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Impact of Microwave Processing on Quality of High Value Shelf Stable Fruit Products

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IMPACT OF MICROWAVE PROCESSING ON QUALITY OF HIGH VALUE SHELF STABLE FRUIT PRODUCTS

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Para mis padres, Carmen y Pedro, quienes me han apoyado e incentivado en todo momento en la búsqueda de mis sueños y realización de mis metas.

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LIST OF ABBREVIATIONS

CAT	Catechin
CLG	Chlorogenic acid
E-LYC	*all trans*-Lycopene
EPI	Epicatechin
E-β-CAR	*all trans*-β-carotene
HPLC	High Performance Liquid Chromatography
LUT	Lutein
MS	Mass Spectrometry
MW	Microwave heating system
M/Z	Mass to charge ratio
NS	No Significant Differences
PB1	Procyanidin B1
PB2	Procyanidin B2
PC1	Procyanidin C1
QCT-3-GL	Querceting-3-glucoside
RT	Room Temperature (~22°C)
RTN	Rutin
SEM	Standard Error of the Mean
SS	Scraped Surface heating system
STD	Standard Deviation
TUB	Tubular heating system
ZEA	Zeaxanthin
Z-LYC	sum of *cis*-lycopene-1, *cis*-lycopene-2 and 5-*cis*-lycopene
Z-β-CAR	sum of 15-*cis*, 13-*cis* and 9-*cis* β-carotene

α-CAR	α-carotene
α-CRP	α-cryptoxanthin
β-CRP	β-cryptoxanthin

ABSTRACT

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Fruits and vegetables are a rich source of health promoting micronutrients and phytochemicals, and their consumption has been associated with reduction of many chronic and degenerative disease. Thermal processing techniques are used to preserve quality and extent of the shelf life of foods, although these traditional processes are associated with specific quality changes in fruits and vegetables. Compared to traditional thermal processing methods, microwave heating provides the potential to improve product quality by virtue of its energy transfer mechanism that provides rapid volumetric heating of food and can potentially enhance overall quality of processed fruit and vegetable products. Though direct comparisons between traditional and microwave heating on quality and nutritional value of commonly consumed fruits and vegetables remain limited. The objective of these studies was to compare high value applesauce and tomato puree products processed by conventional thermal (scraped surface and tubular heat exchangers) and microwave heating systems to better understand the impact of microwave processing on quality characteristics, phytochemical profile and bioaccessibility after process and following storage.

Applesauce products formulated with apple puree were pasteurized at 96°C for 60 seconds by either a tubular (TB) heat exchanger or 915mHz Industrial Microwave System (MW) were compared after process and over 52 weeks of storage for color, viscosity and polyphenol content. Generally, no significant differences in polyphenol content of applesauce were observed between the two heating systems. Applesauce processed by MW was observed to maintain more stable color characteristics over shelf life compared to TB processed product (7.4 compared to 11). However, MW processed products were found to have slightly lower apparent viscosity (71.1Pa.s/°brix) compared to TB processed products (103.3Pa.s/°brix). Similarly, tomato puree (8°brix) was generated by hot-break (85°C) and pasteurization (121°C/4sec) using MW/MW or scraped surface/tubular configuration was compared for color, viscosity, and, carotenoid content and bioaccessibility. In contrast to applesauce findings, color stability was found to be higher in SS/TB processed products samples. Further, no differences were observed in consistency and apparent viscosity at low shear rate (1/s) between the two heating systems. However, higher apparent viscosity was observed for microwave products at higher shear rates suggesting some improvement in product consistency with MW processing.

Interestingly, no significant differences were observed in lycopene content between processes, however, lycopene bioaccessibility was observed to be higher from tubular compared to microwave processed products. Combined these findings suggest product quality attributes are similar between microwave and conventionally processed tomato and apple products.

However, further research is needed to optimize microwave processing parameters to better understand if it can be leveraged to improve product quality, flavor and other parameters for high value fruits and vegetables.

CHAPTER 1. REVIEW OF THE LITERATURE

1.1 Introduction

The growing awareness of the benefits of increased fruits and vegetables consumption and health has driven demand for new fruit and vegetable based products (Gardner, White et al. 2000). Fruit and vegetables are a major dietary source of health promoting phytochemicals, but due to seasonal availability and variability it is challenging to provide fresh, consistent product throughout the year. Processed foods are considered an option to provide consistent high quality products suitable for delivery of the benefits from fruit and vegetable to consumers. However, positive and negative effects have been associated with traditional thermally processed fruits and vegetables. For example, traditional thermal processing exposes the fruits/vegetables to high temperatures known to adversely affect nutrient and phytochemical content (Shi and Maguer 2000) as well as alter the final sensory and physical properties of finished product. Overly "cooked" products are often associated, by consumers, with lower quality. The generation of an overly "cooked" product is directly related to the high temperatures and long times required for processing due to inefficient heat transfer (Vadivambal and Jayas 2007). Thermal "disruption" of the plant tissue does however, have an apparent positive impact by generation of select flavor volatiles as well as higher nutrient/phytochemical bioavailability (Leong and Oey 2012).

Growing consumer interest in preservation of "fresh-like" minimally processed characteristics but improved nutritional quality has driven the development of novel processing methods for high value fruit and vegetable products. The use of microwave heating systems has been established as a potential alternative to traditional thermal processing methods. The ability of microwave energy to penetrate the volume of a material (volumetric heating), offers potential for rapid heating, a reduction of overall processing time; and, by extension, enhancement of overall product quality (Thostenson and Chou 1999). While promising as a technology to deliver more "fresh-like" product with improved sensory qualities, very little is known regarding the impact of this technology on phytochemical profiles and bioaccessibility from high value fruits and vegetables.

1.2 High Value Crops: Fruits and Vegetables

High value crops refer to non-traditional (non-staple) food crops such as fruits, vegetables, flowers, ornamentals, condiments and spices. Most of these high value crops have higher net return per hectare of land than staple crops, as well as higher market value than traditional cereal grains and export crops; for this reason, high value crops represent higher monetary value as emerging products in local and global markets (Temu and Temu 2005). High value crops play an important role in the daily diet and provide nutritional benefits for people around the world.

The World Health Organization has estimated that low fruit and vegetable consumption is responsible for up to 19% of gastrointestinal cancer, 31% of ischaemic heart disease and 11% of stroke cases worldwide accounting for an estimated of 2.7 million (4.9%) deaths worldwide (WHO 2002). The global production of fruits and vegetables has grown approximately 3% over the past 10 years. In 2011, 640 million tons of fruits and more than 1 billion tons of vegetables were harvested around the world. China has become the largest fruit and vegetable producer in the world, providing 20% of the fruits and more than 50% of the total vegetables globally (FAO 2013). When local production is not enough, importation is the best option to fill the gap, over the past 10 years (2000-2010) the fruits and vegetable market has growth of 11% per year worldwide, with Europe being the largest exporter.

1.2.1 Fruits, Vegetables and Public Health

According to the American Heart Association Dietary guidelines (Krauss, Eckel et al. 2000) a lifestyle practice to achieve and maintain a good cardiovascular health, includes a balance between a healthy diet (whole grains, low fat or non-fat dairy products, lean meats, seafood, legumes and fruits and vegetables) and physical activity. Similarly, the U.S. Department of Health and Human Services (2006) recommends a daily intake of 4-5 cups of fruits and vegetables in the Dietary Approach to Stop Hypertension (DASH) eating plan for a 2000 calorie level, since moderately research suggest that fruits and vegetables consumption is inversely proportional to cardiovascular disease (Liu, Manson et al. 2000, Bazzano, He et al. 2002, Hung, Joshipura et al. 2004, He, Nowson et al. 2007).

Low consumption of fruits and vegetables have been demonstrated in the American diet. The World Health Organization [WHO] also estimates that low consumption of fruits and vegetables may contribute to 2.7 million deaths worldwide; for this reason FAO and WHO recommend a minimum intake of 400g of fruits and vegetables per day. The consumption of whole fruit as well as processed forms (canned, frozen and dried) is recommended compared to their non-diet juice/fruitades forms as the consumption of sugar sweetened beverage, sugars and sweets have been observed to decrease the likelihood of meeting the dietary references intake of the nutrients within the juice and have been linked to childhood obesity (Guthrie and Morton 2000, Ludwig, Peterson et al. 2001, Frary, Johnson et al. 2004, Malik, Schulze et al. 2006, USDA 2010) .

Fruits and vegetables contribute a large number of key nutrients including: folate, magnesium, potassium, dietary fiber and vitamins A, C and K. The consumption of such nutrients has been associated with weight management and reduced risk of chronic disease (USDA 2010). Prostate cancer risk has been shown to decrease with the consumption in vitamin C and phytochemical rich vegetables such as bell pepper and broccoli, which had been associated with inhibition of cancer cell growth (Ambrosini, De Klerk et al. 2007). Fruits and vegetables are also a main source of health promoting carotenoids especially β-carotene which act as Vitamin A precursor. Increased consumption of carotenoid rich fruits and vegetables is an important consideration in the diet of children and women around the world. According to the World Health Organization, 21% of all children and pregnant women suffer from vitamin A deficiency, causing primarily measles and diarrhoeal diseases as well as fetal loss, low birth weight, preterm birth and infant mortality (SanJoaquin and Molyneux 2009).

Vitamin A deficiency is responsible for 1.4% of deaths worldwide (WHO 2002). Deficiency of vitamin A also results in visual impairment, increase in severity of respiratory and gastrointestinal infections. In developing countries consumption of fruits and vegetables represent more than 70% of the daily vitamin A intake (Britton, Liaaen-Jensen et al. 2004). A daily intake of 6 mg of carotenoids is estimated to be obtained from the diet, even when these compounds are not nutritionally essential, they play an important role as vitamin A (Rao and Rao 2007).

1.2.2 The Role of Processed Fruit and Vegetables Products in Nutrition

While nutrition is a key consideration in consumer choice, consumer's buying decision about fresh and processed fruits and vegetable products is based on their quality perception, which can be defined from two different points of view: product and consumer. In order to avoid variation in quality of processed product, grades and standard for the final product are used (Shewfelt 1999).

The protective effect from fruits and vegetables is attributed to the phytochemical content with compounds such as carotenoids, flavonoids, isoflavonoids and phenolic acids (Beecher 1998, Rao and Agarwal 1998, Vinson, Su et al. 2001, Gil, Tomás-Barberán et al. 2002, Leontowicz, Gorinstein et al. 2002, Sun, Chu et al. 2002, Frusciante, Carli et al. 2007). The most popular garden vegetable in America is tomato (Schmidt 2013), which is widely produced for fresh market in 20 states in the United States of America. Tomato consumption represents the main dietary source of lycopene and some β-carotene (Frusciante, Carli et al. 2007).

Research showed the potential health benefits of tomato product consumption (Gama, Tadiotti et al. 2009), such as risk reduction of some cancer types, arteriosclerosis and cataract formation (Gerster 1997, Ambrosini, De Klerk et al. 2007) .

Apples also provide a significant amount of flavonoids in people's diet, providing 22% of per capita consumption of fruit phenols (Vinson, Su et al. 2001). Several studies have linked its consumption with reduced risk of lung cancer (Michaud, Feskanich et al. 2000), cardiovascular diseases (Hertog, Feskens et al. 1993, Sesso, Gaziano et al. 2003), diabetes and weight loss (Knekt, Kumpulainen et al. 2002). For these various reasons, several studies have studied tomato and apples health benefits and improvement of processing technologies to maintain their nutritional value.

1.2.3 Tomatoes

Tomatoes (*Lycopersicon esculentum)* are a climacteric berry fruit of the Solanaceae family. Tomatoes were introduced into Europe in the early 16th century, and has become one of the most widely consumed vegetables in the world both as fresh and processed. The tomato fruit has become central to several diets (mostly Mediterranean), contributing many nutritional attributes through its wide consumption as fresh and processed products (Frusciante, Carli et al. 2007).

According to the FAO, in 2011 global tomato production was about 159 million metric tons; with the leading production countries being China, India and the United States of America with 48.5, 16.8 and 12.6 millions of metric tons, respectively.

In the United States of America most of the tomatoes production is destined for processing. California, produces 95% of tomatoes used for processing and 30% of the fresh tomatoes for consumption in the country (Heuvelink 2005). Food processors can also process and pack in tomato paste in bulk and store it for up to 18 months (USDA 2012). The tomato fruit is harvested at different maturity stages depending on the purpose of the product, fully ripe fruit is mechanically harvested for immediate processing, whereas product for fresh consumption is harvested at partially ripe stage (Saltveit 2005) and can be ripened under modified atmosphere environments. The optimal storage conditions for fresh fruit depends of the maturity stage and intended use. At temperatures above 27°C changes in color can be observed (decrease in color) while storage of fully ripe tomatoes at 10°C or less may affect flavor and aroma development (Maul, Sargent et al. 2000).

Interestingly, Americans consume 75% of their tomatoes in processed form. Tomato products can be classified as: tomato preserves (whole peeled tomatoes, tomato juice, tomato pulp, tomato puree, tomato paste, pickled tomato), dried tomatoes (tomato powder, tomato flakes, dried tomato fruits) and tomato based foods (tomato soup, tomato sauces, chili sauce, ketchup) (Costa and Heuvelink 2005). The most commonly consumed processed tomato form is tomato concentrate, which can be used as an ingredient for different popular products and home preparation/applications (pizza, chili). Concentrates result from concentration of juice obtained from mature tomatoes of red or reddish varieties (*Lycopersicum esculentum* P.Mill*)* and/or residual liquid part from partial extraction of juice, or preparation of tomatoes for canning, which usually exclude, the skin and seeds.

Additional ingredients (salt, lemon juice, sodium bicarbonate, water, spices, flavoring) can be added individually or in combination according to FDA regulations. The classification of the final product for labeling is dependent on the percentage of tomato soluble solids. For example, “tomato puree” should contain not less than 8% but no more than 24% tomato soluble solids. In contrast “tomato paste” should not contain less than 24% soluble solids (FDA 2012).

1.2.3.1 Tomato Carotenoids

Tomatoes are a key dietary source of many potentially healthy phytochemicals, including carotenoids (Table 1.1). Carotenoids are plant pigments in plants, responsible for the yellow-orange color in flowers, fruit and roots of plant materials. These pigments can often be masked by the green of chlorophyll until chlorophyll senescence in leaves during fall and ripening in fruit (Britton, Liaaen-Jensen et al. 1995).

Carotenoids are chemically defined as C_{40} isoprenoid compounds, they are secondary plant metabolites and can be classified broadly into two classes, hydrocarbon carotenes such as β-carotene, α-carotene and lycopene, and oxygenated derivatives known as xanthophyll or oxycarotenoids including lutein, zeaxanthin and violaxanthin (Sandmann 2001, Zaripheh and Erdman 2002). Mammals cannot biosynthesize carotenoids and as such, these compounds can only be acquired from the diet. Carotenoids are generally insoluble in water (Rodriguez-Amaya 2001). Their pigmentation is derived from their chromophore of seven or more double bonds responsible for the absorption of light.

This highly conjugated double bond system is sensitive to oxidative degradation and geometrical isomerization by exposition to light, heat and acids (Britton, Liaaen-Jensen et al. 1995).

Table 1.1 Carotenoid content (ug/g Dw) in tomato products.

Product	Carotenoid	Content (ug/g DW)	Study
Fresh tomato	All-*trans*-lycopene	1122.2 ; 1189.4	(Abushita, Daood et al. 2000, Gama, Tadiotti et al. 2009)
	cis-lycopene	78.8 ; 20.6	
	All-*trans*-β-carotene	52 ; 37.2	
	cis-β-carotene	4.7 ; <1	
Tomato Pulp	All-*trans*-lycopene	974.8	(Gama, Tadiotti et al. 2009)
	cis-lycopene	77.2	
	All-*trans*-β-carotene	82	
	cis-β-carotene	27	
Ketchup	All-*trans*-lycopene	465.2	
	cis-lycopene	19.4	
	All-*trans*-β-carotene	23.5	
	cis-β-carotene	9.5	
Tomato Paste	All-*trans*-lycopene	1628.2	(Abushita, Daood et al. 2000)
	cis-lycopene	25.2	
	All-*trans*-β-carotene	26.3	
	cis-β-carotene	9.7	
Red tomato puree	All-*trans*-lycopene	603	(Georgé, Tourniaire et al. 2011)
	All-*trans*-β-carotene	160	
Yellow tomato puree	All-*trans*-lycopene	n.d	
	All-*trans*-β-carotene	20	
Tomato Pulp	All-*trans*-lycopene	846.86 - 854.01	(Akanbi and Oludemi 2004)

As stated before the most important nutritional role of carotenoids in human and animal diets is their ability to act as vitamin A precursors (Damodaran and Parkin 2008). α-Carotene, β-carotene and β-cryptoxanthin are the main provitamin A carotenoids due to their β-ionone rings.

Non-provitamin A carotenoids include lycopene (tomatoes, pink red grapefruit, guavas, papayas, watermelon), lutein (green and dark green leafy vegetables) and zeaxanthin (green and dark green leafy vegetables and some fruits) (Thane and Reddy 1997) are also abundant in the diet but do not contribute to provitamin A content. However, association between total carotenoid consumption, including non-provitamin A carotenoids, and health benefits have been made including the prevention and risk reduction of several types of cancer and cardiovascular diseases as well as age related macular degeneration (Gil, Tomás-Barberán et al. 2002, Campbell, Canene-Adams et al. 2004, Shi, Kakuda et al. 2004).

Lycopene, the most abundant carotenoid in tomatoes has been associated with reduction of specific cancer risk including prostate cancer (Huang, Alberg et al. 2003, Jian, Du et al. 2005), lung cancer (Feskanich, Ziegler et al. 2000, Wright, Mayne et al. 2003), colorectal cancer (Narisawa, Fukaura et al. 1996, Slattery, Benson et al. 2000), gastric cancer (Tsubono, Tsugane et al. 1999, De Stefani, Oreggia et al. 2000), and cervical cancer (Goodman, Kiviat et al. 1998, Schiff, Patterson et al. 2001). In addition to epidemiological associations, animal models have shown organ specific chemopreventive effect from lycopene consumption on prostate and lung cancer (Cohen 2002). Lycopene and α-carotene intake and diets with high concentration of carotenoids have also been associated with a significant reduction in the risk of lung cancer.

However, high intakes of β-carotene, lutein and β-cryptoxanthin does not represent a significance decrease of risk of lung cancer (10-19%) (Michaud, Feskanich et al. 2000). Consumption of tomato and tomatoes products is viewed as a convenient and consumer friendly way to deliver several health related nutrient components like folate, potassium, vitamin A, vitamin C and several carotenoid compounds: lycopene, phytoene, phytofluene, gamma carotene, β-carotene (vitamin A activity) (Beecher 1998).

Interestingly, the consumption of processed/cooked tomato appears to offer the added advantage of enhanced bioavailability of lycopene compared to fresh products (Bugianesi, Salucci et al. 2004, Reboul, Borel et al. 2005, Colle, Lemmens et al. 2010, Colle, Van Buggenhout et al. 2010, Knockaert, Pulissery et al. 2012, Page, Van Stratum et al. 2012).

Absorption of carotenoids such as lycopene is also enhanced from formulated processed products or when consumed with dietary lipids (Brown, Ferruzzi et al. 2004, Goltz, Campbell et al. 2012, Goltz, Sapper et al. 2013). This enhanced bioavailability of lycopene from processed products may have contributed to the observation that increased consumption of tomato-based products with fat such as pizza for which >1 serving/week vs. <0.5 serving/month was associated with a decrease in prostate cancer risk for subjects with prostate cancer family history.

However, the greater consumption of some lycopene/tomato product (spaghetti with tomato sauce, lasagna and chili) consumption does not contribute to the reduction of prostate cancer risk (Kirsh, Mayne et al. 2006). When pizza and tomato sauce was consumed 2-4 times per week a significant reduction of prostate cancer risk of 15% and 34% respectively was shown.

When a combination of several tomato sources were consumed a decrease of 35% in prostate cancer risk associated with >10 serving per week when compared to <1.5 serving per week has been reported (Campbell, Canene-Adams et al. 2004).

While the health benefits of tomato lycopene and its mechanism of action are still under evaluation, addition of tomato products does contribute to the recommended servings of fruits and vegetables (Yan 2010). Even with growing evidence, the U.S. Food and Drug Administration concluded that the scientific evidence related to consumption of tomatoes and tomato products is not enough to claim its action reducing the risk of prostate, gastric, ovarian and pancreatic cancers (Kavanaugh, Trumbo et al. 2007). However, tomato consumption is still associated with the prevention of several chronic diseases (Gahler, Otto et al. 2003, Shi, Kakuda et al. 2004).

Generally, minimal changes in lycopene content have been observed in tomato products through thermal processing (Abushita, Daood et al. 2000, Seybold, Fröhlich et al. 2004). However, with processing demonstrating the ability to potentially enhance bioavailability by releasing the nutritional compound from the cell (Rao and Agarwal 1998, Boileau, Merchen et al. 1999, van het Hof, West et al. 2000) one must assess the potential for novel processing method to provide similar benefits. To date information on the impact of novel processes on carotenoid stability and bioavailability from fruits and vegetables is lacking. This is especially true in the case of microwave processing.

1.2.4 Apples

Apples (*Malus domestica)* are fruits part of the *Rosaceae* family which contains 11 genera and 620 species. The fruit has a low freezing point -2.8°C to 2.5°C which allows it to growth well in colder climates. A new apple tree takes about 6-8 years to produce the first batch of apples. According to the Food and Agricultural Organization [FAO] in 2011, apple global production was estimated at 75.6 million metric tons.

The leading producing countries are: China, United States of America and India with 35.9, 4.2 and 2.8 million metric tons respectively. Apple harvesting is dependent on the maturity of the fruit. For a high quality product and a reasonable shelf life, the fruit should be harvested at the beginning of the ripening process. Premature harvest of apples will affect physical (size, color, firmness) and chemical (flavor, brix and acidity) characteristics, as well as susceptibility to storage disorders. Different test and techniques (standardized color charts, start/iodine test, pressure tests, Near Infrared Spectroscopy, Nuclear Magnetic Resonance) have been developed mostly to test sugar content (Thompson 2008).

Postharvest practices include hydro-cooling, washing, culling, waxing sorting and packaging. Appropriate storage facilities are important in order to maintain the fresh market product post-harvest in controlled atmosphere (approximately 0°C, 1-3% O_2 and 1-3% CO_2). Under appropriate conditions, fresh apples can be stored up to 12 months (Hui, Barta et al. 2008).

Consumer preferences for apples are typically based on physical characteristics (color, texture, and appearance), aroma and sweetness/tartness (brix to acid ratio).

According to the National Nutrient Database (USDA 2008) raw apples with skin contain 85.6% of water, 13.8% carbohydrates, 2.4% total dietary fiber and 10.4% total sugars (2.07% sucrose, 2.43% glucose, 5.90% fructose). Different apple products are found in the market, and new technology allows industry to minimally process the fresh product and keep it refrigerated for about 1 week before processing. The most common apple processed products are apple juice, apple cider, apple slice, dried apple and applesauce (Hui, Barta et al. 2008).

According to the USDA Economic Research Service (2008), between 1970 and 2005 an estimated decrease of 35% in canned fruit consumption was shown. However, processed apple products and applesauce specifically were the most popular canned fruit product in 2005. Applesauce is mainly consumed by children under the age of 5 and its consumption is directly related with the amount of household income. Canned apple and applesauce included in the School Breakfast and National School Lunch Programs as part of the variety of canned, frozen fresh and dried fruits options for K-12 students, in which consumption of 1 cup per day of fruits is required (USDA 2013).

Applesauce: the Code of Federal Regulations in 21CFR145.110 defines applesauce as a "food prepared from comminuted or chopped apples (*Malus domestica Borkhause),* which may or may not be peeled and cored, and which may have added thereto one or more optional ingredients". Golden creamy color and good sweetness and tartness balance is desirable in the final product. The USDA (1982) developed standards for grades of applesauce, explaining the use of additional ingredients and different types of applesauce.

The applesauce-making process involves washing and dicing the apples, finishing and thermal processed to inactivate spoilage microorganisms and increase shelf life. The final product brix is 9.0 and 15.5 for unsweetened and sweetened applesauce, respectively. The product contains 88.22g/100g of water, 9.39g/100g of sugars, 11.27g/100g of carbohydrates, 1.1g/100g of total dietary fiber, 0.10g/100g of lipids and 0.17g/100g of protein, providing 42kcal per 100 grams (USDA 2008).

1.2.4.1 Apple Nutrients and Polyphenols

Apples are a good source of macro and micronutrients including dietary fiber (Gorinstein, Zachwieja et al. 2001, Leontowicz, Gorinstein et al. 2002), sugars (Temma, Hanamatsu et al. 2002, Liu, Ying et al. 2006), proteins (Björkstén, Halmepuro et al. 1980, Hsu, Heatherbell et al. 1989, Wu and Siebert 2002), vitamins (Suárez-Jacobo, Rüfer et al. 2011), and minerals (Perring 1984, Dris, Niskanen et al. 1999, Gorinstein, Zachwieja et al. 2001, Mihalev, Schieber et al. 2004, Renard, Dupont et al. 2007). In addition to these nutrients and micronutrients, apples are a key dietary source of phenolic antioxidants.

Phenolics are secondary plant metabolites that include molecules that have at least one phenol ring in its structure. Polyphenols are a subclass of phenolics and can be broadly defined as a diverse group of organic compounds containing multiple phenol functional groups (a hydroxyl group bonded to an aromatic ring) (Neilson, Ferruzzi et al. 2013). Polyphenol classes vary from simple molecules (phenolic acid) to highly polymerized compounds (proanthocyanidins) (Daayf and Lattanzio 2009).

Polyphenols can be classified into different groups according to the number of phenol rings and the pattern of substitution elements between them: phenolic acids, flavonoids, stilbenes and lignans. Apples are rich in several classes of phenolic and polyphenolic compounds including hydroxycinnamic acids, dihydrochalcones, flavonols (quercetin, phlorizin and their glycosides), flavan-3-ols (monomeric catechins and oligomeric procyanidins)(Gerhauser 2008) (Table 1.2).

- Phenolic acids have the simplest chemical structure of all phenolics and can be divided into two broad classes: derivatives of benzoic acid and derivatives of cinnamic acid. Hydroxybenzonic acid is present in edible plants in low levels except in the case of red fruits, black radish and onions. Hydroxycinnamic acids are more common than hydroxibenzonic acids, representative compounds are chlorogenic acid, coumaric acids, caffeic acids and ferulic acids. These acids are found in free form in products that have received any type of processing (freezing, sterilization or fermentation) (Manach, Scalbert et al. 2004, Neilson, Ferruzzi et al. 2013).
- Flavonoids share a common structure that consist of 2 aromatic rings (A and B) bonded together by 3 carbon atoms forming an oxygenated heterocycle (ring C). Based on heterocycle involved, flavonoids can be divided into 6 subclasses: flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavonols (Manach, Scalbert et al. 2004) (El Gharras 2009).

Table 1.2. Phenolic components and general content in apple products

Product	Compound	Concentration	Study
Whole fruit	Total phenolic	56 - 125	(Łata and Tomala 2007) *mg of (GAE), mg/fruit
	Flavonol	10.6 - 40.8	
Whole Fruit	Epicatechin	0.9	(Gorinstein, Zachwieja et al. 2001) *mg/100g fresh fruit
	Ferulic acid	12.2	
	Gallic acid	16.2	
	p-coumaric acid	41.1	
Pulp	Epicatechin	0.7	
	Ferulic acid	9.8	
	Gallic acid	15.1	
	p-coumaric acid	38.2	
Peel	Epicatechin	1.1	
	Ferulic acid	14.9	
	Gallic acid	19.4	
	p-coumaric acid	53.1	
Whole fruit	Total phenolic	118.3	(Chun, Kim et al. 2005)* GAE/100g, (CE)/100g
	Total flavonoids	62	
Whole fruit	Total Phenolic	140 - 312	(Kevers, Pincemail et al. 2011)*mg (CAE)/100g
Apple puree	Catechin	1.58 - 6.82	(Bengoechea, Sancho et al. 1997) *mg/liter
	Chlorogenic acid	25.08 - 61.47	
	Caffeic acid	0.39 - 4.91	
	Epicatechin	7.40 - 21.16	
	p-coumaric acid	nd	
	Quercetin-3-glucose	0.48 - 2.23	
	Quercetin-3-rutinose	0.24 - 1.14	
Apple Concentrates	Catechin	nd	
	Chlorogenic acid	38.85 - 81.28	
	Caffeic acid	5.57 - 15.34	
	Epicatechin	nd	
	p-coumaric acid	0.92 - 5.38	
	Quercetin-3-glucose	1.08 - 7.97	
	Quercetin-3-rutinose	0.44 - 3.25	

Apple phenolic content can vary widely between variety and growing season. Differences of up to 57% in total phenolic concentration in apple peel had been shown between growing seasons (Łata and Tomala 2007). Despite this variability, the predominant polyphenolic forms found in both flesh and peel are: procyanidins, followed by quercetin-glycosides in the peel and hydroxycinnamic acid esters in the flesh (Tsao, Yang et al. 2003).

Polyphenol accumulation in the apple flesh occurs early in the fruit life, and concentration of flavonols and procyanidin in skin decrease after blooming (Renard, Dupont et al. 2007). However, total and individual levels differ depending on the cultivar and growth due to temperature variation and sun exposition during ripening (McGhie, Hunt et al. 2005).

Generally, beneficial effect of polyphenols on human health have been attributed to their antioxidant properties (El Gharras 2009). However, the biological activity of phenolics is likely much more complex than direct antioxidant function. Compared to other fruit benefits, consumption of apple and apple products had been directly linked to reduction of risk of heart disease (Hertog, Feskens et al. 1993, Knekt, Jarvinen et al. 1996), asthma (Shaheen, Sterne et al. 2001, Woods, Walters et al. 2003) and type II diabetes (Knekt, Kumpulainen et al. 2002, Jacques, Cassidy et al. 2013). *In vitro* and *in vivo* studies have been done to test the ability of apple phenols to inhibit lipid oxidation and lower cholesterol level (Boyer and Liu 2004). The cancer preventive potential of apples has been linked to its procyanidin concentration, which are important compounds in unclarified apple juice.

The cancer preventive potential of unclarified juices is greater than in clear juices, due to the procyanidins that gives the cloudy appearance to the juice (Zessner, Pan et al. 2008). The polyphenol content (hydroxyacinnamics, flanols, catechins, procyanidins, anthocyanins) in apples has been specifically implicated in the ability of these fruits and their products to inhibit progression of cancer (Gerhauser 2008). Presence of apple phenolics at low concentration may protect intestinal cells from reactive oxygen species induced and DNA damage due to its higher antioxidant effect (Bellion, Digles et al. 2010). Combined these studies suggest that apples, by virtue of their macro, micro and phenolic content may contribute to human health. Processes designed to optimize the stability and delivery of these bioactive compounds would contribute to human health outcomes.

1.3 Traditional Thermal Processing of Fruits and Vegetables

Even though the consumption of fresh fruits and vegetables has increased in the past few years (Buzby, Lin et al. 2008), the goal for fruits and vegetable consumption is not achieved (59% of vegetables and 42% of fruits), only a small amount of Americans consume the recommended amounts by the Dietary Guidelines (USDA 2010). While the current situation can lead to nutrient inadequacy (Bodner-Montville, Ahuja et al. 2006), the inability to deliver on the promise of disease prevention and health outcomes could be equally problematic. Processed foods have become an option to provide new or improved products to aid in achievement of the recommended fruit and vegetable servings.

Consumption of processed food products, including tomatoes, apples and derived products, contribute 4% of vitamin A and E, 29.1% of vitamin C and 1.8% vitamin D to the total dietary micronutrient intake of the U.S. population.

While processing level in the food should not be a determinate factor in the diet selection, instead, more attention should be paid to factors like nutrient composition, frequency and amount consumed in order to achieve a healthy diet (Eicher-Miller, Fulgoni et al. 2012). With this consideration, optimization of thermal processes to provide more nutrient and phytochemical dense products while providing fresh like characteristics could enhance consumer preference and nutritional contribution.

Thermal processing is the most widely used methods to maintain quality characteristics, generate new products and extend a product's shelf life of fruit and vegetable products. The main objective of thermal processing is the production of safe foods by inactivation of pathogenic microorganisms along with extension of shelf life through inactivation of spoilage organisms and natural enzymatic processes. Exposure of foods to high temperature and time is dependent of the target microorganism, at the same time trying to maintain the quality of the product. Thermal processes are designed by processing authorities with conditions to be achieved in the product through heat exposure in order to inactivate microorganisms that could potentially grow in the food under regular shelf conditions, in order to deliver commercially sterile and shelf stable products (Awuah, Ramaswamy et al. 2007). The severity of the thermal conditions depend on different variables: container size of the product, thermal resistance of target microorganism and physical-chemical characteristics of the food, which will be altered by the process (Fellows 2000).

Thermal processing regulations vary between low acid (pH≥4.6) and high acid (pH <4.6) foods, and are monitored by a processing authority. Thermal processing of low acid products target at least 12D of *Clostridium botulinum* inactivation to prevent production of botulinum toxins; the treatment should also target C. *sporogenes*, traditionally a 5D is used to avoid any other economic spoilage during handling/distribution and storage, and be considered commercial sterile (Ramaswamy, Abdelrahim et al. 1995, Ramaswamy, Awuah et al. 1997). High acid products (<4.6), on the other hand, are typically treated with less aggressive temperatures such as 100°C of 212°F, as require to achieve destruction of vegetative cells specific to individual foods, spoilage enzymes and other processes typically responsible for spoilage of fruits and vegetables (Bjornsdottir, Breidt et al. 2006, Breidt, Hayes et al. 2007, Breidt Jr, Kay et al. 2013).

The design of thermal processes also includes the understanding of the mechanism of heat transfer at each step of the operation. There are three heat transfer modes: conduction, convection and radiation. The uses of these modes in food industry depend of the type of food product and its matrix (Holdsworth and Simpson 2008). Convection and conduction are traditional heat transfer mechanism used in the food industry, but their inefficiency can lead to significant nutritional and sensorial changes (Chen, Peng et al. 1995, Howard, Braswell et al. 1996, Prochaska, Nguyen et al. 2000, Morris, Barnett et al. 2004, Seybold, Fröhlich et al. 2004). On the other hand, radiation is a different heating mechanism with heating systems including microwave and radio frequency.

The use of these systems in food processing have shown promise in delivery of better quality characteristics in the final product compared to conventional convection and conduction heating (Brewer and Begum 2003, Wang, Wig et al. 2003, Gerard and Roberts 2004).

1.3.1 Impact of Thermal Processing on Quality in Foods

In order to reduce microbial load a food product is exposed to a combination of high temperature and time. The heat exposure, while inactivating spoilage organisms, pathogens and spoilage enzymes will increase the molecular energy and accelerate most chemical reactions. This will lead to physical, chemical and biological changes, affecting appearance, taste and nutritional value in the final product (Nelson 2010).

One of the most common effect of thermal processing on the physical characteristics of a food product is non-enzymatic browning by Maillard reaction occurring from a reaction between proteins, free amino acids or amines with a reducing sugar to form Amadori products, which will lead to the formation of furfural compounds or hydroxymethylfurfural (HMF) resulting in brown pigments (Martins, Jongen et al. 2000). Browning may be desired in some products but can lead to reduction of nutritional value and changes in sensory characteristics in other products (Van Boekel 2006). Non enzymatic browning reactions are driven principally by temperature, following first order reaction kinetic whereby highest degradation occurs at initial stages of the heating process and decrease at 120°C leading to caramelization (Lan, Liu et al. 2010). Typically, high temperature and longtime combination will decrease the lightness (L*), blue-yellow (b*) values and increase in green-red (a*) values (Garza, Ibarz et al. 1999).

Browning reactions can also lead to changes in natural pigments such as anthocyanins, carotenoids, chlorophyll, anthocyanins, and betalains which are all heat sensitive to various extents. These pigments typically degrade during thermal processing by the first order kinetics reaction (Avila and Silva 1999, Ahmed and Shivhare 2001, Ahmed, Shivhare et al. 2004).

Rapid increase in temperature can also affect texture and rheological properties of the food products by lowering enzyme optimum activity and by inducing protein denaturation (Anthon, Diaz et al. 2008). Protein solubility will be affected by the pH and ionic strength, and at temperature ≥40°C most proteins will begin to precipitate (decrease of solubility) (Damodaran and Parkin 2008). High temperature exposure also results in plant cell wall damage (Greve, Shackel et al. 1994) which can result in changes in rheological properties of the food product through release of pectin materials. However, this can be balanced by insufficient inactivation of pectic enzymes during thermal processing which have been found to result in textural and viscosity changes on fruit purees during storage (Tanglertpaibul and Rao 1987, Ahmed, Shivhare et al. 2000, Ahmed and Shivhare 2001, Lagarrigue and Alvarez 2001).

Variation of thermosensitivity of nutritional compounds such as vitamins is common during thermal processing. The loss of vitamins depend on specific vitamin form, the processing temperature and time, the food matrix, presence of oxygen and other reactive compounds, pH, and moisture. Changes in vitamins content can begin prior to arriving at the processing plant due to environmental and storage conditions of the raw material and can be accelerated during processing (Selman 1994).

In vitamin fortified products thiamin mononitrate and folic acid seem to be the most sensitive to heat compared to tocopheryl acetate, riboflavin and nicotinamide (Ryley and Kajda 1994). Fat-soluble vitamins (A,D,E,K) are generally stable to thermal processing with only minimal losses. However these vitamins can be more sensitive to thermal degradation in presence of oxygen (Damodaran and Parkin 2008).

1.3.2 Impact of Traditional Thermal Processing on Phytochemical in Foods

Impact of Processing on Carotenoid Stability.- Heat treatment on carotenoids is well known to induce *cis/trans* isomerization impacting stability, bioavailability and ultimately, in the case of provitamin carotenoids, affecting provitamin A activity. Typically conversion from the trans isomer of b-carotene to the cis form has been associated with a decrease from 13 to 53% provitamin A activity with only minimal effect in the food color (Damodaran and Parkin 2008). Significant differences have been found in color between fresh and processed tomato products, at the same time presenting different ranges of lycopene content between fresh 125.4 ppm and processed 102.3 ppm (Agarwal, Shen et al. 2001), where color changes have been linked to carotenoid degradation (Chen, Peng et al. 1995, Arias, Lee et al. 2000).

Even though carotenoids are generally heat stable, degradation can be observed due, in part, to oxidation during processing and storage. A combination of intense heat treatment and long time, has shown changes in color as well as decrease in provitamin A activity of carotenoid rich fruits and vegetables. Carrot juice heated at 121°C for 30 minutes showed the highest degradation of carotenoids, with color changing from orange to yellow from high β-carotene isomerization (Chen, Peng et al. 1995).

Similarly, in tomatoes exposed to extensive thermal treatment, β-carotene was reported to be unstable (Abushita, Daood et al. 2000). However, lycopene in processed tomatoes (canned, paste and juice) was found to be generally stable with only minimal isomerization or oxidative loss (Nguyen and Schwartz 1998, Nguyen, Francis et al. 2001).

Exposure to high temperature for short time (121°C, 40sec) processes resulted in highest yield of all-*trans* and *cis* form of lutein and lycopene, β-carotene presenting minor changes during the traditional tomato processing (Lin and Chen 2005). Interestingly, thermal sensitivity appears to be fruit specific as pasteurization (90°C/5min) of peaches resulted in a reduction in xanthophylls zeaxanthin and β-cryptoxanthin, and a decrease of 65% of β-carotene, whereas, levels of lutein were not affected by the heat treatment (Leontowicz, Gorinstein et al. 2002).

The use of blanching or milder heat treatments in raw fruit and vegetable products has actually resulted in an increase in carotenoid extraction efficiency and inactivation of degradative enzymes including lipoxygenase. Thermal processes using hot air can cause extensive carotenoid degradation during processing and storage due to compound exposure to oxygen, especially in large surface area products such as carrots or sweet potato flakes (Chen, Peng et al. 1995).

Carotenoids are relatively stable during other associated processes. Freezing of blanched and fresh materials causes little changes in carotenoid content. However, storage of the final product can also affect the total carotenoid content. Peaches after 3 months storage at room temperature show a ~68% reduction, with a 90% and 83% reduction of β-carotene and lutein, respectively (Oliveira, Pintado et al. 2012).

In addition to time, light exposure of the product during storage enhances degradation of all-*trans*-lutein and isomerization to 9-*cis*-lutein; similar effects occur in lycopene generating 13-*cis* and 15-*cis*-lycopene. Storage temperature enhances formation of specific carotenoid isomers including 15-*cis*-lycopene (4°C), 9-*cis* and 13-*cis*lycopene (25°C) and 5-*cis* and 13-*cis*-lycopene (35°C) (Lin and Chen 2005).

Impact of Processing on Carotenoid bioavailability.-The extent to which carotenoids are released during digestion of an ingested food and made available for absorption is defined as bioaccessibility (Failla, Huo et al. 2008, Fernández-García, Carvajal-Lérida et al. 2009). Bioavailability can be defined the portion of a nutrient or phytochemical that is absorbed from a food through normal digestion and made available at the site of action in the body (Neilson, Ferruzzi et al. 2013). Nutrient/phytochemical bioavailability includes multiple steps including digestive release, intestinal absorption, metabolism, tissue distribution and bioactivity of the nutrient/phytochemical (Lemmens, Van Buggenhout et al. 2010). Several factors influence the utilization and absorption of carotenoids in the human body, for example the amount consumed, type and carotenoid form in the diet, fat intake, and fiber content as well as presence of diseases or parasite infections. For these reasons, bioavailability of carotenoids can vary between foods and can be difficult to evaluate systematically (Rodriguez-Amaya and Pablo 1999).

Bioaccessibility is often used as a surrogate measure for bioavailability (Reboul, Richelle et al. 2006). In order for carotenoids to be bioavailable, they have to be released from the bulk food matrix or from disrupted plant cells walls and organelles, and subject to solubilization in bile salt mixed micelles.

The digestive release and solubilization is defined as bioaccessibility, which is completed by mechanical action, usually human mastication (oral phase) and tested by *in vitro* methods simulating human digestion process. Bioaccessibility, assessed by *in vitro* digestive methods has been extensively used to assess the impact of processing and formulation on carotenoid bioavailability from fruits and vegetables (Serrano, Goni et al. 2005, Goñi, Serrano et al. 2006, Granado-Lorencio, Olmedilla-Alonso et al. 2007).

The most common models utilize a two stage (gastric/small intestinal digestion) or three stage (oral/gastric and small intestinal digestion) (Garrett, Failla et al. 1999, Hedren, Diaz et al. 2002, Granado-Lorencio, Olmedilla-Alonso et al. 2007, Jeffery, Turner et al. 2012). These models have demonstrated the ability to positively predict carotenoid absorption in humans (Reboul et al.)

As applied to the question of processing impact on carotenoid bioaccessibility, *in vitro* methods have illustrated *all-E*-β-carotene bioaccessibility decrease when particle size of carrots is increased (Lemmens, Van Buggenhout et al. 2010). Also, increase in carotenoid bioaccessibility has been observed to increase with the increase of cooking time (Hedren, Diaz et al. 2002). Thermal processing aids in the mechanical breakdown of the food matrix resulting in an increased amount of carotenoids to be released during the digestion; however, thermal degradation in carotenoids may occur during processing (Kopsell and Kopsell 2006). Mild heating processes affect the cell and organelle membrane, enhancing the interaction between the enzymes and β-carotene. When the product has been thermally treated, bioaccessibility will typically be higher compared to the raw product (Greve, Shackel et al. 1994, Rich, Bailey et al. 2003, Lemmens, Van Buggenhout et al. 2010).

Thermal processing of tomatoes increased lycopene bioaccessibility by releasing the carotenoid from the matrix. Studies have shown that tomatoes treated at 88°C for 2, 15 and 30 min, increase the contents of *trans*-lycopene at to 54.39%, 171.11% and 164.26% respectively, as along with an increase in *cis*-lycopene of 5.81%, 17.44% and 34.88% respectively (Dewanto, Wu et al. 2002).

Increase of lycopene *in vitro* bioaccessibility has been observed with increasing blanching temperature, whereas an additional thermal treatment does not improve its bioaccessibility (Svelander, Tibäck et al. 2010).

Generally, in vitro methods have consistently demonstrated the ability of thermal processing to enhance carotenoid bioaccessibility. These results are consistent with human studies that have highlighted the ability of thermal processing to increase the bioavailability of carotenoids from carrots (Hedren, Diaz et al. 2002, Lemmens, Van Buggenhout et al. 2010, Tydeman, Parker et al. 2010), spinach (O'Sullivan, Ryan et al. 2008) and tomatoes (Colle, Lemmens et al. 2010, Svelander, Tibäck et al. 2010, Page, Van Stratum et al. 2012) compared to unprocessed or raw controls.

Polyphenols. – By comparison to carotenoids, much less is known in regards to the impact of thermal processing on phenolic levels in fruits and vegetables. Phenolic oxidation, polymerization and overall decrease has been observed from thermal processing. In peaches thermal treatment at 220°F for 10 min and 230°F for 2.4 min resulted in a loss of 21% and 11% of total phenolics. However, treatment at a slightly lower temperature of 213°F for 40 min produced no significant loss of total phenolics. Changes in phenolic levels is also dependent on the storage conditions of the final product.

Fruit treated at 220°F (10min) and 230°F (2.4min) showed a 30-43% reduction in total phenolic content during the first 3 months of storage, whereas the product processed at 213°F (40min) did not present a significant change in total phenolic content between 3-6 months of storage at room temperature (Asami, Hong et al. 2003). These results suggest that mild thermal treatments may enhance phenolic recovery and stability compared to more severe thermal treatments. In carrots, thermal process has resulted in color degradation and loss of sugar and soluble phenolics. Strained carrots in batch tanks heated at 93.4°C showed a decrease of total soluble phenolics compared to those held at 87.9°C and 82.3°C. Lightness and chroma values are reduced and higher total soluble phenolics are shown when carrots reside in batch and holding tanks at 124°C compared to 118.3°C and 121.2°C (Howard, Braswell et al. 1996). Decrease in total phenolics has been reported for common beans and soybeans upon boiling and steam treatment. Observed losses were dependent on beans color. Pinto and black beans lost about 60-70% total phenolics, whereas black soybeans lost about 40-60% of total phenolics compared to raw products. Yellow soybeans lost about 10-30% of initial phenolic content during boiling, whereas, regular steaming treatments increased total phenolic content about 35% due, most likely, to depolymerization of the phenolic compounds under thermal conditions (Xu and Chang 2011).

Changes in anthocyanins from strawberry and blackberry have been reported after thermal process (70°C/2min) as decreases in a* value (redness) due to decrease in anthocyanin content in both commodities compared to unprocessed samples, reduction of pelargonidin-3-glucoside and cyaniding-3-glucoside of 27.9% and ~3%, respectively, this same. thermal process also resulted in a 21% ascorbic acid degradation in both purees (Temu and Temu 2005). During jam production decrease in total phenolics has been observed. Major losses were observed in anthocyanins content 89% to 21%, along with minor losses of other flavonoids, quercetin and kaempferol were observed during thermal treatments with sugar in strawberry jam making. However, overall, processing of berries did not present significant changes of other individual phenolics (Kim and Padilla-Zakour 2004).

Thermal process causes breakdown of antioxidant compounds, decreasing the antioxidant activity. This has been observed in processed beans (Xu and Chang 2011), but not other products. For example, fresh broccoli exposed to steam treatment increased the total phenolic content by 6.39% and 18.19% resulting in an increase of antioxidant capacity of 94.7% and 146.7% when steam processed for 5 and 10 min respectively (Roy, Juneja et al. 2009). Thermal treatment of tomatoes at 88°C for 2, 15 and 30 min increase the antioxidant activity in 27.93%, 33.88% and 62.09% respectively compared to the raw product (Dewanto, Wu et al. 2002). However, in peaches, pasteurization at 90°C for 5 min resulted in a significant reduction of antioxidant activity after storage for 90 days, as well as a decrease of ~51% total phenolic content including a reduction of epicatechin and quercetin-3-glucoside of ~70% and ~40%, respectively.

On other hand an increase of ~31% procyanidins, 35% chlorogenic acid and 43% neochlorogenic acid has been reported (Oliveira, Pintado et al. 2012). Overall the impact of processing on phenolic composition of fruits and vegetables remains unclear as the balance of release from bound forms with degradative processes has made systematic assessment difficult Additional insight into the impact of thermal processing on phenolic stability in fruits is needed in order to better optimize processing strategies for recovery of these bioactive compounds. Furthermore, stability of phenolics through storage of processed products in generally lacking. Additional insight is needed in order to provide consumers with superior products with optimal levels of phenolic compounds.

Impact of Processing on Polyphenols bioavailability. In order to be absorbed phenolics and polyphenols should be released from the food matrix during digestion and remain stable and soluble in the intestinal tract. The phenolic compounds should be stable to saliva, gastric juice and intestinal secretions and soluble in the gastrointestinal milieu in order to facilitate diffusion into the enterocyte surface. Polyphenols must then pass the enterocytes in order to access the bloodstream (Neilson, Ferruzzi et al. 2013).

Food processing (heating and freezing) is believed to aid in digestive extraction of phenolics by virtue of its impact to the fruit and vegetable texture and cellular structure. Release of anthocyanins following thermal processing of select fruits has been observed resulting in higher concentration after processing compared to fresh product (Leong and Oey 2012). In the assessment of bioaccessibility *in vitro* models have been employed to assess phenolic bioaccessibility.

Many of these studies have demonstrated that phenolics and flavonoids are mainly released when reaching the gastric phase (65%) with a further <10% release during the intestinal digestion (Saura-Calixto, Serrano et al. 2007, Bouayed, Hoffmann et al. 2011). The total polyphenol absorption in the small intestine is poor (0.3-43%) with the total amount ingested depending of the compound, meaning that the majority of the polyphenols pass thru the small intestine directly into the colon without being absorbed. The wide absorption rate present by the polyphenols demonstrates the great variability in their bioavailability (Manach, Williamson et al. 2005).

Although the absorption of these compounds is poor, their bioavailability increases with increasing intake (Silberberg, Morand et al. 2006). Several studies have showed losses of flavonoids and reduction of total antioxidant activity as result of conventional thermal processing in apple juice (Mihalev, Schieber et al. 2004, Kahle, Kraus et al. 2005, Oszmianski, Wolniak et al. 2007, Candrawinata, Golding et al. 2013), although applesauce has been observed to retain the most total phenols (>60%) and antioxidant capacity (>40%) after thermal processing compared to juice and cider (Spanos and Wrolstad 1992, Bonsi and Padilla-Zakour 2005). Improvement in the processing methods have been encouraged to minimize phenolic loses during thermal treatments (Spanos and Wrolstad 1992, Bengoechea, Sancho et al. 1997, Le Bourvellec, Bouzerzour et al. 2011).

1.3.3 Novel Heating Technologies in Food Industry and its Impact in Foods

The use of novel technologies have been proposed for food processing, such as high intensity electric field pulse treatment, high hydrostatic pressure and microwave heating. Attention to these technologies has increased as a result of their proposed advantages such as application of low temperatures that lead to less quality changes in the food product as well as reducing energy waste in the processing plant (Knorr 1999). For example high intensity electric field pulses technology has been studied for microbial, protein and enzyme inactivation, to prevent oxidative reactions, off flavors and color changes in the food product (Qin, Pothakamury et al. 1996, Jeyamkondan, Jayas et al. 1999, Heinz, Alvarez et al. 2001, Toepfl, Heinz et al. 2007).

In conventional thermal processing a product is heated by energy transfer from the heating medium to the food product by convection, conduction and radiation; this energy exchange is driven by thermal gradients which may result in an increased thermal processing time and losses of nutritional properties of the product. In contrast, microwave processing products heat through molecular interactions with the electromagnetic field. The direct interaction of incident radiation with the molecules of the food and the ability of the microwaves to penetrate into food materials results in volumetric heating of the food (Maskan 2000). This energy transfer has the potential to reduce of processing time and enhance of quality of finished products (Venkatesh and Raghavan 2004). In order to determine if the microwave treatment can be applied as an alternative, value added, processing method, a more complete understanding of the impact to a food product should be made.

Some of the effects that may occur during microwave heating are: changes in the ability of certain compounds to dissolve in the product, texture generation due to gas expansion, starch gelatinization, protein denaturation, steam generation from water, non-enzymatic browning reactions, changes in viscosity and textural properties, enzyme inactivation due to protein denaturation by temperature increase (Brewer and Begum 2003).

The rate at which a food product will heat during microwave processing depends on the dielectric properties of the product, which should be considered when stablishing processing conditions. However, during microwave processing the time required to reach the treatment temperatures will be reduced compared to conventional thermal methods and in most of the cases, the quality of the final product processed by microwave will be improved (Zhang, Tang et al. 2006). Increase in total phenolic and flavonoid content, soluble solids and turbidity in apple juice and cider has been shown when the raw material has been pre-heated using microwaves before mash, these effects increase as the temperature used increase (Gerard and Roberts 2004).

Microwave blanching was reported to be effective for the inactivation of polyphenol oxidase (PPO) from kiwi fruit and strawberry purees, with a range of inactivation from 32% to 70%. Microwave power is directly related to the increased PPO inactivation. In strawberry puree treated at 475W/45sec anthocyanin concentration was not affected , whereas, in kiwi puree microwave process promoted chlorophyll degradation, when treated at 475W for 60s a decrease of 72% of xanthophyll, 84% of chlorophyll a and 75% of β-carotene was shown.

Total color difference in fruit puree increases with increasing microwave power, except in papaya which only present slight decrease of a* and increase of b* value (de Ancos, Cano et al. 1999). Similar reaction in color properties of celery leaves when undergoing microwave heating have been shown, decrease of L*, b*, chroma and hue angle is observed with the increase of microwave power, whereas a* and total color differential increase (Demırhan and Özbek 2011). Application of microwave power to orange peel and leaves have been found to result in color changes, color value preservation and are inversely proportional to power applied and directly related to enhancement of phenolic compounds extractability from the peel (Bejar 2011).

1.4 Industrial Microwave Processing

Microwave heating is the result of polarization effect at frequencies between 300 MHz and 300 GHz (wavelengths between 1m and 1mm) occurring into a dielectric material (Decareau 1985). The Federal Communications Commission (FCC) assigned the frequencies for industrial, scientific and medical: *radio*: 13.56MHz±6.68kHz, 27.12MHz±160kHz, 40.68MHz±20kHz, *microwaves:* 915MHz±13MHz, 2450MHz±50MHZ, 5800MHz±75MHz, 24125MHz±125MHz. In the industrial food processing the microwave frequencies used are 2450 (household ovens) and 915 MHZ (industrial systems) (FDA 2012).

Microwaves are non-ionising radiations due to its location between low (radio) and high (infrared and visible light) frequencies in the electromagnetic spectrum, with its band of 300MHz to 300 GHz.

The three main components of a microwave system are: microwave source, waveguide and the actual applicator (Regier and Schubert 2005), whereas circulator, tuner and directional coupler are additional components of the microwave system (Thostenson and Chou 1999):

Microwave source: most common microwave source are vacuum tube: magnetrons, traveling wave tubes (TWTs) and klystrons. Magnetron is the lowest cost source of microwave available and is mostly used for industrial and domestic applications. Its consist of a "vacuum tube with a central electron-emitting cathode of highly negative potential which is surrounded by a structured anode" (Regier and Schubert 2005).

Cavities (microwave resonant frequency) formed by the anode are surrounded by the surface of the cathode, which has lower potential compared to the anode, this potential differential is used to remove electrons from the cathode creating heat (Regier and Schubert 2005). An external magnet creates a magnetic field orthogonal to the electric field which will result in a circumferential force on the electron and acceleration towards the anode, causing the electron to travel in spiral direction under high electrical voltage (4000-6000 volts) through the resonant cavities where oscillations are set up in the electron cloud. With the contact of the electron with the anode heat is dispersed by the kinetic energy transformation into heat. Higher temperatures in the anode should be avoided by using cool air or liquid in order to avoid overheating. Control of the power output from the magnetron tubes can be achieved by adjustments in the operation period, cathode or magnetic field (Thostenson and Chou 1999).

Waveguide: is the connection between the microwave sources (usually magnetron) with transmission lines to the single or multi-mode applicators.

"Waveguides are hollow conductors of normally constant cross-section, rectangular and circular forms being of most practical use" (Regier and Schubert 2005). The size, appropriate boundary conditions (for no surface charge, no surface content, ideally conduction wall: metallic) and solution of wave equation (1) can be used to define its minimum frequency f_c below which waves do not propagate. The wave equation utilize the material equation for the electric field $\vec{D} = \epsilon_0 \epsilon \cdot \vec{E}$, the Maxwell's equation $\nabla \cdot \vec{D} = \rho$ and the vector identity $\nabla \times \left(\nabla \times \vec{X}\right) = \nabla\left(\nabla \cdot \vec{X}\right) - \Delta\vec{X}$:

$$\Delta\vec{E} - \mu_0 \mu \epsilon_0 \epsilon \frac{\partial^2 \vec{E}}{\partial t^2} = 0 \qquad (1)$$

In the case of rectangular waveguides, a derivation of the minimum frequency (f_c) equation can be used (2), in which a is the width and b is the height, m is a constant, n is the refractive index (constant) and f is the frequency (constant):

$$f \geq \frac{\sqrt{\left(\frac{m^2}{a}\right)+\left(\frac{n^2}{b}\right)}}{2\sqrt{\mu\mu_0\epsilon\epsilon_0}} \min_{=} \left\{ \begin{matrix} \frac{1}{2a\sqrt{\mu\mu_0\epsilon\epsilon_0}}, a \geq b \\ \frac{1}{2b\sqrt{\mu\mu_0\epsilon\epsilon_0}}, a \leq b \end{matrix} \right\} = f_c \qquad (2)$$

The electromagnetic field distribution into the waveguide is defined by the so-called modes, which can be split into transversal electric (TE) and transversal magnetic (TM), and describe the direction and magnetic field (Erle, Regier et al. 2000). The propagation direction will determine the mode, if magnetic intensity is zero the mode is transversal magnetic and if the electric intensity is zero the mode is transversal electric. The TE_{10} mode is commonly used, the name denomination indicates the direction of propagation (TE or TM) and the limit number of each field in a waveguide (Thostenson and Chou 1999).

Microwave applicators: are used to transfer the microwave energy to the materials, common type of applicators are: single-mode, multi-mode and near-field applicators. The type used depend of the material to be processed and can include waveguides, single and multiple mode cavities, as well as traveling wave applicators. In near field applicators the microwaves are transmitted and completely absorbed directly by the product to be heated, usually by conversion of dielectric loads, these applicators work best with materials with high losses. Single mode applicators are used for materials will low dielectric losses, consist of a feeding waveguide and a microwave resonator (range of wavelength). The electric field (wave yields) is used to heat the product. Multi-mode applicators are made by the changing (increasing) in dimensions of the cavity in single mode applicators, consequently the increase in possible resonant mode (Erle, Regier et al. 2000).

The most commonly used applicators are the multi-mode type because of its easy construction, cost, easy connection with multiple microwave inlet ports, and large dimensions. The biggest disadvantage with the multi-mode applicators is the need of insulation and the lack of information based on the Maxwell's equation describing the electromagnetic field. In contrast with the multiple mode, the single mode applicators are easier to install and can provide more homogeneous electromagnetic field at specific zones, but due to its high field density it is a more expensive alternative (Leonelli and Mason 2010).

Circulator: acts as a microwave equivalent to a diode in an electrical circuit, protecting the microwave source from the excess power that is reflected back allowing only one direction microwave pass, the three part circulator is connected to the microwave source, applicator and dummy load (Thostenson and Chou 1999).

Directional coupler: designed to separate the small amount of forward and reflected waves to be measured by power meters (Thostenson and Chou 1999).

Tuners: used to adjust the impedance and load of the microwave source, in order to maximize the power absorption by the load (Thostenson and Chou 1999).

1.4.1 Dielectric Properties of Foods

In order to determine a microwave processes one should consider the dielectric properties of the food, food matrix and temperature (Venkatesh and Raghavan 2004).

Dielectric properties determine how the energy from microwaves exposure is either reflected, transmitted or/and absorbed in the surface of the material. Such interactions can be described by the relative permittivity of the material ε^*, and its expressed as:

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad (3)$$

Where ε' is the dielectric constant, ε'' is the dielectric loss factor and j is the complex constant (j=$\sqrt{-1}$) (Venkatesh and Raghavan 2004).

The dielectric constant (permittivity) refers to the interaction between the electric field and the conducting matter. In the processing of food products there is polarization and losses into heat which originate from ion conductivity and dipole-orientation (Erle, Regier et al. 2000).

Microwave frequency, temperature, moisture and salt content, and physical state are some of the factors that affect the dielectric properties of the food. Research shows that the amount of solids in the food matrix do not represent a big contribution to the dielectric properties of the dried food products (solids, fats and oils) regardless the frequency and temperature used (Hasted, Ritson et al. 1948), but instead its chemical composition (water and salts concentration) present strong interaction with microwaves, the interactions with components after than ion and dipoles are weak with small permittivity (Erle, Regier et al. 2000).

Microwave heating results from the interaction of the chemical components of the food matrix with the electromagnetic field, due to the disruption of the hydrogen bonds (dipole rotation of free water molecules) and the electrophoretic migration of free salts in the rapid polarity changing environment (Decareau 1985). The combination of the dielectric properties of the material and the electromagnetic field results in the conversion of the electromagnetic energy to heat (Thostenson and Chou 1999).

1.4.2 Application of Microwave Processing to Fruits and Vegetables

Even though several studies have focused on the effect of microwave heating system in raw fruits and vegetables based products (de Ancos, Cano et al. 1999, Maskan 2000, Brewer and Begum 2003, Gerard and Roberts 2004, Regier and Schubert 2005, Vadivambal and Jayas 2007), little literature is found on the effect of this technology in physical and phytochemical characteristics of tomato and apple based food products.

Peng, Tang et al. (2013) observed that dielectric properties of tomatoes are different within the tissue and salt addition in commercial tomato canned product, and are not affected by them. On the other hand, Heredia, Peinado et al. (2010) observed that a combination of osmotic pre-treatment and microwave heating limited the trans-cis-isomerization with increasing microwave power. Microwave heating system had been studied in the processing of apple based products. Gerard and Roberts (2004) observed an improvement in juice yields and phytochemical extraction with the increase of microwave power. Picouet, Landl et al. (2009) observed minimal effects of microwave heating system on total polyphenol during processing and storage of minimally processed granny smith apple puree, whereas, changes in product viscosity and oxidation during storage were observed as result of inefficacy of the treatment to inactivate the enzymes.

1.5 Objectives

Compared to traditional thermal process, microwave heating system has been observed to better maintain quality characteristics of food product (de Ancos, Cano et al. 1999, Steed, Truong et al. 2008, Demırhan and Özbek 2011) as the high temperature exposure in traditional thermal processing had been found to result in serious damage to flavor, color and nutrient content in thermally processed products (Lin, D Durance et al. 1998, Drouzas, Tsami et al. 1999). Based on results reported by several authors on the effects of microwave heating system in food products and need to identify processing methods that can deliver safe products with improved physical and nutritional profiles we set out to assess the impact of traditional (tubular and scraped surface heating) and microwave processing on the quality of processed fruit products.

Specifically, we will provide information on the profile of phytochemicals from fresh apples and tomatoes and their corresponding commercial products. Then we will assess the impact of a continuous microwave heating system on product quality attributes (color, viscosity) as well as phytochemical stability and bioaccessibility.

CHAPTER 2. MATERIALS AND METHODS

2.1 Applesauce Production

Processing trials were performed at the Purdue University Pilot Plant over a two week period. Applesauce with ascorbic acid, to be used as a base for the formulated product, was pasteurized by microwaves or tubular heat exchanger (trial 1) and packaged aseptically. One week later, the applesauce from previous runs was mixed with 4% sucrose and 0.025% ascorbic acid and pasteurized by microwave or double tube heat exchanger (trial 2). All apple products obtained from these trials were aseptically processed. The processing line used for both heating methods was exactly the same for applesauce manufacture, holding tank, product pump, holding tube, cooling system and aseptic filler. The only difference was the heating system used for each trial as described in the following section. The tests were designed to simulate current production protocol by an industrial processor with some modifications.

Trial 1: One ton (1000 kg) of Golden Delicious apples was processed each day. Apples were rinsed in a water bath, diced, crushed with a hammer mill (1in screen) and passed through a finisher (Langsenkamp Mod. 57, 0.045in/1.14mm screen). Ascorbic acid solution was diluted in water at a rate 25:100 and sprayed on the apples along various stages.

Applesauce from the finisher was poured into a 125 gallon steam jacketed mixing tank and heated to 37.8ºC (100ºF) for deareation prior to thermal process. Processed product was aseptically packaged into 5 gallon laminated bags (Scholle Packaging, Northlake, IL)

Trial 2: Approximately 420 kg of apple puree from trial 1 were mixed with sucrose (4%) and ascorbic acid (0.025%) into a 125 gallon steam jacketed mixing tank. The product was mixed for approximately 30 minutes and heated to 37.8ºC (100ºF) for deareation prior to thermal process (Figure 2.1).

2.1.1 Applesauce Thermal Processing

Microwave Processing (MW): products processed in trial 1 were heated by a 100 kW cylindrical microwave (915MHZ) heating system (IMS, Raleigh, NC). This system has two applicators. Each applicator comprises a 30 cm long ceramic tube (2.5in diameter) transparent to microwave radiation. Thus, heating only occurs along these two applicators. One static mixer is located between both applicators, and another is used before entering the holding tube to minimize temperature differences within the product. Flow rate was set at 2.0 gal/min (7.6 L/min). An average holding time of 60 seconds were used and the target temperature at the end of the holding tube was set at 93ºC (Trial 1) and 96ºC (Trial 2).

Double tube heat exchanger (TUB): a coiled double tube heat exchanger (Stork) heated by steam was used on tests conducted on trial 2. Flow rate was set at 2.0 gal/min (7.6 L/min). The target temperature at the end of the holding tube was set at 93ºC (trial 1) and 96ºC (trial 2). An average holding time of 60 seconds was used.

The holding tube was insulated and the temperature monitored at the center of the tube at the outlet of the holding tube.

Aseptic packaging: processed product was aseptically packaged, using a Scholle TrueFill® 900 filler (Foothill Ranch, CA) in laminated bags (5 gallon bags for trial 1 and 1 gallon bags for trial 2). Headspace was flushed with nitrogen during packaging. The processed applesauce product was stored at three different temperatures (4°C, 50°C and room temperature ~22°C) to conduct the storage study. Analyses were performed using three bags (1 gallon each) of applesauce from each storage temperature each week.

A storage study: for the formulated product obtained from trial 2 was designed for 52 weeks. Samples were stored at 4^{o}C, room temperature (~22^{o}C) and 50^{o}C. Samples were retrieved at different time points and evaluated for color, Bostwick consistency, rheological properties and phytochemical content.

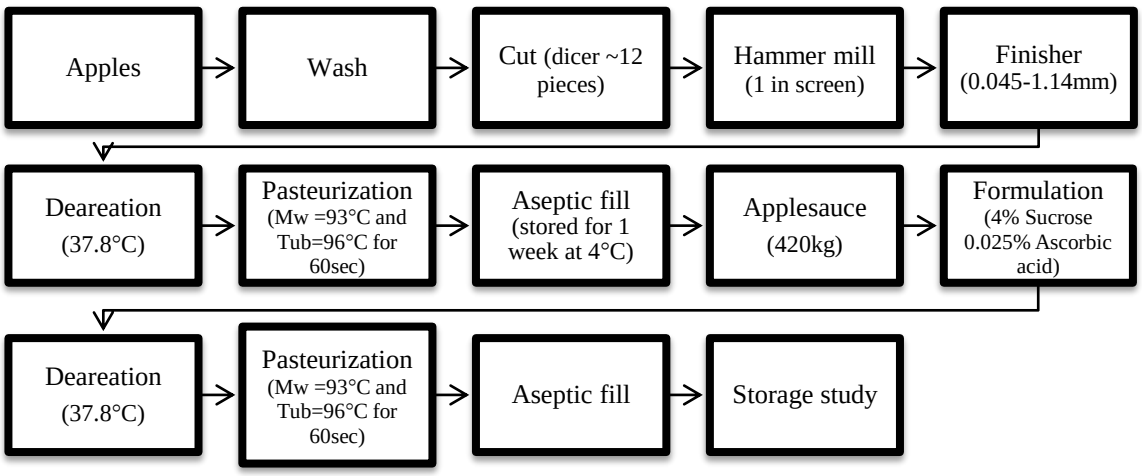

Figure 2.1. Applesauce work flow for processing by microwave and tubular heating system.

2.2 Tomato Puree Making

Processing trials were performed at the Purdue University Pilot Plant, over two days period, on day one samples were processed by microwave/microwave and in day 2 samples were processed by scraped surface/tubular, tomatoes were received, washed and cut in pieces before hot break for enzyme inactivation by either microwave or scraped surface heat exchanger and passed through a finisher, evaporated and formulated with salt prior pasteurization by either microwave or tubular heat exchanger at 121°C during 4 seconds and packaged by hot filled at 85°C into 250mL plastic bottles. The processing methods and temperatures were designed to simulate current production protocol by an industrial processor with some modifications.

2.2.1 Tomato Trial #1

A total of 280kg of Heinz tomatoes (140kg per treatment) were thermally processed and formulated with 0.3% salt, sieved to remove tomato juice and passed through a finisher concentrated in a vacuum kettle at 72°C to 7°Brix hot filled in 250 mL plastic bottles. The product was stored at three different temperatures (4°C, 50°C and room temperature ~22°C) on day 1 for microwave samples and day 2 for scraped surface for enzyme inactivation and tubular for processing. Analyses were performed as a triplicate of the composite of two bottles of product from each storage temperature each week. A storage study for the formulated product was conducted over 6 months. Samples were stored at 4^{o}C, room temperature (~22^{o}C) and 50^{o}C.

Samples at 50°C were retrieved at 1, 2, 4, weeks; whereas samples from 4°C and room temperature were retrieved at 4, 12 and 24 weeks and evaluated for color, Bostwick consistency, rheological properties and carotenoid content (Figure 2.2).

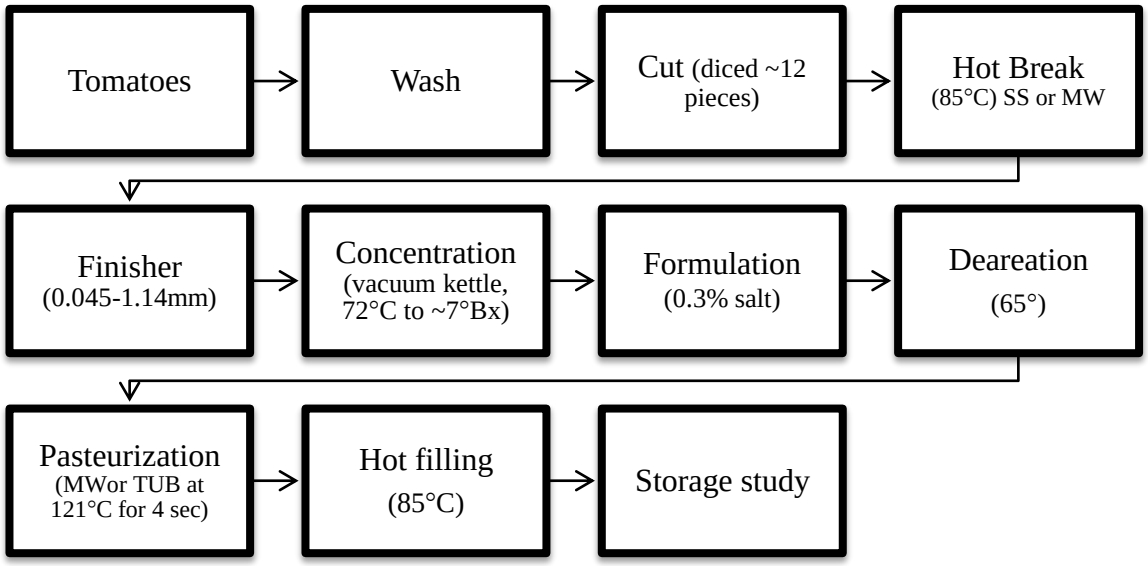

Figure 2.2 Tomato puree work flow for processing by microwave and scraped surface/tubular heating system.

2.2.1.1 Thermal Treatment of Product for Trial 1

Microwave Processing (MW): tomatoes were heated by a 6 kW microwave unit 2450MHZ (Industrial Microwave Systems, Morrisville, N.C.,U.S.A), the unit includes a high voltage power supply 6kw, magnetron, rectangular waveguide and ceramic applicator. No holding time was used and the target temperature at the end of the unit was set at 85ºC during hot break, for pasteurization a target temperature at the end was 121°C and holding time of 4seconds.

Double tube heat exchanger (TUB): a coiled double tube heat exchanger (Stork) heated by steam was used. Flow rate was set at 2.0 gal/min (7.6 L/min). The target temperature at the end of the holding tube was set at 85^{o}C (hot-break with scraped surface heat exchanger) and 121^{o}C (pasteurization).

An average holding time of 4 seconds was used. The holding tube was insulated and the temperature monitored at the center of the tube at the holding tube outlet.

2.2.2 Tomato Trial #2

A total of 200kg of Heinz tomato were processed in a one day period and thermally treated by microwave and scraped surface heating system. Tomatoes were rinsed in a water bath, diced and mixed with reconstituted tomato juice (~20% for microwave and ~30% scraped surface of the total fresh tomato weight), to aid in pumping through the hot-break (microwave or scraped surface) for enzyme inactivation. The product was then passed through a finisher (Langsenkamp Mod. 57, 0.045in/1.14mm screen), packaged and frozen in 50mL polypropylene tubes for determination of carotenoid profile and bioaccessibility.

2.2.2.1 Thermal Processing of Tomato Puree

Microwave Processing (MW): during this trial only hot-break process was performed, the tomato product was heated by a 100 kW cylindrical microwave (915MHZ) heating system (IMS, Raleigh, NC). This system has two applicators.

Each applicator comprises a 30 cm long ceramic tube (2.5in diameter) transparent to microwave radiation. Flow rate was set at 2.0 gal/min (7.6 L/min). No holding time was applied and a temperature of 85°C was targeted.

Double tube heat exchanger (TUB): a coiled double tube heat exchanger (Stork) heated by steam was used on tests conducted on tomato trial 2.

Flow rate was set at 2.0 gal/min (7.6 L/min) for hot-break process. The target temperature at the end of the holding tube was set at 85^{o}C. No holding time was used.

Samples for analysis were taken from the starting material (diced tomato), product after hot-break and product after finisher, each step was sampled at 3 time points (beginning, middle and end); samples were flushed under nitrogen and stored at -20°C until carotenoid analysis.

2.3 Physical Analyses

Color measurement: color of microwave and tubular heated products was measured using a LabScan XE spectrophotometer (HunterLab, Reston, VA) with 0^{o}/45^{o} optical geometry, illuminant D65 and 10^{o} observer. CIELAB color parameters (L*, a*,b*) were measured. The instrument was calibrated against standard black and white tiles reference (L=94.02, a=-1.01, b=0.63).

The *L** value indicates the lightness (100=white and 0=black), the *a** value represents (greenness (-) and redness (+)) and the *b** (blueness (-) and yellowness (+)). Chroma (C*) and hue angle (h) were calculated, total color difference (ΔE value) was calculated.

Total color differences were made in comparison to values from the formulated applesauce processed on Trial 2 (microwave and tubular respectively) at time 0. Tomato puree processed in Trial 1 (microwave and scraped surface respectively) was named as Time 0.

$$\Delta E = \sqrt{(L_1^* - L_0^*)^2 + (a_1^* - a_0^*)^2 + (b_1^* - b_0^*)^2}$$

Bostwick consistency: the consistency of applesauce and tomato puree was evaluated by using a Bostwick consistometer. Product was poured into the chamber up to the top of the product gate.

The gate was released and product allowed to flow for 30 seconds. The values reported are the distance in cm traveled by the product in the consistometer at 25°C.

Rheological Properties: flow properties of apple purees were measured using a controlled stress DHR-3 rheometer (TA Instruments, New Castle, DE), controlled by the commercial computer software TA Instruments Trios (Version 2.3.3.1485). A controlled stress mode was used with a concentric cylinder geometry with a cup diameter of 30.32mm, a bob diameter of 27.99 mm and a bob length of 42mm. A three steps procedure was used:

1) Sample conditioning. – Samples were pre-sheared at $100s^{-1}$ for 2 minutes followed by 1 minute equilibration of the sample at 25°C.

2) Flow Sweep. - Steady state flow curves were collected at 25°C in log mode. Ten points per decade were recorded within a shear rate range of 0.1 to 1000/s (0.01 to 1000/s in samples from tomato trial #2). A 5% tolerance level between consecutive measurements and a maximum time of 60 seconds were set as equilibration criterion.

3) Conditioning-End of test. - After finishing the data collection, a temperature equilibration was set at 25°C for the end of the test. One flow curve was obtained for each applesauce bag (3 bags from each storage temperature at each time point) for a total of n=3 for each sample. A pooled sample from two bottles of tomato puree was measured and three flow curves were obtained from each mixture corresponding to each storage time.

Moisture content: was determined by spreading a thin layer of sample across 90% of a fiberglass pad and placing it into a microwave system (CEM Smart System ^{5TM} Moisture/Solids Analyzer Model AVC-MP, CEM Corp., PO Box 200, Matthews, NC 28106, USA). The instrument automatically calculates the total solids percentage through changes in weight samples during drying. Moisture content was used for reporting phytochemical results in a dry weight basis.

2.4 Phytochemical Analysis

2.4.1 Extraction and Analysis of Phenolic Compounds

Phenolic compounds were extracted using a modified method from Wojdyło, Oszmiański et al. (2008). Five grams of fresh applesauce sample were spiked with 0.5 mL of 206µM of ethyl gallate, homogenized using a Polytron® System PT 2100 with 10mL of the extraction solution (79% methanol:19% water:2% formic acid) for 30 seconds. The homogenate was centrifuged (2360xg, 10 min, 4°C) and the supernatant was recovered preventing contamination with the pellet.

The extraction was repeated, two times total and diluted to 25 mL with 2% formic acid. Then 2mL aliquots of extract were filtered through a 0.45µm filter and analyzed by HPLC-MS, with an injection volume of 10µL. Ethyl gallate recovery was observed to be 97%.

2.4.1.1 Phenolic Compounds Identification and Quantification

Samples were analyzed using an HPLC system (Waters 2695) coupled with a photodiode array detector (Waters 2996) and a ZQ Mass Selective Detector. A reversed-phase C_{18} column (100 x 2.1mm i.d.; particle size 3.5 µm) (X-terra RP18, Waters) with a guard column containing the same stationary phase was used.

Three mobile phase were used 0.4% formic acid in water (A); 0.4% formic acid - 4% isopropanol in methanol (B); methanol (C); elution started with 98% A/B, which remained isocratic until 30 minutes, at 30 minutes a linear gradient was used to reach 55%A/B, and then 20% A/B at 35 min, and 98%A/B at 37min. the column was then washed with 100% C during 5 minutes. The flow rate was 0.3mL/min and peak spectra were recorded from 210 to 500nm. Electrospray mass spectrometric analyses were performed in the negative mode using a Waters MicromassZQ system. The phenolic compounds in applesauce were identified by their ionization pattern (Table 2.1) and retention time comparisons with authentic external standards Chromadex (Irvine,CA).

Table 2.1. Mass to Charge ratios (m/z) used to quantify specific phenolic compounds by HPLC-ESI-MS from peaches and tomatoes phenolics

Phenolic	HPLC-ESI-MS (m/z)
Catechin	289
Chlorogenic acid	353
Epicatechin	289
Procyanidin B1, B2	577
Procyanidin C1	867
Quercetin 3-glucoside	463
Rutin	609

2.4.2 Extraction and Analysis of Carotenoids

All extractions procedures were completed under yellow light to minimize photo-oxidative reactions. Carotenoids were extracted using a modified method from Lessin, Catigani et al. (1997), five grams of the homogenized tomato samples, combined with 5 grams of sodium bicarbonate and celite, 50 ml of extraction solution (acetone:petroleum ether with 0.1% BHT) was added, and homogenate at 10,000 rpm for 30 seconds using Polytron® System PT 2100.

The mixture was vacuum filtered through two pieces of #1 filter paper into a vacuum flask, the filtrate was quantitatively collected and the extraction was repeated, 4 times for tomatoes samples. Then 20mL of 40% Potassium Hydroxide in Methanol (weight/weight) was added to the extract, covered and placed on magnetic stirring plate for 30 minutes at room temperature. The extract was transferred into a separatory funnel and 10mL of saturated sodium chloride and distilled water was added and mixed. The petroleum ether layer was quantitatively collect and poured through a funnel covered with crystalline sodium sulfate and diluted to 250mL using petroleum ether, 4mL of the extracts were dried under nitrogen.

The extract was resolubilized in 1mL ethyl acetate, vortexed, then 1mL methanol was added and vortexed. HPLC analysis followed the method of Kean, Hamaker et al. (2008), with and injection volume of 10µL.

2.4.2.1 Carotenoids Analysis by LC-PDA

Samples were analyzed with an HP 1090A HPLC system with model 79880Adiode array detection. Carotenoid separation was completed on a YMC C30 carotenoid reversed phase (2.0 x 250 mm) with a guard column containing the same stationary phase (Waters Corp.) with a gradient elution profile of 15 min using 98:2 methanol/1M ammonium acetate (Phase A) and ethyl acetate (Phase B). A flow rate of 0.37mL/min at 100% A with a linear gradient to 80:20 A/B over 6 min held for 2 min then followed by a 3 min linear gradient to 100% A and back to initial conditions for 4 min. Quantification of carotenoids were possible using multilevel response curves constructed at 450nm.

The identification and quantification of carotenoids were accomplished by using authentic standards from Sigma Aldrich (St. Louis, MO) *all-trans* lutein, zeaxanthin, β-cryptoxanthin, and β-carotene. *Cis*-isomers of β-carotene were quantitated using the response of *all-trans*-β-carotene. β-Carotene was reported both as *all-trans* isomer only and as the sum of *all-trans-, 9-cis-, 13-cis-,* and *15-cis-* isomers. Lutein and zeaxanthin are reported as the sum of *all-trans-* and *cis-* isomers formed during thermal processing of the samples, Lycopene was reported both as *all-trans-* isomer only and as the sum of - *all-trans-, cis-lycopene-1, cis-lycopene-2, 5-cis-lycopene.*

2.4.3 Carotenoid Bioaccessibility

Sample material. - Tomato products were obtained from the pilot plant trial 2 for both microwave and scraped surface heating system. Samples for analysis were taken at three processing step: the beginning material (diced tomato), product after hot break and product after finisher, each step was sampled at 3 time points (beginning, middle and end), and samples were stored at -20°C until analysis. A composite sample of the three time points was used for analysis and the results are presented in this report.

2.4.3.1 *In vitro* Digestion

Simulated digestion experiments were conducted under yellow lights to minimize photo-oxidative reactions. Simulated oral, gastric, and intestinal digestion used the conditions described by Garrett, Failla et al. (1999). To 8 g of homogenate tomato sample, 0.4 g canola oil were added to facilitate micellarization. During each set, one 0.5 g homogenized green vegetable salad (spinach, tomato, carrots and lettuce) containing 5% canola oil was simultaneously digested as a control.

Approximately 8g of homogenate tomato sample was combined with 6 mL oral phase base solution including α-amylase (3000 units) and vortexed for 3 minutes, then incubated under nitrogen at 37°C for 10 min in a shaking water bath at 90 rpm. For the gastric phase, porcine pepsin (final concentration 0.5 g/L) was added, the pH adjusted to 2.5 with 1 M HCl, the volume brought to 40 mL with saline (0.9% NaCl), and the mixture incubated under nitrogen at 37°C for 60 min at 90 rpm. For the intestinal phase, porcine pancreatin (final concentration 0.8 g/L), lipase (0.4 g/L), and porcine bile extract (1.8 g/L) were added, the pH was adjusted to 6.5 with 1 M $NaHCO_3$.

The mixture was incubated under nitrogen at 37°C for 120 min at 90 rpm. Following incubation, aliquots of final digesta (DG) were collected and frozen under nitrogen at -20°C until analysis. The aqueous micellar fraction (AQ) containing bioaccessible carotenoids were subsequently isolated from fresh digesta by centrifugation at 10,000xg for 60 min at 4°C. The resulting aqueous fraction (AQ) was collected, filtered (0.22 µm pore size), and frozen under nitrogen at-20°C until analysis.

2.4.3.2 Extraction of Carotenoids from *In vitro* Digestion Fractions

Four mL of digesta (DG) and aqueous (AQ) fractions were thawed and directly subjected to liquid-liquid extraction: 1mL acetone and 3 mL petroleum ether (with 0.1% w/v BHT) were added, the tubes were vortexed for 1 minute followed by centrifugation at 3,500 rpm for 3 minutes to facilitate phase separation. Extraction was repeated a total of 3 times. The organic layers were combined and dried under a stream of nitrogen in a water bath at 30°C.

Resolubilized with methanol: ethyl acetate (1:1) in total 150µL for aqueous samples total 2000µL for digesta samples and analyzed by HPLC as described above with an injection volume of 10 µL. Average extraction recovery of β-apo-8-carotenal was 90.79% and 96.36% for aqueous and digesta fraction, respectively.

2.5 Data Analyses

Color values L* a* b* were used to calculate total color difference (ΔE). Color values, apparent viscosity and phytochemical content differences in applesauce and tomato puree were compared by One way ANOVA and analyzed by Tukey's Studentized Range Test (SAS version 9.3, SAS Inst. INC., Cary, NC, USA), using a significance level of $P<0.05$. The comparison were made from time zero for physical (color, consistency and rheological properties) and phytochemical (each independent compound) changes within the same thermal treatment across storage time and between thermal treatments used (MW or TUB) within the same week. Apparent viscosity in both applesauce and tomato puree was normalized by °Brix content of each independent sample. Phytochemical content in applesauce and tomato puree was expressed as mean±SEM, calculated from three independent extractions normalized by total soluble solids. Preliminary bioaccessibility results from tomato product are presented as relative and absolute carotenoid bioaccessibility and expressed as micellarization percent and mean ±SEM respectively from two independent *in vitro* digestions. Relative bioaccessibility was expressed as the percentage of carotenoid transferred from tomato product digesta to the aqueous micellar fraction during simulated digestion (aqueous fraction/digesta fraction)*100, whereas absolute carotenoid bioaccessibility was calculated by multiplication of micellerization percent and total carotenoid content in the raw material.

CHAPTER 3. RESULTS

3.1 Applesauce Processed by Microwave and Tubular Heating System

3.1.1 Impact of Thermal Treatment on Color Properties Over Long-Term Storage

The color values: L*, a*, b* and total color difference (ΔE) of applesauce products were assessed using a Hunter LAB system were plotted against storage time (weeks), to monitor changes in overall color. Color values L* indicates lightness (0=black, 100=white), a* indicates redness (+) and green (-) and b* indicates yellowness (+) and blue (-), usual limits for a* and b* are + or -80. At time 0 the sample treated by tubular heating system was 5% lighter and presented 3% more redness and 6% more yellowness compared to microwave processed samples (Figure 3.1).

The L* value of microwave samples stored at 4°C (Figure 3.1) decreased by only 3% (from 54.2 to 52.8) after 52 weeks as compared to a decrease of 7% (56.9 to 53) for tubular heated samples. Similar behavior was observed for a* and b* values, with a drop of 4% (7.9 to 7.5) and 17% (37.9 to 31.6), respectively, for samples treated by tubular heating system. However, at the end of week 52, only minimal changes in a* (<1%) and a significant difference of 11% decrease in b* (35.8 to 31.7) value in microwave samples were observed.

While statistically significant differences ($P<0.05$) between microwave and tubular heated samples were observed at initial time 0 for L* and b* values, the differences was reduced and significance was lost after the full 52 weeks storage. On the other hand, the differences in a* values between microwave and tubular heated samples were not statistically significant from time 0 to 52 weeks of storage at 4˚C. The magnitude of total color difference (ΔE) between the samples at time zero and after 52 weeks was 7.4 and 4.3 for tubular and microwave heated samples respectively. The differences in color difference (ΔE) between the two heating systems was 41% when stored at 4°C for 52 weeks (Table A.1); with samples from tubular heating showing the largest change in color over time.

No statistically significant differences ($P<0.05$) in L* and b* were found for samples processed by microwave treatment during the first 4 weeks of storage at room temperature (RT). However, a total increase of 15% in a* (7.7 to 9.2) as well as a decrease of 12% and 11% in L* (54.2 to 47.6) and b* (35.8 to 31.7) values, respectively, were observed after 52 weeks of storage compared to time 0. Tubular samples showed statistically significant ($P<0.05$) differences in L* value at time 0 and after 1 week of storage as compared to microwave heated samples. However, after 52 weeks of storage the differences in L* and b* between heating methods lost statistically significance. Tubular heated samples decreased a total of 16% in L* and b* value at the end of 52 weeks of storage (Figure 3.1). Although an increase of 16% in a* was observed at the end of the storage study, statistically significant ($P<0.05$) differences were only observed in a* and ΔE values between the two heating systems at the end of the storage study.

Total color difference for microwave and tubular heated samples was 7.9 and 11, respectively, representing a difference of 29% between the two thermal systems (Table A.2).

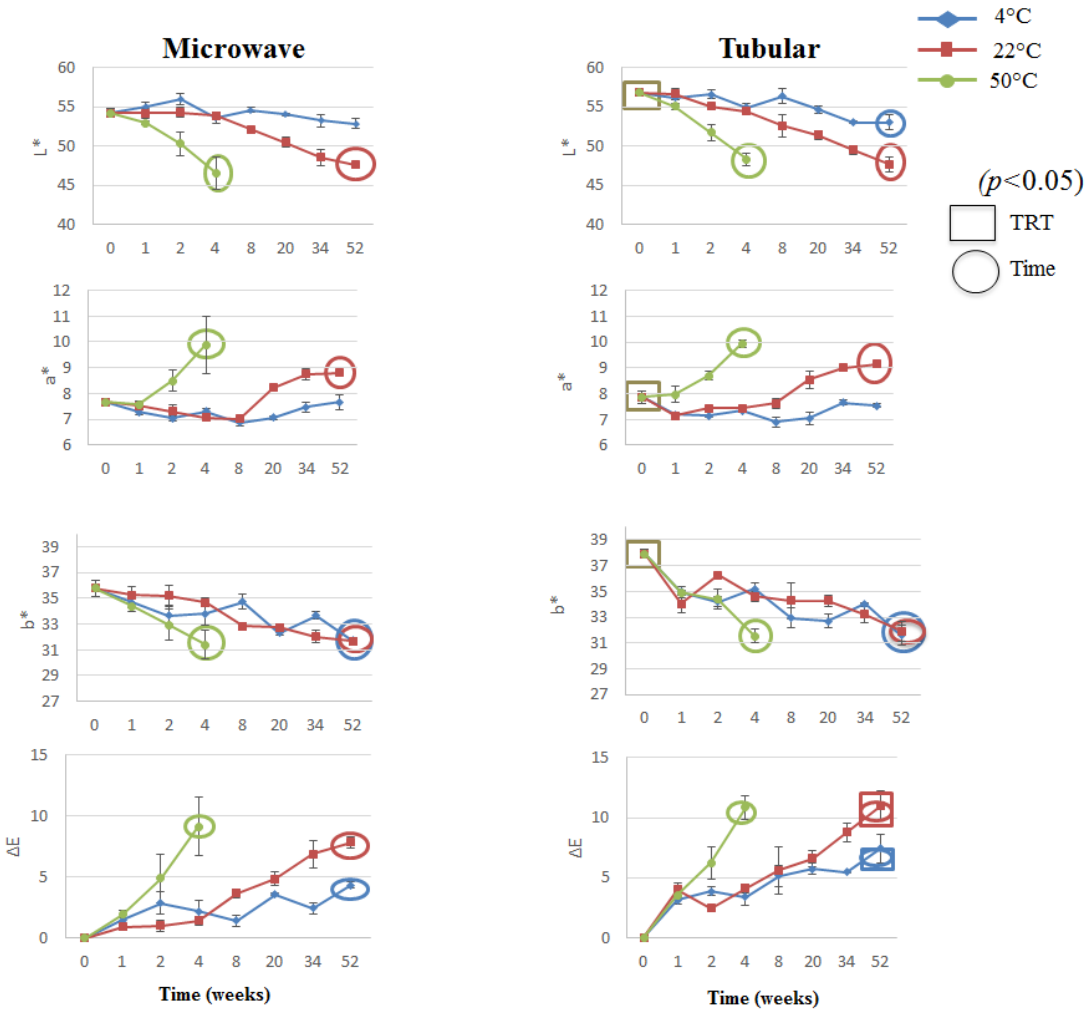

Figure 3.1 Color values (L*, a*, b*, ΔE) of applesauce thermally treated by microwave and tubular heating system during storage conditions.
Mean (±Std) when samples were stored at 4°C, 22°C during 52 weeks and 50°C during 4 weeks. Initial significant differences ($P<0.05$) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

Storage of applesauce at 50°C decreased the L* value in 14% for microwave and 15% for tubular samples. High storage temperature increased the a* value between 26% and 29% for both samples and decreased b* value in of 12% and 17% for microwave and tubular heated samples respectively after 4 weeks of storage (Figure 3.1). No statistically significant differences ($p<0.05$) in color values were observed at 2 and 4 weeks between both heating systems. Total color difference values in week 4 were 9.2 for microwave and 10.8 for tubular heating systems, although the difference of 15.6% in ΔE value observed between both systems at the end of the storage time was not statistically significant (Table A.3). There appeared to be general agreement between accelerated conditions was effective to mimicked storage of applesauce at room temperature during one year, as ΔE values obtained from both microwave and tubular heated samples were similar at both storage temperature at the end of storage (Figure 3.4).

A linear correlation between samples stored at room temperature and 50°C total color differences was observed as a function of time, and it was observed a 98% probability to predict changes at room temperature in microwave heated samples and 96% in tubular samples by an accelerated study at 50°C, where 1, 2 and 4 weeks at 50°C can predict total color changes at 8, 20 and 52 weeks at room temperature respectively (Figure 3.2).

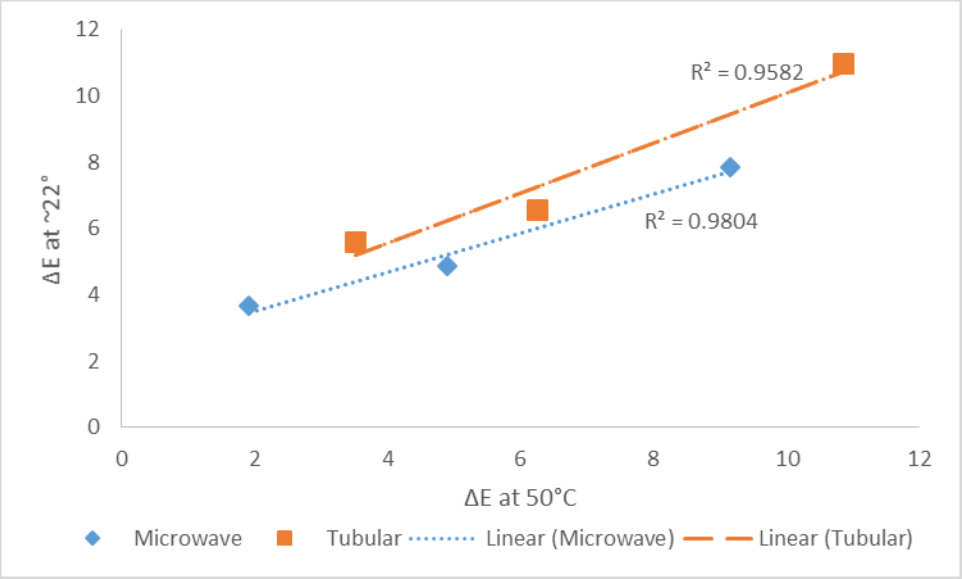

Figure 3.2. Correlation of total color differences between samples stored at 50°C and room temperature as a function of storage time.

3.1.2 Consistency and Normalized Apparent Viscosity

Consistency of applesauce ranged from 4 to 7 cm/(30s) during the storage study (Figure 3.3). Statistically significant differences ($P<0.05$) were observed between heating systems at the study for samples stored at all temperatures. At the end of the study, microwave heated samples were observed to be thinner compared to tubular heated samples regardless the storage temperature. Consistency across time was mostly non-significantly affected over time for both heating systems (Table A.4).

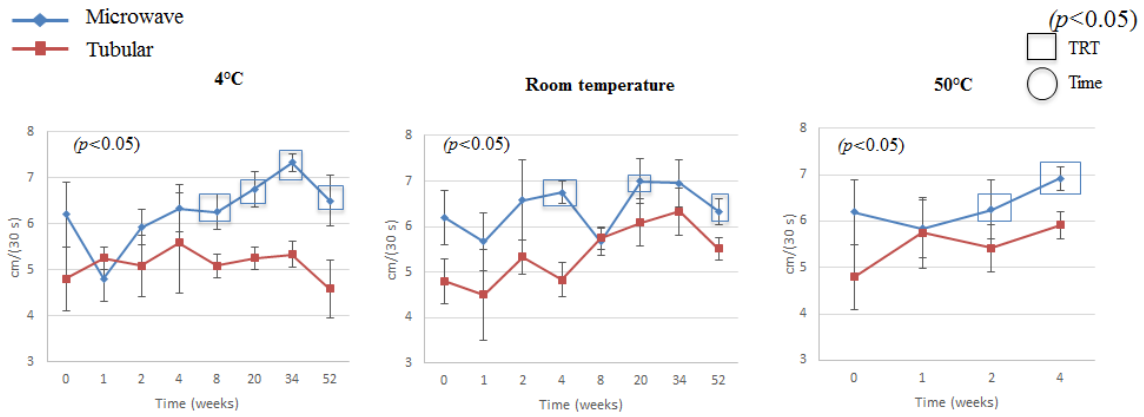

Figure 3.3. Bostwick consistency (cm/(30s)) of applesauce processed by microwave and tubular heating system.
*Mean (±Std) when samples were stored at 4°C, room temperature and 50°C. Significant differences ($P<0.05$) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

Apparent viscosity was measured using a controlled stress rheometer. In general, apparent viscosity at low and high shear rate was statistically higher in tubular samples compared to microwave samples by the end of the storage study regardless of storage temperature (Figure A.1).

At low shear rate (1/s) microwave samples stored at 50°C decreased by 47% compared to 35% in tubular samples after four weeks; similar behavior was shown at higher shear rate (100/s) in both samples with a decrease of 25% and 16%, for microwave and tubular samples, respectively. At low shear rate, microwave samples stored at room temperature and 4°C decreased their apparent viscosity in 35% and 38% respectively, in contrast to a decrease of 17% and 22% in tubular samples. At high shear rate (100/s) the decrease in apparent viscosity was 19% and 20% for microwave samples and 13% and 10% for tubular samples stored at room temperature and 4°C respectively.

The apparent viscosity of samples stored at 50°C decreased continuously over storage time regardless of the thermal treatment used or the shear rate applied (Figure 3.4).

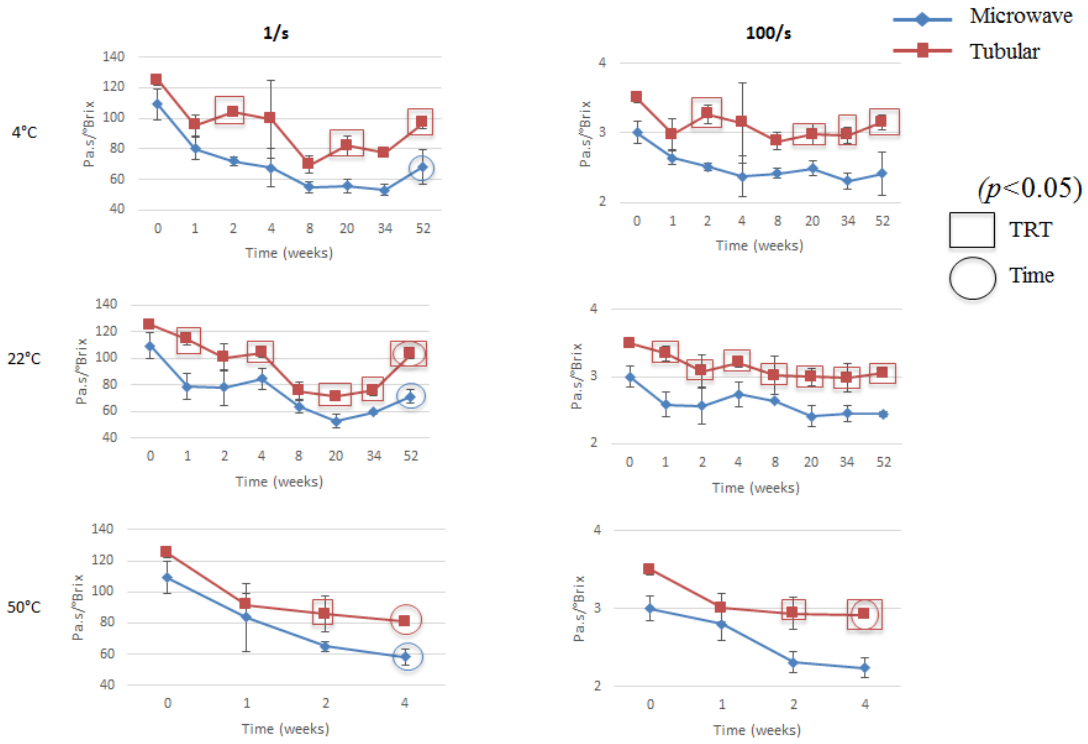

Figure 3.4. Apparent viscosity (Pa.s/°Brix), at 1/s (1) and 100/s (2) shear rate in applesauce processed by microwave and tubular heating system.
*Mean (±Std) when samples were stored at 4°C, 22°C and 50°C during given time points. Significant differences (P<0.05) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

3.1.3 Phytochemical Content on Applesauce During Storage Study

Applesauce phytochemicals were assessed using LC-MS. Phenolic constituents identified in apples sauce products included: chlorogenic acid, catechin, epicatechin, rutin, procyanidin B1, B1 and C1, quercetin-3-glucoside. These results are consistent with previously published phytochemicals characterized in apple products (van der Sluis, Dekker et al. 2001, van der Sluis, Dekker et al. 2005, Drogoudi, Michailidis et al. 2008, Oszmiański, Wolniak et al. 2008, Vieira, Borges et al. 2009, Candrawinata, Golding et al. 2013) (Figure 3.5).

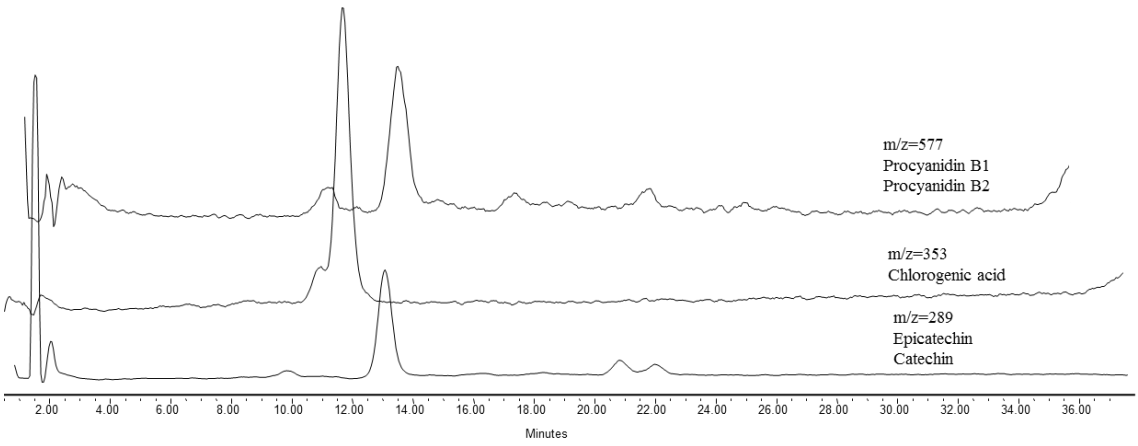

Figure 3.5. Representative chromatogram of phenolic compounds from applesauce by LC/MS at time 0.

At time zero chlorogenic acid content was 175mg/kg Dry weight (DW) and 163.4mg/kg DW for microwave and tubular heated samples respectively. Whereas catechin and epicatechin content was 3.2 and 60.7 mg/kg DW in microwave heated samples and 2.9 and 52.3 mg/kg DW in tubular heated samples respectively.

Rutin content of 0.6mg/kg DW in microwave heated samples and 0.5mg/kg DW in tubular heated samples were found. Quercetin-3-glucoside content was found to be 65.1mg/kg DW in microwave heated samples and 56.6mg/kg DW in tubular heated samples. Procyanidin B1, B2 and C1 content in microwave heated samples was 19.3, 30.6 and 28.9mg/kg DW respectively; whereas procyanidins content in tubular heated samples was 17.1, 26.2 and 27.7mg/kg DW respectively.

Although microwave samples tended to have higher phenolic content, no significant differences in polyphenol content were found at time zero between heating systems. The stability of these phenolic constituents was then followed over storage at 4°C, room temperature for 52 weeks and 50°C for 4 weeks (Table A.5-A.6).

Recovery of phenolic constituents was not consistent between individual species. Samples stored at 4°C showed an overall increase in chlorogenic acid content for both, microwave (23%) and tubular (29%) heated samples after 52 weeks. Epicatechin content decreased by 44% in microwave and 37% in tubular treated samples with differences being significant ($P<0.05$) at the end of 52 weeks of storage. Loss of procyanidin B1 was modest in both samples over time with 8% decrease in microwave and 2% decrease in tubular samples, and small significant differences ($P<0.05$) after 52 weeks. Procyanidin B2 concentration increased at week 1 but decreased during the following weeks; a total loss of 23% and 13% in microwave and tubular samples as shown in Table A.8. No significant differences were observed in procyanidin C1 content form microwave treated samples over storage. In contrast, procyanidin C1 was found to decrease over time in tubular samples with losses of 13% and significant ($P<0.05$) differences were observed at 8 weeks of storage.

Quercetin-3-glucoside in microwave samples remained generally stable during 34 weeks storage, showing a significant ($P<0.05$) increase of 85% in concentration after 52 weeks. Similar increase was found in tubular heated samples with 88%, and higher number of significance differences over storage time (Figure 3.6).

Catechin showed a 27% loss of concentration in both samples over time; however, microwave treated samples did not show significant differences after week one. Rutin increased by 136% in tubular heated samples. Higher rutin levels were observed in tubular samples (136%) compared to microwave (85%) heated after 20 weeks (Figure 3.6).

No significant differences in chlorogenic acid content were observed between heating systems in samples stored at room temperature within the same week. However, some phenolic compounds were significantly higher in tubular samples including: catechin at 4 weeks, epicatechin at 4, 8 and 34 weeks, procyanidin B2 at 1 and 8 weeks, rutin at 4 and 8 weeks. On other hand, microwave treated samples showed higher phenolic concentration of procyanidin B1 at 52 weeks, procyanidin C1 at 20, 34 and 52 weeks and querceting-3-glucoside at 20 and 52 weeks (Table A.6).

At room temperature tubular heated samples showed significant ($P<0.05$) increase of 19% in chlorogenic acid content at 52 weeks, resulting 163.4mg/kg DW compared to 194.2mg/kg DW at time zero. Similar increase (12%) in chlorogenic acid was observed in microwave heated samples at the end of the study. Epicatechin showed a 72% loss in microwave heated samples with differences starting at 1 week; whereas in tubular heated samples final loss was 68% but significant differences started at 4 weeks.

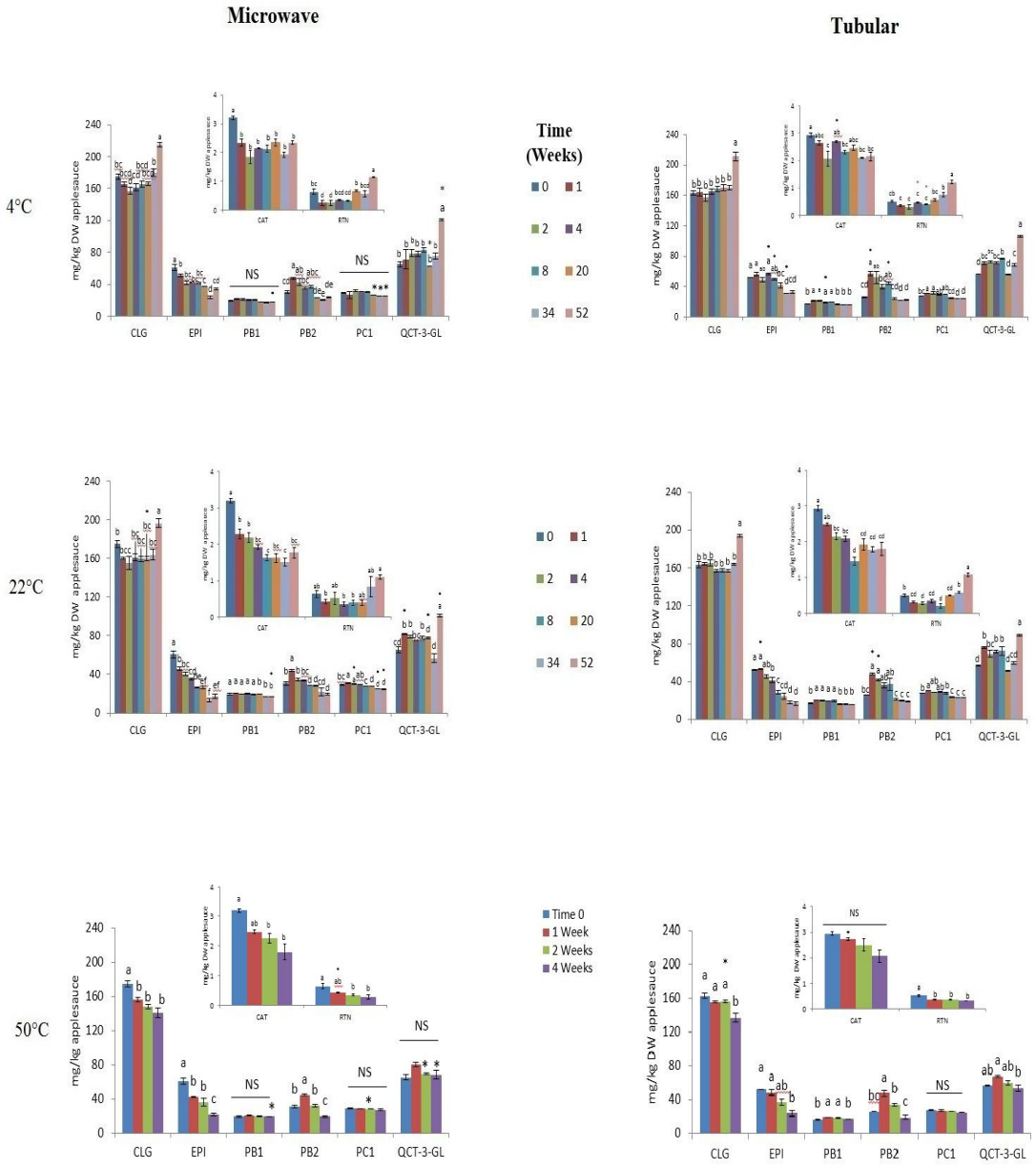

Figure 3.6. Phenolic content (mg/kg DW) in applesauce processed by microwave and tubular heating system stored at 4°C, 22°and 50°C.

*Significant differences (Tukey test: $P<0.05$) in phenolic content (mean±SEM) within one process (MW or TUB) during storage time are indicated with different letters (a,b,c,d), whereas the symbol (*) indicates significant differences between thermal processes (MW or TUB).

Procyanidin C1 did not show significant differences after week 8 in both samples, whereas at the end of 52 weeks, losses of 14% in microwave and 17% in tubular were observed (Table A.9). Quercetin-3-glucoside showed significant (P<0.05) increase at the end of the study with 55% in microwave and 57% in tubular heated samples compared to time zero, although final content was significantly (P<0.05) higher in microwave heated samples. Microwave treated samples did not show changes in catechin concentration from week 8 to 52, but a total loss of 45% was found at the end of the study. Similar behavior was shown by tubular samples which did not show significant changes after week 8, but with final loss of 39% at 52 weeks was observed. Similar to results obtained from samples at 4°C, tubular samples showed an increase of 111% and 74% in microwave heated samples at the end of the storage study (Figure 3.6).

No significant differences were found in catechin and rutin levels between heating systems within the same week in samples stored at room temperature. Microwave heated samples showed higher (P<0.05) concentration of chlorogenic acid at 20 weeks; procyanidin B1 at 52 weeks; procyanidin C1 at 2, 34 and 52 weeks; quercetin-3-glucoside at 1 and 20 weeks. Tubular heated samples were significantly (P<0.05) higher in epicatechin at 1 week; procyanidin B2 at 1 and 2 weeks. However, as described previously, no significant differences in phenolic content were found between heating systems at time zero. At the end of the study only statistical differences were found for procyanidin B1, C1 and quercetin-3-glucoside (Table A.7). Phytochemical content in applesauce was also compared across storage after four week time in samples at 50°C (Table A.6 and Table A.7).

After four weeks of storage at 50°C, chlorogenic acid content decreased, 19% and 16% for microwave and tubular samples, respectively. Statistical differences for microwave treatment samples were shown at week 1, after which no significant drop in concentration was observed in samples treated by tubular heating. Chlorogenic acid content did not significantly change during the first 2 weeks, however, concentration decreased significantly ($P<0.05$) at 4 weeks from 175 to 137.5mg/kg DW. No significant differences during storage were found for procyanidin B1 and quercetin-3-glucoside content in microwave treated samples as compared to time 0; although a slight increase of 5% was shown on quercetin-3-glucoside by the end of the study.

Catechin concentration decreased 44% and 30% in microwave and tubular heated samples respectively. No significant differences in catechin content were found at the end of the study in tubular samples compared to time 0. However, statistically significant differences were detected in microwave heated samples after 2 weeks of storage at 50°C with catechin levels dropping from 3.2 to 1.8mg/kg DW ($P<0.05$). Procyanidin C1 concentration on microwave and tubular samples was not significantly different during the study, with decrease in concentration was 6% and 9%, respectively.

Epicatechin content in microwave heated samples showed the lowest stability across time with a loss of 64% compared to 54% in tubular samples in which significant differences ($P<0.05$) were observed by the end of the study week 4. Rutin concentration showed significant differences on week 1 for tubular heated samples, but no significant differences ($P<0.05$) were observed the following weeks.

At the end of the study, a 40% loss of rutin was shown for tubular samples. Microwave heated samples showed a 57% loss in rutin, showing significant differences ($P<0.05$) after week 2 of storage. Changes in procyanidin B2 concentration were not significantly different in microwave and tubular heated samples, losses of 36% and 25%, respectively at the end of the study were shown (Figure 3.6). When compared to samples stored at room temperature, polyphenols losses across time was similar, although more variation within the same compound during 52 weeks of storage was observed at room temperature.

3.2 Tomato Trial #1

3.2.1 Color Properties Affected by Thermal Treatment During Storage Study

As with applesauce products, tomato puree color values: L*, a*, b* and total color difference (ΔE) were plotted as a function of storage time. At time 0, samples treated by tubular heating system were found to be 5% darker with L* 28.8 and presented 5% more redness with a* of 30.9 and 14% more yellowness with b* 31 as compared to microwave samples (L*=30.2, a*=29.6, b*=27) (Figure 3.7).

During storage at 4°C microwave treated samples showed significant differences ($P<0.05$) in L* value at 11 weeks, with a final decrease in lightness of 6% at 24 weeks (from 30.2 to 28.3). Interestingly, tubular treated samples did not show a statistically significant changes in L* value across storage time. Significant changes in a* value of microwave samples were shown after 4 weeks, with an increase of 29.6 to 30.7 representing a 4% increase. By comparison; tubular samples showed a more stable behavior with no change in a* value through storage compared to time zero.

No significant differences were observed in b* value of tubular heated samples. However, microwave heated samples began to increase in b* value after week 4 and showed an increase of 14% by the end of the study increasing from 27.0 to 30.7 (Table A.10). However, this increase did not reach significance ($P<0.05$) and thus no differences in b* value were observed between the two heating systems (Table A.11).

While individual color parameters did not show major differences, total color difference (ΔE) was found to be 4.3 for microwave samples and 0.8 for tubular samples at 24 weeks, resulting in statistically significant ($p<0.05$) differences were observed between the two treatments at each week of the study following initial conditions (Figure 3.7). Although minimal total color differences (<3.5) may not by perceivable by human eye.

During storage at room temperature microwave samples showed similar behavior in color changes as samples stored at 4°C, with only a 6% decrease in L* value, with significant ($P<0.05$) differences observed by 4 weeks of storage. Tubular samples showed no change L* over 24 weeks of storage. Both treatment samples showed a significant ($P<0.05$) increase in a* value during the first 4 weeks, followed by a gradual decrease in a* value over time. At the end of the study, tubular samples showed higher a* value than microwave samples (31.1 compared to 30.7 respectively) (Table A.11). An initial increase of b* value in microwave heated samples was observed during the first 4 weeks followed by a slight decrease over time; however, at the end of 24 weeks the b* value in microwave heated samples increased by 11% compared to time zero, whereas in tubular heated samples the b* remain relatively stable for the duration of the study (Table A.7).

Total color difference (ΔE) was significantly higher in microwave samples with a value of 3.6 compared to 0.6 in tubular samples (Figure 3.7). These data suggest only modest changes in color overall for both microwave and tubular heated samples.

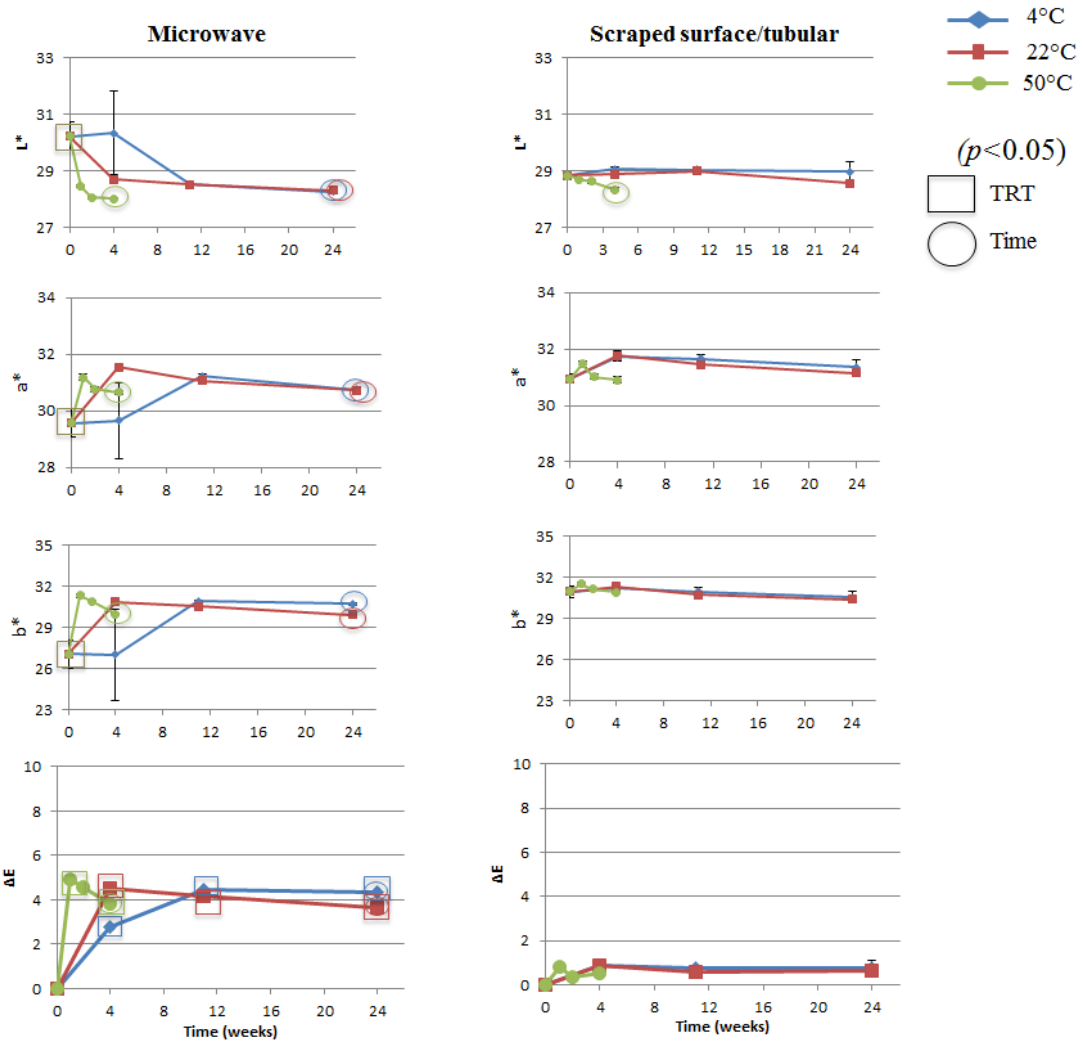

Figure 3.7. Color values (L*, a*, b*, ΔE) of tomato puree thermally treated by microwave and scraped surface/tubular heating system during storage conditions.
Mean (±Std) when samples were stored at 4°C, 22°C and 50°C. Significant differences (P<0.05) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

Samples stored at 50°C during 4 weeks showed a faster decrease in L* value at week 1, for microwave heated samples than for tubular samples. Microwave treated samples decreased by 6% compared to no significant change in tubular heated samples. At the end of the study, microwave samples were significantly ($P<0.05$) darker than tubular samples. Significant ($P<0.05$) increases for both, a* and b* values were observed in microwave samples (4% and 11%). However; tubular treated samples at 4 weeks (Table A.12). However, when comparing between heating systems, significantly higher a* and b* values were observed for tubular samples at each time point (Table A.13). Total color difference (ΔE) was 3.8 for microwave samples compared to 0.53 for tubular samples, representing 14% of changes in microwave, which was statistically different at each time point (Figure 3.7). However, practically these differences suggest only modest color deterioration in microwave and general stability in tubular treated samples.

Overall, these data suggest that modest color differences can be found in tomato puree processed by microwave heating system, and that tubular heating system generate generally more stable color in the product over storage time independently of the storage temperature, although consumers may not be able to perceived these color changes. Microwave heated samples stored at 50°C showed high linear correlation with samples at room temperature ($R^2=0.98$), suggesting than the accelerated study at 50°C can predict color changes of tomato puree stored at room temperature for 24 weeks, whereas no correlation was observed between tubular heated samples.

3.2.2 Consistency and Normalized Apparent Viscosity

Tomato puree consistency values ranged between 4.5 and 6cm/(30s) (Figure 3.8). Tubular samples generally had higher consistency values compared to microwave samples. In samples stored at room temperature consistency values decreased 18% for microwave and 9% for tubular samples over the stage period. No significant changes were found in tubular samples during storage, but consistency remained significantly higher ($P<0.05$) than microwave heated samples over 24 weeks. At 4°C the consistency value of microwave samples decreased by 18% (5.5 to 4.5cm/30s) compared to 16% (5.7 to 4.8cm/30s) decrease for tubular samples. The only statistically significant difference between heating systems was observed at 4 weeks of storage. When samples were stored at 50°C, an opposite trend was observed; consistency values remained generally stable over 4 weeks of storage (Figure 3.8, table A.13).

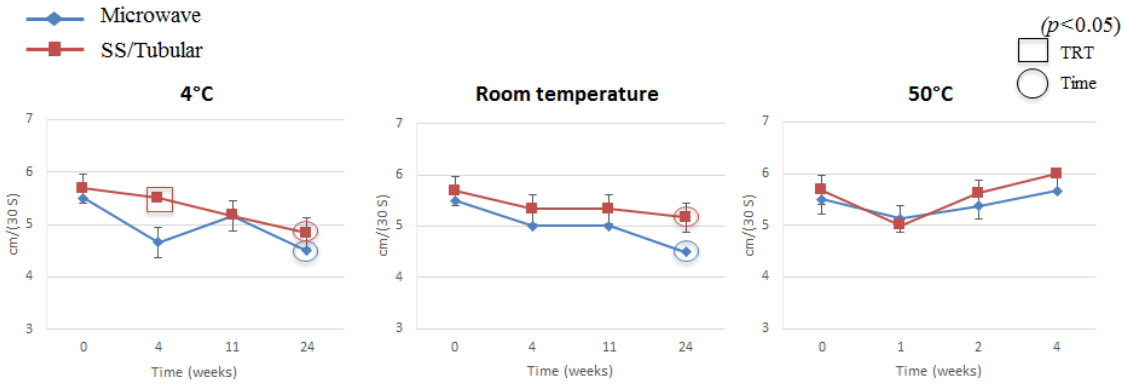

Figure 3.8. Consistency values (cm/(30s)) of tomato puree processed by microwave and tubular heating system.
*Mean (±Std) when samples were stored at 4°C, room temperature and 50°C. Significant differences ($P<0.05$) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

Apparent viscosity at low shear rate (1/s) increased from 102 to 167 Pa.s/°Brix for microwave and 110 to 146Pa.s/°Brix for tubular heated samples when stored at room temperature (Figure 3.9). This represented a 64 and 33% increase over time. Similar trends were observed for other storage temperatures by the end of the study, whereby the increase for microwave samples was 33% and 23% at 4°C and 50°C, respectively. Tubular heated samples also showed an increase in apparent viscosity with 23% and 16% increase at 4°C and 50°C respectively. At high shear rate (100/s), only tubular samples at room temperature showed 4% increase in apparent viscosity, whereas samples stored at 4°C and 50°C decreased by 7% and 3% compared to time zero. Microwave heated samples at high shear rate showed 11% and 13% decrease when stored at room temperature and 50°C, whereas at 4°C the viscosity reduction was 7% at the end of 24 weeks (Figure 3.9).

Interestingly, significant differences ($P<0.05$) were observed at high shear rate ($100s^{-1}$) between both heating systems, at this condition microwave heated samples showed an apparent viscosity of 5.4 Pa.s/°Brix compared to apparent viscosity of 4.6 Pa.s/°Brix in tubular samples at $100s^{-1}$. Microwave heated samples stored at 4°C showed significantly ($P<0.05$) higher apparent viscosity during every single time point of storage; however in microwave samples at RT and 50°C this significant difference was lost at 24 and 4 weeks of storage respectively. At 50°C microwave samples showed statistically higher apparent viscosity than tubular heated samples during the first 2 weeks.

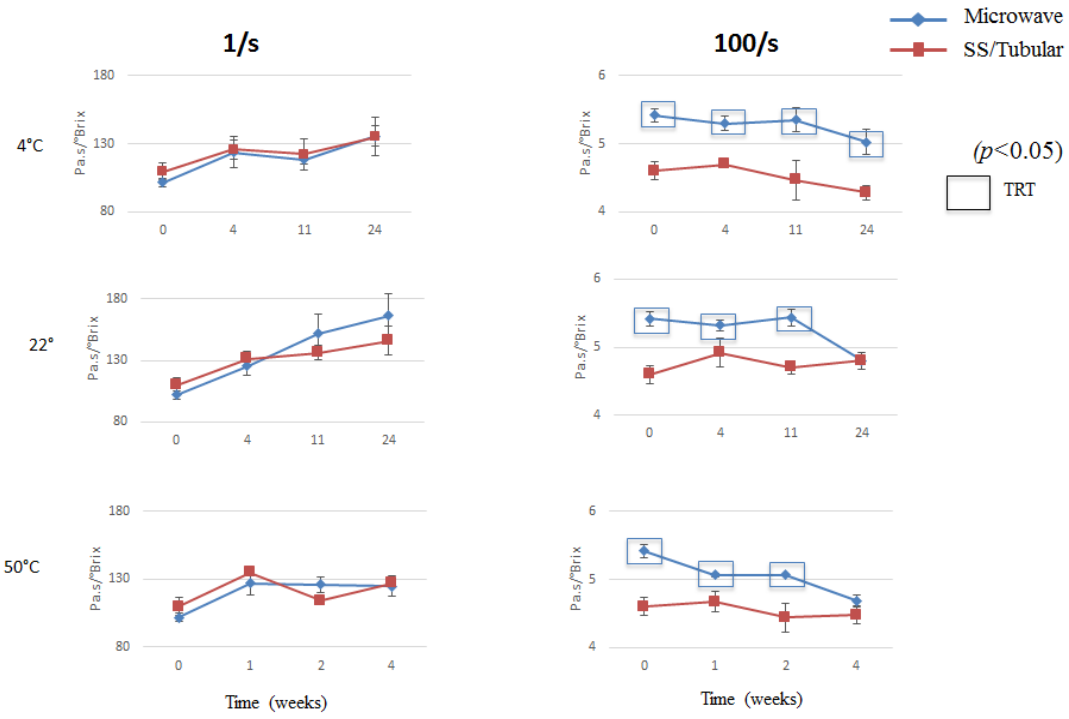

Figure 3.9. Normalized apparent viscosity (Pa.s/°Brix), at 1/s and 100/s shear rate in tomato puree processed by microwave and tubular heating system.
*Mean (±Std) when samples were stored at 4°C, 22°C and 50°C during storage time. Significant differences (P<0.05) between heating systems are presented with square (□), whereas significant differences across time compared to time zero within the same heating system are presented with oval (○).

Samples stored at 50°C during 4 weeks showed an apparent viscosity of 4.7 and 4.5 Pa.s/°Brix for microwave and tubular heated samples. However, samples at room temperature the apparent viscosity after 24 weeks was 4.8Pa.s/°Brix for both heating systems, these results suggest the effectiveness of accelerated storage conditions to predict the changes in viscosity of tomato puree at room temperature.

No significant differences on apparent viscosity over time were observed for tubular samples evaluated at high shear rate; however, microwave samples showed significant decrease in apparent viscosity by the end of the study.

At low shear rate tubular samples stored at 4°C did not show significant differences during 24 weeks, but at room temperature, a significant increase was shown on week 4, and then remained unchanged up the end of the study (Figure 3.9). Microwave heated samples showed a significant increase for samples at 4°C, room temperature and 50°C when tested at low shear rate (Figure A.2).

3.2.3 Carotenoid Content in Tomato Puree

Carotenoid profile from tomato puree was assessed (Figure 3.10), for lutein, α-cryptoxanthin, β-cryptoxanthin, α-carotene, *z* and *E*-β-carotene, z and *E*-lycopene.

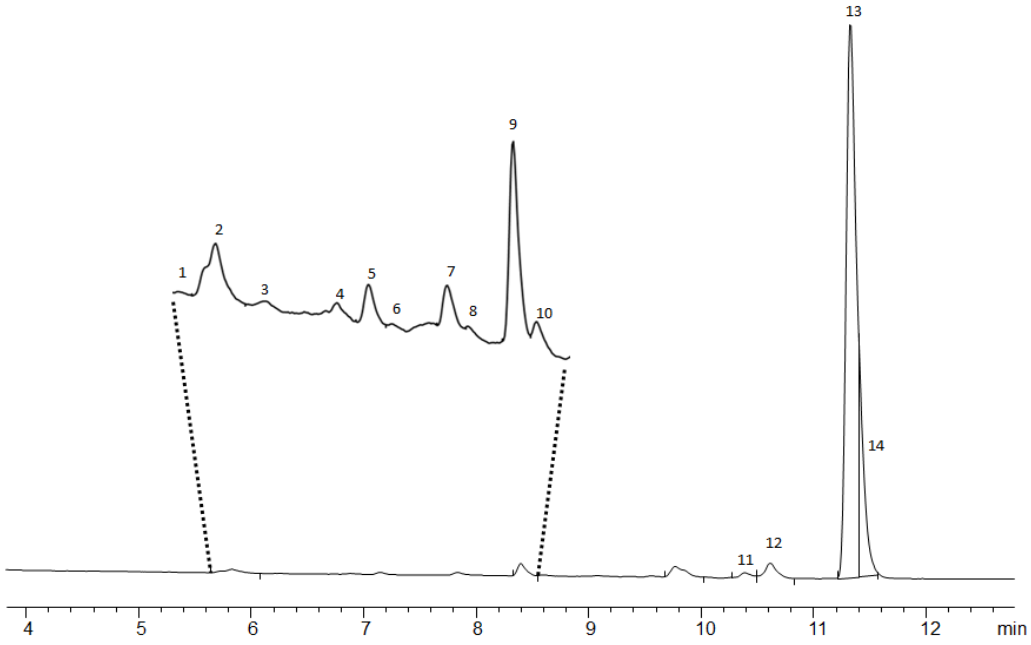

Figure 3.10. Representative carotenoid profile of tomato puree at time 0.
Peak Identification: 1 =*z*-lutein; 2 =lutein; 3 = zeaxanthin; 4 = α-cryptoxanthin; 5= β-cryptoxanthin; 6= 15-*z*-β-carotene; 7= 13-*z*-β-carotene; 8= α-carotene; 9 = *E*-β-carotene; 10= 9-*z*-β-carotene; 11=*z*-lycopene-1; 12=*z*-lycopene-2; 13=lycopene; 14=5-*z*-lycopene.

Microwave and tubular samples stored at 4°C during 24 weeks showed a decrease in all-*trans* lycopene concentration of 21% and 29% respectively. A slight increase of 8% for *z*-lycopene was observed for both samples at 24 weeks of storage; however, this difference was not statistically significant (Figure 3.12). Significant differences in both microwave and tubular were observed in lutein and zeaxanthin. An increase of 559% for microwave and 756% for tubular was observed in lutein by the end of week 24.

Similar decrease in zeaxanthin concentration in microwave (80%) and tubular (82%) was found at the end of the study (Figure 3.11). These changes may be a result of zeaxanthin degradation and isomers formations that eluted at the same retention time than lutein, for this reason results are presented as sum of lutein and zeaxanthin. No statistically significant differences in carotenoid content were observed between microwave and tubular samples at any given time point (Figure 3.11).

When stored at room temperature, *E*-lycopene content decreased 53% in microwave and 49% in tubular samples, showing significant decrease by 11 weeks (Figure 3.12). Microwave and tubular samples showed significant changes in concentration of lutein 0.3 to 1.8mg/kg DW for microwave and 0.2 to 1.8 mg/kg DW for tubular heated samples. As well as changes in zeaxanthin content from 1.3 to 0.1mg/kg DW for microwave heated samples and 1.2 to 0.1mg/kg DW and β-cryptoxanthin content from 0.3 to 1mg/kg DW for both microwave and tubular heated samples. Additionally, α-carotene concentration showed significant differences only in tubular samples (Figure 3.11). Concentration of β-carotene was significantly ($P<0.05$) higher in tubular heated samples at week 11 compared to initial levels. No other significant differences in carotenoid content were found between heating systems.

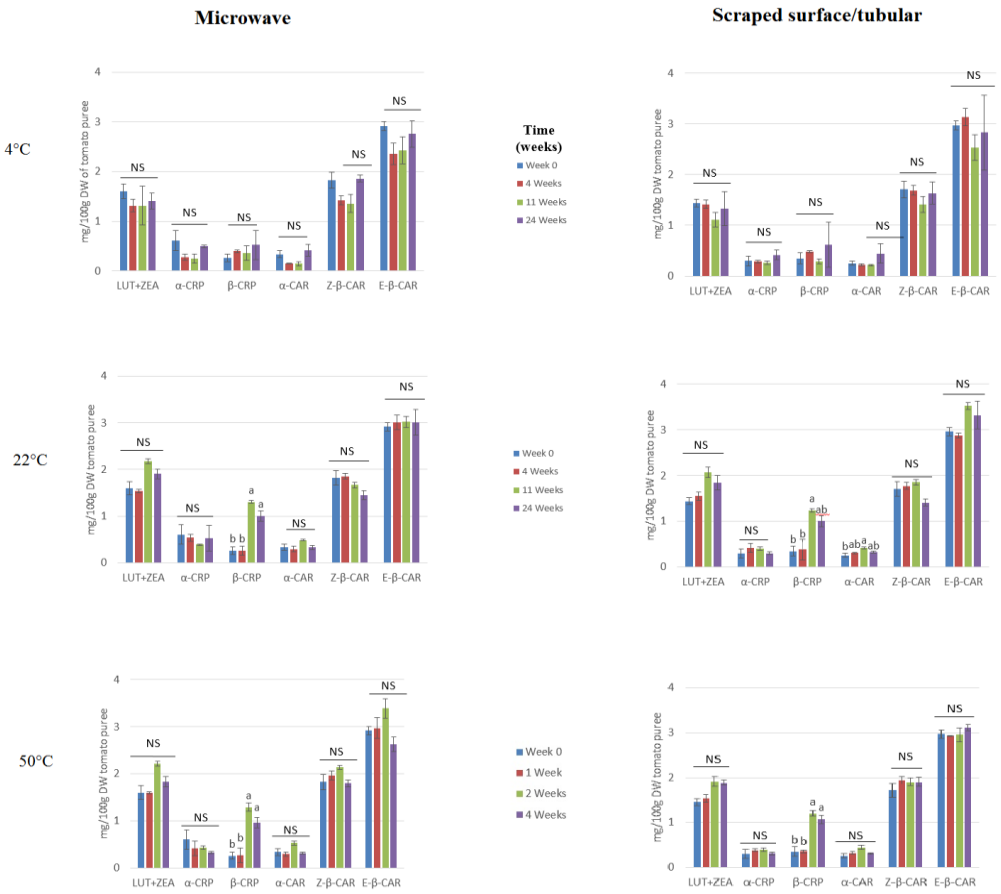

Figure 3.11. Carotenoid content (mg/kg DW) in tomato puree processed by microwave and scraped surface/tubular heating system and stored at 4°C, 22°C and 50°C.
*Significant differences (Tukey test: $P<0.05$) in carotenoid content (mean±SEM) within one process (MW or TUB) over storage time are indicated with different letters (a,b,c), whereas the symbol (*) indicates significant differences between thermal processes (MW or TUB).

Carotenoid content in tomato puree was compared within heating system across storage time (Figure 3.11-12; Table A.16). In samples stored at 50°C concentration of *E*-lycopene in microwave and tubular samples decreased by 54% and 46%, respectively. Significant increase of 30% of *z*-lycopene in tubular samples was observed at 4 weeks.

In contrast, increase of 16% in concentration of *z*-lycopene (from 10 to 11.6mg/100kg DW) was observed in microwave samples (Figure 3.12), but this was found to be non-significant ($P<0.05$). Additional compounds that presented significant differences in microwave and tubular samples were lutein, zeaxanthin and β-cryptoxanthin; whereas in tubular samples differences in α-carotene were also observed. The only significant difference observed for tubular samples was at 4 weeks with higher *E*-β-carotene concentration compared to microwave samples evaluated at the same time point (Figure 3.11).

Even when some statistically significant ($P<0.05$) changes were observed in the rest of the carotenoid compounds in the tomato puree stored at different temperatures, changes in lycopene content have the major impact in total carotenoid contents as lycopene ~95% of the total carotenoid content in the tomato puree. Higher carotenoid stability was observed in samples stored at 4°C regardless the thermal treatment used.

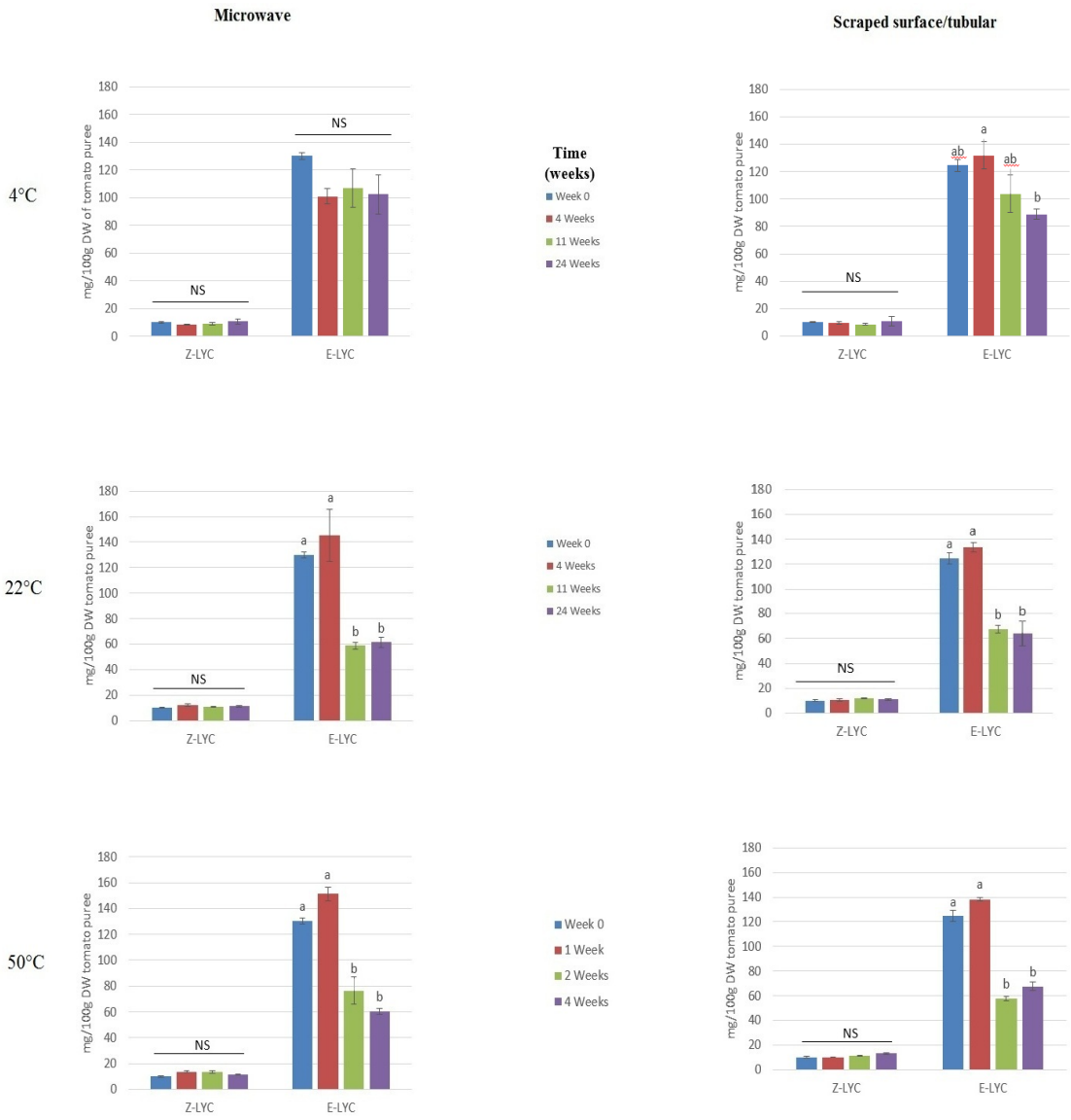

Figure 3.12. Lycopene content (mg/kg DW) in tomato puree processed by microwave and scraped surface/tubular heating system and stored at 4°C, 22°C and 50°C.
*Significant differences (Tukey test: $P<0.05$) in carotenoid content (mean±SEM) within one process (MW or TUB) over storage time are indicated with different letters (a,b,c), whereas the symbol (*) indicates significant differences between thermal processes (MW or TUB).

3.3 Tomato Trial #2

3.3.1 Carotenoid Content Affected During Processing Stages

Based on outcomes from the first trial, a second tomato trial was conducted to determine if thermal treatment would impact carotenoid content during specific processing stages (dicing, hot break or finisher). Microwave samples did not show significant differences on *E* and *z*-lycopene concentration, but a decrease of 15% and 14%, respectively was shown after finisher compared to diced tomato. Significant decrease in β-carotene of 37% and increase of *z*-beta carotene of 73% was shown after finisher, lutein decrease of 15%, small changes were found in β-cryptoxanthin, zeaxanthin and α-carotene. Tubular samples showed a non-significant decrease in *E*-lycopene of 12% with a significant ($P<0.05$) decrease of 22% in *z*-lycopene after finisher. Significant decreases of β-carotene (40%) and lutein (35%) compared to diced tomato were shown, but no significant changes between hot break and finisher were found for these compounds. Significant increase of α-cryptoxanthin and *z*-β-carotene by 9% and 121%, were found after hot break and finisher. Zeaxanthin, β-cryptoxanthin and α-carotene showed minimal changes during the process (Figure 3.13).

Carotenoid degradation was similar in both heating systems (Table A.14). During tomato processing final recovery of the product after finisher was 95.23% and 96.58% for microwave and tubular respectively (peels and seeds recovered from the finisher were 2.51% and 1.26% of the final product for microwave heating and tubular heating system respectively).

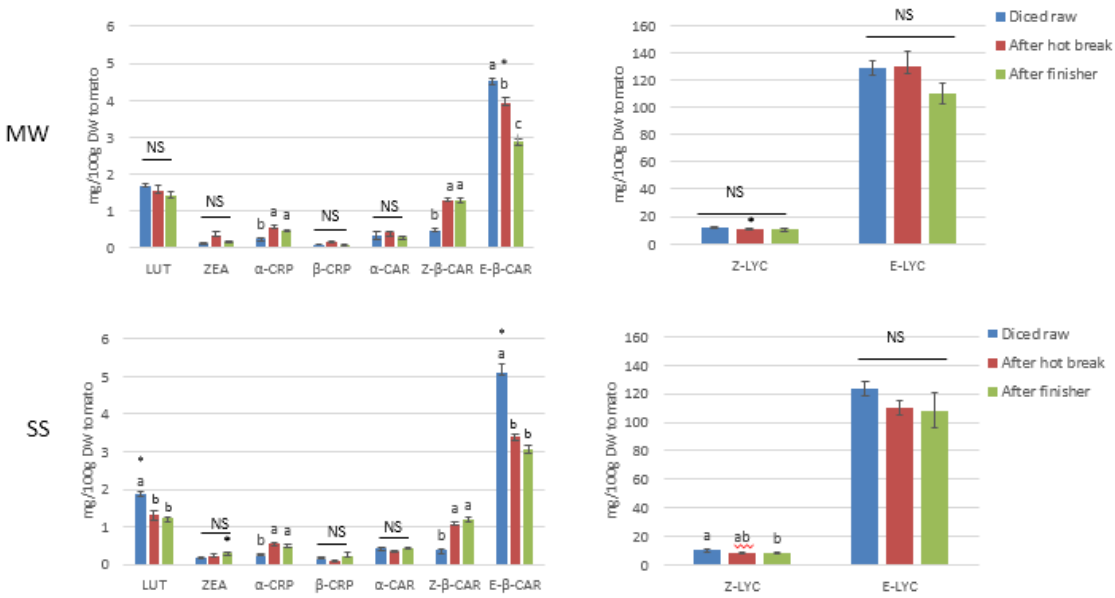

Figure 3.13. Carotenoid content (mg/100g DW) in tomato thermally treated by (A) microwave and (B) tubular heating system during processing stages: diced (fresh), after hot break and after finisher (Mean±SEM).
*Significant differences (Tukey test: $P<0.05$) in carotenoid content within processing stages (diced, hot-break, finisher) are indicated with different letters (a,b,c), whereas the symbol (*) indicates significant differences between thermal processes (MW or TUB).

3.3.2 Preliminary Study on Carotenoid Bioaccessibility Affected During Processing Stages

Relative bioaccessibility of carotenoids was assessed form tomato intermediates using an in vitro digestion model (Garrett, Failla et al. 1999). Bioaccessibility in microwave samples was enhanced after hot break, but a decrease was observed after product was passed through a finisher. Although *z* and *E*-lycopene bioaccessibility (%micellarization) showed a significant decrease of 1% and 19% respectively, after finisher compared to hot break. The relative loss of bioaccessibility ranged from 15% to 50% after finisher compared to hot break samples.

In tubular samples, the relative carotenoid bioaccessibility was consistently enhanced through the processing stages, a total increase from 4.3 to 21.8 percent micellarization for *z*-lycopene and 1.2 to 2.7 percent micellarization for *E*-lycopene after finisher in comparison to diced tomato. Increase in bioaccessibility of other carotenoid compounds was observed including for lutein (20% diced to 44% micellarization after finisher) (Figure 3.14).

The increase in absolute bioaccessibility of lycopene in tubular samples after finisher suggests a more extensive destruction of peel and tomato tissue observed by preliminary scanning electron microscopy (Figure A.3-A.4).

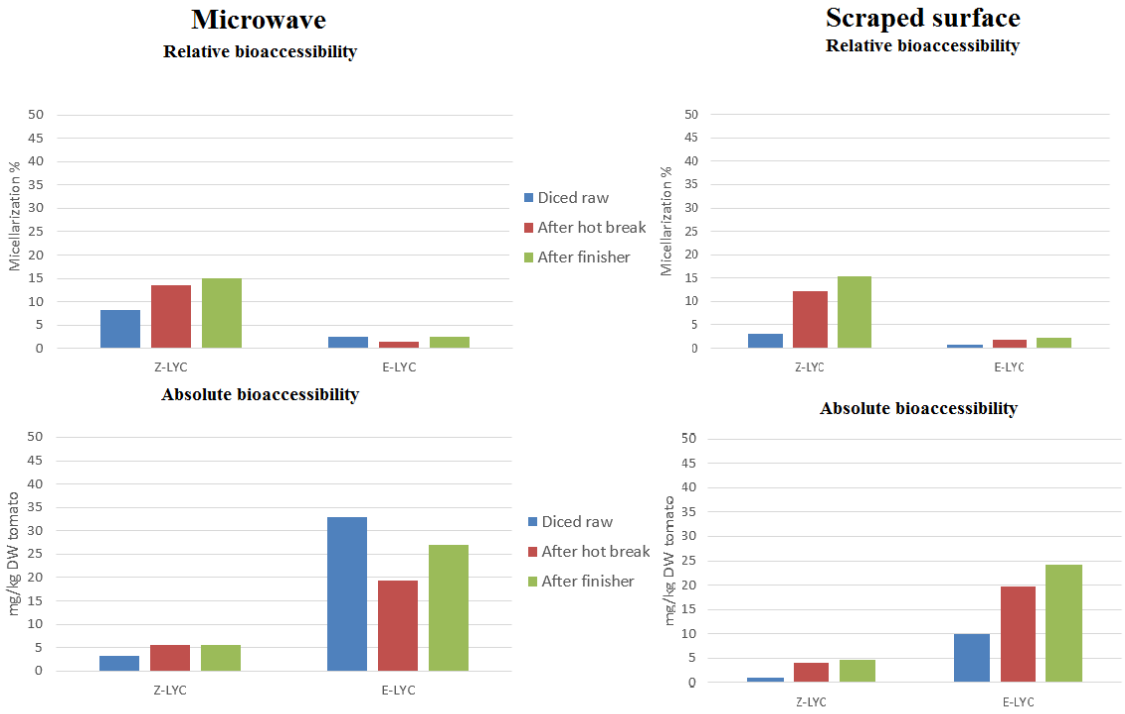

Figure 3.14. Relative bioaccessibility (micellarization percentage) and absolute bioaccessibility (mg/kg DW) of lycopene from tomato intermediate product after microwave and scraped surface heating system in different processing stages: diced (fresh), after hot break and after finisher.
*Data represent the mean of two independent *In vitro* digestions.

Absolute bioaccessibility (mg of bioaccessible carotenoids per kg tomato) was highest in microwave hot break tomatoes and scraped surface treated and finished tomatoes. No differences in lycopene absolute bioaccessibility were observed in microwave treated samples after the finisher, although changes in lutein and *E*-β-carotene were observed suggesting a potential partitioning of carotenoid materials through the finisher or extraction of soluble fibers through the finisher that could impair carotenoid bioaccessibility, whereas a trend showing an increasing in absolute bioaccessibility after finisher on samples processed samples was observed (Palafox-Carlos, Ayala-Zavala et al. 2011) (Figure 3.15).

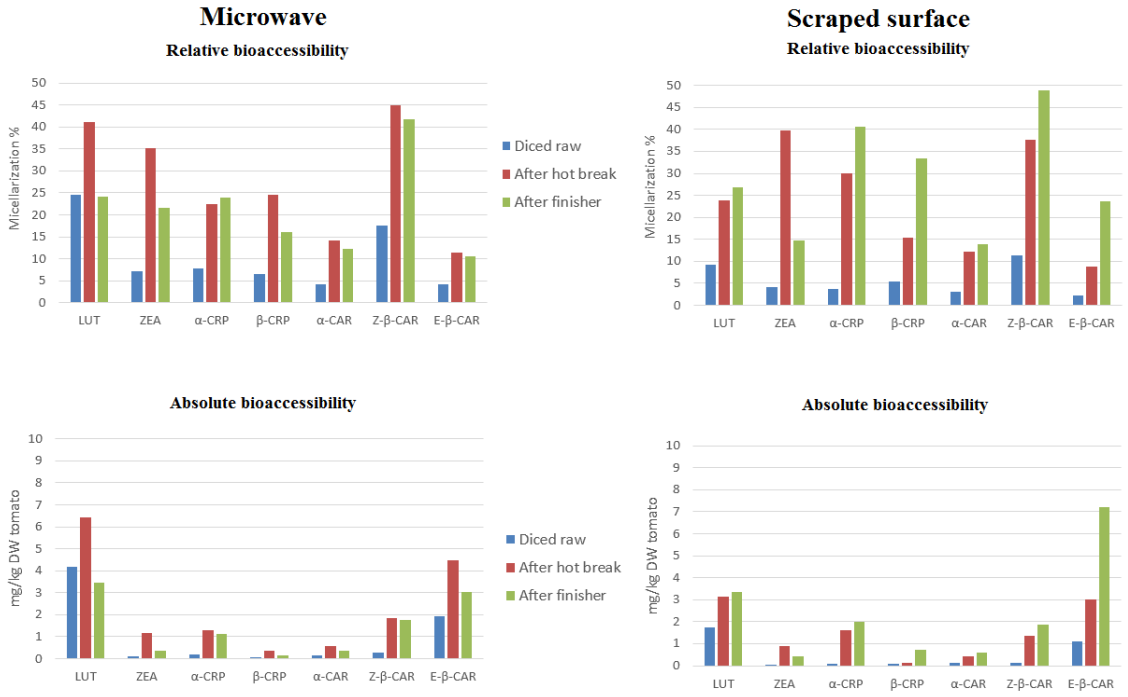

Figure 3.15. Relative bioaccessibility (micellarization percentage) and absolute bioaccessibility (mg/kg DW) of carotenoids from tomato intermediate product after microwave and scraped surface heating system in different processing stages: diced (fresh), after hot break and after finisher.
*Data represent the mean of two independent *In vitro* digestions.

CHAPTER 4. DISCUSSION

Both production and consumption of fruits and vegetables has increased globally in the past 10 years (FAO 2013). In developed countries, this is due in part to the increased awareness and scientific research supporting the health benefits related to the regular consumption of fruits and vegetables. (Krauss, Eckel et al. 2000, Sesso, Gaziano et al. 2003, Liu 2004, Ellinger, Ellinger et al. 2006). These benefits are believed to be due to a combined effect of the nutritive and non-nutritive constituents in plants, specifically the phytochemicals (Rao and Agarwal 1998, Dimitrios 2006, Reboul, Richelle et al. 2006, Canene-Adams, Lindshield et al. 2007). While promising, consumption of fruits and vegetables among Americans still remains below the amount recommended in the Dietary Guidelines for Americans 2010 (USDA 2010). One potential strategy to improve fruit and vegetable consumption is development of new fruit and vegetable based products that provide improved flavor, quality and nutritional content compared to traditional processed products.

Traditional thermal processing techniques have been widely applied in fruit and vegetable products for reduction of microbial load (Breidt Jr, Kay et al. 2013), preservation of nutritional quality (Zenker, Heinz et al. 2003, Awuah, Ramaswamy et al. 2007) and shelf life extension (Gould 1996, Sepulveda, Góngora-Nieto et al. 2005, Mosqueda-Melgar, Raybaudi-Massilia et al. 2008).

It is well known that exposure of fruits and vegetables to thermal treatments may change the physico-chemical, nutritional and organoleptic properties of the final product. Several studies have reported changes in total phytochemical content and their bioavailability after processing (Asami, Hong et al. 2003, Anthon, Diaz et al. 2008, Oey, Lille et al. 2008, Roy, Juneja et al. 2009). Similarly, a decrease in vitamins and nutrient after traditional thermal processing has been observed by several investigators (Abushita, Daood et al. 2000, Gahler, Otto et al. 2003). Consumer demand for "fresh like" characteristics in processed products has stimulated interest in new processing technologies to achieve benefits of thermal processing (safety, shelf life and long term preservation of nutritional quality) but deliver improved color, flavor and textural attributes more aligned with freshly prepared products. In addition, improvements in stability of biologically active phytochemicals due to processing would be desirable. Novel thermal processing systems including microwave heating technology which allows for rapid heating in continuous mode have been the subject of intense investigation for application to high value fruit and vegetable products (Cañumir, Celis et al. 2002, Zhang, Tang et al. 2006, Picouet, Landl et al. 2009). Several benefits have been reported in the past such as increase in polyphenolic content, no effect in antioxidant activity and no overall color changes after processing, suggesting a high quality retention by microwave processing (de Ancos, Cano et al. 1999, Brewer and Begum 2003, Hart, Menzies et al. 2007, Steed, Truong et al. 2008, Demırhan and Özbek 2011). While promising technology, very little is known about the impact of this technology on the impact of color, texture and phytochemical stability/bioaccessibility from commonly consumed and economically important high value fruits and vegetables such as apples and tomatoes.

The overall objective of this project was to assess the impact of traditional (tubular or scraped surface heating) and microwave processing on the quality of high value processed apple (sauce) and tomato (puree) products. The impact of a continuous microwave heating system on product quality attributes (color, viscosity) as well as phytochemical content, stability and bioaccessibility (in select products) were assessed and compared to traditional processing treatments.

4.1 Comparison of Microwave Processing on Color Stability in Apple and Tomato Products

Color can be defined as the brain interpretation of the signals sent from cells in the retina, obtained by the impact of the wavelengths of light in the visual spectrum (Francis 1995). Color influences acceptability, preference of foods and ultimately, choice by the consumers. Color also influences the overall perception of sensory characteristics such as sweetness, salt and overall flavor (Clydesdale 1993). While color can be used to estimate chemical composition of fruits and vegetables (Francis 1995), it is generally used as a direct estimate of quality of a fruit and vegetable product.

Color can be evaluated by descriptive sensory analysis (Villarino, Dy et al. 2007) as well as instrumental techniques including RGB (red, green and blue) and Hunter LAB with L*(white=100 to black=0) a*(green(-) and red(+)) b*(blue(-) and yellow (+)) (Jha, Chopra et al. 2007), trying to mimic the human eye response (Abbott 1999).

In the current study, the impact of processing on color of finished products was assessed by Hunter LAB colorimeter immediately before and after processing and then at predetermined time intervals over 52 weeks and 24 weeks of storage for apples and tomatoes (4 weeks for samples at 50°C), respectively. In applesauce products, initial color was found to differ between traditional and microwave processing treatments by higher L*, a*, b* values (5%, 3% and 6%) compared to tubular heated samples. The lower L* (slightly darker color) in microwave heated samples may have resulted of a non-uniform heating of the sample as the heating rates are different for various locations with center points absorbing more energy than the surface points (Deng, Singh et al. 2003), and faster degradation of oxidative compounds (Kus, Gogus et al. 2005) as result of the volumetric heating effect of microwaves occurring in the applesauce (Oliveira and Franca 2002).

In general, color degradation in applesauce is primarily driven by non-enzymatic browning (NEB). In the case of apples NEB would include oxidative reactions associated with the degradation of polyphenols, ascorbic acid and other oxidative sensitive compounds that would degrade leading to the formation of large brown pigments (Garza, Ibarz et al. 1999, Roig, Bello et al. 1999, Queiroz, Mendes Lopes et al. 2008, Picouet, Landl et al. 2009). These NEB reactions are accelerated at high storage temperatures and over time (Buedo, Elustondo et al. 2000, Selen Burdurlu and Karadeniz 2003). The color stability of both microwave and tubular heated samples when stored at 4°C compared to room temperature (RT) or 50^{o}C is consistent with these degradative processes (Figure 3.1).

Similar color degradation (browning), has been shown consistently in several fruit purees (Ahmed, Shivhare et al. 2000, Ahmed and Shivhare 2001, Ahmed and Shivhare 2001, Ahmed, Shivhare et al. 2002, Ahmed, Shivhare et al. 2002, Ahmed and Ramaswamy 2004, Ahmed, Shivhare et al. 2004, Tsanova-Savova, Ribarova et al. 2005, Chutintrasri and Noomhorm 2007).

Changes in color of applesauce over 52 weeks of storage were observed to be similar between microwave and traditional tubular processes regardless of storage temperature. While tubular samples seem to be more sensitive to color degradation over time, this may be a result of the large difference in color observed just following process and at initial storage time. Sensitivity over time can be a result of higher ascorbic acid degradation and production of reactive carbonyls compounds forming brown pigments such as 5-hydroxymethylfurfural (5-HMF) (Damasceno, Fernandes et al. 2008) as a result of the high reducing sugar content of the applesauce (Bengoechea, Sancho et al. 1997) and the higher degree of severity of heating likely applied in less efficient tubular heating (Roig, Bello et al. 1999, Buedo, Elustondo et al. 2000, Ibarz, Pagan et al. 2000, Apaiah and Barringer 2001).

Therefore, while initial color of microwave products was darker (L* value of 54.2 compared to 56.8 in tubular heated samples) the less severe heat treatment may have had created less 5-HMF accumulation in the sample resulting in overall color stability over storage for microwave processing (de Ancos, Cano et al. 1999, Brewer and Begum 2003, Selen Burdurlu and Karadeniz 2003).

While this mechanism was not directly investigated, the lack of significant differences in polyphenol profiles between microwave and tubular treated samples (Figure 3.8-3.10) would be indicative of alternative substrate consumption, such as sugars or ascorbic acid, in generation of brown color.

In tomato puree, color degradation has also been reported to follow first order kinetics (Nisha, Singhal et al. 2011). Similarly, NEB reactions have been reported to contribute to loss of color and generation of brown off colors. However, oxidative degradation of lycopene, the primary pigment in tomato, is also a key marker of tomato color degradation and quality loss (Arias, Lee et al. 2000, Apaiah and Barringer 2001, Bicanic, Dimitrovski et al. 2010). In the present study, samples processed by tubular heating generally showed a significant ($P<0.05$) decrease in color (measured as ΔE) over time compared to microwave processed samples (Figure 3.7). Consistent with first order processes, color in all samples was found to be more stable at 4°C compared to higher temperatures. Interestingly, even with higher color degradation in microwave compared to tubular processed samples, the decrease in lycopene content was not significant during the first 4 weeks of the study regardless of storage temperature (Figure 3.11).

Further, the decrease in *E*-lycopene and *z*-lycopene was not significantly ($P<0.05$) different between both heating systems. Moreover, color changes were not related to the carotenoid content as showed in previous studies in which the color degradation was correlated to the loss and degradation of carotenoids during processing and storage (Avila and Silva 1999).

Arias et al. (2000) showed the relationship of L*, a* and b* values with lycopene content in Laura tomatoes at maturity stage, an increase in a* and b* values was observed as the result of lycopene and β-carotene synthesis, while a decrease in L* value was observed with an increase in lycopene concentration. More recent studies have shown linear correlation of chroma C* value with *trans*-lycopene concentration in tomato homogenates (Bicanic, Dimitrovski et al. 2010).

These results suggest that other chemical reactions, including additional NEB processes may have contributed to color degradation in our study, since carotenoid stability was similar between traditional tubular and microwave processed products. However, it is important to point out that while color changes in microwave samples were significantly higher than tubular, these changes are still relatively modest (ΔE of 3.6). Even though color changes were observed in both microwave and tubular heated samples, low ΔE have been reported to have not been perceive by human eye, some authors have reported little detection of color changes in green vegetables at 3.5 ΔE by sensory panels (Gnanasekharan, Shewfelt et al. 1992) although without color changes other appearance factors influence in consumer preferences (Mor-Mur and Yuste 2003).

Accelerated shelf life testing in food has been used in the past to predict shelf life at lower temperatures than tested (Hough, Garitta et al. 2006) by the relationship of the temperature used and the reaction rate over time (Lee and Krochta 2002). Accelerated shelf life test are based on Arrhenius equation where *k* is temperature-dependent meaning that the higher the temperature the higher degradation level (Pedro and Ferreira 2006). In our study, accelerated storage conditions were applied (50°C for 4 weeks).

These conditions appear to be generally predictive of changes occurring when product is stored at RT during one year in applesauce for both microwave and tubular systems (Figure 3.3). Our results are thus in agreement with other authors whom using lineal model and Arrhenius have been able to predict shelf life of several food products (Petersen, Tønder et al. 1998, Lee and Krochta 2002, Garitta, Hough et al. 2004).

Overall, results from the current study thus suggest that color stability in applesauce is promoted by the lower storage temperature, with higher stability when stored at 4°C. Although microwave heated samples presented lower color values as compared to tubular heated samples, applesauce processed by microwave heating system showed more subtle color changes over time compared to tubular heated samples, suggesting that microwave heating system may better retained color characteristics in applesauce compared to tubular heating system. On the other hand, tomato puree color results suggest that microwave heated samples may be slightly less stable over time, showing higher color changes compared to tubular heated samples, with major changes when stored at high temperatures (RT and 50°C). Although significant changes were observed in both applesauce and tomato puree, the color degradation ($\Delta E < 7$) may not be perceived by human eye.

4.2 Comparison of Microwave Processing on Rheology of Applesauce and Tomato Product

Rheology is defined as the study of the deformation of a solid under the action of a stress (force per unit area) and flow of a liquid in response to stress (Borwankar 1992).

Assessment of rheological properties of fruit purees are important in product development, design and evaluation of unit processes.

Rheology describes textural characteristics of the food which impact directly on the consumer perception as well as stability, portioning and convenience (Fischer and Windhab 2011). Fruit purees are pseudoplastic fluids that exhibit a yield stress (Tabilo-Munizaga and Barbosa-Cánovas 2005) and their rheological changes are used to control quality in finished food products (Espinosa, To et al. 2011).

Consistency, on the other hand, refers to the ability of a semi liquid solid to hold the liquid section in suspension. The Bostwick consistometer is used to evaluate consistency and to observe flowing and liquid separation of food products (Tehrani and Ghandi 2007). Bostwick consistency measurements are commonly used as part of quality assurance testing during processing of apples and tomato products and its results can be directly related to apparent viscosity (Milczarek and McCarthy 2006). Consistency is one of the most important quality attributes in applesauce and tomato products, affecting its appearance and mouth feel (Schijvens, Vliet et al. 1998, Bayod, Willers et al. 2008).

Rheological properties, including viscosity and consistency of fruit purees can be affected by total solids, total soluble solids, particle size and temperature (Rao 1977) as well as by processing conditions, treatments applied and storage temperatures and time (Usiak, Bourne et al. 1995, Schijvens, Vliet et al. 1998, Espinosa, To et al. 2011, Espinosa-Muñoz, Symoneaux et al. 2012).(Marsh, Buhlert et al. 1980, Ahmed, Shivhare et al. 2000, Ahmed and Ramaswamy 2004, Tehrani and Ghandi 2007). In tomato concentrates, consistency, has been reported to decrease as the °Brix increased (Tehrani and Ghandi 2007).

Further, when food materials are pureed through finer screens the surface area increases, such as in the production of applesauce or tomato puree. By decreasing particle size, larger resistance to flow is created which typically results in increased viscosity (Ahmed, Shivhare et al. 2000).

In the present study, applesauce samples processed by microwave heating showed lower apparent viscosity relative to tubular processed products (Figure 3.4). This may be related to differences in extraction, solubilization and/or stability of pectic polysaccharide content in ripe apples that can act as strengthener of the cellulose network preventing deformation and disorganization of cellular structure (Redgwell, Curti et al. 2008).

Degradation of pectin may also contribute to differences in viscosity between processing methods in apples. Residual pectin methylesterase (PME) activity after thermal treatment has been demonstrated regardless of the temperatures achieved in process for several fruit products (De Sio, Dipollina et al. 1995, Castaldo, Servillo et al. 1996, Castaldo, Laratta et al. 1997). In apple and apricot purees residual PME activity about 10^{-3} U/mL have been found (Castaldo, Laratta et al. 1997). While pectinase activities were not measured in these products, it is plausible that microwave processing may not have been optimized to inactivate these enzymes as efficiently as the tubular process. However, in our study no change in soluble solids content was observed in any samples of applesauce or tomato puree during storage. This would suggest that if pectic enzymes remained active they were likely degrading soluble pectin and not releasing additional materials.

In contrast to applesauce results, tomato puree processed by tubular heating system showed lower apparent viscosity compared to microwave processed samples when assessed at high shear rates (Figure 3.9). Several factors may be related to this observation including the longer effective thermal treatment in tubular processed samples relative to microwave samples. This longer effective processing time may affect the rigidity of the particles and promote the degradation of cell walls and consequently degrading and solubilizing pectins or extraction of other components through the microwave heating process. Pectic substances can be further hydrolyzed by heat making cell walls less rigid and smaller (Tanglertpaibul and Rao 1987). Espinosa, To et al. (2011) reported that an increase in duration of thermal treatment applied to apple puree, resulted in decrease of particle size, and by extension viscosity. While complete analysis for pectic substances needs to be completed, differences in heating efficiency could therefore explain the observed differences in apparent viscosity between microwave and tubular processes.

It is also plausible that the decrease in apparent viscosity over time in both applesauce and tomato puree is likely related to the structural breakdown of these molecules (Balestra, Cocci et al. 2011), and PME thermo-resistance (De Sio, Dipollina et al. 1995). Further, the slight increase in viscosity observed in applesauce after 8 weeks at 4°C and 20 weeks at RT, regardless the processing method used (Figure 3.4), may be simply a result of additional release of soluble pectin. PME activity would decrease viscosity over time. No increase in apparent viscosity of applesauce stored at 50°C and tomato puree at all storage temperatures (Figures 3.4 and 3.9) was observed.

However, this may be due to either degradation of pectic substances (in the case of applesauce) or the shorter storage time of tomato samples (24 versus 52 weeks). Similar effects were noted by Usiak, Bourne et al. (1995) whom reported a decrease of thickness of applesauce during the first three months of storage at room temperature followed by a slight increase over the next three months.

As the system starts flowing from low to high shear rate the network is eventually disrupted by the stress applied into apparent aggregates (Bayod, Månsson et al. 2007). The results from tomato puree at high shear rate suggest that structure of tubular heated samples was more susceptible to disruption under shear, probably as a result of irreversible changes in fiber orientation during longer heat exposure on tubular heating system (Rao and Cooley 1992, Marti, Höfler et al. 2005), suggesting that microwave heating system may not have had as severe a treatment to fiber network as direct relationship between fiber level and viscosity have been reported in the past by Switzer III and Klingenberg (2003). Our results are also in concordance with Bayod, Månsson et al. (2007) whom found higher deformation with the increase in shear rate on tomato products after homogenization.

Overall these results suggest that, both applesauce and tomato puree samples, when processed by microwave heating system has a different effect of the structure of apples and tomato tissues potentially impacting the release and/or the stability of rheologicaly important structural molecules, such as pectin. The results suggest that under the current, non-optimized conditions, tubular heated samples maintained better consistency and viscosity over time compared to samples processed by microwave heating system.

4.3 Impact of Microwave and Tubular Processing on Phytochemical Content of Apple and Tomato Products

Polyphenols and carotenoids have been linked to the prevention of chronic and degenerative diseases (Negri, La Vecchia et al. 1991, Scalbert and Williamson 2000) by virtue of their anti-oxidant and anti-inflammatory actions (Gardner, White et al. 2000, Wojdyło, Oszmiański et al. 2008, Bellion, Digles et al. 2010). These associations have stimulated interest in the presence of these compounds in fruits and vegetable products, their stability through food processing and ultimate bioavailability from food products.

Apples are believed to provide a significant portion (>20%) of the total daily polyphenols in the U.S. diet (Vinson, Su et al. 2001). Apples are a rich source of polyphenols due to its high procyanidins content (Oszmianski, Wolniak et al. 2007) and are the second common fruit with highest phenolic concentration and antioxidant activity with 272.1mg/100g and 97.6µmol/g just behind cranberries (Sun, Chu et al. 2002). Chlorogenic acid, 4-*p*-coumaroylquinic acid, procyanidin B2 and epicatechin are the major phenolics compounds in apples (Kahle, Kraus et al. 2005). The health benefits from apple and apple products consumption have been widely reported (Boyer and Liu 2004, Gerhauser 2008, Adyanthaya, Kwon et al. 2010). On the other hand, tomatoes are the second most produced and consumed vegetable in the U.S. (Willcox, Catignani et al. 2003). Tomatoes are also a rich source of dietary phytochemicals including carotenoids, tocopherols, ascorbic acid and flavonoids. Around 20 carotenoids have been characterized in tomatoes but lycopene is found in highest concentration (80%-90%) (Shi, Kakuda et al. 2004).

Tomatoes are the main dietary source of lycopene and their consumption have been related to reduction in the incidence of several chronic diseases including cancer, and cardiovascular disorders (Clinton 1998, Agarwal and Rao 2000, Willcox, Catignani et al. 2003).

4.3.1 Processing Effect on Polyphenolic Content of Apple Product

Phenolics and polyphenolic compounds were the main phytochemical class targeted for assessment in applesauce products. Previous studies did not report changes in phenolic content during applesauce processing but instead changes were observed from the processing of cloudy and clear apple juice (ław Markowski and Płocharski 2006).

In our study, differences in total phenolic content were noted between products obtained from tubular (346.7 mg/kg) and microwave heating systems (383.4mg/g) at time 0 (Figure 3.6). These differences may be attributed to slight differences in raw product characteristics as well as differences in process induced loses. Microwave heating has previously been reported to induce faster inactivation of enzymes including peroxidase (POD) and polyphenol oxidase (PPO) involved in phenolic oxidation (Matsui, Granado et al. 2007, Oszmiański, Wolniak et al. 2008, Picouet, Landl et al. 2009) resulting in reduced loses of phenolic content during processing. While unprocessed phenolic content and residual enzyme levels were not assessed in this study this remains a possibility.

While typical degradative processes including release of oxidative and hydrolytic enzymes can result in loss of phenolic acids (Chang, Lin et al. 2006), an overall increase in accessibility of phenolics for extraction would, in fact, seemed to have increased total phenolic content after processing and during increased storage time.

In accordance with these previous studies, an increase in chlorogenic acid and rutin from applesauce samples stored at room temperature and 4°C was observed in products made from both microwave a tubular heating systems (Figure 3.6). This increase in phenolic content over time can be linked to the changes in apparent viscosity observed during the study (Section 3.1.2), as the degree of phenolic crosslinking in the cell walls decreased, likely as a result PME activity and cell separation resulting in changes in textural quality of the product (Waldron, Smith et al. 1997). A general decrease in all other polyphenols analyzed was observed over time with loss occurring in a predictable first-order type process.

Losses are most likely related to similar oxidative processes that contributed to polyphenol oxidation and browning as described previously. Therefore, color degradation and polyphenol degradation in these products does not appear to be improved significantly by application of novel microwave processing methods.

Overall, results suggest that polyphenols in applesauce are more stable when the product is stored at 4°C. In samples processed by microwave and tubular heating systems, the polyphenol concentration seem to be maintained without major differences in concentration or effects between the heating systems. While microwave processed samples showed more procyanidin B1 and C1 compared to tubular heated samples, this increase is not likely impactful to overall quality.

4.3.2 Processing Effect on Carotenoid Content of Tomato Product

It has been previously reported that *E*-lycopene is the most abundant carotenoid in tomato and the significant decrease of lycopene and increase of (Z) isomers of β-carotene (<1% vs ~27%) after thermal processing (Seybold, Fröhlich et al. 2004). While the ability of thermal processing to induce isomerization of lycopene under typical processing conditions remains controversial (Nguyen and Schwartz 1998), never the less oxidative breakdown of lycopene, a process that can lead to isomerization, through processing and storage remains a constant issue for tomato nutritional/health quality as well as color and consumer driven attributes. In concordance with Seybold, Fröhlich et al. (2004), in tomato product from tomato trial #2, lycopene and β-carotene content decreased after both microwave and tubular hot-break (Figure 3.13).

Interestingly, no differences were found in *z*-lycopene content and a significant increase in *z*-β-carotene in our study is supported by previous studies suggesting that isomerization was not the main product of lycopene loss through processed tomato products (Abushita, Daood et al. 2000, Nguyen, Francis et al. 2001). While final breakdown products were not characterized in this study many possibilities do exist including apocarotenoids (lycopenoids) (Khachik, Goli et al. 1992, Lindshield, Canene-Adams et al. 2007, Kopec, Riedl et al. 2010) which may continue to impact color and thus not negatively affect ultimate color properties of the finished product.

Breakdown of lycopene in tomato through thermal processing is known to be very difficult (Colle, Van Buggenhout et al. 2010, Anese, Mirolo et al. 2012). However, the losses documented in this study may be directly related to phenomena previously described.

Lycopene is typically found in crystalline form packaged into organelles known as chromoplasts (Shi and Maguer 2000). While in crystalline state in these plant organelles, the lycopene molecule is well protected from oxidative or other degradative mechanism. A breakdown of cellular and subcellular structures during thermal processing would drive the lycopene out of the crystalline form and into an amorphous form more susceptible to long-term degradation (Colle, Van Buggenhout et al. 2010). Therefore, the loss of protection from the tomato matrix during processing may result in increasing isomerization of the compounds as well as differences in stability of the molecules during thermal treatment (Nguyen and Schwartz 1998). In addition to typical process losses, decrease in lycopene and β-carotene was found to be particularly high after the finishing step when processed by microwave heating system.

This may be due in part to the lower yield and increased loss of waste (mainly skin and seed) obtained from these samples. Skins would contain a large portion of lycopene and loss of skins from the finished products would result in lower overall lycopene content (Koh, Charoenprasert et al. 2012).

Decrease of bioactive compounds as a result of conventional thermal processing has been reported for total carotenoids in mango puree (Vásquez-Caicedo, Schilling et al. 2007, Djioua, Charles et al. 2009, Kim, Lounds-Singleton et al. 2009), whereas no significant carotenoid changes were found when microwave heating system was used (Wong and Kitts 2001, Sajilata and Singhal 2006). In contrast to these findings, samples from tomato trial #1 did not show significant difference in carotenoid content between the heating systems used (Figure 3.12).

Akanbi and Oludemi (2004) also reported higher lycopene degradation in samples stored at 40°C compared to 29°C and 35°C, with a lycopene decrease of 45%, 10% and 25% after 10 weeks of storage, respectively. Similar results were found in our study, where lycopene was more stable over time in samples at 4°C, suggesting lycopene instability at high storage temperatures, although, stability of β-carotene in tomato paste over 12 months have been previously reported at room temperature, and lycopene decrease of 40% during the first 3 months of storage, remaining stable after that period of time (Koh, Charoenprasert et al. 2012).

Overall these results suggest that lycopene stability in tomato puree are not likely affected by application of different heating system used during processing. Further, changes in stability over storage were similar between microwave and tubular processed samples.

4.4 Preliminary Assessment of Microwave and Tubular Processing on Lycopene Bioaccessibility from Tomato Products

Bioaccessibility is defined as the amount of a phytochemical released from the food matrix by normal digestion, remains stable and soluble in the gut lumen and is therefore made available for subsequent absorption by the intestinal epithelia (Goñi, Serrano et al. 2006). It is an established predictor of bioavailability in vivo (Boileau, Merchen et al. 1999, Agarwal, Shen et al. 2001), and as such, several in vitro digestive models have been applied to estimate food matrix effects on bioaccessibility of carotenoids (Van Buggenhout, Alminger et al. 2010, Knockaert, Pulissery et al. 2012) and phenolics from foods.

Understanding the behavior of phytochemicals from foods through the digestive process can lead to a better interpretation of the importance of these compounds in the human health and determination of processing and formulation factors that can be leveraged to enhance the delivery of the health promoting compounds.

Several factors can affect carotenoid bioaccessibility and bioavailability including the physical properties of the food matrix (Faulks and Southon 2005). Thermal processing in food products are usually used for preservation and increased shelf life but at the same time thermal treatment ruptures cell walls (Kunzek, Kabbert et al. 1999), and has been documented to positively impact carotenoid bioaccessibility form fruits and vegetables (Svelander, Tibäck et al. 2010, Chanforan, Loonis et al. 2012). In tomato products several studies have shown beneficial effects of thermal processing on lycopene bioaccessibility specifically (Anese, Mirolo et al. 2012, Knockaert, Pulissery et al. 2012, Page, Van Stratum et al. 2012).

Lycopene bioaccessibility has been reported to increase by up to ~200% (Svelander, Tibäck et al. 2010, Van Buggenhout, Alminger et al. 2010) through application of thermal treatments applied to tomato products. Bioaccessibility increases after treatment mainly due to the failure of cell wall materials which are fibrous and compact and interfere with bioavailability by reducing efficiency of micelle formation (Jeffery, Turner et al. 2012) as well as preventing the release of phytochemical from the food matrix. As result of the rupture, the nutritional value of tomato is increased by increase in bioaccessibility of lycopene (Dewanto, Wu et al. 2002).

In our study, bioaccessibility of carotenoids from tomato intermediate processed products (hot break and finisher) was investigated. This was due, in part, to the limited availability of evaporation capacity that did not allow us to finalize sufficient final puree product for assessment. Tomatoes after dicing, hot-break and finisher were collected in order to compare the impact of microwave versus traditional scrape-surface hot break on lycopene bioaccessibility. Similar thermal loads were applied between treatments and the only differences in the processes were the mechanism of thermal treatment (Figure 3.15).

Consistent with previous reports on impact of processing (Colle, Van Buggenhout et al. 2010, Svelander, Tibäck et al. 2010, Knockaert, Pulissery et al. 2012) lycopene bioaccessibility was found to increase after both microwave and tubular hot break. However, after the finisher, tubular heated samples showed an increase in lycopene bioaccessibility, whereas, no changes were observed in microwave heated samples (Figure 3.14). While microwave results are in concordance with Ryan, O'Connell et al. (2008), who did not find changes in lycopene micellarisation after microwave cooking, the differential effect of scraped surface compared to microwave process was interesting.

Although decrease in lutein, zeaxanthin and *E*-β-carotene bioaccessibility was observed after finisher in microwave heated samples, whereas these compounds increased their bioaccessibility in scraped surface heated samples, the increase of carotenoids bioaccessibility from scraped surface heated samples after finisher may be a result of the physical disruption of the food matrix and protein denaturation during the exposure to high temperatures for longer time (Ryan, O'Connell et al. 2008).

The results suggest that a softer tissue with higher extractability levels is obtained from scraped surface heated samples which became even easier to extract after finisher.

In contrast microwave processed samples may not have had the thermal extraction/breakdown of tomato fibers. These results are consistent with viscosity results observed from the same samples (Figure 3.8 and 3.9). Higher apparent viscosity of microwave treated samples at high shear may be related to decreased accessibility of carotenoids, in part due to the nature and amount of soluble fiber present in the system. Soluble fiber has been documented to prevent the release and solubilization of the lipophilic compounds by the bile salts in the small intestinal digestion (Colle, Van Buggenhout et al. 2010, Palafox-Carlos, Ayala-Zavala et al. 2011). These results can be linked back to apparent viscosity results from tomato test #1 where microwave heated samples showed higher viscosity at high shear rate, probably as a result of less damage to the fiber strength network during processing (Section 3.2.2), as digestion of lipophilic molecules is affected by rheological properties of the food (McClements, Decker et al. 2008, Porrini and Riso 2008, Pérez-Jiménez, Serrano et al. 2009) .

Although relative bioaccessibility after finisher of lycopene and *z* and *E*-β-carotene was slightly higher in tubular samples. Isomerization from *E*-lycopene to *z*-lycopene between 5% and 20% after thermal treatment have been reported (Qiu, Jiang et al. 2006, Heredia, Peinado et al. 2010) probably the longer exposure time in tubular heating system created a more severe cell and organelle membrane damage and carotenoids isomerization (Abushita, Daood et al. 2000, Lemmens, Van Buggenhout et al. 2010).

These changes in β-carotene content do not cause a significant impact in total carotenoid content in tomato puree as its presence in the product represent ~5% of the total carotenoids.

Overall these results suggest that thermal treatment increased the carotenoid bioaccessibility in tomato products, where the heating system used did not show significant ($P<0.05$) effect on increase relative bioaccessibility from these compounds. Slightly higher lycopene bioaccessibility was observed from scraped surface processed samples as a result of more severe damage of the cell microstructure suggesting that a less fresh product was obtained. This would suggest that optimized microwave processing may be used to deliver a more “fresh like” product, although that would translate to a lower bioavailability for health promoting phytochemicals such as lycopene.

CHAPTER 5. CONCLUSSIONS AND FUTURE WORK

The increased awareness of the nutritional content and health benefits from fruits and vegetables consumption has stimulated interest in the effect of processing techniques on the final product. Conventional thermal processing of fruits and vegetables are known to produce color and viscosity changes, as well as decrease the phytochemical content after exposure to a combination of high temperatures and longtime intervals. Although thermal processing effects vary depending on the food matrix, as well as fruit variety and maturity stage, strategies to minimize changes in quality during processing are being investigated by using emerging processing technologies such as microwave heating systems. This novel processing technology has been observed to maintain and/or enhance shelf life by ensuring sufficient microbial and enzyme inactivation during processing, but, at the same time, maintaining the quality and nutritional characteristics of the product.

The overall objective of this study was to assess the impact of traditional and microwave processing on the quality of apple and tomato based products due to the importance of these two commodities in the US, and economic and the nutritional value provided by them.

In order to achieve this objective applesauce and tomato puree were processed in the Purdue University pilot plant by both heating systems and subjected to 52 weeks and 24 weeks of storage study respectively, trying to mimic regular industrial processing conditions. Because appearance and textural properties are an important factor in the quality of the food products, as consumers based their preferences and buying decisions on the physical characteristics of the product, color, consistency and apparent viscosity were measured in both products applesauce and tomato puree.

The first objective of this study was to compare the quality characteristics: color, consistency, apparent viscosity and phytochemical content of applesauce processed by tubular and microwave heating system over storage conditions.

In this part of the study it was observed that color degradation in both applesauce and tomato puree are driven by non-enzymatic browning (Bengoechea, Sancho et al. 1997, Damasceno, Fernandes et al. 2008). Initial applesauce color was found to differ between tubular and microwave heated samples, where tubular heated samples showed higher lightness after processing, whereas in tomato puree, samples obtained from tubular heating process were darker compared to microwave heated samples. The initial color differences between heating systems in applesauce became insignificant over time, as the color degradation for tubular samples over time was higher than microwave heated samples which showed more stable color with less total color differences (ΔE) during storage.

Applesauce processed by tubular heated samples was observed to be thicker and have higher apparent viscosity than microwave heated samples at both low and high shear rate.

The results from the applesauce study showed a similar decrease in polyphenols content within tubular and microwave heating systems. Most abundant polyphenol in applesauce was chlorogenic acid, followed by epicatechin, procyanidin B2, C1 and B1. Although no significant differences in polyphenol content were observed between the heating system a slightly higher total polyphenol content by LC-MS (383.4mg/kg DW) was observed in microwave heated samples compared to tubular heated samples (346.7mg/kg DW). During storage, an increase on chlorogenic acid, quercetin-3-glucoside and rutin content was observed over time, these increases were in similar proportions between the heating systems.

The heating system used during processing was not to a determinant factor in polyphenol changes in applesauce over time; storage temperature was the main factor influencing degradation reactions, where storage at 4°C was the most effective condition to minimize polyphenols changes over time in both microwave and tubular heated samples.

High correlation of total color differences was observed between accelerated storage study at 50°C to storage at room temperature for 52 weeks in applesauce for samples processed by both microwave (R^2=0.98) and tubular (R^2=0.96) heating system.

Even though, the heating system used did not cause differences in polyphenol content on applesauce, similar degradation behavior at higher temperatures and increasing in extraction yield over have been previously reported (Chang, Lin et al. 2006, Steed, Truong et al. 2008).

The second objective of this study was to compare the quality characteristics: color, consistency, apparent viscosity and phytochemical content of tomato puree processed by scraped surface/tubular and microwave heating system over storage conditions, as well as compare carotenoid content and bioaccessibility in tomato intermediate process product from microwave and scraped surface heating systems.

Contrary to the results obtained from the applesauce study, the color seem to be more stable over time on tomato puree processed by tubular, where microwave heated samples got darker after processing and showed higher total color differences during storage. The color degradation in tomato puree from this study was not related to lycopene degradation as reported in the past (Arias, Lee et al. 2000, Brewer and Begum 2003, Bicanic, Dimitrovski et al. 2010, Stinco, Rodríguez-Pulido et al. 2013). In both, applesauce and tomato puree, the color was most stable when product was stored at 4°C, and results from samples at 50°C were comparable to storage at room temperature. Although total color differences obtained may not be high enough to be perceived by the human eye.

No differences between heating systems in consistency and apparent viscosity at low shear rate were observed in tomato puree, although at high shear rate microwave heated samples showed higher apparent viscosity, probably as a result of less fiber damage during processing, which is interacting with the carotenoids and preventing the release and assimilation of these compounds during digestion and preventing deformation at high shear rate during rheological tests (De Sio, Dipollina et al. 1995, Balestra, Cocci et al. 2011).

The results from tomato puree study showed a similar decrease of carotenoids after processing, where the total carotenoid content of the final product was similar between microwave (127.7mg/100g) and tubular (141.8mg/100g) heated samples. It was also observed that thermal processing did not cause lycopene isomerization; whereas an increase in β-carotene isomers was observed for both heating system as reported in the past by Seybold, Fröhlich et al. (2004), Abushita, Daood et al. (2000) and Nguyen and Schwartz (1998). It was observed that lycopene degradation during storage was not influenced by the storage temperature used. The preliminary bioaccessibility showed a increase of lycopene bioaccessibility after thermal processing was expected as reported in the past by several authors (Saura-Calixto, Serrano et al. 2007, Colle, Van Buggenhout et al. 2010, Lemmens, Van Buggenhout et al. 2010, Jeffery, Turner et al. 2012, Page, Van Stratum et al. 2012), from this part of the study an increase of lycopene bioaccessibility after thermal processing regardless the heating system used was observed, although a steady slight increase was noted in tubular heated samples after finisher, whereas microwave heated samples bioaccessibility remained unchanged.

The findings in apparent viscosity from tomato puree can be linked to carotenoid bioaccessibility preliminary study results, as microwave heated samples showed slightly low bioaccessibility probably as a result of more tight fiber network and less damage of cell walls, preventing the release of carotenoids for absorption during digestion.

Taken together the results from this study it was observed that applesauce processed by microwave heating system resulted in more stable color over compared to tubular heated samples, whereas tomato puree processed by tubular heating system were observed to have less color differences over time.

The consistency and apparent viscosity in applesauce was not improved by microwave heating system, whereas in tomato puree better rheological properties are observed only at high shear rate.

Microwave heating system in applesauce and tomato puree does not minimize phytochemical degradation during processing and storage, although slightly higher lycopene bioaccessibility from tomato puree processed by tubular heating system.

The trend of increased bioaccessibility by scraped surface after finisher in tomato puree suggest more severe microstructure damage caused by scraped surface heating system, and less damage in dietary fiber by microwave heating system, resulting in less fresh product compared to microwave heated samples.

For a better understanding of the effect of microwave heating system in foods, a comparison of optimized microwave and traditional process in quality characteristic of applesauce and tomato puree. It is necessary to preform analysis for detection of pectin methylesterase which can be responsible of viscosity changes, as well as dietary fiber content which could be related with the low carotenoid bioaccessibility from microwave heated samples. It may be also beneficial to determine changes in flavor profiles and odor active volatiles thru storage time and correlate them with results from obtained from trained sensory panel, to certainly conclude which heating system is better minimizing quality changes in food products.

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APPENDIX

APPENDIX

Table A.1. Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at 4°C during 52 weeks in applesauce thermally treated by microwave and tubular heating system.

Time 0						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.2±0.5b	7.67±0.1b	35.8±0.6b	36.6±0.6b	77.9±0.0b	0.00±0.0a
TUB	56.8±0.4a	7.9±0.2a	37.9±0.3a	38.7±0.4a	78.3±0.3a	0.00±0.0a
1 Week						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	55.0±0.5a	7.3±0.1a	34.7±0.7a	35.4±0.7a	78.1±0.2b	1.5±0.8b
TUB	56.1±0.3a	7.2±0.1a	35.0±0.4a	35.7±0.4a	78.4±0.2a	3.1±0.4a
2 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	56.0±0.7a	7.0±0.1a	33.7±0.6a	34.4±0.7a	78.2±0.1a	2.9±1.0b
TUB	56.5±0.5a	7.1±0.0a	34.2±0.4a	34.9±0.4a	78.2±0.2a	3.9±0.3a
4 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	53.3±0.7a	7.3±0.1a	33.8±0.9a	34.6±0.9a	77.8±0.3a	2.2±0.9a
TUB	54.9±0.5a	7.3±0.0a	35.2±0.5a	35.9±0.5a	78.2±0.1a	3.4±0.7a
8 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.5±0.3b	6.9±0.1a	34.7±0.6a	35.4±0.6a	78.8±0.1a	1.4±0.4b
TUB	56.4±1.0a	6.9±0.2a	33.0±0.8b	33.7±0.8b	78.2±0.1b	5.1±0.9a
20 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.0±0.1a	7.0±0.1a	32.3±0.1a	33.0±0.1a	77.7±0.2a	3.6±0.1b
TUB	54.6±0.5a	7.0±0.3a	32.7±0.6a	33.5±0.6a	77.9±0.2a	5.8±0.5a
34 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	53.2±0.8a	7.5±0.2a	33.7±0.3a	34.5±0.3a	77.5±0.3a	2.4±0.5b
TUB	53.0±0.2a	7.7±0.1a	34.1±0.1a	34.9±0.1a	77.3±0.2a	5.5±0.1a
52 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	52.8±0.7a	7.7±0.3a	31.7±0.1a	32.6±0.1a	76.4±0.5a	4.3±0.2b
TUB	53.0±0.6a	7.6±0.1a	31.6±0.8a	32.5±0.7a	76.6±0.4a	7.4±1.2a

^Mean±Standard deviation (n=3). Significant differences (Tukey test: P <0.05) for color values between thermal treatments are indicated with different letters (a,b).

Table A.2- Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at room temperature (~22°C) during 52 weeks in applesauce thermally treated by microwave and tubular heating system^.

Time 0						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.2±0.5b	7.66±0.1b	35.78±0.6b	36.6±0.6b	77.9±0.0b	0.00±0.0a
TUB	56.8±0.4a	7.87±0.2a	37.90±0.3a	38.7±0.4a	78.3±0.3a	0.00±0.0a
1 Week						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.2±0.6b	7.5±0.2a	35.3±0.7a	36.1±0.6a	77.9±0.5a	0.9±0.2b
TUB	56.6±0.7a	7.1±0.0b	34.1±0.7a	34.8±0.7a	78.2±0.2a	4.4±0.6a
2 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	54.3±0.7a	7.3±0.2a	35.2±0.7a	36.0±0.7a	78.3±0.6a	1.0±0.5b
TUB	55.0±0.1a	7.4±0.0a	36.3±0.1a	37.0±0.1a	78.4±0.0a	2.5±0.2a
4 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	53.9±0.4a	7.1±0.1b	34.7±0.3a	35.4±0.3a	78.5±0.2a	1.4±0.3b
TUB	54.4±0.1a	7.4±0.0a	34.6±0.4a	35.4±0.4a	77.9±0.1b	4.1±0.4a
8 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	52.2±0.2a	7.0±0.1b	32.8±0.3a	33.6±0.3a	77.9±0.1a	3.7±0.4a
TUB	52.6±1.4a	7.6±0.2a	34.3±1.4a	35.1±1.4a	77.4±0.7a	5.6±2.0a
20 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	50.5±0.6a	8.2±0.1a	32.8±0.2b	33.8±0.2b	75.9±0.1a	4.9±0.6b
TUB	51.4±0.6a	8.6±0.3a	34.3±0.4a	35.4±0.4a	76.0±0.5a	6.6±0.7a
34 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	48.6±1.0b	8.7±0.2a	32.0±0.5a	33.2±0.4b	74.7±0.6a	6.9±1.1a
TUB	49.5±0.6a	9.0±0.1a	33.2±0.6a	34.4±0.6a	74.8±0.4a	8.9±0.8a
52 Weeks						
	L*	a*	b*	Chroma	Hue angle	Delta E
MW	47.6±0.3a	8.8±0.1b	31.7±0.3a	32.9±0.2a	74.5±0.3a	7.9±0.4b
TUB	47.7±0.9a	9.2±0.1a	33.9±0.8a	33.2±0.7a	74.0±0.4a	11.0±1.2a

^Mean±Standard deviation (n=3). Significant differences (Tukey test: P <0.05) for color values between thermal treatment are indicated with different letters (a,b).

Table A.3 - Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at 50°C during 4 weeks in applesauce thermally treated by Microwave and Tubular heating system^.

Time 0						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	54.2±0.5b	7.7±0.1b	35.8±0.6b	36.6±0.6b	77.9±0.0b	0.00±0.0a
TUB	56.8±0.4a	7.9±0.2a	37.9±0.3a	38.7±0.4a	78.3±0.3a	0.00±0.0a
1 Week						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	52.9±0.3b	7.6±0.1a	34.4±0.1b	35.2±0.1b	77.6±0.2a	1.9±0.3b
TUB	55.0±0.4a	8.0±0.3a	34.9±0.2a	35.8±0.2a	77.1±0.5a	3.5±0.2a
2 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	50.3±1.5a	8.5±0.4a	32.9±1.2a	34.0±1.1a	75.5±1.1a	4.9±2.0a
TUB	51.7±1.1a	8.7±0.2a	34.4±0.8a	35.5±0.7a	75.8±0.5a	6.2±1.3a
4 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	46.5±2.1a	9.9±1.1a	31.4±1.1a	32.9±0.9a	72.5±1.2a	9.2±2.4a
TUB	48.3±0.8a	9.9±0.2a	31.5±0.6a	33.1±0.5a	72.5±0.5a	10.9±1.0a

^Mean±Standard deviation (n=3). Significant differences (Tukey test: $P < 0.05$) for color values between thermal treatments are indicated with different letters (a,b).

Table A.4 - Consistency of applesauce (cm/30seconds) at given time points from samples storage at room temperature, 4°C and 50°C ^.

Time (weeks)	Room temperature				4°C				50°C			
	Pulp		Liquid		Pulp		Liquid		Pulp		Liquid	
	Microwave	Tubular	Microwave	Tubular	Microwave	Tubular	Microwave	Tubular	Microwave	Tubular	Microwave	Tubular
0	6.2±0.6ab	4.8±0.5bc*	6.5±0.7ab	5.4±0.7abc*	6.2±0.6b	4.8±0.5a	6.5±0.7ab	5.4±0.7a	6.2±0.6a	4.8±0.5b	6.5±0.7ab	5.4±0.7a
1	5.7±0.6b	4.5±1.0c*	6.3±0.6ab	4.8±0.8c*	4.8±0.4c	5.3±0.3a	5.3±0.5c	5.5±0.3a	5.8±0.4a	5.8±0.0ab	6.1±0.3b	6.0±0.0a
2	6.6±0.9ab	5.3±0.4abc*	6.9±0.6ab	5.5±0.5abc*	5.9±0.4b	5.1±0.6a*	6.1±0.4bc	5.3±0.7a	6.3±0.4a	5.4±0.1ab*	6.5±0.3ab	5.8±0.0a*
4	6.8±0.3ab	4.8±0.4bc*	7.0±0.3a	4.9±0.3bc*	6.3±0.6ab	5.6±1.0a	6.7±0.5ab	5.8±1.1a	6.9±0.5a	5.9±0.1a*	7.3±0.5a	6.2±0.1a
8	5.7±0.3b	5.8±0.3abc	5.8±0.4b	5.9±0.3abc	6.3±0.3b	5.1±0.1a*	6.2±0.4bc	5.3±0.3a*	nsa	nsa	nsa	nsa
20	7.0±0.5a	6.1±0.5ab*	7.3±0.7a	6.2±0.4ab*	6.8±0.4ab	5.3±0.3a*	6.9±0.4ab	5.3±0.3a*	nsa	nsa	nsa	nsa
34	7.0±0.5a	6.3±0.5a	7.0±0.5a	6.5±0.4a	7.3±0.2a	5.3±0.3a*	7.3±0.2a	5.8±0.3a*	nsa	nsa	nsa	nsa
52	6.6±0.5ab	5.5±0.7abc*	6.3±0.3ab	5.5±0.3abc*	6.8±0.6ab	4.8±0.6a*	6.5±0.3ab	4.6±0.4a*	nsa	nsa	nsa	nsa

^Significance differences (Tukey $p<0.05$) across time points are represented by different letters (a,b,c). Differences between heating systems are present by (*).
nsa: no sample analyzed at given time points.

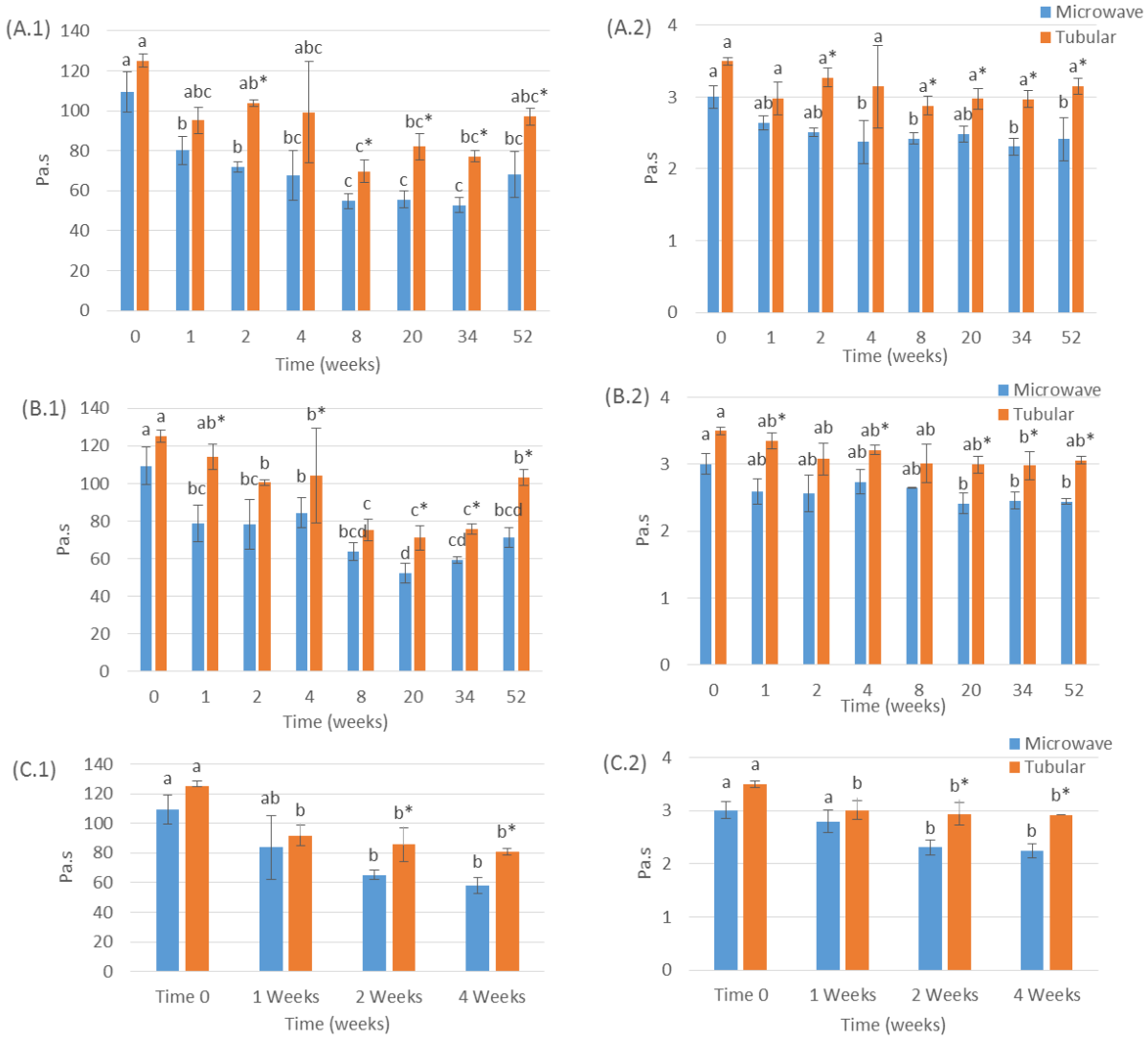

Figure A.1- Apparent viscosity (Pa.s) normalized by °Brix, at 1/s (1) and 100/s (2) shear rate in tomato puree processed by microwave and tubula heating system and stored at 4°C (A), room temperature (B) and 50°C (C

Table A.5- Phenolic content (mg/kg DW) of applesauce thermally treated by microwave heating system and storage given temperatures during given time points^.

4°C								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	175.0±3.6bc	3.2±0.1*	60.7±3.7a	19.3±0.9ab	30.6±1.6cd	28.9±0.7	0.6±0.09cb	65.1±3.1
1	165.6±2.4bcd	2.3±0.1	51.0±2.0b	21.4±0.4a	47.6±1.3a	26.7±4.6	0.3±0.09d	71.2±12.0
2	157.1±4.6d	1.9±0.2	41.8±2.5bc	21.2±0.8a	42.9±4.8ab	31.9±1.0	0.3±0.09d	79.0±4.0
4	161.5±4.4cd	2.1±0.0	42.3±0.8bc	20.1±0.4a	35.2±1.3bc	30.6±0.2	0.4±0.01bcd	78.3±3.4
8	165.5±3.5bcd	2.1±0.1	41.9±1.3bc	20.3±0.2a	37.3±1.7abc	30.4±0.1	0.3±0.01cd	83.0±2.4
20	166.0±2.0bcd	2.4±0.1	37.3±0.3c	17.3±0.2b	23.3±0.3ed	26.5±0.2	0.7±0.0b	62.7±0.2
34	180.0±5.2b	1.9±0.1	24.1±1.8d	17.3±0.2b	20.8±0.5e	25.2±0.0	0.6±0.1bcd	75.0±3.9
52	215.0±2.5a	2.3±0.1	33.7±1.0cd	17.8±0.1b	23.7±0.4de	25.6±0.2	1.1±0.0a	120.7±0.9*
Room temperature								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	175.0±3.6b	3.2±0.1a	60.7±3.7a	19.3±0.9a	30.6±1.6b	28.9±0.7bc	0.6±0.1ab	65.1±3.1cd
1	160.1±0.8bc	2.3±0.1b	45.8±1.6b	20.7±0.2a	44.1±0.7a	30.8±0.2a	0.4±0.1b	81.5±0.5b
2	155.3±6.5c	2.2±0.1b	40.3±1.8bc	19.7±0.1a	34.5±1.7b	30.0±0.1ab	0.5±0.2ab	79.2±1.5b
4	161.0±3.8bc	1.9±0.1bc	35.2±0.6cd	20.0±0.1a	33.4±0.7bc	29.4±0.1ab	0.4±0.1b	75.2±0.6bc
8	159.9±2.7bc	1.6±0.1c	26.5±0.7de	19.4±0.4a	28.5±0.3d	27.9±0.2c	0.4±0.1b	77.9±1.7b
20	163.8±2.1bc	1.8±0.1bc	20.6±1.2ef	16.6±0.2b	20.1±0.5d	24.4±0.3d	0.6±0.1ab	59.5±1.1d
34	164.0±5.1bc	1.5±0.1c	13.3±1.9f	16.9±0.2b	22.0±4.0cd	24.6±0.1d	0.8±0.3ab	56.5±4.5d
52	196.7±4.5a	1.8±0.1bc	17.2±2.3ef	16.8±0.1b	19.4±0.6d	24.7±0.2d	1.1±0.1a	101.0±1.6a
50°C								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	175.0±3.6*	3.2±0.1*	60.7±3.7a	19.3±0.9	30.6±1.6b	28.9±0.7	0.6±0.1a	65.1±3.1
1	156.5±2.3	2.5±0.1	42.0±0.5b	20.5±0.1	44.6±1.1a	28.6±0.2	0.4±0.0ab	79.9±2.6
2	147.9±2.5	2.3±0.2	36.3±4.7b	19.7±0.3	32.1±1.5b	28.2±0.3	0.4±0.0b	69.2±1.2
4	141.0±5.7	1.8±0.3	22.1±1.6c	19.1±0.3	19.5±1.0c	27.2±0.8	0.3±0.1b	68.1±5.1

^Mean±SEM deviation (n=3). Significant differences (Tukey test: P <0.05) during time are indicated with different letters (a,b), one significance difference across time is indicated with symbol (*).

Table A.6- Phenolic content (mg/kg DW) of applesauce thermally treated by tubular heating system and storage given temperatures during given time points.

4°C								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	163.4±2.9	2.9±0.1a	52.3±0.1a	17.1±0.1b	26.2±0.1cd	27.7±0.4bc	0.5±0.0bc	56.6±0.6d
1	164.2±5.0	2.6±0.1abc	56.6±2.3a	21.1±0.4a	57.3±2.7a	31.0±0.3a	0.4±0.0c	71.2±1.5bc
2	156.9±5.1	2.1±0.3c	49.3±2.7ab	21.4±0.9a	52.5±8.1ab	31.7±1.4a	0.3±0.1c	72.7±1.1bc
4	165.4±3.5	2.7±0.0ab	56.8±1.1a	19.6±0.5a	40.0±2.7bc	29.1±0.6ab	0.5±0.0c	71.2±1.0bc
8	168.8±3.4	2.3±0.1bc	49.8±1.4ab	19.9±0.2a	44.4±1.4ab	30.0±0.2ab	0.4±0.0c	77.0±0.8b
20	169.9±4.0	2.5±0.1abc	41.7±3.5bc	17.0±0.3b	24.2±1.3cd	25.2±0.3cd	0.6±0.0bc	56.1±1.1d
34	169.9±2.8	2.1±0.0bc	31.1±0.2d	16.8±0.1b	21.9±0.1d	24.1±0.1d	0.8±0.1b	68.3±1.8c
52	211.3±6.0*	2.2±0.2bc	33.0±1.8cd	16.7±0.1b	22.8±0.7d	24.1±0.2d	1.2±0.1a	106.2±1.0a
Room temperature								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	163.4±2.9	2.9±0.1a	52.3±0.1a	17.1±0.1b	26.2±0.1b	27.7±0.4b	0.5±0.0b	56.6±0.6d
1	164.1±1.6	2.5±0.0ab	53.2±0.2a	20.5±0.1a	47.9±0.6a	30.7±0.1a	0.3±0.0cd	75.9±1.2b
2	165.0±3.5	2.2±0.1bc	45.5±2.1ab	19.9±0.1a	41.7±0.8a	28.8±0.3ab	0.3±0.0d	69.3±3.8bc
4	157.2±1.3	2.1±0.1bc	41.5±2.4b	19.5±0.2a	36.1±2.6ab	28.9±0.4ab	0.4±0.0cd	71.9±1.5b
8	157.4±2.2	1.5±0.1d	28.6±1.4c	19.5±0.9a	37.0±6.3ab	27.9±1.0b	0.2±0.1d	72.4±4.6b
20	156.8±1.3	1.9±0.2cd	24.6±3.1cd	16.3±0.3b	20.9±1.0c	23.6±0.3c	0.5±0.0bc	51.1±0.4d
34	164.2±1.0	1.8±0.1cd	18.2±1.5d	16.2±0.2b	19.5±0.5c	23.3±0.1c	0.6±0.0b	59.6±1.6cd
52	194.2±1.4*	1.8±0.2cd	16.6±2.0d	16.0±0.1b	18.6±0.5c	23.1±0.1c	1.1±0.0a	89.1±1.0a
50°C								
Time (weeks)	**CLG**	**CAT**	**EPI**	**PB1**	**PB2**	**PC1**	**RTN**	**QCT-3-GL**
0	163.4±2.9a	2.9±0.1	52.3±0.1a	17.1±0.1b	26.2±0.1bc	27.7±0.4a	0.5±0.0*	56.6±0.6ab
1	156.0±1.5a	2.7±0.1	48.8±3.3a	19.8±0.4a	48.1±3.5a	27.3±0.9ab	0.3±0.0	68.0±1.0a
2	156.7±1.9a	2.5±0.2	37.6±3.6ab	19.1±0.2a	34.6±1.8b	26.3±0.1ab	0.4±0.0	60.2±2.5ab
4	137.5±5.2b	2.1±0.2	24.1±3.5b	17.7±0.3b	19.6±2.4c	25.2±0.2b	0.3±0.0	53.8±3.3b

^ Mean±SEM deviation (n=3). Significant differences (Tukey test: P <0.05) during time are indicated with different letters (a,b), one significance difference across time is indicated with symbol (*).

Table A.7- Phenolic content (mg/kg DW) of applesauce thermally treated by microwave and tubular heating system and storage at 50°C during 4 weeks^.

Time 0								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	175.0±3.6	3.2±0.1	60.7±3.7	19.3±0.9	30.6±1.6	28.9±0.7	0.6±0.09	65.1±3.1
TUB	163.4±2.9	2.9±0.1	52.3±0.1	17.1±0.1	26.2±0.1	27.7±0.4	0.5±0.0	56.6±0.6
1 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	156.5±2.3	2.5±0.1	42.0±0.5	20.5±0.1	44.6±1.1	28.6±0.2	0.4±0.0*	79.9±2.6*
TUB	156.0±1.5	2.7±0.1*	48.8±3.3	19.8±0.4	48.1±3.5	27.3±0.9	0.3±0.0	68.0±1.0
2 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	147.9±2.5	2.3±0.2	36.3±4.7	19.7±0.3	32.1±1.5	28.2±0.3*	0.4±0.0	69.2±1.2*
TUB	156.7±1.9*	2.5±0.2	37.6±3.6	19.1±0.2	34.6±1.8	26.3±0.1	0.4±0.0	60.2±2.5
4 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	141.0±5.7	1.8±0.3	22.1±1.6	19.1±0.3*	19.5±1.0	27.2±0.8	0.3±0.1	68.1±5.1
TUB	137.5±5.2	2.1±0.2	24.1±3.5	17.7±0.3	19.6±2.4	25.2±0.2	0.3±0.0	53.8±3.3

^Mean±Std error of the mean (n=3). Significant differences (Tukey test: P <0.05) in phenolic content between thermal treatments are indicated with symbol (*).

Table A.8- Phenolic content (mg/kg DW) of applesauce thermally treated by microwave and tubular heating system and storage at 4°C during 52 weeks^.

Time 0								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	175.0±3.6	3.2±0.1	60.7±3.7	19.3±0.9	30.6±1.6	28.9±0.7	0.6±0.09	65.1±3.1
TUB	163.4±2.9	2.9±0.1	52.3±0.1	17.1±0.1	26.2±0.1	27.7±0.4	0.5±0.0	56.6±0.6
1 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	165.6±2.4	2.3±0.1	51.0±2.0	21.4±0.4	47.6±1.3	26.7±4.6	0.3±0.09	71.2±12.0
TUB	164.2±5.0	2.6±0.1	56.6±2.3	21.1±0.4	57.3±2.7*	31.0±0.3	0.4±0.0	71.2±1.5
2 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	157.1±4.6	1.9±0.2	41.8±2.5	21.2±0.8	42.9±4.8	31.9±1.0	0.3±0.09	79.0±4.0
TUB	156.9±5.1	2.1±0.3	49.3±2.7	21.4±0.9	52.5±8.1	31.7±1.4	0.3±0.1	72.7±1.1
4 Weeks								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	161.5±4.4	2.1±0.0	42.3±0.8	20.1±0.4	35.2±1.3	30.6±0.2	0.4±0.01	78.3±3.4
TUB	165.4±3.5	2.7±0.0*	56.8±1.1*	19.6±0.5	40.0±2.7	29.1±0.6	0.5±0.0*	71.2±1.0
8 Weeks								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	165.5±3.5	2.1±0.1	41.9±1.3	20.3±0.2	37.3±1.7	30.4±0.1	0.3±0.01	83.0±2.4
TUB	168.8±3.4	2.3±0.1	49.8±1.4*	19.9±0.2	44.4±1.4*	30.0±0.2	0.4±0.0*	77.0±0.8
20 Weeks								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	166.0±2.0	2.4±0.1	37.3±0.3	17.3±0.2	23.3±0.3	26.5±0.2*	0.7±0.0	62.7±0.2*
TUB	169.9±4.0	2.5±0.1	41.7±3.5	17.0±0.3	24.2±1.3	25.2±0.3	0.6±0.0	56.1±1.1
34 Weeks								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	180.0±5.2	1.9±0.1	24.1±1.8	17.3±0.2	20.8±0.5	25.2±0.0*	0.6±0.1	75.0±3.9
TUB	169.9±2.8	2.1±0.0	31.1±0.2*	16.8±0.1	21.9±0.1	24.1±0.1	0.8±0.1	68.3±1.8
52 Weeks								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	215.0±2.5	2.3±0.1	33.7±1.0	17.8±0.1*	23.7±0.4	25.6±0.2*	1.1±0.0	120.7±0.9*
TUB	211.3±6.0	2.2±0.2	33.0±1.8	16.7±0.1	22.8±0.7	24.1±0.2	1.2±0.1	106.2±1.0

^Mean±Std error of the mean (n=3). Significant differences (Tukey test: P <0.05) in phenolic content between thermal treatments are indicated with symbol (*).

Table A.9- Phenolic content (mg/kg DW) of applesauce thermally treated by microwave and tubular heating system and storage at room temperature during 52 weeks^.

Time 0								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	175.0±3.6	3.2±0.1	60.7±3.7	19.3±0.9	30.6±1.6	28.9±0.7	0.6±0.1	65.1±3.1
TUB	163.4±2.9	2.9±0.1	52.3±0.1	17.1±0.1	26.2±0.1	27.7±0.4	0.5±0.0	56.6±0.6
1 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	160.1±0.8	2.3±0.1	45.8±1.6	20.7±0.2	44.1±0.7	30.8±0.2	0.4±0.1	81.5±0.5*
TUB	164.1±1.6	2.5±0.0	53.2±0.2*	20.5±0.1	47.9±0.6*	30.7±0.1	0.3±0.0	75.9±1.2
2 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	155.3±6.5	2.2±0.1	40.3±1.8	19.7±0.1	34.5±1.7	30.0±0.1*	0.5±0.2	79.2±1.5
TUB	165.0±3.5	2.2±0.1	45.5±2.1	19.9±0.1	41.7±0.8*	28.8±0.3	0.3±0.0	69.3±3.8
4 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	161.0±3.8	1.9±0.1	35.2±0.6	20.0±0.1	33.4±0.7	29.4±0.1	0.4±0.1	75.2±0.6
TUB	157.2±1.3	2.1±0.1	41.5±2.4	19.5±0.2	36.1±2.6	28.9±0.4	0.4±0.0	71.9±1.5
8 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	159.9±2.7	1.6±0.1	26.5±0.7	19.4±0.4	28.5±0.3	27.9±0.2	0.4±0.1	77.9±1.7
TUB	157.4±2.2	1.5±0.1	28.6±1.4	19.5±0.9	37.0±6.3	27.9±1.0	0.2±0.1	72.4±4.6
20 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	163.8±2.1*	1.8±0.1	20.6±1.2	16.6±0.2	20.1±0.5	24.4±0.3	0.6±0.1	59.5±1.1*
TUB	156.8±1.3	1.9±0.2	24.6±3.1	16.3±0.3	20.9±1.0	23.6±0.3	0.5±0.0	51.1±0.4
34 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	164.0±5.1	1.5±0.1	13.3±1.9	16.9±0.2	22.0±4.0	24.6±0.1*	0.8±0.3	56.5±4.5
TUB	164.2±1.0	1.8±0.1	18.2±1.5	16.2±0.2	19.5±0.5	23.3±0.1	0.6±0.0	59.6±1.6
52 Week								
	CLG	CAT	EPI	PB1	PB2	PC1	RTN	QCT-3-GL
MW	196.7±4.5	1.8±0.1	17.2±2.3	16.8±0.1*	19.4±0.6	24.7±0.2*	1.1±0.1	101.0±1.6*
TUB	194.2±1.4	1.8±0.2	16.6±2.0	16.0±0.1	18.6±0.5	23.1±0.1	1.1±0.0	89.1±1.0

^Mean±Std error of the mean (n=3). Significant differences (Tukey test: P <0.05) in phenolic content between thermal treatments are indicated with symbol (*).

Table A.10- Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at 4°C during 24 weeks in tomato puree thermally treated by microwave and tubular heating system^.

Time 0						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	30.21±0.5a	29.58±0.5b	27.05±1.0b	40.08±1.1b	42.43±0.6b	0.00±0.0a
SCR	28.84±0.0b	30.95±0.2a	30.96±0.4a	43.77±0.2a	45.01±0.5a	0.00±0.0a
4 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	30.21±0.9a	29.76±0.8b	27.09±1.9b	40.24±1.9b	42.26±1.3b	1.56±1.4a
SCR	29.07±0.1b	31.71±0.1a	31.21±0.2a	44.49±0.1a	44.54±0.3a	0.86±0.1a
11 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.52±0.1b	31.23±0.1b	30.89±0.1a	43.92±0.1b	44.68±0.1a	4.44±0.1a
SCR	29.05±0.1a	31.66±0.1a	30.95±0.3a	44.27±0.2a	44.36±0.3b	0.79±0.1b
24 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.26±0.1b	30.75±0.1b	30.71±0.1a	43.46±0.1a	44.97±0.1a	4.31±0.1a
SCR	28.98±0.3a	31.35±0.3a	30.55±0.4a	43.78±0.5a	44.26±0.2b	0.78±0.3b

^Mean±Standard deviation (n=3). Significant differences (Tukey test: P <0.05) for color values between thermal treatments are indicated with different letters (a,b).

Table A.11- Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at room temperature during 24 weeks in tomato puree thermally treated by microwave and tubular heating system^.

Time 0						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	30.21±0.5a	29.58±0.5b	27.05±1.0b	40.08±1.1b	42.43±0.6b	0.00±0.0a
SCR	28.84±0.0b	30.95±0.2a	30.96±0.4a	43.77±0.2a	45.01±0.5a	0.00±0.0a
4 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.71±0.0b	31.55±0.0a	30.85±0.0b	44.12±0.0b	44.36±0.0b	4.54±0.0a
SCR	28.89±0.1a	31.75±0.2a	31.35±0.0a	44.62±0.1a	44.63±0.2a	0.90±0.2b
11 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.52±0.1b	31.07±0.1b	30.55±0.1a	43.57±0.1b	44.52±0.1a	4.17±0.1a
SCR	29.00±0.1a	31.46±0.1a	30.72±0.2a	43.97±0.1a	44.32±0.2a	0.61±0.1b
24 Weeks						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.30±0.0b	30.72±0.0b	29.93±0.0b	42.89±0.0b	44.26±0.0a	3.64±0.0a
SCR	28.56±0.0a	31.14±0.0a	30.42±0.0a	43.53±0.1a	44.33±0.1a	0.64±0.0b

^Mean±Standard deviation (n=3). Significant differences (Tukey test: P <0.05) for color values between thermal treatments are indicated with different letters (a,b).

Table A.12- Color values (Reflectance measurements L*, a*, b*, H°, C* and Delta E) at 50°C during 4 weeks in tomato puree thermally treated by microwave and tubular heating system^.

Time 0						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	30.21±0.5a	29.58±0.5b	27.05±1.0b	40.08±1.1b	42.43±0.6b	0.00±0.0a
SCR	28.84±0.0b	30.95±0.2a	30.96±0.4a	43.77±0.2a	45.01±0.5a	0.00±0.0a
1 Week						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.45±0.0b	31.18±0.1b	31.32±0.1b	44.19±0.2b	45.12±0.0a	4.89±0.1a
SCR	28.71±0.0a	31.41±0.1a	31.54±0.1a	44.55±0.1a	45.06±0.1a	0.79±0.1b
2 Week						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.06±0.0b	30.77±0.1b	30.93±0.0b	43.62±0.1b	45.15±0.0a	4.59±0.0a
SCR	28.63±0.1a	31.02±0.1a	31.17±0.0a	43.97±0.1a	45.15±0.1a	0.32±0.1b
4 Week						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**Delta E**
MW	28.02±0.0b	30.66±0.1b	29.97±0.1b	42.87±0.1b	44.34±0.0b	3.81±0.1a
SCR	28.33±0.1a	30.90±0.1a	30.91±0.1a	43.70±0.1a	45.00±0.2a	0.53±0.1b

^Mean±Standard deviation (n=3). Significant differences (Tukey test: $P < 0.05$) for color values between thermal treatments are indicated with different letters (a,b).

Table A.13- Consistency of tomato puree (cm/30seconds) at given time points from samples storage at room temperature, 4°C and 50°C ^.

Time (weeks)	Room temperature			
	Pulp		Liquid	
	Microwave	Tubular	Microwave	Tubular
0	5.5±0.0a	5.7±0.3a	5.5±0.0a	5.7±0.3a
4	5.0±0.0b	5.3±0.3a	5.0±0.0b	5.3±0.3a
11	5.0±0.0b	5.3±0.3a	5.0±0.0b	5.5±0.0a*
24	4.5±0.0c	5.2±0.3a*	5.0±0.0b	5.7±0.3a*
Time (weeks)	4°C			
	Pulp		Liquid	
	Microwave	Tubular	Microwave	Tubular
0	5.5±0.0a	5.7±0.3a	5.5±0.0a	5.7±0.3a
4	4.7±0.3b	5.5±0.0a*	5.0±0.0b	5.7±0.3a*
11	5.2±0.3a	5.2±0.3ab	5.2±0.3ab	5.2±0.3a
24	4.5±0.0b	4.8±0.3b	5.0±0.0b	5.3±0.3a
Time (weeks)	50°C			
	Pulp		Liquid	
	Microwave	Tubular	Microwave	Tubular
0	5.5±0.0ab	5.7±0.3a	5.5±0.0b	5.7±0.3a
1	5.1±0.3b	5.0±0.0b	5.1±0.3b	5.7±0.3a*
2	5.4±0.3ab	5.6±0.3a	5.4±0.3b	6.0±0.0a*
4	5.7±0.3a	6.0±0.0a	6.2±0.3a	6.0±0.0a

^Significance differences (Tukey $p<0.05$) across time points are represented by different letters (a,b,c). Differences between heating systems are present by (*).

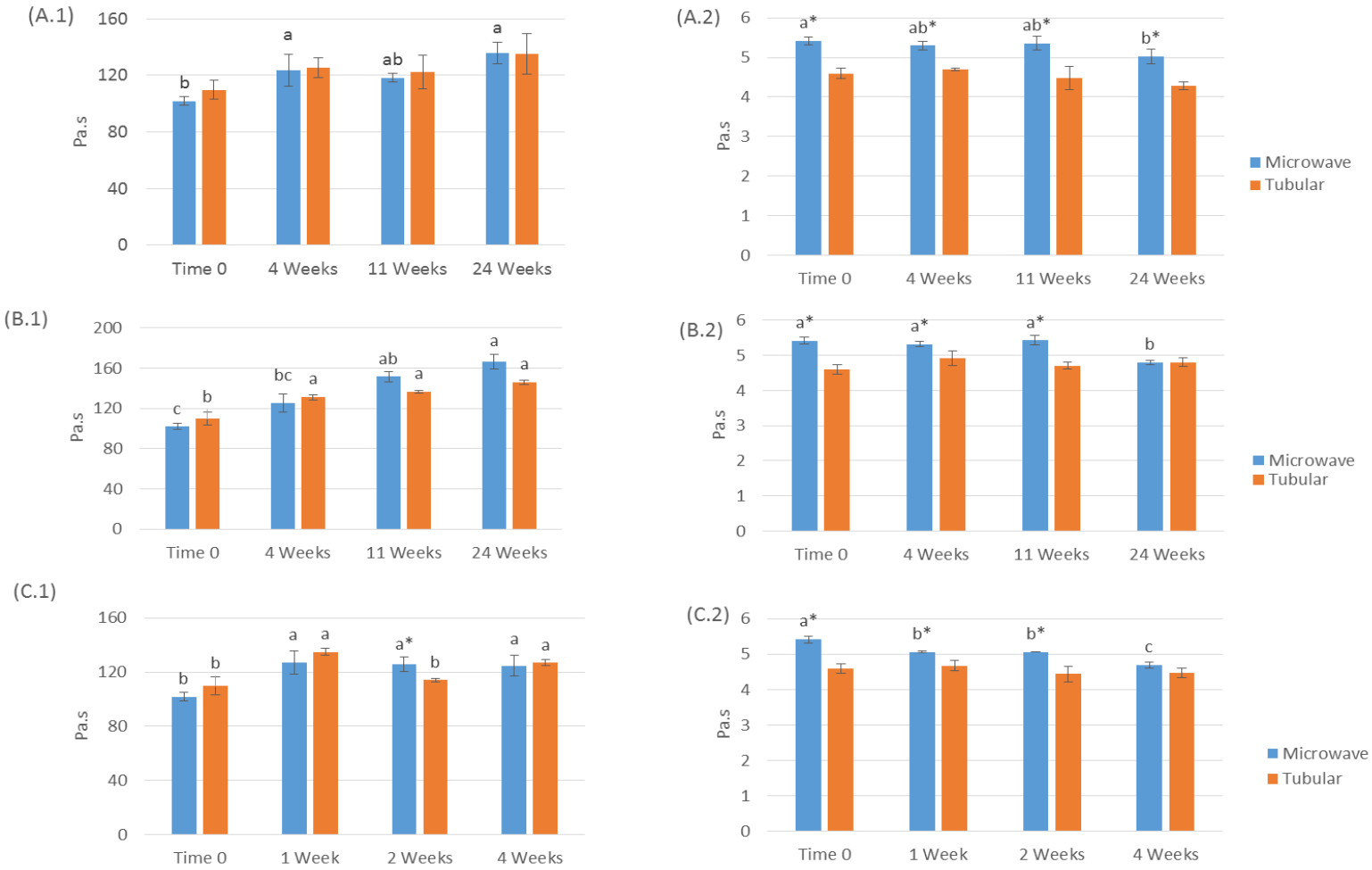

Figure A.2- Apparent viscosity (Pa.s) normalized by °Brix, at 1/s (1) and 100/s (2) shear rate in tomato puree processed by microwave and tubula heating system and stored at 4°C (A), room temperature (B) and 50°C (C).

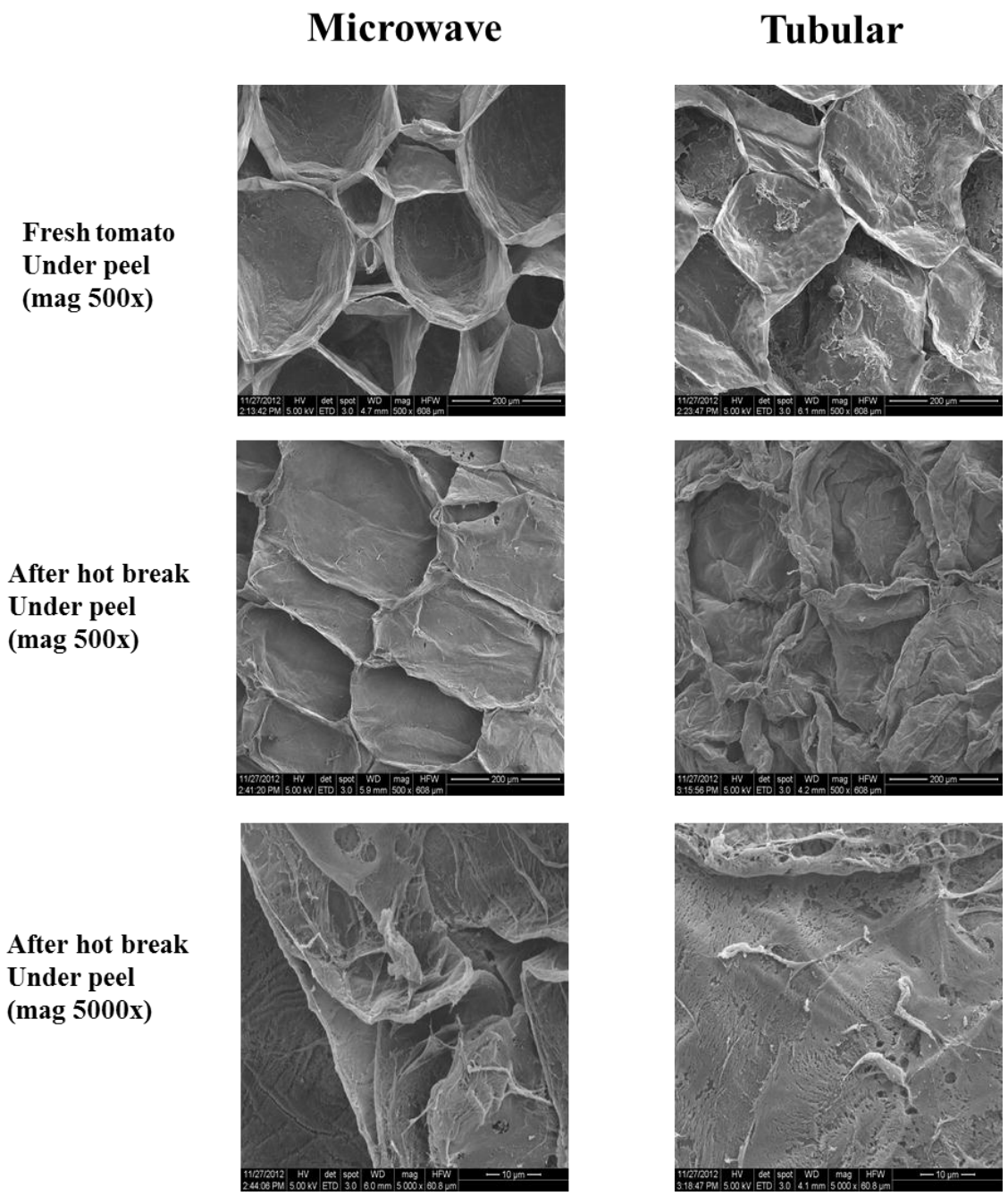

Figure A.3- Scanning Electron Microscopy image of tomato peels after microwave and tubular hot break. Samples were treated by chemical fixation prior analysis.

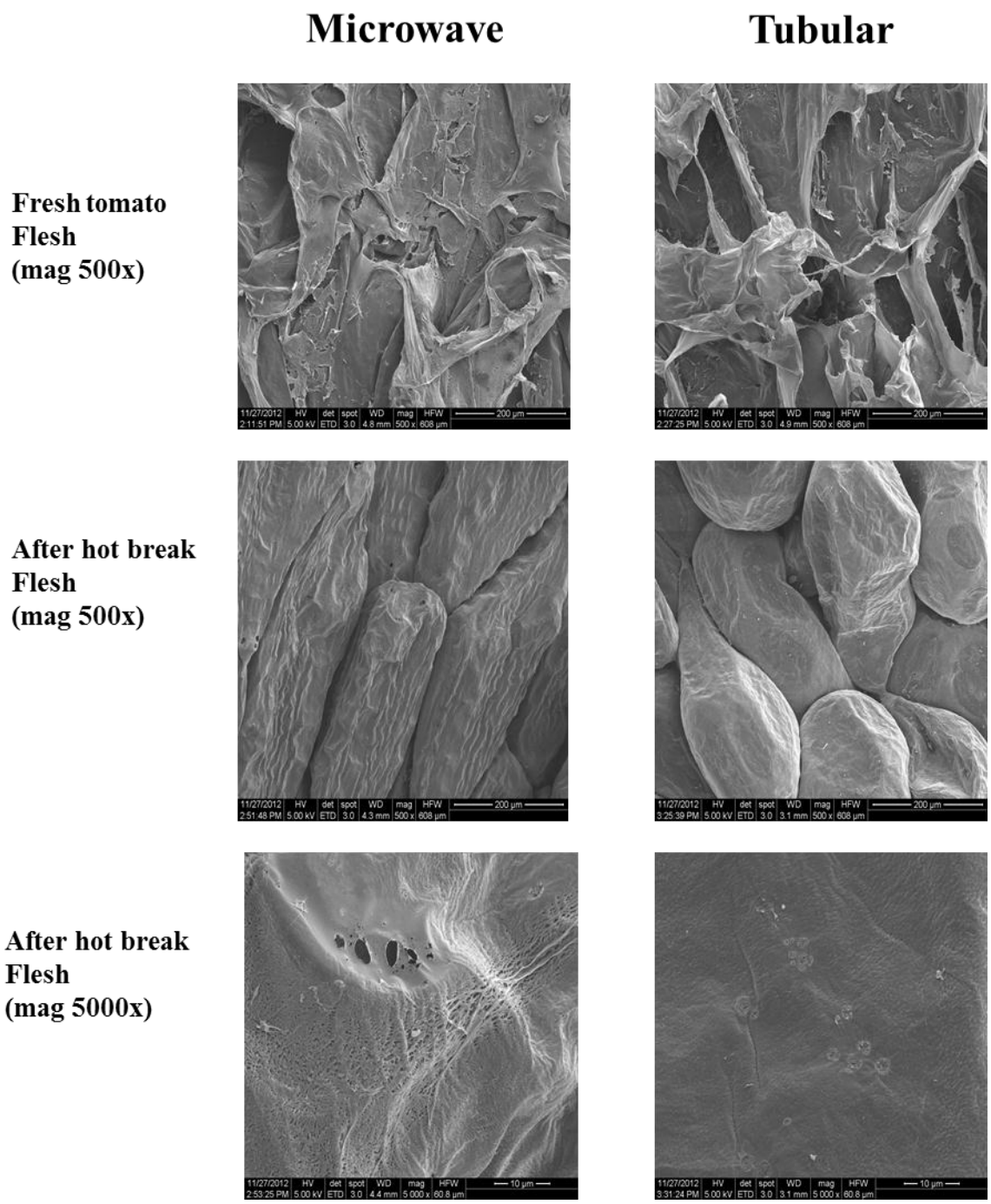

Figure A.4. Scanning Electron Microscopy image of tomato flesh after microwave and tubular hot break. Samples were treated by chemical fixation prior analysis.