resulted in no significant differences in the two flour samples, as the deviations were higher. The higher standard deviations may be due to the volatility and sensitivity of the reagents used in the assays. Additionally, the impact of dilution factors may have affected the standard deviation and results of the assays. Bolling et al (2012) analyzed the extent to which dilution factors had an impact on the results of some common antioxidant assays, including the DPPH and ORAC assays. Testing three different juices, the group found that increasing dilution factors had a linear effect on the DPPH assay. While concentrations differed for all tests using different dilution factors, as to be expected considering errors between tests, the effects appeared minimal as the slope of the best fit lines were small. However, in terms of the ORAC assay, the effect of the dilution factor was much more apparent. As the dilution factor rose, some samples increased significantly in value, with others decreased. Bolling et al (2012) proposes these differences may be due to molecular interactions, non-competitive interaction, or concentration dependent synergy or antagonisms in antioxidant compounds. These

results may explain some of the standard deviation in the ORAC assay; a dilution factor of 1:500 was used. They also further the belief that although the values of assays are reported as exact numbers, it is more worthwhile to use reported values as comparisons between foods tested against the same method and not as the true value of the radical scavenging capacity of a food.

While the values of individual assays can be compared to values of other foods from the corresponding assay, this may not be a useful final evaluation strategy. Since more than one assay is required to analyze and correlate antioxidant and radical scavenging capacity (Prior, Wu, and Schaich, 2005), assessing the assays individually doesn't fully encapsulate the meaning of these results. Instead, understanding where these values lie for all assays compared to other foods may be more useful. The results indicate much higher than average values for some assays and lower for others. The tomato seed flour had higher values than some foods commonly thought of as having beneficial properties, such as onions, peas, carrots, and common flours such as barley and wheat. This gives a perspective as to where about tomato seed flour might rank when considering all possible ways to quantify antioxidants. The ORAC assay indicated that it contained very high radical scavenging capacity, but other assays indicated that it was more similar to common vegetables. Tomato seed flour has similarities in antioxidant and radical scavenging capacity compared to common vegetables such as broccoli, potatoes, onions, peas, and millet; indicating it may be as good of a source of antioxidants as these commonly used and researched

foods. The seed flour also contained more phenolic contents and higher radical scavenging capacities in µmol TE/g according to two of the three assays when compared to three studies looking at tomato seeds or tomato wastes (Toor and Savage, 2004; Valdez-Morales et al, 2014; Silva et al, 2018). This may indicate that the hydrophilic portion of the tomato seed contains most of the phenolics and free radical scavenging capacities.

UHPLC-HRMS tentatively identified some phenolic and radical scavenging compounds in tomato seed flour. The low vitamin C values indicate it is likely not a main source of antioxidant activity. Identifying quercetin, kaempferol, and benzoic acid derivatives, along with salicylic acid and resveratrol, may indicate the main sources of antioxidant and free radical scavenging activity in tomato seed flour.

Conclusion

While research has been done regarding the beneficial properties of whole tomatoes, the negative effects of tomato processing are just beginning to be looked at. Many variables can contribute to a decrease in beneficial properties, but one of these is certainly the removal of tomato seeds in many common tomato products. In a study conducted by Capanoglu et al (2010) of negative health consequences of tomato processing, the removal of seeds was listed as a possible negative consequence of tomato paste processing, and it was noted that studies of seed wastes especially have been limited. Besides commercial processing, consumers may prefer to remove the

seeds of tomatoes and keep the skin on before consumption (Capanoglu et al, 2010), making additional studies of tomato seeds worthwhile.

The results of this study showed that the tomato seed flour contained significant amounts of phenolics and radical scavenging capabilities, and the potential compounds the activity is derived from. These findings are important because minimal research has been conducted on tomato seed flour's beneficial health properties when compared to tomato seed oil. This is likely due to how the oil and flour are produced; tomato seed flour is a waste product of oil production, so the oil is seen as the primary product. More studies should be conducted regarding the properties and potential use of tomato seed flour, considering its potential to improve human health. The high radical scavenging capacity and phenolic content of tomato seed flour indicate that it may be useful in blocking pathways associated with cancer, obesity, diabetes, and other various health conditions.

Chapter 3: Tomato Seed Oil

Introduction

Abstract

Tomato seeds are a major waste product of the tomato processing industry. One way to utilize tomato seeds is to turn them into an edible oil. In order to study their potential health beneficial properties, tomato seed oil samples were extracted with 80% methanol and evaluated for their phenolic contents, radical savaging capacities, beta-carotene content, and color. The two oils contained an average of 0.09946 mg gallic acid equivalents/g in total phenolics, more than some common plant seed oils, such as sesame. The oils had an average ORAC value of 16.59 µmol Trolox equivalents/g, higher than the reported values for commonly consumed oils like canola and extra virgin olive oil. The two oils were significantly different in beta carotene content at 107.69 and 85.07 µg beta-carotene/mL, but both were ten to 100 times higher than reported values for most common oils. The two oil samples differed significantly in radical scavenging capacities and beta-carotene content, possibly showing the effect of tomato seed variety or production method on the resulting compounds in the final product. The results of this study show that tomato seed oil may be beneficial to health when compared to other commonly used oils and could be utilized as a value-added product.

Introduction

Many tomato products, such as tomato sauce, paste, and juice, involve the removal of tomato seeds during processing. Tomato seeds are often treated as a waste product of processed tomato products. In order to reduce waste and increase profits, companies could find a way to turn tomato seeds into a value-added product. For instance, tomato seeds may be used to produce an edible oil.

Recently, there has been an increased interest in foods for health. It's well known that maintaining a diet high in plant foods is associated with positive health benefits, and these benefits are most commonly linked to their phytochemicals (Willett, 2002). Phytochemicals, such as phenolics, vitamins and carotenoids, have been linked to antioxidant and free radical scavenging capacities (Pandey and Rizvi, 2009). The ability of a compound to act as an antioxidant or a free radical scavenger may be beneficial in the prevention or treatment of chronic disease, as at least 70 disorders have been linked to reactive oxygen species in the body, including cancer, diabetes and neurogenerative disorders (Ferrari and Torres, 2003).

Previous studies have shown that agricultural wastes, such as cherry tomato wastes, contained high amounts of antioxidants (Deng et al, 2012). It's reasonable to believe that an oil produced from tomato seeds would also have antioxidants or other beneficial health compounds. Previously, tomato seed oils have been studied

regarding its fatty acid profile, sterol contents, and physicochemical properties by a number of groups (Lazos et al, 1998; Malecka, 2002; Eller et al, 2010). Tomato seed oil has been demonstrated to reduce reactive oxygen species in cells (Muller et al, 2012) and to have some antibacterial activity against the model bacterial strands (Ma et al, 2013). Limited groups have tested tomato seed oil against multiple radical scavenging capacity assays, though one reported the ORAC value of differently extracted tomato seed oils to be between 0.96−1.47 μmol TE/g (Eller et al, 2010).

The objective of the present study was to test two tomato seed oils for their total phenolic, radical scavenging capacity and beta-carotene contents, as well as their colors, to evaluate their potential use as a value-added and health beneficial product.

Analytical Methods

Materials

Tomato seed oil samples were gifted from Botanic oil innovations (Spooner, WI, USA). Methanol, 3,4,5-trihydroxybenzoic acid (Gallic acid), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin and Ciocalteu's phenol reagent (FC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), manganese oxide, 2,2′-Azinobis (2-amidinopropane) dihydrochloride (AAPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), and Fluorescein (FL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl acetate was purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was prepared by an ELGA

Purelab ultra Genetic polishing system with >5 ppb TOC and resistivity of 18.2 mΩ (Lowell, MA, USA). Two different samples of tomato seed oil were used for the study. The samples were referred to as Tomato Seed Oil "1" or "2."

Methods

Seed Oil Extraction. The oil was extracted using 80% methanol. Three grams of oil were accurately weighed and combined with 9 mL of the solvent. The mixtures were centrifuged at 600 rpm for 5 minutes and supernatant was collected. The two oil samples, 1 and 2, were extracted in triplicate.

Total Phenolic Contents. The TPC assay was completed according to a laboratory procedure (Moore et al, 2005). 20% Na_2CO_3 (w/v) solution was prepared. Gallic acid standards were prepared in the solvent. 3 mL of ultrapure water, 50 µL of the sample, standard, or blank (solvent), and 250 µL Folin-Ciocalteu reagent were added to a test tube. The mixture was vortexed for five seconds. After one minute, 750 µL 20% Na_2CO_3 was added. The tubes were covered and allowed to sit in the dark for two hours at ambient temperature. A spectrophotometer was blanked at 765 nm using the blank sample. The absorbance of all standards and samples were measured. A standard curve was developed using the standards. Results were reported as milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

ABTS•+ Scavenging Capacity. The ABTS scavenging capacity assay was completed according to a laboratory procedure (Moore et al, 2005). ABTS working solution was prepared by reacting ABTS with manganese oxide and diluting the mixture to an absorbance of 0.700 ± 0.005 at 734 nm. 2 mL of ABTS and 160 µL of sample extract were added to a test tube. After centrifuging for 30s, absorbance was read at 734 nm after a total of 90 seconds. Trolox was used as the standard and results were reported as µmol Trolox Equivalent per gram of sample (µmol TE/g).

Relative DPPH Radical Scavenging Capacity. The Relative 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay was completed according to a laboratory procedure (Cheng, Moore, and Yu, 2006) adapted for a microplate reader. DPPH solution absorbance was read at 515 nm and diluted so that the absorbance read between 0.9 and 1.0. Trolox standards were prepared. A 96 well plate reader was used to complete the assay. 200 µL of solvent was added to the blank wells. 100 µL of solvent, standard, and sample was added in triplicate to its respective well. 100 µL of DPPH was added to the solvent, standard, and sample wells. The assay was carried out with the lab protocol on a Victor multilabel plate reader (Perkin-Elmer, Turku, Finland) for 1.5 hours. The area under the curve was generated from the program and a standard curve developed from the Trolox standards. Results were reported as µmol TE per gram of oil (µmol TE/g).

Oxygen Radical Absorbing Capacity (ORAC). The ORAC assay was completed according to the lab's established protocol (Moore et al, 2005). AAPH solution, working fluorescein solution, and Trolox standards were prepared. Samples were diluted using a dilution factor from previous preliminary experimental data. Oil extracts were diluted in a 1:10 ratio. A Victor multilabel plate reader (Perkin-Elmer, Turku, Finland) was preheated to 37 °C. The final reaction mixture consisted of 225 μL of 8.16×10^{-8} M FL, 30 μL of the extract or solvent blank or standard, and 25 μL of 0.36 M AAPH. The fluorescence of the mixture was recorded every 2 min over 2 h at 37 °C. Excitation and emission wavelengths were 485 and 520 nm, respectively. A standard curve was developed from the AUC data from the Trolox standards. Data from the samples were used to obtain μmol of Trolox per gram of oil (μmol TE/g).

Beta-Carotene Quantification. Beta-carotene was measured spectrophotometrically using an established laboratory standard curve. Beta-carotene was measured at varying concentrations at 450 nm to develop a curve with the equation $y=0.0394x+0.2465$. The $R^2= 0.9958$. Pure oil samples were combined with pure ethyl acetate in a 50/50 ratio. The spectrophotometer was blanked using 1 mL of ethyl acetate. The absorbance of the oil/ethyl acetate samples was then measured. The results are reported as μg of beta-carotene per mL of oil.

Color Measurements. Color of the oils was measured using a HunterLab ColorFlex spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA). Color value was obtained using daylight 65 illuminant/10° observer setting. 15 mLs of oil was pipetted onto a sample cup for measurement. Measurements were completed in triplicate.

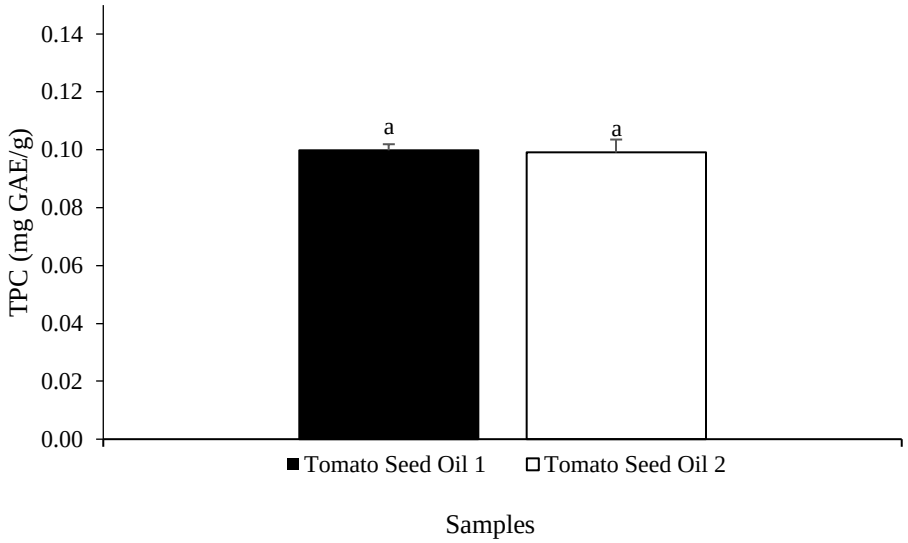
Statistical Analysis. Statistical analysis was performed using SAS programming. Means plus the standard deviation (n = 3 unless otherwise noted) were used for all data points. For comparison, a t test was completed to compare the two samples. Significant difference is declared at $P < 0.05$.

Results and Discussion

Phenolics and Radical Scavenging Capacity

Total Phenolic Contents. The results of the TPC assay are shown in **Figure 3.1**. The average mg GAE/g sample for Tomato Seed Oil 1 was 0.0998 and the average mg GAE/g sample for Tomato Seed Oil 2 was 0.0991. The two oils were not significantly different from each other and give an average of 0.09946 mg GAE/g of phenolic compounds in tomato seed oil.

Figure 3.1. Total phenolic Contents (TPC) of Tomato Seed Oils. GAE is gallic acid equivalent. Error bars represent standard deviation (n = 3). Different letters indicate significant difference at $P < 0.05$.



The lipophilic total phenolic content of tomato seeds has been previously reported (Toor and Savage, 2005). The sample was prepared by separating the lipophilic portion of seeds from the hydrophilic portion. The reported value was 0.035 mg GAE/g of oil, almost three times lower than the tomato seed oils tested in the current study. This could be due to the preparation of the samples. Tomato seed oil may contain more concentrated lipophilic phenolics since it is manufactured to only contain the lipophilic portions of the seed; an extract prepared from the seeds may not be as concentrated.

When comparing this to available data on the TPC of other oils, tomato seed oil is not very rich in total phenolics. Xuan et al (2018) examined the phenolic contents of 14

commonly consumed oils and found that the oils with the lowest phenolic content was safflower at 1.76 mg GAE/g. Wu et al (2004) took samples from commonly eaten foods in the United States and found that the foods with the lowest total phenolic contents were cucumbers with no peel (0.24 mg GAE/g), poppy seeds (0.2 mg GAE/g), and chilchen (0.11 mg GAE/g). Tomato seed oil is lower than all of these reported values. However, in a study of 10 plant oils, Japanese quince and sesame seed oils were reported to have the highest total phenolics of all tested, with 0.064 and 0.028 mg GAE/g, respectively (Gornas et al, 2014).

Radical Scavenging Capacity. **Table 3.1** shows the scavenging capacities of the oil extract against the three free radicals. Different letters in different columns show significant difference between samples.

Table 3.1. Free Radical Scavenging Capacities of Tomato Seed Oils. TE is Trolox equivalent. Scavenging capacities reported as mean ± SD (n = 3). Letters in a column indicate significant difference at $P < 0.05$.

	DPPH (μmol TE/g)	ORAC (μmol TE/g)	ABTS (μmol TE/g)
Tomato Seed Oil 1	$0.0561_b \pm 0.0043$	$16.35_a \pm 1.289$	$0.0125_b \pm 0.0035$
Tomato Seed Oil 2	$0.1216_a \pm 0.0039$	$16.83_a \pm 2.402$	$0.0358_a \pm 0.0024$

The DPPH and ABTS radical scavenging assays showed significant difference between samples, while the ORAC assay showed no significant difference. This could be due to differences in the tomatoes used in preparing the oil samples or due to the assays themselves. The ORAC assay is typically preferred at measuring hydrophilic antioxidants (Karadag, Ozcelik, and Saner, 2009), which would be limited in a tomato seed oil product processed so that only the hydrophobic components remained. Since the oils would be produced similarly in this regard, it's likely that they wouldn't differ in hydrophilic antioxidant components.

The differences in the DPPH and ABTS assays could be due to differences between lots of tomato seed oils, caused by different tomatoes used in the two oils. There are currently no standards at which tomato seed oil is produced, so seeds from tomatoes grown in different areas at different times could be used interchangeably. The effect of cultivar (variety) of tomato on tomato seed oils has been studied in regard to its physicochemical properties. In a comparison study done on three extracted tomato seed oils from three different Italian cultivars, the different seed types significantly changed the values for nearly all of a reported 40 parameters, especially color, fatty acid composition, and free acidity (Giuffre and Capocasale, 2016). If the tomato variety would significantly affect physicochemical characteristics, it's reasonable to assume that it would also affect free radical scavenging properties, potentially causing the significant difference in the two samples studied.

The radical scavenging capacity of tomato seeds extracted for lipophilic components against the ABTS radical has been previously reported. The value was nearly three times higher than Tomato Seed Oil 2 in the present study at 0.094 µmol TE/g. Similarly, the present study shows values ten or more times lower than the lowest value reported by Valdez-Morales et al (2014) at 0.351 µmol TE/g for whole seed byproducts. The value for seed byproducts this previous study reports for radical scavenging capacity against the DPPH radical was 0.836 µmol TE/g (Valdez-Morales et al, 2014), again higher than that observed in the present study. However, in the ORAC assay, the previous study reports four of five seed types with lower µmol TE/g than that of the present study; the seeds ranged from 10.283-18.250 µmol TE/g (Valdez-Morales et al, 2014). These reports may show the effect of seed type on the radical scavenging properties of the oil. The oil making process may also affect the types and availability of active compounds remaining in the oil.

Pellegrini et al (2003) reported the antioxidant activity of common foods using the ABTS assay. The lowest reported value for a food was endive with 0.30 µmol TE/g, and the lowest for an oil was peanut oil with 0.61 µmol TE/g. Both tomato seed oils contained much less µmol TE/g against the ABTS radical. The DPPH radical scavenging capacity assay for the tomato seed oils showed they contained 0.05613 and 0.1216 µmol TE/g. This would equate to about 5.6 or 12.2 µmol of Trolox per 100 grams, respectively, far below the lowest reported value by Miller et al (2000), which was celery containing 50 µmol TE/100 grams. Though the ABTS and DPPH

assays show significant difference in the tomato seed oil samples, possibly indicating the effect of seed variety on free radical scavenging capacity, both values are below reported values for common foods. Also, different assays involve different chemical reactions, which may alter the overall antioxidant or radical scavenging capacity estimations.

The two tomato oils were not significantly different when analyzed by the ORAC assay at 16.35 and 16.83 µmol TE/g for Tomato Seed Oil 1 and 2, respectively. These values are higher than those previously reported by Eller et al (2010), at 0.96-1.47 µmol TE/g. This may be because the tested tomato seed oils were made by laboratory extraction, which could differ from larger scale productions of tomato seed oil, as well as different tomato seeds.

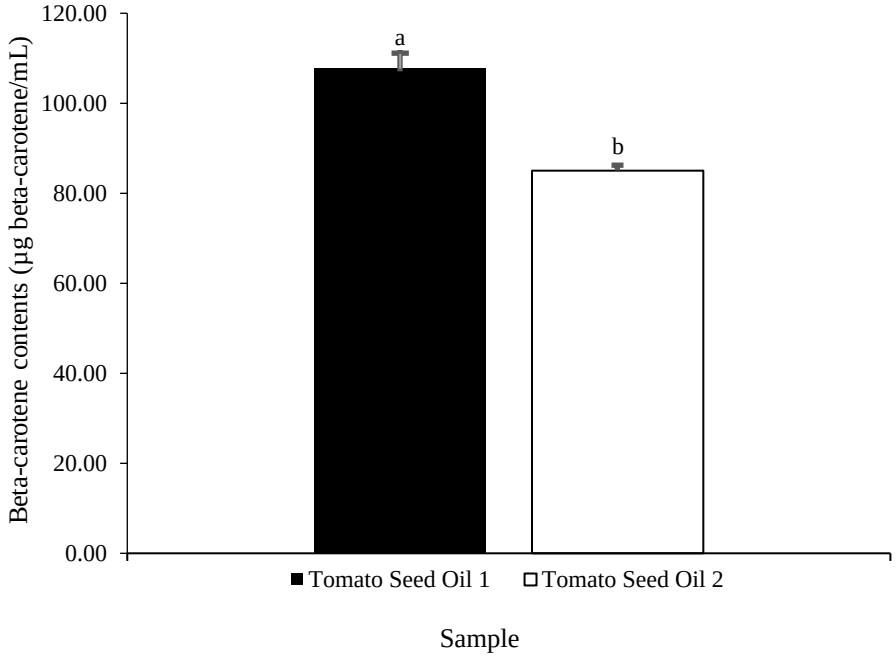
Previously, the ORAC value of 169 samples of tomatoes was reported to range between 33 and 112 µmol TE/g (Ou et al, 2002). While tomato seed oil shows less activity than the whole tomato, previous research on pressed canola, extracted canola, and extra virgin olive oils shows tomato seed oil has higher ORAC values, as they all contain between 1.60 – 11.06 µmol TE/g (Szydlowska-Czerniak et al, 2008).

Beta-carotene and Color

Beta-carotene contents. Beta carotene contents of the tomato seed oils were measured spectrophotometrically. The two samples were significantly different from each other, with Tomato Seed Oil 1 containing 107.69 µg beta-carotene/mL and Tomato Seed Oil

2 containing 85.07 µg beta-carotene/mL (**Figure 3.2**). Palm oil and coconut oil were found to have no beta-carotene contents using a similar spectrophotometric method (Dauqan et al, 2011). HPLC analysis of some consumable oils has found that sesame contains 0.1-0.2, safflower oil contains 0.2-0.4, olive oil contains 0.4-2.6, linseed contains 1.7-1.8, and wheat germ contains 1.7-6.4 µg beta-carotene/mL (Luterotti, et al, 2002).

Figure 3.2. Beta-carotene Contents in Tomato Seed Oils. Error bars represent standard deviation (n = 3). Different letters indicate significant difference at $P < 0.05$.



Color of oils. Color measurements taken on a HunterLab Colorimeter are listed in **Table 3.2.** L is a measure of lightness, a* is a measure of red (positive values) to

green (negative values) and b* is a measure of yellow (positive values) to blue (negative values). All values were significantly different between the two oil samples. Tomato seed oil made from processing waste had previous reported L, a*, and b* values of 36.69, 1.57, and 38.08, respectively (Yilmaz et al, 2015). The values for Tomato Seed Oil 2 are close to these reported values.

Table 3.2. HunterLab color measurements of tomato seed oils. Color measurement parameters: D65/10° illuminant/observer. "L," measure of lightness, increasing from 0 (dark) to 100 (light); "a," measure of red (+) to green (–); "b," measure of yellow (+) to blue (–). Values reported as mean ± SD (n = 3). Letters in a column indicate significant difference at $P < 0.05$.

	L*	**a***	**b***
Tomato Seed Oil 1	$33.15_b \pm 0.91$	$19.20_a \pm 0.27$	$56.18_a \pm 1.32$
Tomato Seed Oil 2	$40.99_a \pm 0.60$	$1.51_b \pm 0.14$	$46.72_b \pm 3.03$

Visually, Tomato Seed Oil 1 looked orange and Tomato Seed Oil 2 looked yellow, and the results in the **Table 3.2** confirm the visual inspection. Tomato Seed Oil 1 had significantly greater a* and b* values. Though it had a higher value for yellow, it also had a much higher value for red (19.20 versus 1.51), so it had a visually orange color coming through. Tomato Seed Oil 2 was significantly lighter, with an average

lightness value of 40.99 compared to 33.15. It had a higher b* value than a* value, so the visually yellow observed yellow color makes sense, as there are fewer red pigments.

It has been reported that extracting tomato seed oil by Soxhlet acetone extraction resulted in a yellow oil, while supercritical acetone extraction of oil resulted in a reddish yellow color (Demirbas, 2010). Another reported the visual color of cold break seed oil was golden yellow, while hot bread seed oil was darker red (Cantarelli et al, 1993). While it's unlikely that the two oils sourced from the same place are produced differently, it's possible that production methods could have also contributed to the recorded color differences.

Discussion

Other unconventional seed oils have been tested for their potential antioxidant and radical scavenging capabilities. Tomato seed oil has less total phenolics and radical scavenging capacity against the ABTS cation radical and in the ORAC assay when compared to caraway, carrot, cranberry, and hemp seed oils (Yu et al, 2005). It is the closest to hemp seeds, which were reported to contain 0.44 mg GAE/g total phenolics and have a value of 28.2 μmol TE/g in the ORAC assay (Yu et al, 2005).

Though tomato seed oil tests resulted in low values for radical scavenging capacity assays, it may be comparable or better than some other oils in terms of total phenolics

and beta-carotene content. Additionally, the significant difference in samples indicated that while Tomato Seed Oil 2 had more radical scavenging capacity, Tomato Seed Oil 1 had significantly more beta-carotene, indicating some other compound in tomato seed oil must have contributed to the overall radical scavenging capabilities, especially since the two oils were not significantly different in total phenolics. The results of the color measurements of the oil may indicate why these two oils were different in some of these tests; Tomato Seed Oil 1 had a significantly larger value for a* on the CIE L*ab color scale, indicating it has more red pigments. Beta-carotene is associated with orange pigments in foods, so the mix of orange and yellow pigments in the oil may indicate the prevalence of the beta-carotene. While the yellow color in Tomato Seed Oil 2 did not contribute to beta-carotene, some other yellow-associated phenolic pigment may be contributing to the higher radical scavenging capacity of this oil sample. It's likely that different varieties of tomato seeds were used to produce these two oils, leading to differences in radical scavenging capacity, beta-carotene content, and color. However, as other studies have observed color differences due to oil production differences, it's also possible that the method in which the oils were produced affected the color change, thus the types of phenolics and radical scavenging capacity that ended up in the final oil product.

Conclusion

Tomato seed oils have been found to contain compounds beneficial to human health. Significant difference in the two oils tested shows possible differences in seed varieties used may affect the amount of these beneficial compounds. Additional

studies on tomato seed oils may be needed to quantify specific antioxidant compounds and determine which seed varieties are used and most beneficial. Since tomato seed oil is produced from a byproduct of many tomato processing plants, the use of the oil may also increase profits for agricultural companies and aid in sustainability efforts to lessen the amount of waste produced by food processing activities.

Chapter 4: Tomato Seed Flour as a Functional Food Ingredient

Abstract

Tomato seed flour was previously identified to have more beneficial health properties than tomato seed oil. However, tomato seed flour is used and investigated much less in the food industry, as it is seen as a waste product of a waste product. In the present study, tomato seed flour was added to two different ketchup samples to test how it affects a food system over time. Total phenolics, radical scavenging capacity, texture, pH, and color were all analyzed. Overall, tomato seed flour did not significantly affect how ketchup performed in any of the experiments conducted. However, the samples with tomato seed flour also appeared to age in regard to shelf life in a similar matter. The tomato seed flour may also have functioned as a thickener in some samples. Tomato seed flour may be an effective hydrocolloid or functional food ingredient in ketchup or other food samples.

Introduction

Nutraceuticals differ from pharmaceuticals and food; they provide some sort of benefit to human health beyond general nutrition. Nutraceuticals can be used to prepare functional foods (Daliri and Lee, 2015). As previously stated, the FDA does not define functional foods. It is a term commonly used in the market to describe foods that function to improve health from their bioactive components or value (Ross, 2000). Bigliardi and Galti (2013) compiled a list of 39 different definitions of

functional foods as reported on from various sources of literature. They concluded that nearly all definitions mentioned one or all of three key concepts: functional foods have health benefits, functional foods have something added or taken away from their original form to make them a functional food, and functional foods have a health beneficial function.

The presence of polyphenols and antioxidants in a food sample has both been cited as possible components that can boost food health-promoting properties (Ghosh, Das, and Sen, 2019). While both tomato seed oil and flour contain polyphenols and antioxidant activity, tomato seed flour showed significantly more in regard to both. This makes tomato seed flour a good candidate as a functional food ingredient. It has been shown to contain compounds that benefit human health, and as a dry meal/flour, there is vast potential for use in food products.

The growing market for functional food ingredients and products is most likely due to a positive correlation between their healthful properties and the consumer's willingness to purchase (WTP). A study conducted by Pappalardo and Lusk (2016) confirmed the results of previous studies by finding that health is the most important feature of the high WTP of functional foods. They also found that health claims positively affect WTP. Even though the consumers in the study proved to seek out healthfulness, they were not willing to accept products that didn't also align with their standards for other factors, such as taste and safety. New functional foods must

therefore highlight the health benefits while also competing with the current standard for other food products.

Tomato seeds are a waste product of the processed tomato industry. Besides prevention, processing for reuse is considered the waste strategy with the lowest environmental impact (Ravindran and Jaiswal, 2015). One of the main concerns and challenges for waste processing reuse is the availability and amount of incoming raw materials needed for a viable functional food ingredient (Ravindran and Jaiswal, 2015; Banerjee et al, 2017). Both the area harvested and production of tomatoes has been rising globally since at least 1996 (FAO, 2016). During this time, America was second in tons of tomatoes produced (FAO, 2016). Using the available 2016 data, Lu et al (2019) estimated that 5.4-9 x 10_6 tons of tomato pomace are wasted annually. Considering that the different climates and growing seasons in the United States (Florida produces tomatoes in the winter, California during all times but the winter) and the use of greenhouses to extend the tomato season (Guan, Biswas, and Wu, 2017), it is reasonable to believe that the availability of tomato pomace should not limit initial studies into the processing of tomato seeds for reuse.

Some research has been conducted regarding using tomato wastes as a value-added product. The antioxidant activity of tomato powders has been studied, but the tomato seeds were removed before testing (Lavelli, Hippeli, and Dornisch, 2001). Sarkar and Kaul found that cabinet dried, defatted tomato seeds showed significant antioxidant activity and may be a potential source of plant-based protein (Sarkar and Kaul, 2014).

Others have attempted to mix tomato wastes with other ingredients to produce an extruded snack food. Tomato pulp, not including the seeds, has been extruded with corn grits to make an acceptable snack food (Caltinoglu, Tonyali, and Sensoy, 2013). Another group (Yagci and Gogus, 2008) extruded durum flour, defatted hazelnut flour, rice grits, orange peel wastes, grape seeds, and tomato pomace. Tomato pomace is typically composed of the waste skins, seeds, and pulp from production. It was found that the fruit waste, which was defined as an 80%, 10%, and 10% mixture of the orange peel, grape seed, and tomato pomace, respectively, did not have a significant effect on the sensory properties of the product. Barely flour and dried tomato pomace have also been found to produce an acceptable snack food, with varying levels of pomace yielding different physical and sensory properties (Altan, McCarthy, and Maskan, 2008).

Isik and Yapar (2017) specifically looked at how tomato seed wastes would affect the physical, chemical, and sensory properties of tarhana, a soup made of wheat flour, yogurt, tomato puree, paprika, onion, salt, yeast, and mint. The group replaced 15, 25, and 35 percent of the wheat flour with dried tomato seeds and analyzed the differences in the protein, oil, fiber, amino acids, and minerals found in the soup. Additionally, total phenolic contents, free radical scavenging activity against DPPH radicals, color, and sensory properties were analyzed. As the amount of tomato seed increased, the protein, oil, fiber, ash, and mineral content increased. The measured

total phenolic content and antioxidant activity also increased. Some amino acids were increased significantly. All samples received high overall acceptance scores, but the control with no tomato seeds and the formulation with 15% tomato seeds scored the highest. This study showed that tomato seeds can be a nutritionally beneficial and acceptable ingredient in food products (Isik and Yapar, 2017).

There has also been some research regarding tomato waste as a potential ingredient in ketchup. Tomato pomace containing the seed, peel, and pulp was added to a ketchup formulation and compared to commercial ketchup products. While the goal of this study was to use the waste product as a way to add fiber, the final product did indicate that tomato wastes may be able to be incorporated into an acceptable ketchup product (Torbica et al, 2016). Tomato pulp powder made of waste peels and seeds have also been studied as an ingredient in ketchup. Different concentrations of the powder were added to a commercial ketchup formulation. It was found that even at low concentrations, the powder may be able to successfully compete with/replace hydrocolloids in ketchup products (Farahnaky et al, 2008).

Since the hydrophilic portion of tomato seeds can be made into a meal or finely ground flour, the potential applications for this ingredient are vast. Dried fruit and vegetable wastes have been investigated for supplementation use due to their many beneficial properties, including their low caloric value, water holding and retention properties, oil absorption capacity, fiber content, bioactive compounds, and

antioxidant properties (Sahni and Shere, 2018). Tomato seed protein isolate derived from tomato seed flour has specifically been found to have good emulsion stabilization properties (Szabo, Catoi, and Vodnar, 2018). Dried fruit and vegetable wastes have been used to supplement a variety of products including baked goods, spices, frozen foods, and imitation cheeses and meats (Sahni and Shere, 2018).

In addition to the beneficial antioxidant properties, tomato seed flour has the potential to be a source of fiber when used in food products. The limited composition studies completed on tomato seed flour found that laboratory extracted tomato seed flour contained 54.1% total dietary fiber (Liadakis et al, 1995), while commercial tomato seed flour contained 21% dietary fiber (Rao, 1990). While these studies aren't recent and would need to be updated according to new dietary fiber standards, they still confirm that tomato seed flour has a significant amount of fiber. Belovic et al (2018) also concluded that ketchup made with pomace powder had an increased fiber content due to the tomato seeds in the pomace. The fiber content of tomato seed flour may contribute to additional health and functional benefits. Fiber can lower the risk of cardiovascular disease, cancer, and lower low density lipoproteins and overall cholesterol (Abuajah, Ogbonna, and Osuji, 2015).

Tomato seed flour may be able to serve as an effective hydrocolloid. Most food hydrocolloids are also considered dietary fibers (Li and Nie, 2016). Hydrocolloids are polymers added to foods that change the system to form gels, thicken the product,

stabilize emulsions, coat, or otherwise stabilize the food system (Phillips and Williams, 2009). The protein content may make it beneficial in food emulsions (Szabo, Catoi, and Vodnar, 2018). Mehta et al (2018) found that tomato seed pomace from processing waste served as a beneficial hydrocolloid in bakery products due to its ability to absorb water and reduce starch retrogradation. Tomato seed flour may be a less expensive alternative to current commercial hydrocolloids, as xanthan seaweed based and synthetic hydrocolloids involve multi-step, complicated extraction processes (Li and Nie, 2016).

One of the most common foods where hydrocolloids are utilized to control the viscosity is ketchup (Li and Nie, 2016). Farahnaky et al (2008) prepared a ketchup using tomato pomace as a replacement for conventional hydrocolloids. The group found that using the pomace at levels as low as 1-2% greatly improved the expected viscosity of ketchup. The pomace that was used included the seeds and skins, so tomato seed flour on its own has still not been tested as a possible hydrocolloid replacement in ketchup. This replacement would be beneficial, as the addition of the skins would add back in the nutrients lost when tomato paste is processed, as also stated by Farahnaky et al (2008) stated in their conclusion.

While some work has been done in trying to add tomato seeds to foods as a functional ingredient, none of them focused on just tomato seed flour. All of the studies also focused on the chemical and physical properties of the ketchup. Since this work has

been done, understanding how potential beneficial health properties are affected by the addition of tomato seed flour is worthwhile to investigate.

Ketchup is a processed tomato food in which the seeds are removed, potentially removing the beneficial health components. There has been some previous work in which tomato seed waste was used to formulate ketchup. However, all tests were done with the entire wasted pomace, not focusing on the seeds. Additionally, most research focused on the physical properties of the fortified ketchup and not the antioxidant properties. For these reasons, this food product was chosen to evaluate as a potential functional fortified food.

The objective of the present study was to successfully make homemade ketchup using tomato seed flour that is comparable to commercial ketchup and evaluate the ketchups for any possible changes caused by the addition of tomato seed flour.

Materials and Analytical Methods

Materials. Ingredients for ketchup preparation and the control ketchup were purchased from a local grocery store (College Park, MD, USA). Tomato seed flour samples were gifted from Botanic oil innovations (Spooner, WI, USA). Methanol, 3,4,5-trihydroxybenzoic acid (Gallic acid), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin and Ciocalteu's phenol reagent (FC), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), manganese oxide, and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), were purchased from Sigma-Aldrich (St.

Louis, MO, USA). Acetone and pH strips were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water was prepared by an ELGA Purelab ultra Genetic polishing system with >5 ppb TOC and resistivity of 18.2 mΩ (Lowell, MA, USA).

Formulation. A homemade version of ketchup was prepared. Although not completely similar to commercial ketchup, it can serve as a good standard to prepare a functional food version of ketchup. A recipe was obtained online that was most similar to the ingredients found in commercial ketchup (Mike, 2015). The recipe was converted to grams and was prepared as according to **Table 4.1**.

Table 4.1. Formulation for Prepared Ketchup. Recipe was based on a homemade recipe posted by Mike (2015) and converted to grams. The recipe was made twice for the two prepared ketchup samples.

Ingredient	**Grams**
Tomato paste	110.00
Light corn syrup	80.00
White Vinegar	75.00
Water	38.45
Sugar	8.13
Salt	3.69
Onion Powder	0.38

Garlic Powder	0.27
TOTAL	**315.92**

After combining all of the ingredients, the mixture was heated until boiling and allowed to simmer for twenty minutes.

The ingredients and their relative amounts reflect the information on the label of Heinz ketchup, selected as a comparison as it claims the majority market share for ketchup sold in America (David, 2013), making it one of if not the most popular brands.

To control for all variables, four samples of ketchup were prepared for testing. Two samples were prepared using the formulation above, one as is and one with additional tomato seed flour. 5.25 grams of tomato seed flour was added to the formula before heating, equating to approximately 1.6% (w/w) of the recipe. A goal of 1-2% tomato seed flour was set due to the previous research by Farahnaky et al (2008) in which this low concentration showed physical viscosity benefits. If this same lower amount also showed health benefits, small monetary investments could be made to make tomato seed flour an easily incorporated ingredient for ketchup.

Two samples of Heinz ketchup were also prepared. One was set aside as is. Another sample was made using the same ratio as the homemade ketchup, measuring out the

commercial sample so that tomato seed flour made up 1.6% (w/w) of the total sample. Approximately 1.65 grams of the tomato seed flour was combined with 99.97 grams of the commercial ketchup.

The four samples were prepared to account for both the heating of the tomato seed flour in the prepared ketchup and to account for any interactions in commercial ingredients in the commercial ketchup. Although no sample can completely replicate the behavior of the ketchup when prepared commercially, with tomato seed flour, these four samples should give a better understanding of the effect of the addition of tomato seed flour on ketchup samples.

Analytical Methods

To replicate the conditions in which a consumer may encounter ketchup, samples were split into two portions and stored both in the refrigerator and at room temperature. It is common for restaurants to store ketchup at room temperature on tables, as its assumed that the bottle is consumed before it spoils.

To test for phenolic and antioxidant properties, the TPC, ABTS, and DPPH assays were used. As previously stated, it is beneficial to use several different assays to represent the antioxidant and radical scavenging capacity of a food sample. Since these three assays can be performed spectrophotometrically, making it quicker and easier to do consistently, they were chosen for the ketchup analysis. These three

assays were performed biweekly, along with extractions of each sample, to determine any shelf life trends.

Extraction. To extract the ketchups, the samples were combined with 50% acetone and centrifuged for 3 mins at 13200 rpm. Acetone was selected for extraction since this solvent was initially used to test the antioxidant properties of tomato seed flour.

Total Phenolic Contents. The TPC was examined according to a laboratory procedure (Moore et al, 2005). A 20% Na_2CO_3 w/v solution was prepared. Gallic acid stock solution and working solutions for the standard curve were prepared using each of the two solvents. For the assay, 3 mL of ultrapure water, 50 μL of the sample, standard, or blank (solvent), and 250 μL FC reagent were added to a test tube. The mixture was vortexed for five seconds and allowed to stand for at least a minute. 740 μL 20% Na_2CO_3 was then added. The tops of the tubes were covered and allowed to sit in the dark for two hours at room temperature. After this time, a spectrophotometer was blanked at 765 nm using the solvent blank. The absorbance of all standards and samples was measured. A standard curve was developed using the varying concentrations of standards. Results were reported as milligrams of gallic acid equivalent per gram of sample.

$ABTS^{\bullet+}$ Scavenging Capacity. The ABTS scavenging capacity assay was completed according to laboratory procedures (Moore et al, 2005). A 1.5 mM phosphate buffer

was prepared and adjusted to pH 7.4. ABTS stock and working solution were prepared. Trolox primary, secondary, and working standards were prepared in each of the two solvents. When the ABTS working solution was prepared, a spectrophotometer was blanked at 734 nm using 2 mL of phosphate buffer and 160 µL ultrapure water. Each sample was measured individually. 2 mL of ABTS and 160 µL of the sample to be measured were added to a test tube. A timer was immediately set for 90 seconds. For the first 30 seconds, the test tube was vortexed. After the timer was up, the mixture was transferred into a cuvette and the absorbance was read. A standard curve was developed for each solvent using the varying concentrations of Trolox. Results were reported as µmol Trolox equivalent (TE) per gram of sample.

Relative DPPH Radical Scavenging Capacity. The DPPH assay was modified to be completed on a spectrophotometer but was still based on previous lab procedures (Cheng, Moore, and Yu, 2006). DPPH stock solution and working solution were prepared using the solvent, and the working solution was diluted until the absorbance at 515 nm was between 0.9 and 1. The spectrophotometer was first blanked with acetone. Trolox standards and sample dilutions were still prepared using the solvent. Equal parts blank, standard, and samples and DPPH working solution were added to test tubes and allowed to sit in the dark for 40 mins. Absorbance at 515 nm was recorded and the absorbance of the blank was subtracted for standard curve preparation and sample analysis. Results were reported as µmol TE per gram of sample.

The assays were performed biweekly on all four samples for both storage conditions, resulting in eight weekly samples. The samples were extracted weekly before testing. The first extraction and sampling began at week "0," to represent the time at which the prepared ketchup was just made, and the commercial ketchup was just opened. For week "0," only four samples were tested; there was no environmental effect to test, so the samples were immediately extracted once prepared. All samples were tested in triplicate unless otherwise noted.

pH Measurements. pH strips were used to quantitatively asses the pH of the samples after 10 weeks. The strips were dipped into each sample as quick as possible, excess ketchup wiped off, and the strips were placed on white paper towels to assess their color compared to the key and to each other.

Texture Analysis. Exponent Stable Micro Systems software with TA XT Plus Texture Analyzer (Stable Micro Systems, London, UK) was used for texture analysis. The texture of the ketchups was measured after 10 weeks. The probe pre-test speed was set at 2 seconds, the test speed at 5 seconds, and the post-test speed at 5 seconds. The distance was 6 mm and the count (repetitions) was three.

Color Measurements. Color of the ketchups was measured twice, at week 4 and week 8, using a HunterLab ColorFlex spectrophotometer (Hunter Associates Laboratory,

Inc., Reston, VA). Color value was obtained using daylight 65 illuminant/10 ° observer setting. Measurements were completed in triplicate.

Statistical Analysis. Statistical analysis was performed using SAS programming. Means plus the standard deviation (n = 3 unless otherwise noted) were used for all data points. For comparison, both a one-way ANOVA ($p \leq 0.05$) followed by a post hoc Tukey test were completed on the data . Significant difference is declared at $p < 0.05$.

Results and Discussion

Phenolic and Radical Scavenging Capacity

The total phenolic contents of the ketchups and radical scavenging capacity assed by the ABTS and relative DPPH assays were performed. The samples were first assayed at the time of preparation. Samples were assayed biweekly to asses any major differences caused by the tomato seed flour addition. **Table 4.2** shows the results of the assays performed initially and at week 8. The effects of storage temperature were tested throughout the weeks, so the number of samples doubled for all assays after the initial tests. Complete data for the weekly results of all assays can be found in the Appendix B. While the results of all assays generally trended downward, especially when comparing the initial and final weeks, all three tests had weeks where the results spiked. This could be a due to a number of factors, including the weekly preparation of assay reagents. It could also be due to the low amount of phenolics and

low radical scavenging capacity of the ketchups, so small numerical deviations could show as significant. Additionally, the ketchups with added tomato seed flour only contained 1% by weight of the flour. While the samples were mixed until visually homogenous, it's possible that some samples in some weeks had more or less flour included, as they were all mixed and sampled by hand. Still, **Table 4.2** shows some significant differences for the three assays that are useful in determining the effect of added tomato seed flour.

In measuring the total phenolic contents, all of the original ketchup samples were not significantly different from each other. Besides the commercial samples with tomato seed flour stored at the different temperatures (room and fridge) were significantly different from each other. In addition, these two samples, none of the other week 8 samples were significantly different from each other. Generally, all week 8 samples had less phenolics than the initial samples. Phenolics were lost over time but were not affected in this amount of time by storage temperature. The ketchups contained between 0.49 – 0.21 mg GAE/g, much lower than the value previously reported by Wu et al (2004) at 2.49 mg GAE/g. However, this study only reported one sample of ketchup tested and did not state the brand and had different extraction procedures. Some foods reported by Wu et al (2004) that fall into the range of the reported ketchup values in this study include garlic powder and cucumbers with no peel at 0.42 and 0.24 mg GAE/g, respectively.

For both the ABTS and DPPH radical scavenging capacities, the initial samples generally had more μmol TE/g than the week 8 samples. The refrigerated week 8 samples also had higher radical scavenging capacities than the room temperature samples, although these results weren't always significant. At the same storage temperature, the commercial with no flour, commercial with flour, and prepared with flour were never significantly different from each other.

The values obtained against the ABTS and DPPH radicals were much less than the lowest reported values for common foods, with endive containing 0.30 μmol TE/g against the ABTS radical and celery containing 0.50 μmol TE/g against the DPPH radical (Pellegrini et al, 2013; Miller et al, 2000). However, the values obtained for all ketchups, including the samples with added tomato seed flour, contained greater radical scavenging capacities than tomato seed oil tested in the same assays.

These results show that the added tomato seed flour did not significantly alter the potential antioxidants in ketchup. Although, at such a low inclusion level (1% of the total recipe by weight) and with such low results and standard deviations, it may be hard to detect significant differences under the experimental conditions. Additionally, ketchup is not typically considered a food high in antioxidants or other health beneficial properties, so it does not make the ketchup unacceptable to have low levels of such components. While both the commercial ketchup with added flour and

homemade ketchup with added flour were arbitrarily higher than the commercial sample for some tests at some temperature, no differences were significant.

	TPC (mg GAE/g)	ABTS (µmol TE/g)	DPPH (µmol TE/g)
Initial			
Commercial	$0.45_{a} \pm 0.013$	$0.091_{abc} \pm 0.0011$	$0.34_{c} \pm 0.0045$
Commercial Flour	$0.43_{a} \pm 0.018$	$0.090_{abc} \pm 0.0029$	$0.31_{c} \pm 0.0033$
Prepared	$0.49_{a} \pm 0.0055$	$0.11_{a} \pm 0.0019$	$0.62_{a} \pm 0.015$
Prepared Flour	$0.42_{ab} \pm 0.0028$	$0.092_{ab} \pm 0.0039$	$0.47_{b} \pm 0.026$
Week 8			
Commercial Room Temperature	$0.26_{cd} \pm 0.015$	$0.041_{e} \pm 0.0047$	$0.071_{e} \pm 0.0064$
Commercial Flour Room Temperature	$0.21_{d} \pm 0.016$	$0.042_{e} \pm 0.0073$	$0.068_{e} \pm 0.0096$
Prepared Room Temperature	$0.27_{cd} \pm 0.012$	$0.064_{cde} \pm 0.015$	$0.12_{de} \pm 0.0059$*
Prepared Flour Room Temperature	$0.24_{cd} \pm 0.036$	$0.044_{e} \pm 0.015$	$0.076_{e} \pm 0.026$
Commercial Fridge	$0.26_{cd} \pm 0.0022$	$0.058_{de} \pm 0.012$	$0.10_{de} \pm 0.026$*
Commercial Flour Fridge	$0.33_{bc} \pm 0.093$	$0.057_{de} \pm 0.011$	$0.11_{de} \pm 0.019$*
Prepared Fridge	$0.29_{cd} \pm 0.033$	$0.073_{bcd} \pm 0.013$	$0.12_{d} \pm 0.0042$
Prepared Flour Fridge	$0.25_{cd} \pm 0.031$	$0.049_{de} \pm 0.00096$	$0.092_{de} \pm 0.00058$

Table 4.2. Total Phenolic Content, ABTS Radical Scavenging, and Relative DPPH Radical Scavenging capacity of ketchups. Initial samples taken at time of preparation. Week 8 samples taken after 8 weeks of storage at noted conditions. Values are listed as mean ± standard deviation (n=3; * notation indicates n=2). Significant difference is declared at $P < 0.05$. Letters in the same column (same assay) indicate significant difference. The first four samples listed in the first column (commercial, commercial flour, prepared, and prepared flour) are the initial four samples that were prepared and tested before being held in the two different storage conditions. The remaining samples after the week 8 indication are the eight samples that resulted from storing these four initial samples at two different temperatures for eight weeks, with assay testing happening during the eighth week of storage.

Physical Characteristics

pH. The pH of the samples was qualitatively assessed using pH strips. See **Figures 4.1 and 4.2.**

Figure 4.1. pH Test Strips from Ketchups Stored at Room Temperature. In descending order: Commercial ketchup, commercial ketchup with added tomato seed flour, prepared ketchup, and prepared ketchup with tomato seed flour.

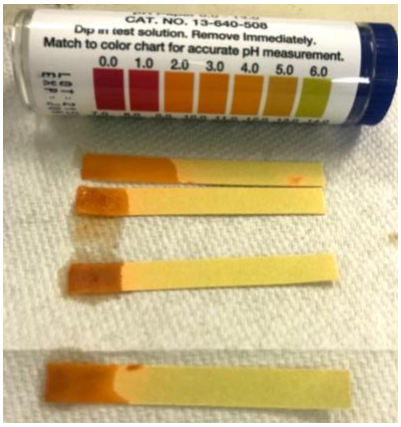


Figure 4.2. pH Test strips From Ketchups Stored in the Fridge. In descending order: commercial ketchup, commercial ketchup with added tomato seed flour, prepared ketchup, and prepared ketchup.

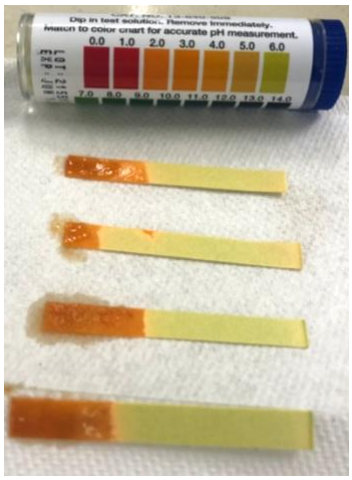
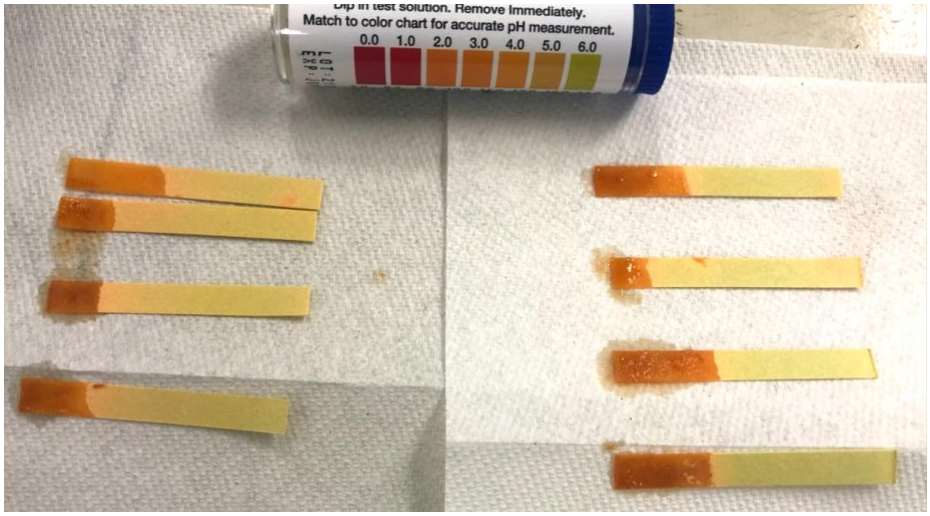


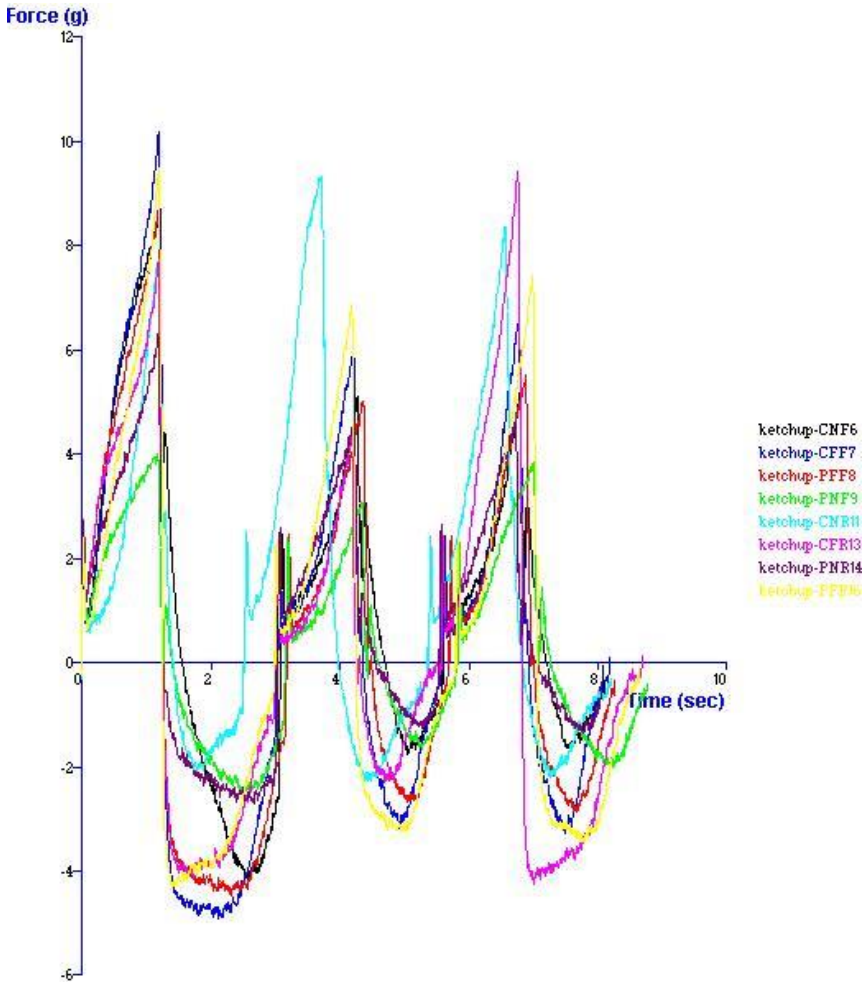
Figure 4.3. All pH Test Strips. Room temperature samples are on the left and fridge samples on the right. In descending order starting from the top strip, the samples are commercial ketchup, commercial ketchup with added flour, prepared ketchup, and prepared ketchup with added flour.



As shown in **Figures 4.1, 4.2, and 4.3**, the ketchups retained an acidic pH while showing little differences among differently prepared and stored ketchups. The pH estimate given the strips is between 3 and 4 for all samples. While the color of the ketchup slightly interfered with the strips, they all appear to have an orange color. The importance of this test was to establish that all samples remained acidic and likely safe to eat after 10 weeks of storage.

Texture. Analysis with the texture analyzer was completed for all samples. The graphs reflect the force required to break through the sample on the positive y axis and the force required to remove the probe from the sample on the negative y axis.

Figure 4.4. The Effects of Tomato Seed Flour on Ketchup Texture. Sample codes: first letter indicates ketchup type (C is commercial, P is prepared), second letter indicates tomato seed flour (N is not added, F is added) and last letter indicates storage (R is room temperature, F is fridge). Numbers were generated by the program and do not indicate samples. For example, CNF is the commercial sample with no added tomato seed flour stored in the fridge.



As shown in **Figure 4.4** The samples that required the highest force to break or pull out of were the refrigerated commercial sample with tomato seed flour, the refrigerated commercial sample without tomato seed flour, the room temperature commercial sample with tomato seed flour, and the refrigerated prepared sample with tomato seed flour. The samples that required the least force to break or pull out of were the two prepared samples with no flour added.

Figure 4.5. Texture Analyzer Data for All Room Temperature Samples. Blue is commercial with no flour, pink is commercial with flour, dark purple is prepared with no flour, and yellow is prepared with flour.

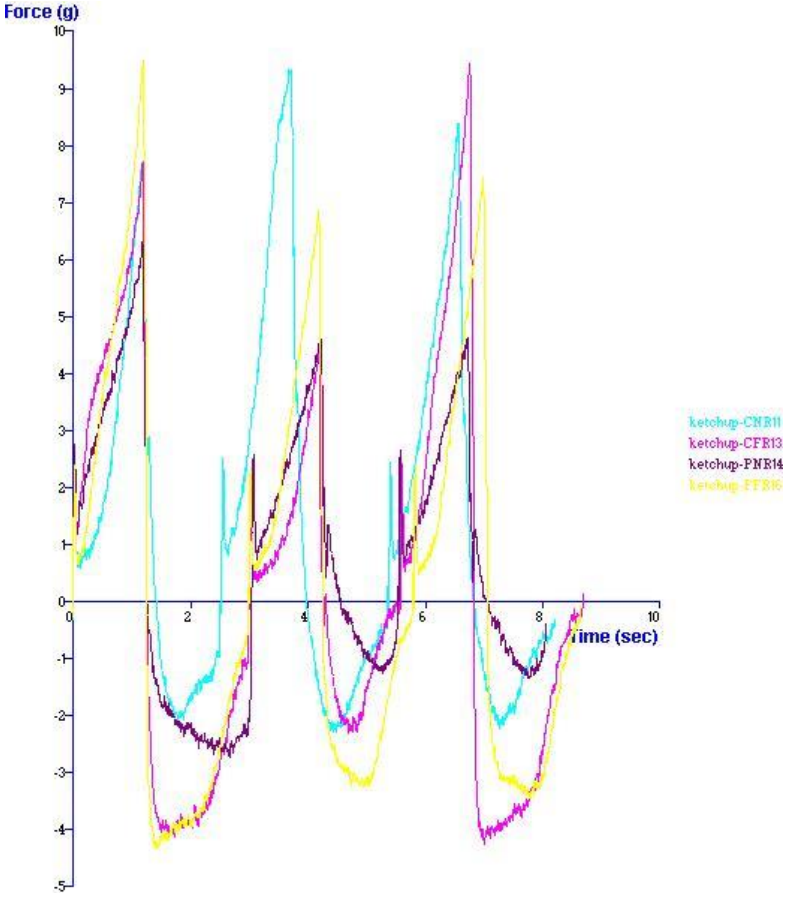
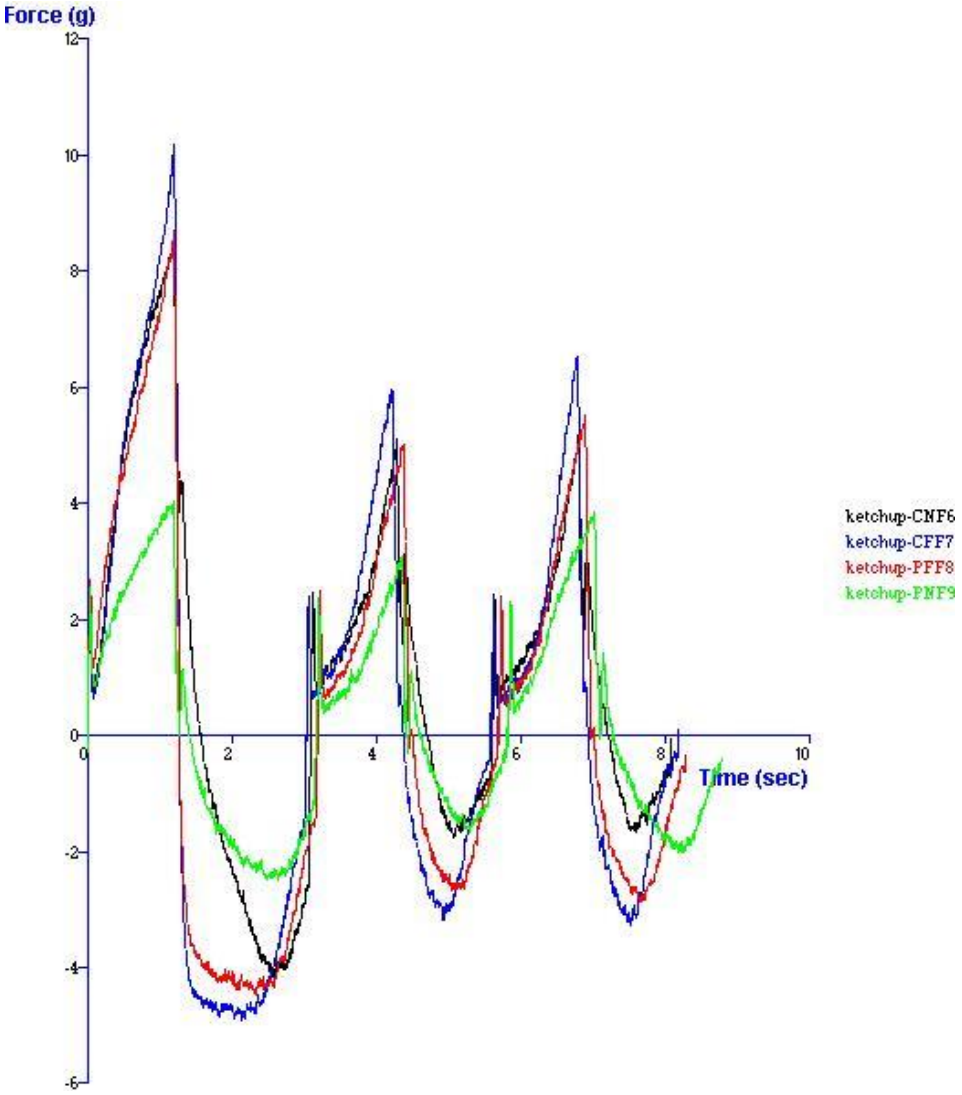


Figure 4.6. Texture Analyzer Data for All Samples Stored in the Fridge. Black is commercial without flour, blue is commercial with flour, red is prepared with flour, and green is prepared without flour.



Figures 4.5 and **4.6** divide the texture analyzer data by storage conditions. As seen in **Figure 4.5**, showing all room temperature samples, both commercial samples and the prepared sample with added flour required similar force to break through the sample

for at least one of the repeats. Less force was required for all repeats for the prepared sample with no flour. The same can be observed in **Figure 4.6,** where all refrigerated samples are shown. All samples except the prepared ketchup with no added flour required a similar amount of force to break through and pull out of the sample. Notably, the commercial sample with added flour always required the most amount of force. The samples stored in the fridge also showed that they required more consistent force when compared to the room temperature samples. **Figure 4.5** does not have as consistent results for each sample as **Figure 4.6**; the former shows different samples requiring the most amount of force for any given replicate while the later shows the samples remained consistent in their force requirements compared to one another.

Figures 4.7 and **4.8** show a direct comparison with the commercial sample with no added flour and the prepared sample with added flour, the first at room temperature and the second at refrigeration temperature. **Figure 4.9** shows the commercial and prepared samples with no added flour at room temperature. Given that the prepared sample with no added flour required less force, these graphs show that the added flour to the prepared sample increased the thickness of the ketchup. Adding 1% tomato seed flour allowed the prepared samples to very closely mimic the texture of the commercial sample. The tomato seed flour thickened the ketchup.

Figure 4.7. Texture Analyzer Data for Two Room Temperature Samples. Blue is commercial with no flour and yellow is prepared with added flour. The graph shows the effect tomato seed flour has on texture, as the prepared sample more closely matches the commercial sample when tomato seed flour is added.

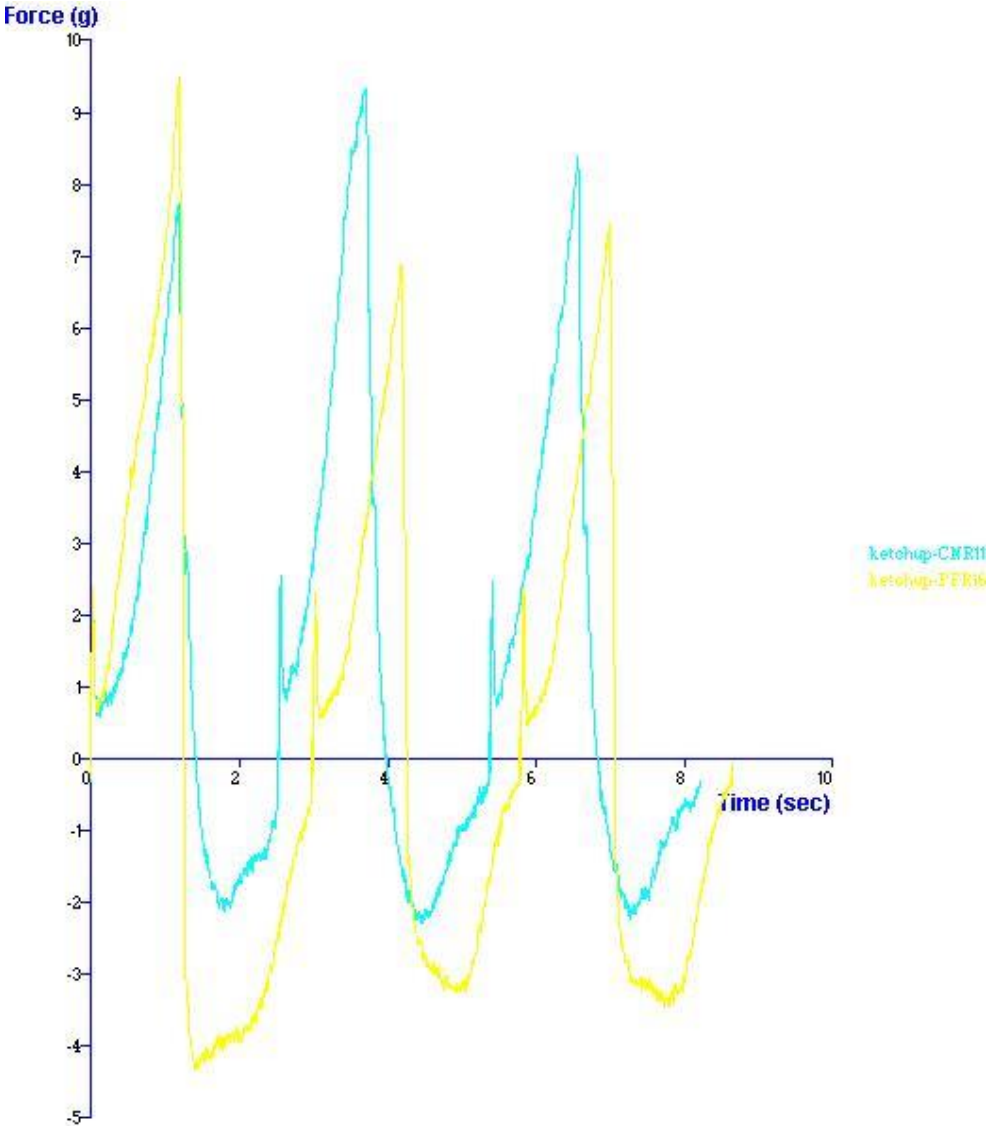


Figure 4.8. Texture Analyzer Data for Two Samples Stored in the Fridge. Black is commercial with no flour and red is prepared with flour. The graph shows the effect tomato seed flour has on texture, as the prepared sample more closely matches the commercial sample when tomato seed flour is added.

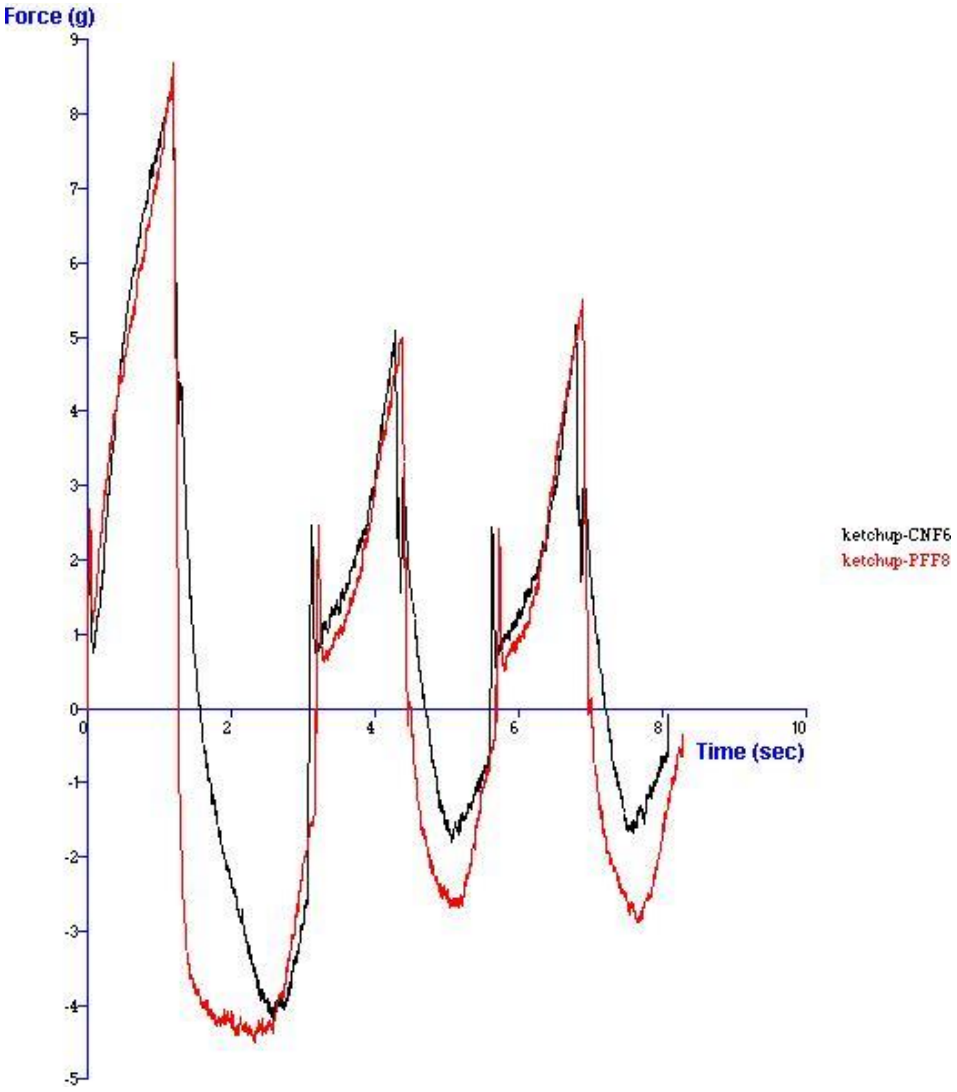
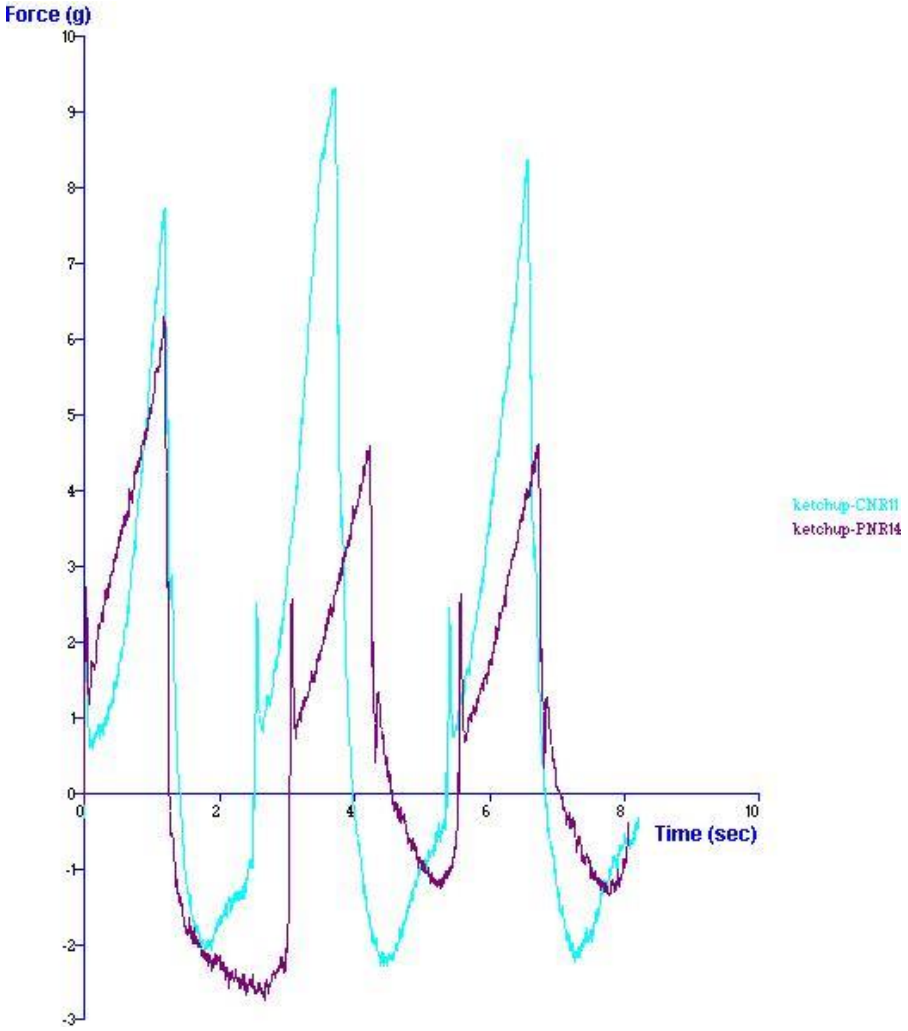


Figure 4.9. Texture Analyzer Data for Commercial and Prepared Samples Stored at Room Temperature with No Added Flour. Blue is commercial with no added flour and purple is prepared with no added flour. Compared to **Figure 4.7**, the prepared sample with no flour does not as closely match the texture of the commercial sample.



Color. Pictures of the ketchups were taken upon preparation.

Figure 4.10. Commercial Ketchup with Added Tomato Seed Flour (left) and Without (right).

Figure 4.11. Prepared Ketchup with Added Tomato Seed Flour (left) and Without (right).

The small flecks of tomato seed flour were more noticeable in the commercial samples (**Figure 4.10)** likely because the prepared samples were not as homogenous

(**Figure 4.11)**. Immediate observations show that the prepared samples are a little lighter, likely because they went through a less intense heat treatment. Adding tomato seed flour to both commercial and prepared samples allowed the effects of the flour on color to be more apparent.

After four and eight weeks of storage, the color of the ketchups was analyzed using a Hunter Colorimeter. The values and analysis are shown in **Table 4.3.**

Table 4.3. Color Values and Statistical Analysis of Ketchups. HunterLab color measurements of ketchups are listed. Color measurement parameters: D65/10° illuminant/observer. "L*," measure of lightness, increasing from 0 (dark) to 100 (light); "a*," measure of red (+) to green (–); "b*," measure of yellow (+) to blue (–). Values reported as mean ± SD (n = 3). Letters in a column indicate significant difference at $P < 0.05$. Values are reported as mean plus or minus standard deviation (n=3). Room means stored at room temperature; fridge means stored in the fridge (4 degrees Celsius). TF means contained tomato seed flour. Different letters in the same column in the same week indicate significant difference. Significant difference is declared at $p < 0.05$. Delta E 1 is delta E76; = $\text{sqrt}[(L_2-L_1)_2 + (a_2-a_1)_2 + (b_2-b_1)_2]$, where L_2, a_2, and b_2 are the commercial room and commercial fridge samples compared to other samples stored at the same temperature. Delta E 2 is the same formula, but where L_2, a_2, and b_2 are Week 4 and L_1, a_1, and b_1 are Week 8 for the same sample.

Sample	Week 4				Week 8				
	L*	a*	b*	Delta E 1	L*	a*	b*	Delta E 1	Delta E 2
Commercial Room	22.65$_{d}$ ± 0.08	28.18$_{c}$ ± 0.34	28.89$_{bc}$ ± 0.29	N/A	22.91$_{d}$ ± 0.01	28.52$_{c}$ ± 0.20	29.98$_{c}$ ± 0.15	N/A	1.17
Commercial Room TF	23.74$_{c}$ ± 0.03	27.15$_{e}$ ± 0.05	30.08$_{a}$ ± 0.27	1.91	23.31$_{c}$ ± 0.02	26.92$_{d}$ ± 0.64	31.26$_{b}$ ± 0.12	2.09	1.28
Prepared Room	13.65$_{g}$ ± 0.03	29.49$_{a}$ ± 0.12	21.87$_{ef}$ ± 0.27	11.49	13.42$_{f}$ ± 0.12	30.22$_{b}$ ± 0.18	21.94$_{e}$ ± 0.13	12.55	0.77
Prepared Room TF	23.87$_{c}$ ± 0.02	27.93$_{cd}$ ± 0.10	29.18$_{ab}$ ± 0.31	1.28	22.83$_{d}$ ± 0.12	27.26$_{d}$ ± 0.03	29.42$_{c}$ ± 0.17	1.28	1.26
Commercial Fridge	24.70$_{b}$ ± 0.20	28.93$_{b}$ ± 0.22	27.45$_{d}$ ± 0.60	N/A	24.52$_{b}$ ± 0.14	30.89$_{a}$ ± 0.13	34.26$_{a}$ ± 0.54	N/A	7.09
Commercial Fridge TF	27.09$_{a}$ ± 0.04	27.36$_{e}$ ± 0.05	27.83$_{cd}$ ± 0.12	2.88	26.46$_{a}$ ± 0.12	28.45$_{c}$ ± 0.20	34.76$_{a}$ ± 0.31	3.16	7.04
Prepared Fridge	13.91$_{f}$ ± 0.04	29.29$_{ab}$ ± 0.07	21.69$_{f}$ ± 0.19	12.24	13.41$_{f}$ ± 0.07	30.94$_{a}$ ± 0.26	22.55$_{e}$ ± 0.10	16.14	1.93
Prepared Fridge TF	14.66$_{e}$ ± 0.01	27.50$_{de}$ ± 0.09	22.88$_{e}$ ± 0.68	11.12	14.21$_{e}$ ± 0.05	28.50$_{c}$ ± 0.07	23.72$_{d}$ ± 0.27	14.93	1.38

For delta E values, less than one is considered not perceptible by human eyes, 1-2 is perceptible through close observation, 2-10 is perceptible at a glance, and at 11-49 colors are more similar than opposite (Schuessler, 2019).

Individual color values yielded many significant differences between samples. This is likely due to the low standard deviation. This makes sense as the standard deviation only reflects deviation in the machine since the triplicate samples were taken in sequence. It is more useful to analyze the delta E values obtained to compare whole color differences in samples. While prepared and commercial samples without added flour were very different in both weeks at both temperatures, it appears that the addition of flour gave the prepared samples a color closer to that of the commercial sample, especially in the room temperature sample across the two tested weeks. Additionally, adding tomato seed flour did not affect how the color of the ketchup changes over time. Delta E 2 shows that color differences between weeks can mostly be categorized as perceptible through close observation, likely barely noticeable as it occurs over four weeks. The prepared samples had less of a color change in the fridge when compared to the commercial samples, but this is likely due to the preparation or ingredients in the ketchup base, as the addition of tomato seed flour didn't slow color change in the commercial sample or speed it up in the prepared sample.

Overall, the addition of tomato seed flour in ketchup did not significantly change the color of ketchup between samples or over time. While individual color parameters are

significantly different, the color of ketchup with tomato seed flour may not be perceivably different, especially when kept at room temperature.

Conclusion

Direct Effects of Added Tomato Seed Flour

To show the effects of tomato seed flour on ketchup formulations, two samples with and two samples without added tomato seed flour were held at room temperature and refrigeration temperature. The effect of tomato seed flour was observed by directly adding it to a commercial sample and incorporating it during the cooking of a prepared ketchup sample; these two were able to be compared to their counterparts that had no added tomato seed flour.

The results of the present study showed that tomato seed flour can be added into ketchup and produce an acceptable and similar product to a commercial sample currently available on the market. The pH of the ketchup did not change between sample. This is important as the acidity of the ketchup keeps it from spoiling, especially at room temperature. The color of the ketchup also did not much. Adding tomato seed flour to commercial ketchup at most changed the color to be perceivable at a glance or through close observation. Adding tomato seed flour to homemade ketchup made the sample closer in color to the commercial sample compared to the homemade sample without tomato seed flour. Homemade samples may also be more color stable when stored in the fridge over time. For all samples, the added tomato

seed flour did not affect how the color changes over time. The actual look of the ketchups was visibly different. The tomato seed flour was noticeable in the samples where it was added. Depending on consumer perceptions, this may be an acceptable change, or the flour may need to be ground finer to lessen the appearance of visible flecks. For both the pH and color analysis, the addition of tomato seed flour in ketchup did not drastically change the samples.

The tomato seed flour was tested in these products due to its antioxidant properties found previously. In the phenolic and radical scavenging assays used, the addition of tomato seed flour did not significantly increase the amount of phenolics or radical scavenging compounds in the samples. While the results were not significant, for some tests, the values did increase when comparing samples with and without added tomato seed flour. The values obtained by all tests were very low. It's possible that they may not be accurate or precise at such low levels. It is likely difficult for to obtain significant differences between results for such low values.

The results of the texture analyzer show that the tomato seed flour was able to thicken both commercial and prepared ketchup samples. The prepared ketchup sample required much less force to break through when it did not contain tomato seed flour, but with added tomato seed flour the texture analyzer graph nearly mimicked the commercial sample. These results are in agreement with Farahnaky et al (2008), who showed that at 1% of the total recipe, tomato pomace was an effective hydrocolloid

thickener in ketchup. The current results show that tomato seed flour on its own is just as effective as the tomato pomace. This information may be beneficial to the food industry, as some current hydrocolloids used in foods are expensive and labor-intensive to produce (Li and Nie, 2016). As shown in previous results, tomato seed flour may also have more beneficial health properties when compared to some synthetic hydrocolloids currently used in the food industry.

Chapter 5: Future Studies

Tomato Seed Flour

Further studies are needed to understand if tomato seed flour may be viable ingredients in commercialized products. The limited studies performed are mostly limited to their properties as individual products, not in a complex food system. Some suggestions for supplementation using these products may include snack foods and bakery products. Supplementation in tomato products may also be a useful area, as it would be adding the original nutrients back into these food products.

In the initial tomato seed flour investigation, UHPLC-HRMS was conducted and some tentative compounds identified. However, other peaks could not be identified due to time constrictions and lack of relevant journals. In the future, additional study could be run to identify the remaining peaks. Additionally, only one flour sample was analyzed. Since the two flour samples had significantly different phenolic contents, it's possible that their chemical makeup could be different. This could also be a reflection of the number of compounds in the flour, which would require quantification of the chemical compounds.

In order to better understand the health beneficial properties of tomato seed flour when it is mixed into a food system, additional antioxidant screening methods could be performed. The ORAC assay was not completed in the present study due to the

time and resources it takes. Additionally, the DPPH assay was done spectrophotometrically and not using a microplate reader. Both these assays could be conducted and may produce better results that provide clear significant differences in the samples due to the number of readings taken during microplate assays.

It's also possible that the low percentage of tomato seed flour used didn't have a significant effect on the antioxidant and free radical scavenging properties of the ketchup. Higher amounts of the flour could be incorporated into food samples to see at what inclusion rate they make a significant difference. However, adding more flour could have negative effects on color and texture that were not seen in the current results.

At any inclusion rate, ketchup with added tomato seed flour would need to be analyzed for consumer acceptability. The flecks of flour were noticeable in the ketchup. While it's possible to mill them down to smaller sizes, it's unlikely that they would be unnoticeable in a ketchup. They may also contribute to a grainy mouthfeel. These sensory attributes could be offset by giving consumers the knowledge that the added ingredient is beneficial for health and the environment, however, this would need to be tested.

Finally, as suggested in the present study, tomato seed flour may potentially be an effective thickener in foods. Additional studies on other food systems could prove that tomato seed flour is an effective, natural hydrocolloid. Some of these foods,

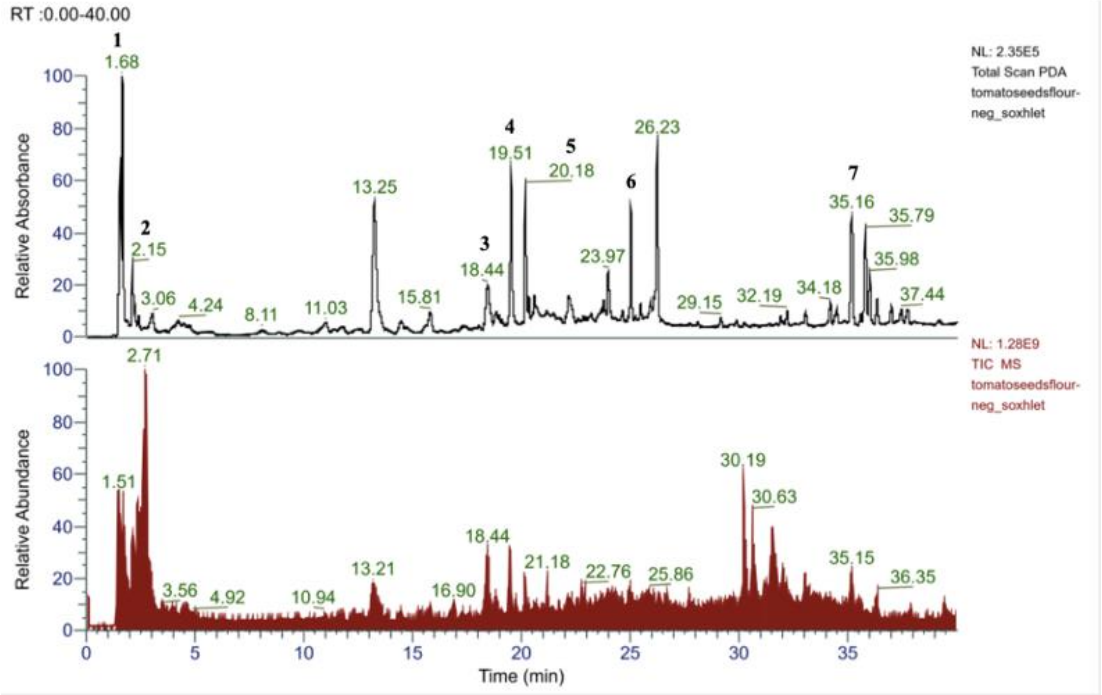
mainly ones that use hydrocolloids as a thickener, may include sauces (Li and Nie, 2016), puddings, cakes, soups, gravies, and dessert fillings (Saha and Bhattacharya, 2010).

Additional studies using tomato seed flour in different food systems may reveal its usefulness as a functional food ingredient. This is significant for human health, businesses, and environmental health. Tomato seed flour has antioxidant and radical scavenging properties and presents a possible source of income for tomato processing facilities while reducing food processing wastes.

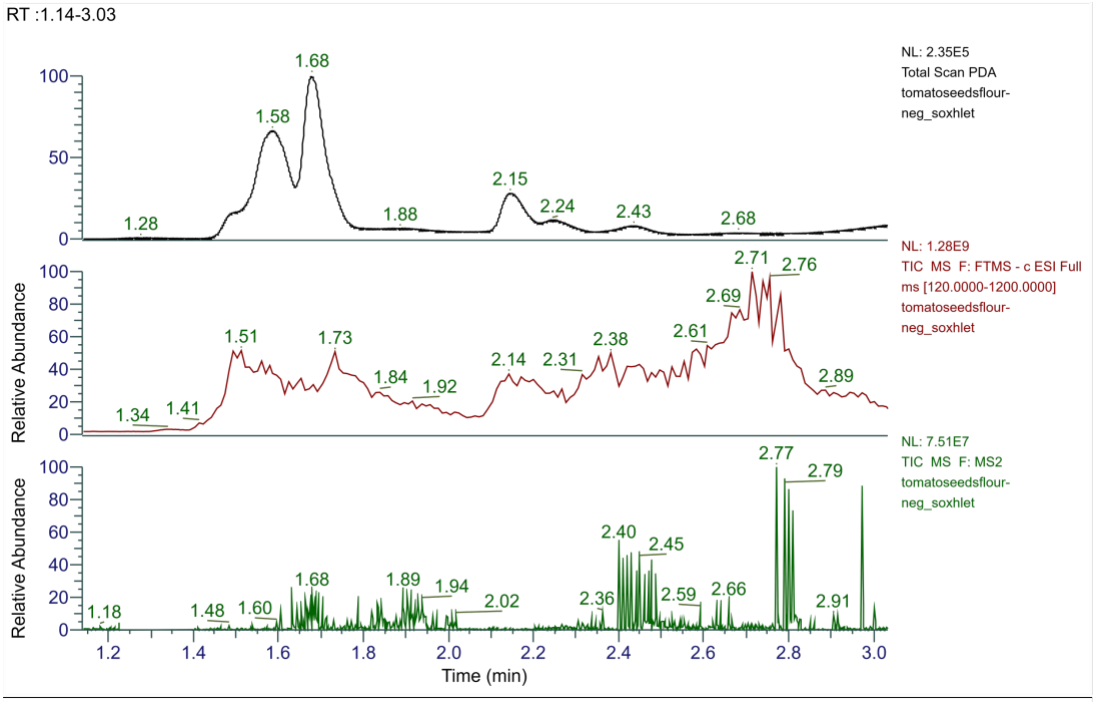
Appendices

Appendix A

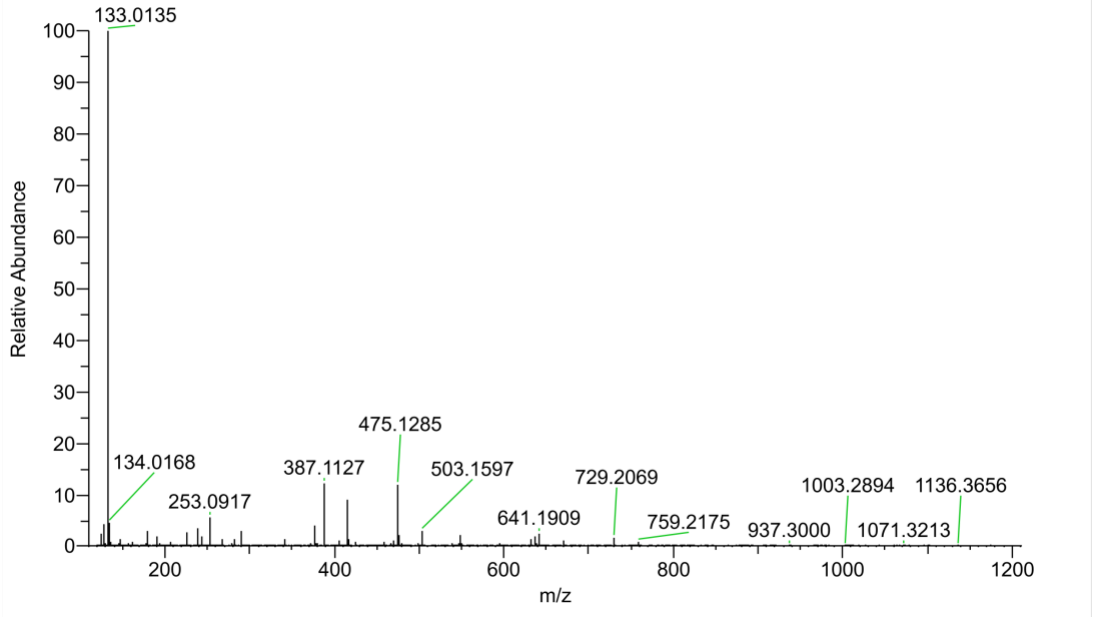
The total UHPLC-HRMS spectrum data is shown below. PDA is photo diode array spectra and MS is mass spectra. Labeled numbers on peaks on total scan PDA correlate to peaks listed in **Table 2.2**.



Individual peak data is shown below. RT on each image indicates retention time. PDA is photodiode array spectra, MS is mass spectra, and MS2 is tandem MS spectra.

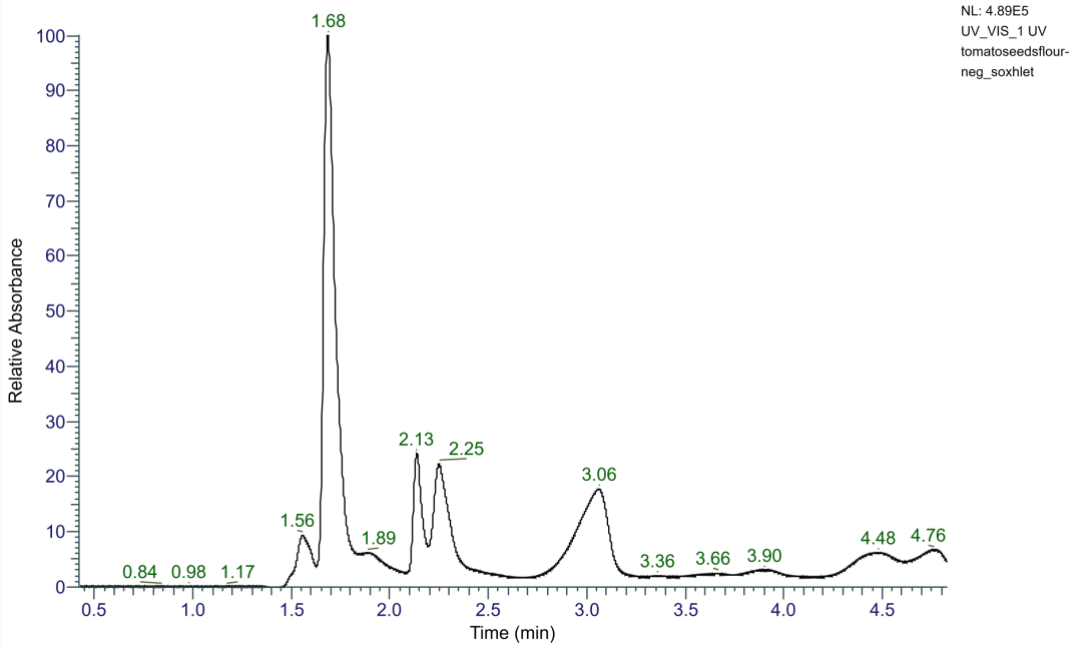


tomatoseedsflour-neg_soxhlet #577 RT: 1.68 AV: 1 NL: 1.38E+008
T: FTMS - c ESI Full ms [120.0000-1200.0000]

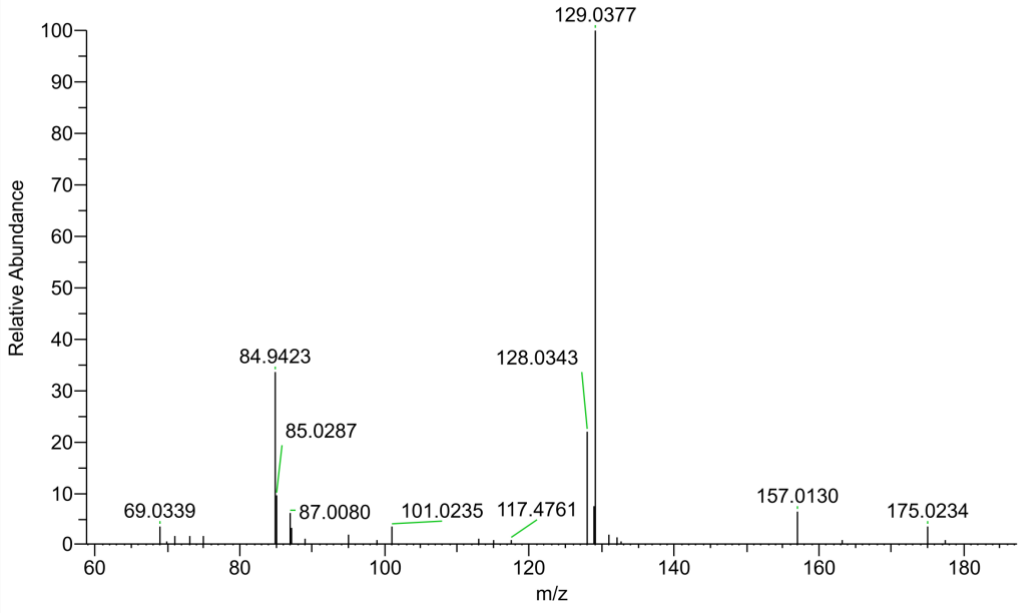


C:\Users\User\Desktop\tomatoseedsflour-neg_soxhlet.raw 3/6/2020 11:24:16 AM

RT :0.42-4.83



tomatoseedsflour-neg_soxhlet #854 RT: 2.13 AV: 1 NL: 3.31E+005
T: FTMS - c ESI d Full ms2 175.0240@hcd35.00 [60.0000-186.0000]



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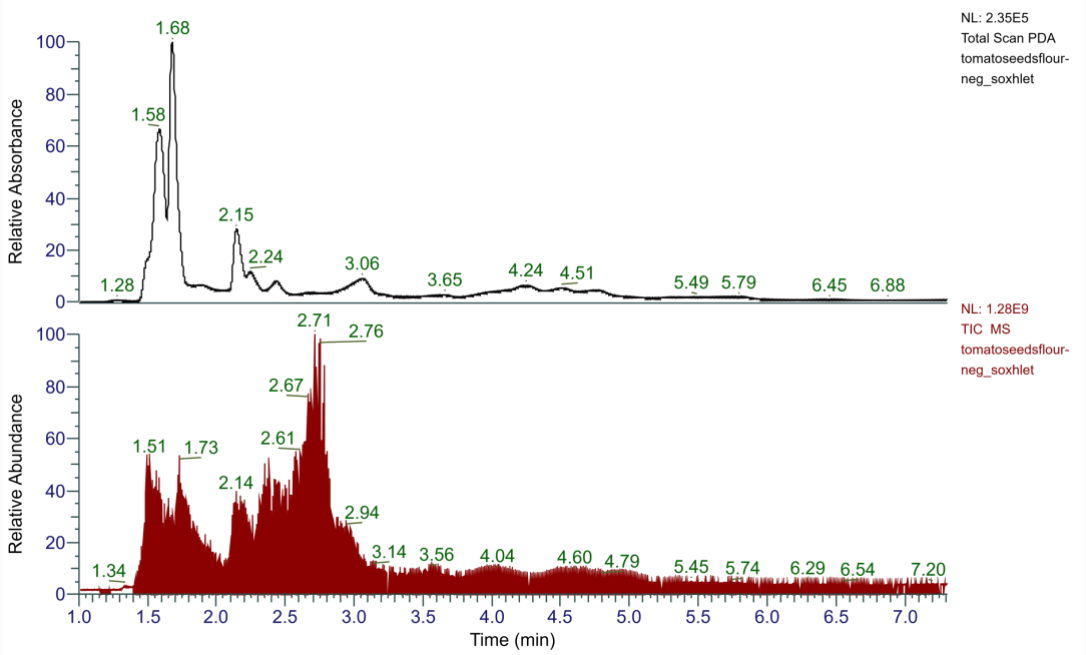
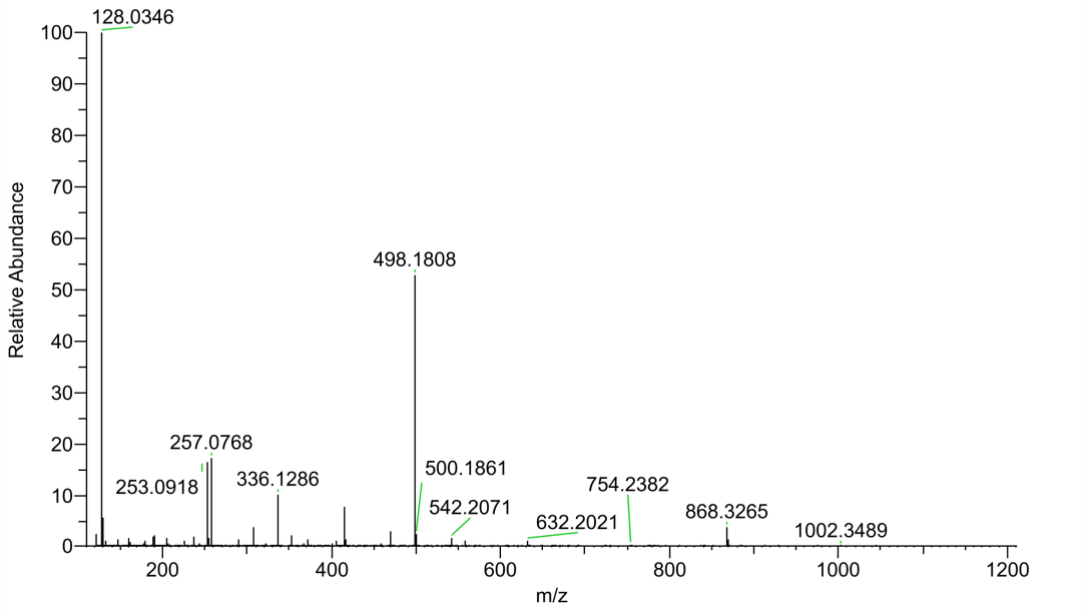
RT :1.00-7.30

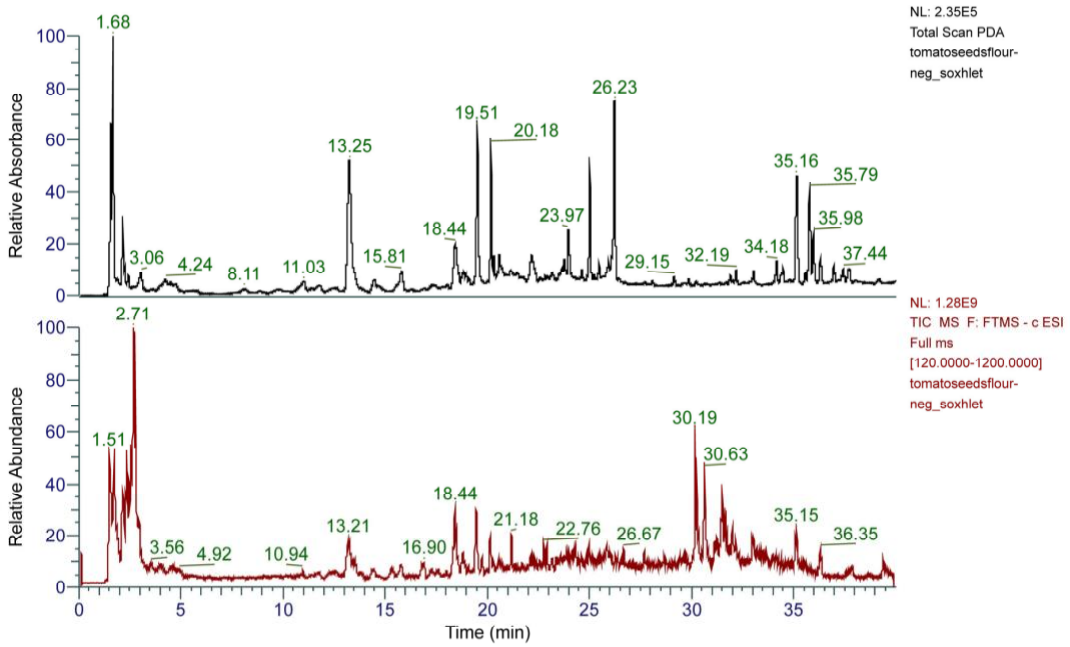


tomatoseedsflour-neg_soxhlet #865 RT: 2.15 AV: 1 NL: 1.24E+008
T: FTMS - c ESI Full ms [120.0000-1200.0000]

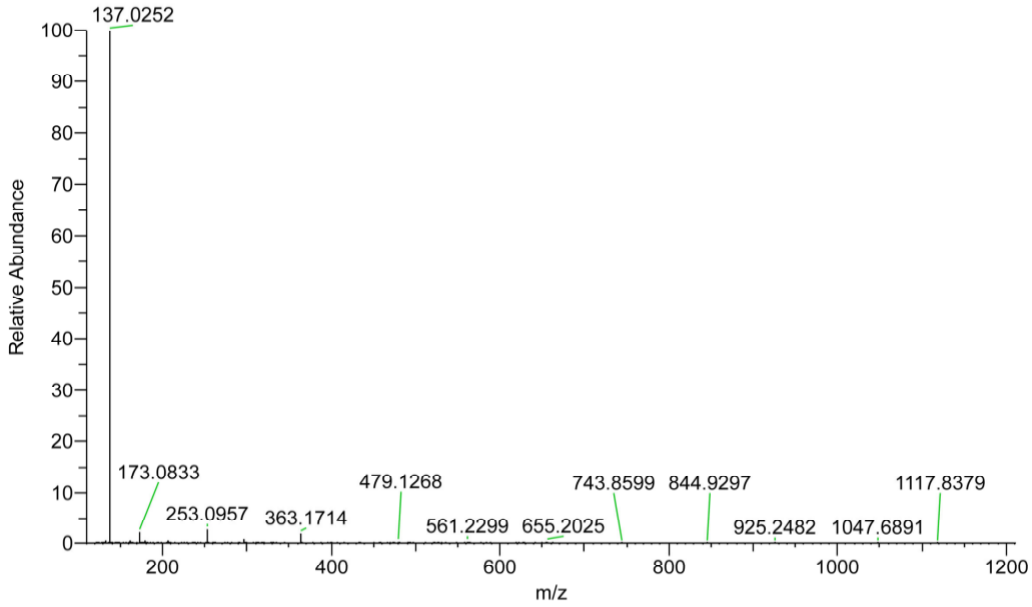


C:\Users\ericc\Desktop\tomatoseedsflour-neg_soxhlet.raw 9/18/2019 10:50:22 AM

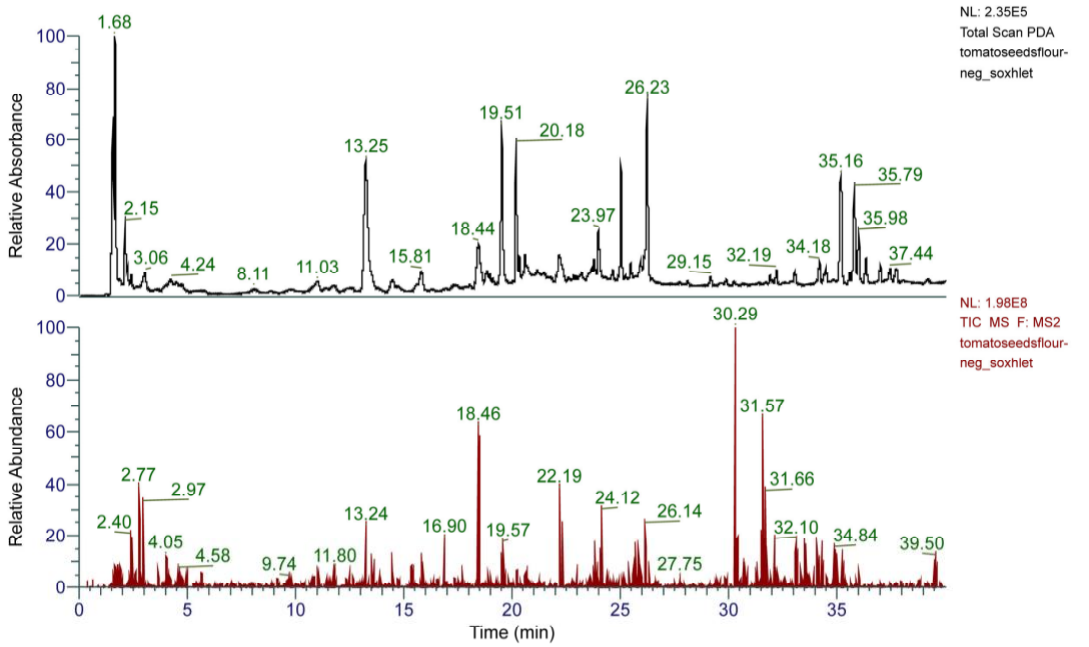
RT :0.00-40.00



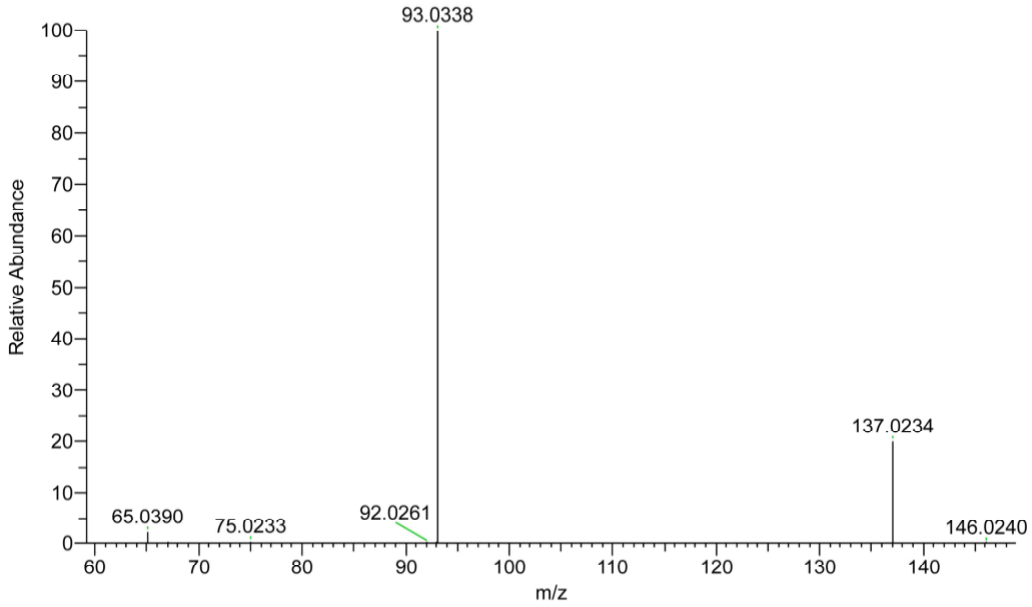
tomatoseedsflour-neg_soxhlet #10384 RT: 18.44 AV: 1 NL: 1.54E+008
T: FTMS - c ESI Full ms [120.0000-1200.0000]



RT :0.00-40.00



tomatoseedsflour-neg_soxhlet #10397 RT: 18.46 AV: 1 NL: 9.39E+007
T: FTMS - c ESI d Full ms2 137.0253@hcd35.00 [60.0000-148.0000]



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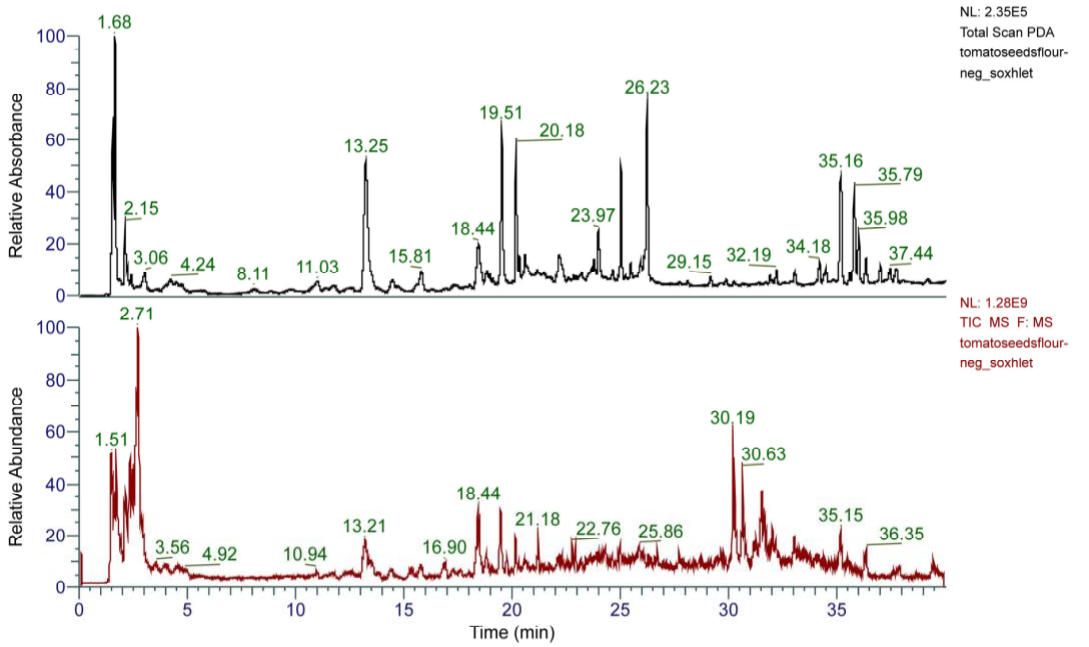
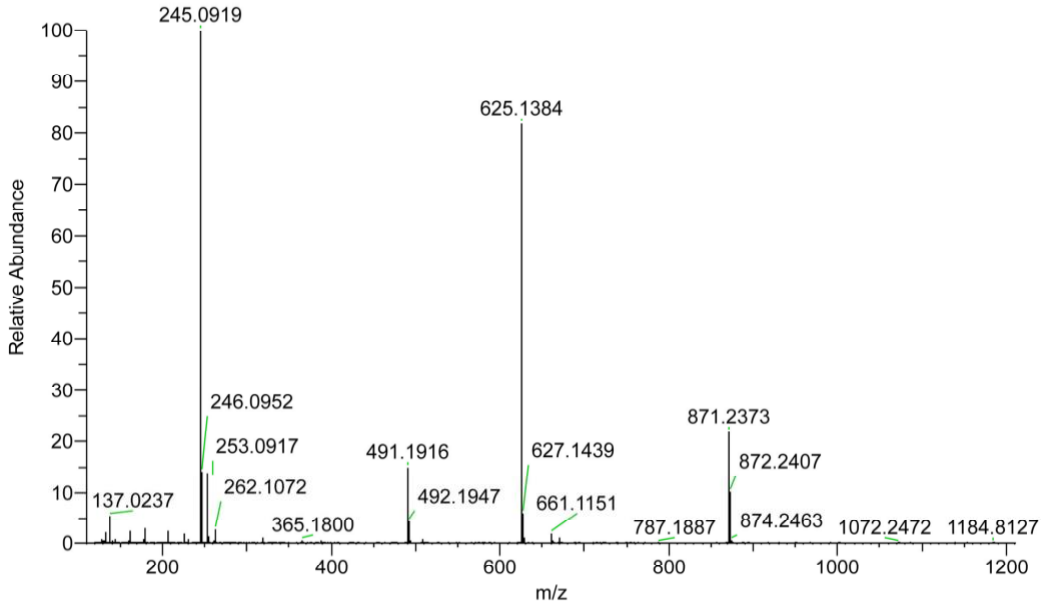
RT :0.00-40.00



tomatoseedsflour-neg_soxhlet #11029 RT: 19.51 AV: 1 NL: 6.61E+007
T: FTMS - c ESI Full ms [120.0000-1200.0000]



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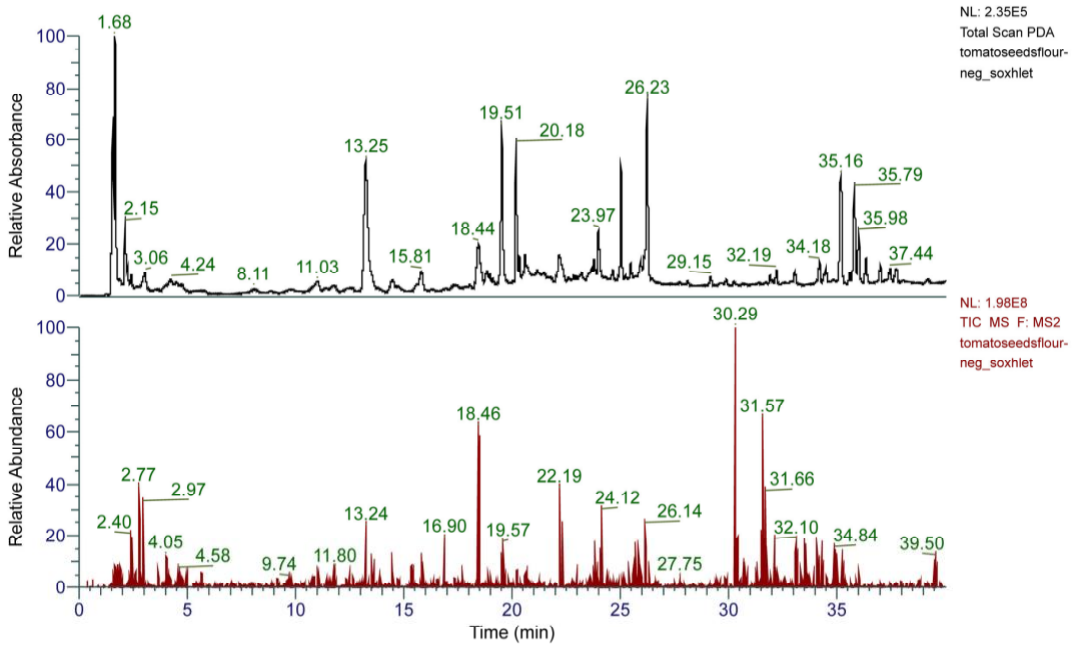
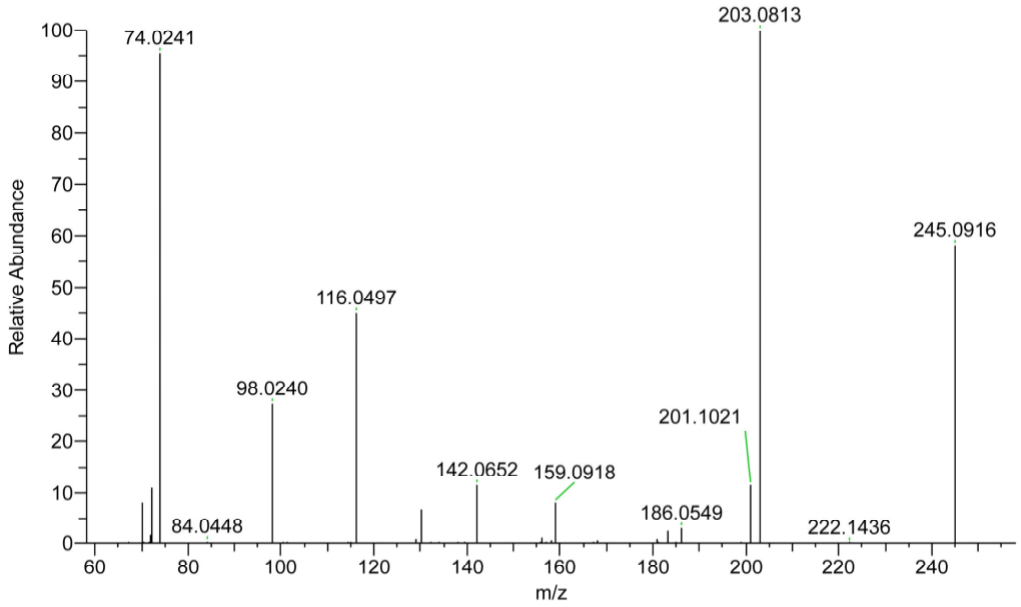
RT :0.00-40.00



tomatoseedsflour-neg_soxhlet #11036 RT: 19.52 AV: 1 NL: 4.75E+006
T: FTMS - c ESI d Full ms2 245.0919@hcd35.00 [60.0000-256.0000]



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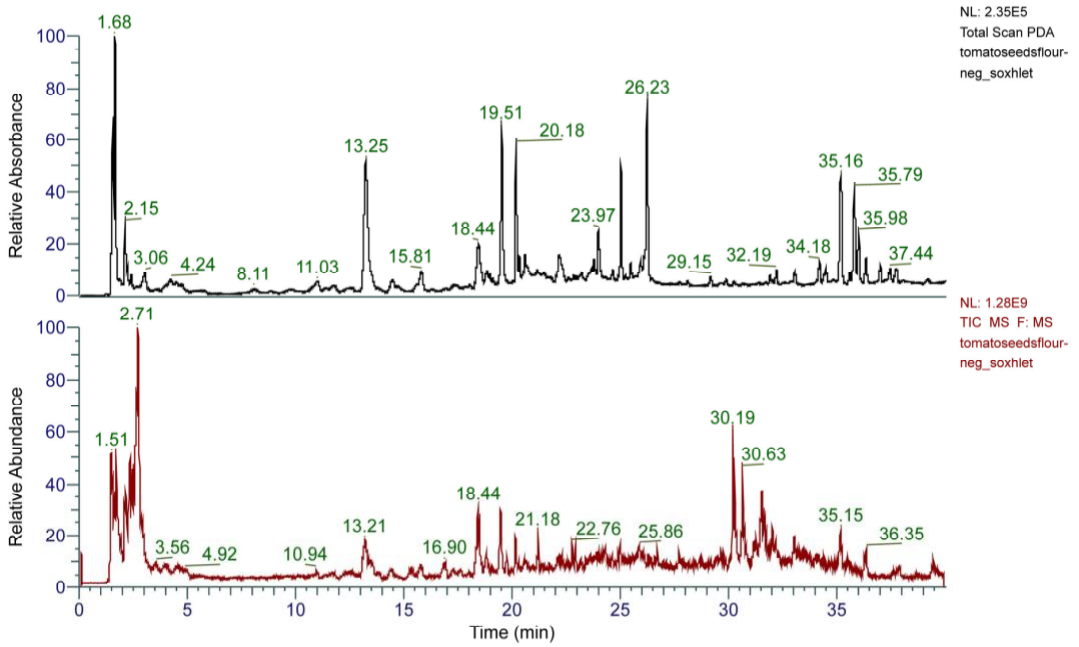
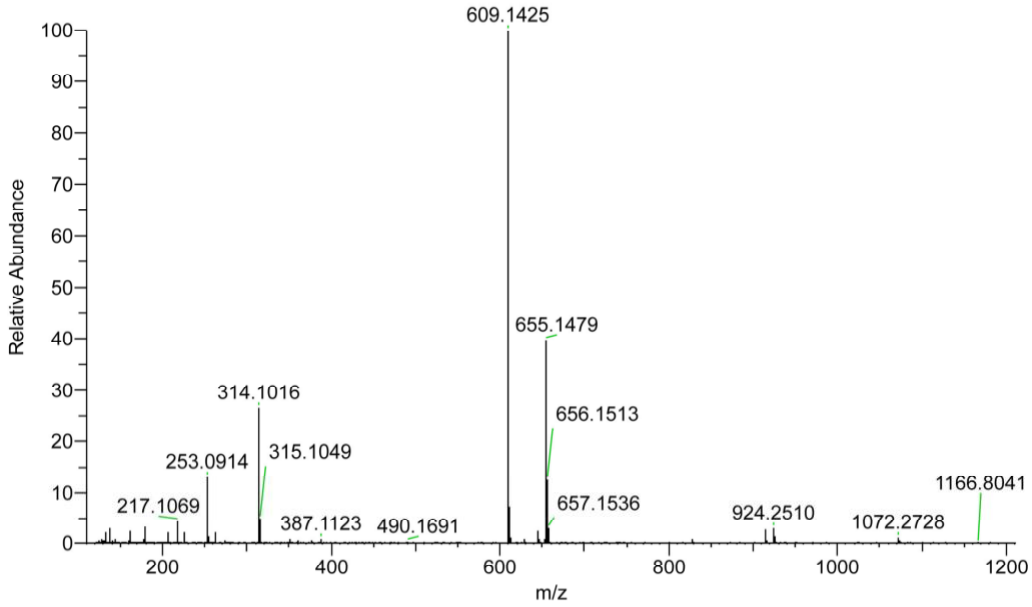
RT :0.00-40.00



tomatoseedsflour-neg_soxhlet #11431 RT: 20.17 AV: 1 NL: 6.77E+007
T: FTMS - c ESI Full ms [120.0000-1200.0000]



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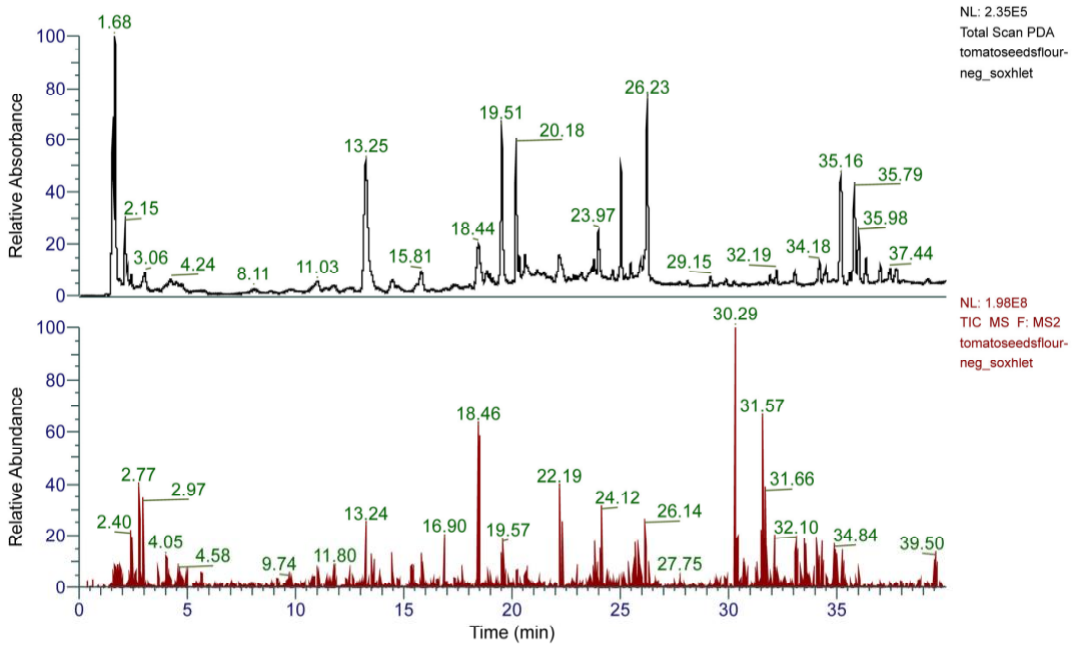
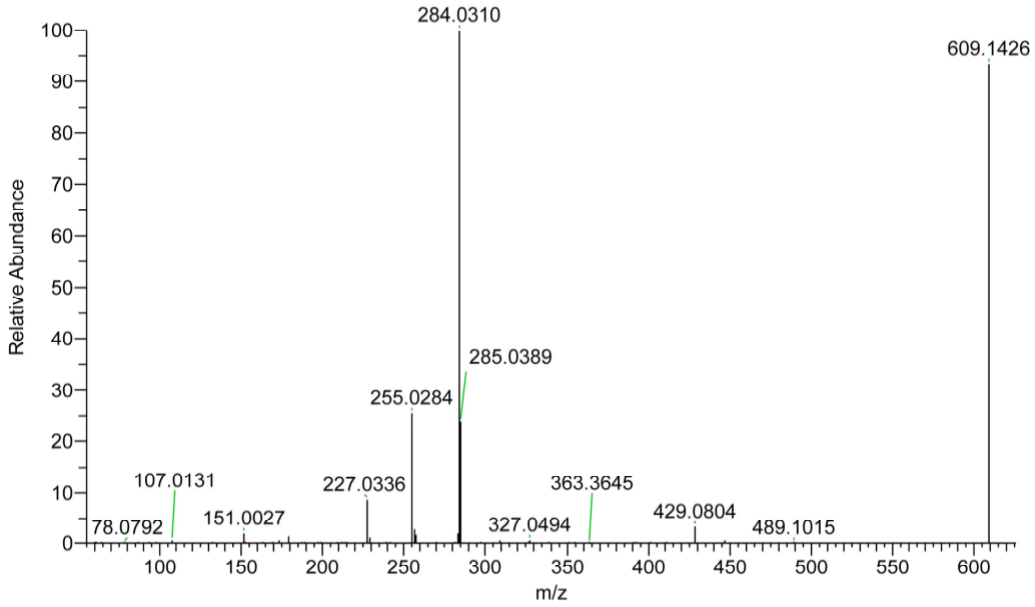
RT :0.00-40.00



tomatoseedsflour-neg_soxhlet #11462 RT: 20.22 AV: 1 NL: 4.51E+006
T: FTMS - c ESI d Full ms2 609.1429@hcd35.00 [60.0000-620.0000]



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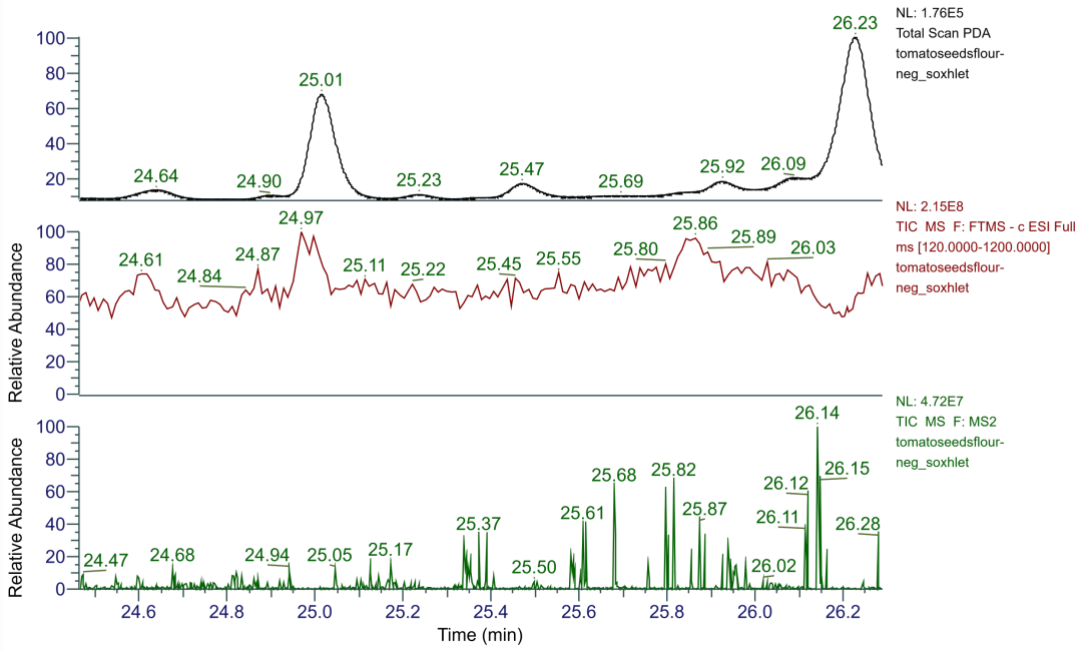
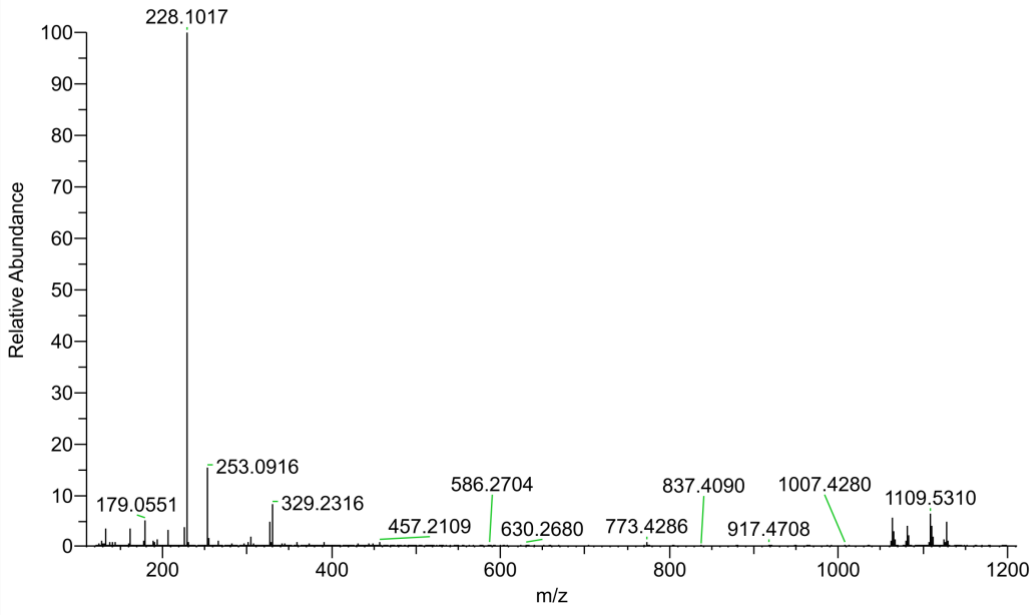
RT :24.46-26.29



tomatoseedsflour-neg_soxhlet #14382 RT: 25.01 AV: 1 NL: 5.42E+007
T: FTMS - c ESI Full ms [120.0000-1200.0000]



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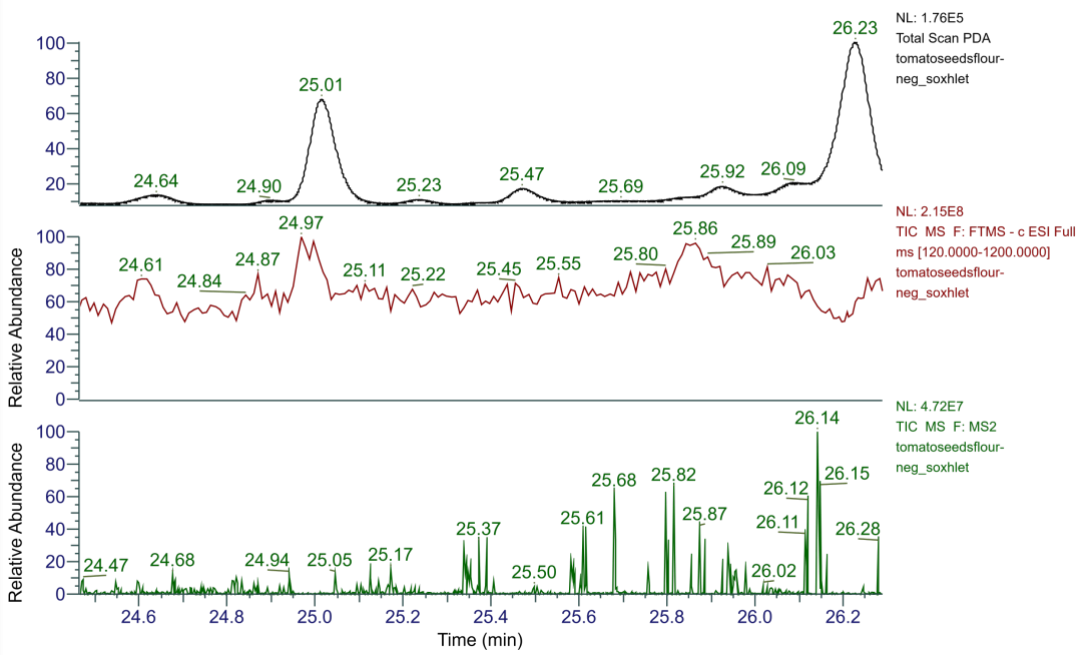
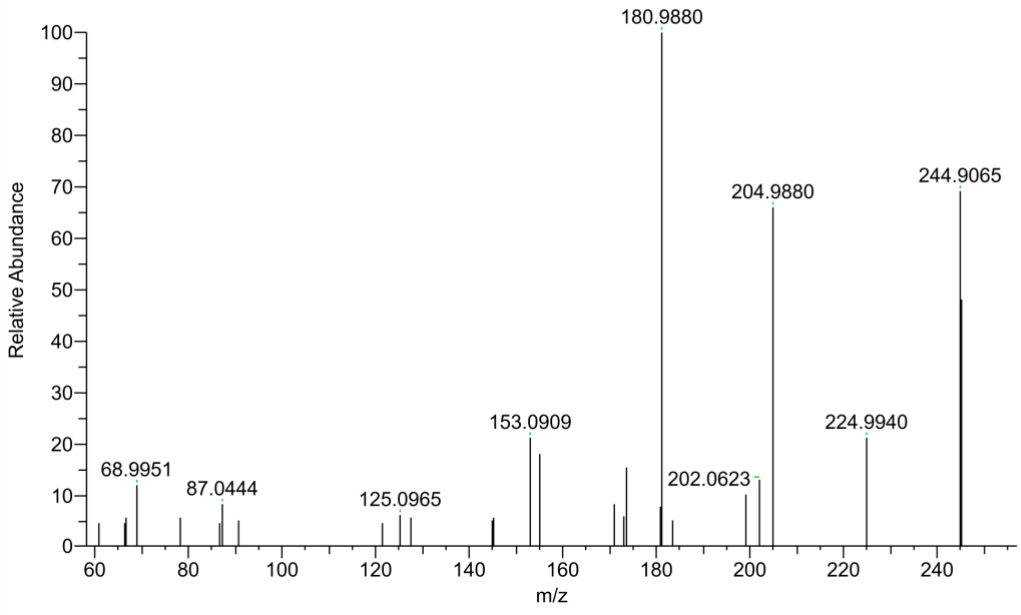
RT :24.46-26.29

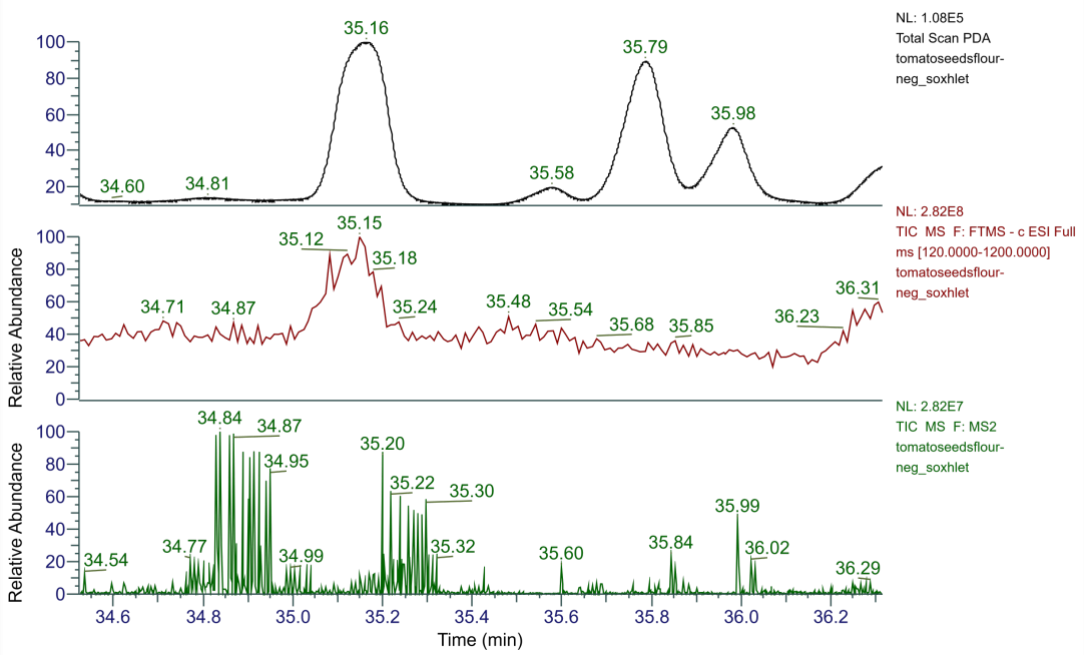


tomatoseedsflour-neg_soxhlet #14383 RT: 25.01 AV: 1 NL: 5.31E+004
T: FTMS - c ESI d Full ms2 244.9842@hcd35.00 [60.0000-255.0000]

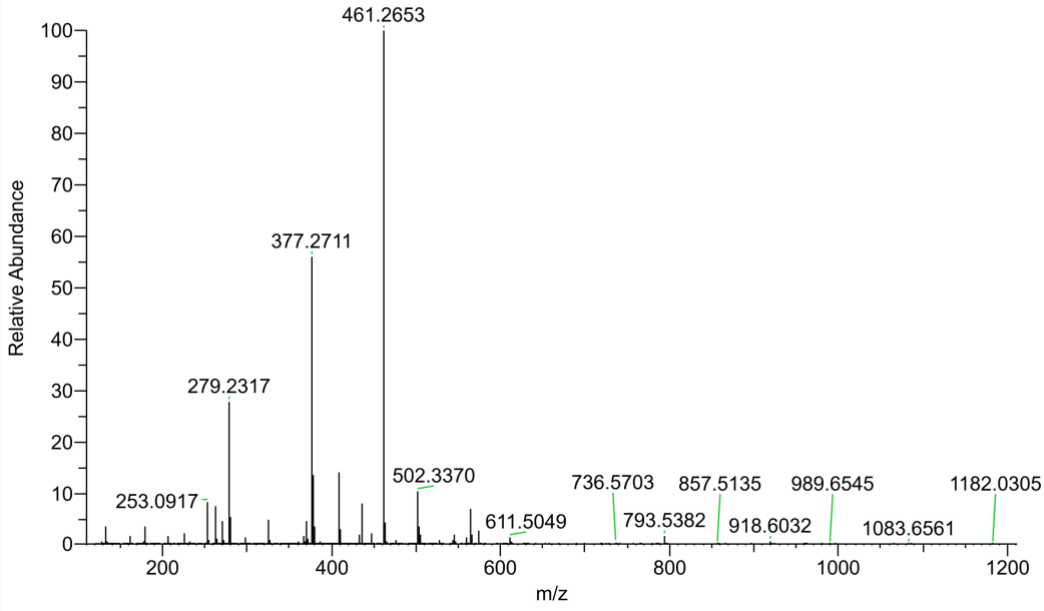


C:\Users\User\Desktop\tomatoseedsflour-neg_soxhlet.raw 3/6/2020 12:43:40 PM

RT :34.52-36.31



tomatoseedsflour-neg_soxhlet #20603 RT: 35.16 AV: 1 NL: 6.04E+007
T: FTMS - c ESI Full ms [120.0000-1200.0000]



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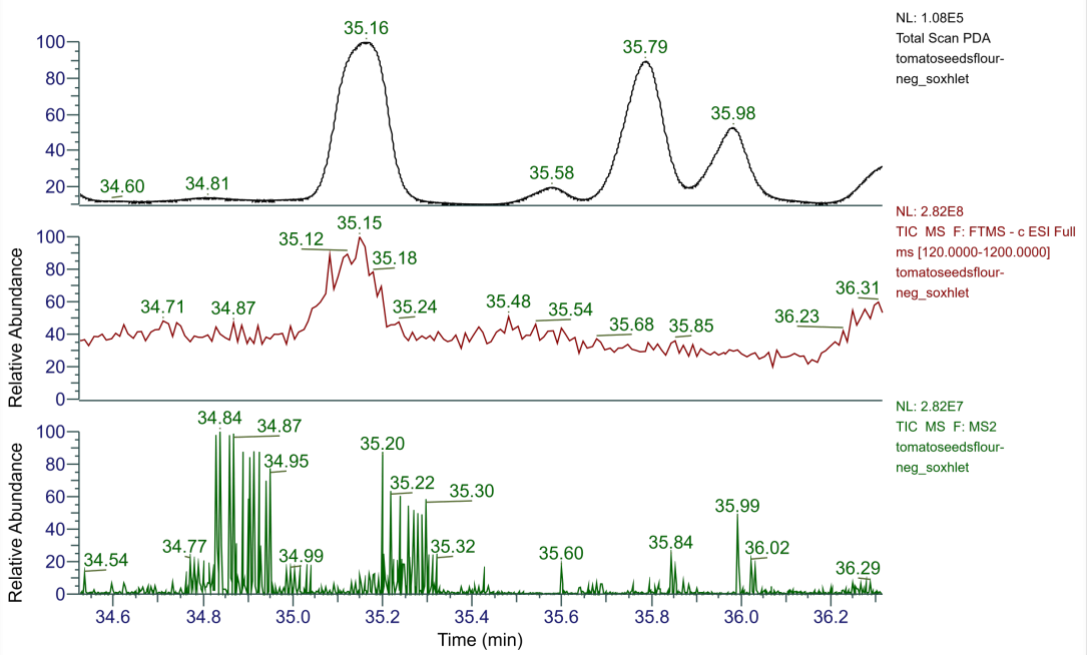
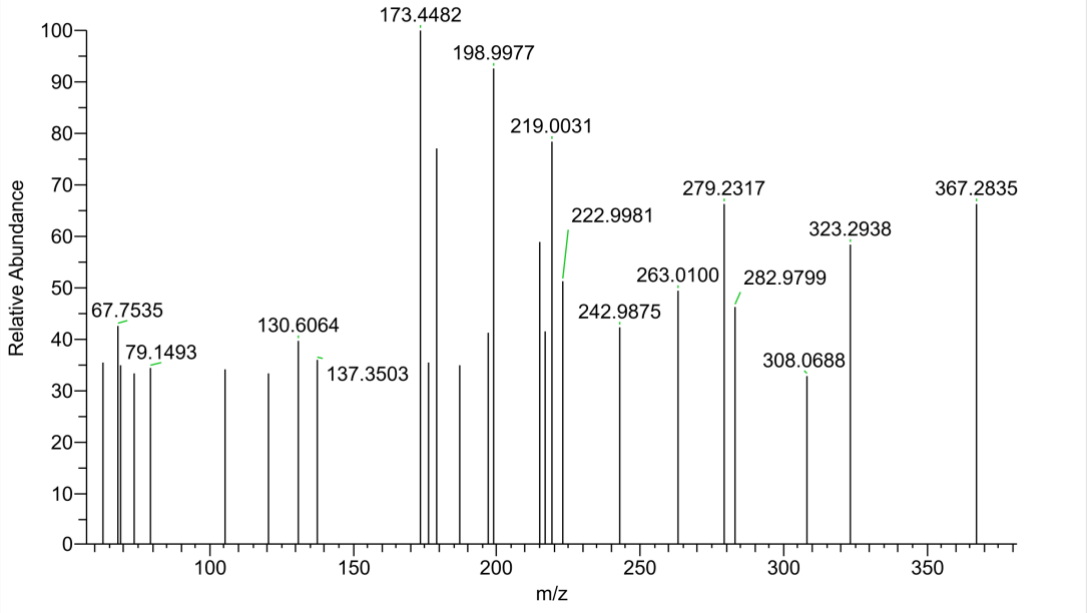
RT :34.52-36.31

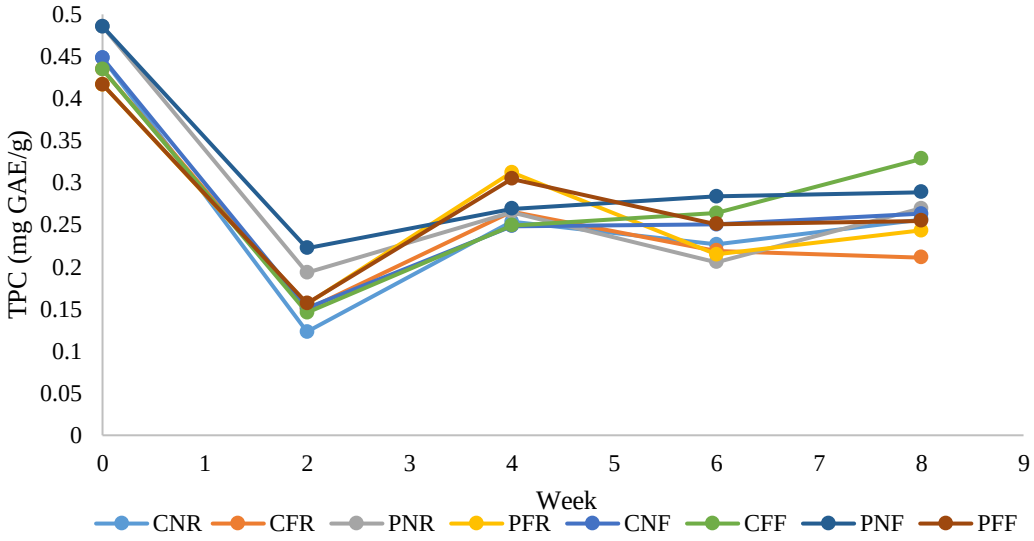


tomatoseedsflour-neg_soxhlet #20601 RT: 35.16 AV: 1 NL: 7.56E+003
T: FTMS - c ESI d Full ms2 367.2426@hcd35.00 [60.0000-378.0000]

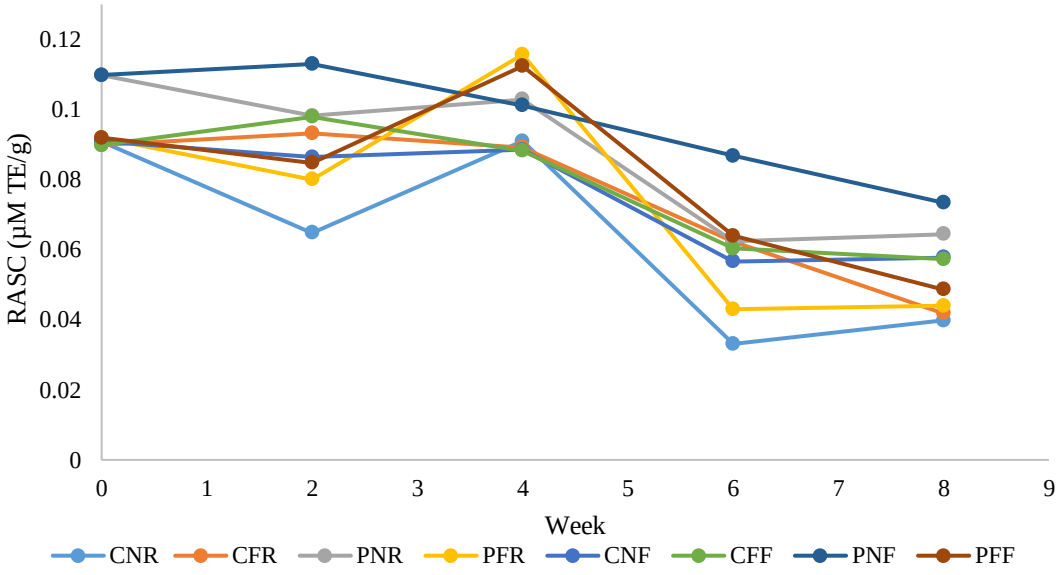


Appendix B

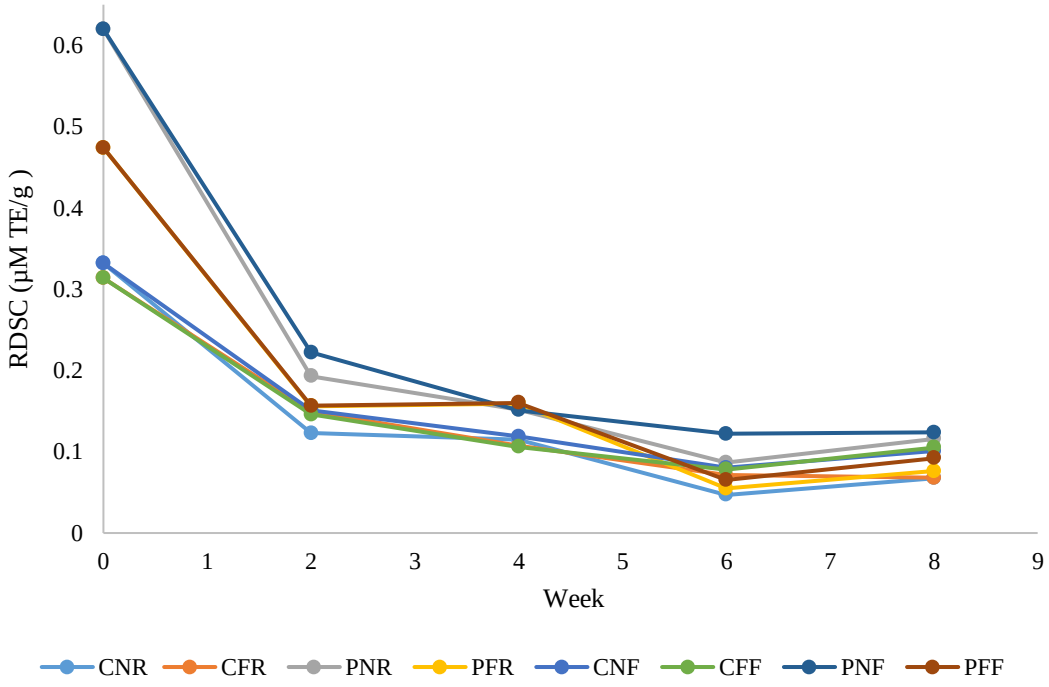
Total phenolic contents of all ketchup samples over 8 weeks. TPC is total phenolic content, GAE is gallic acid equivalent. For sample coding, C/P is commercial or prepared, F/N is flour or no flour, and R/F is room temperature or fridge.



Relative ABTS radical scavenging capacity of all ketchup samples over 8 weeks. RASC is relative ABTS radical scavenging capacity, TE is Trolox equivalent. For sample coding, C/P is commercial or prepared, F/N is flour or no flour, and R/F is room temperature or fridge.



Relative DPPH radical scavenging capacity of all ketchup samples over 8 weeks. RDSC is relative DPPH radical scavenging capacity, TE is Trolox equivalent. For sample coding, C/P is commercial or prepared, F/N is flour or no flour, and R/F is room temperature or fridge.



There were no discernable trends when the total phenolic contents and radical scavenging capacities of the ketchup samples were tested biweekly for eight weeks. Some weeks produced general peaks and some samples had peaks or low weeks. Standard deviation is not shown, but there was little significance between samples, especially at week eight. Some samples were generally higher, but due to the inconsistency of results over time, it is hard to pull out trends. The DPPH radical scavenging of the samples seemed to be the most consistent, with a visible downward trend in all samples.

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