Rapid Assessment of Quality Parameters in Processing Tomatoes Using Handheld and
Bench-top Infrared Spectrometers and Multivariate Analysis

THESIS

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Abstract

Two portable infrared sensors were evaluated for the rapid determination of quality parameters in processing tomatoes. A total of 370 hot-break juices were prepared from ~40 processing tomato varieties grown in 5 California counties. The levels of sugars (glucose and fructose), acids (citric, malic, and glutamic), soluble solids, titratable acidity, and pH in these juices were determined using standard reference methods. For FT-IR analysis, juices were centrifuged, filtered through Whatman® filter (0.45µm), and directly applied to the FT-IR crystal (15-40µL) to obtain spectra. Partial least squares regression (PLSR) was used to generate correlation models, both calibration and validation, between the component concentration and the sample spectra. The PLS validation models showed good ability in estimating the sugars, acids and especially soluble solids in tomato for both the transmission DialPath portable system and bench-top unit using a triple-bounce ATR, judging by the high correlation coefficient of validation ($R_{\text{val}} > 0.80$) and low standard error of predictions (SEP). Prediction errors of 1.3 g/L for estimation of sugars (fructose, glucose), 0.22 °Brix, and 0.22 g/L for glutamic and citric acids were determined using the 1800-900cm⁻¹ infrared region. The IR portable unit may provide the tomato processing industry with an efficient method for in-field, high throughput quantification of quality parameters in tomatoes.

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Rapid Assessment of Quality Parameters in Processing Tomatoes using Portable Infrared
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Table of Contents

Abstract	ii
Acknowledgments.....	iii
Vita.....	iv
Publications.....	iv
Fields of Study	iv
List of Tables	ix
List of Figures	x
Chapter 1: Literature Review	1
1.1 The tomato crop industry	1
1.1.1 Taxonomy, origin, history, and uses of tomato	1
1.1.2 Overview of worldwide tomato industry.....	2
1.1.3 Tomato industry in the U.S.A.....	3
<i>1.1.3.1 Fresh tomato industry.....</i>	<i>4</i>
<i>1.1.3.2 Processing tomato industry</i>	<i>5</i>
1.2 Tomato Physiology	6
1.2.1 Fruit Characteristics.....	7

1.2.1.1 <i>Composition</i>	8
1.2.2 <i>Nutrients</i>	10
1.2.2.1 <i>Sugars</i>	11
1.2.2.2 <i>Organic and Amino Acids</i>	12
1.2.2.3 <i>Vitamins and Antioxidants</i>	14
1.3 <i>Quality Characteristics</i>	15
1.3.1 <i>Quality assessment of processing tomatoes</i>	15
1.3.2 <i>Traditional quality control techniques</i>	17
1.4 <i>Infrared spectroscopy</i>	18
1.4.1 <i>Equipment and Tools for Mid-Infrared Spectroscopy Analysis</i>	19
1.4.1.1 <i>Transmission Techniques</i>	22
1.4.1.2 <i>Attenuated Total Reflectance (ATR)</i>	23
1.4.1.2 <i>Multivariate analysis and chemometrics</i>	24
1.4.2 <i>Current Technologies</i>	26
1.4.2.1 <i>Portable spectrometers</i>	27
1.4.2.2 <i>Transmission accessories</i>	28
1.4.2.2 <i>Reflectance accessories</i>	29
1.5 <i>References</i>	31

Chapter 2: Rapid Assessment of Quality Parameters in Processing Tomatoes Using Handheld and Bench-top Infrared Spectrometers and Multivariate Analysis	36
2.1 Abstract	37
2.2 Introduction	38
2.3 Materials and Methods	41
2.3.1 Tomato plant material.....	41
2.3.2 Sample preparation	42
2.3.3 Reference Analysis: Sugars, Acids, Soluble Solids	44
2.3.3.1 <i>Enzymatic Determination of Sugars and Acids</i>	44
2.3.3.2 <i>pH and Titratable Acidity</i>	45
2.3.3.3 <i>Soluble Solids (°Brix) Determination</i>	45
2.3.4 Infrared Spectroscopy Analysis.....	45
2.3.5 Multivariate Analysis: Partial Least Squares Regression (PLSR).....	47
2.4 Results and Discussion.....	49
2.4.1 Quality parameters of tomato fruit	49
2.4.2 Calibration model development using tomato juice samples from the 2010 growing season	50
2.4.3 Re-calibration model development using tomato juice samples from the 2010 and 2011-growing season by selected FT-IR systems.....	54

2.4.4 External validation using an independent set of tomato juices	58
2.5 Conclusion.....	59
2.6 References	60
Combined References	65

List of Tables

Table 1.1. The top ten tomato-producing countries with production and area harvested data from 2010 (source: FAO).....	3
Table 1.2. Composition of ripe tomato fruit	10
Table 1.3. Composition of dry matter in ripe tomato.....	11
Table 1.4. Relationship between the level of sugars and titratable acids on the taste and flavor of fresh tomato fruit.....	17
Table 2.1. Selected tomato varieties from 5 California counties for 2010 and 2011 growing seasons.....	43
Table 2.2. Reference method results of quality parameters in tomato samples from the 2010 and 2011 growing seasons.....	50
Table 2.3. Performance statistics for PLS regression models generated for quality parameters in processing tomatoes from the 2010 growing season on FT-IR systems...	53
Table 2.4. Calibration and validation performance statistics for PLS regression models generated for quality parameters in processing tomatoes from the 2010-2011 growing season on bench-top (triple-bounce ATR) and Cary portable systems.....	57

List of Figures

Figure 1.1. Growth characteristics of tomato types.....	8
Figure 1.2. Cross-section of two (A) and seven (B) locule tomato fruits. Two-locule fruits are more common in the processing market whereas larger locule-containing fruits are found in the fresh tomato market.....	9
Figure 1.3. Molecular structure of predominant sugars in tomato fruits.....	12
Figure 1.4. Molecular structure of citric acid (C ₆ H ₈ O ₇).....	13
Figure 1.5. Molecular structure of glutamic acid (C ₅ H ₉ NO ₄).....	13
Figure 1.6. Molecular structure of lycopene (C ₄₀ H ₅₆).....	15
Figure 1.7. General set-up of an infrared spectrometer and design of a Michelson interferometer.....	20
Figure 1.8. An illustration of how an interferogram is Fourier transformed to generate a single beam infrared spectrum.....	22
Figure 1.9. Basic illustration of transmission FTIR sampling, where the infrared beam passes through the sample then strikes the detector.....	23
Figure 1.10. Schematic of a typical attenuated total reflectance crystal.....	24
Figure 1.11. Cary 630 FTIR from Agilent Technologies.....	27
Figure 1.12. Transmission tools from Agilent Technologies (Connecticut, USA).....	28
Figure 1.13. Diamond ATR accessory used with Agilent Cary 630 FTIR.....	30
Figure 2.1. Five California counties where the processing tomato samples were grown	42
Figure 2.2. Infrared absorption spectrum of the tomato samples on bench-top (single-bounce ATR in pink, triple-bounce ATR in purple), FlexScan handheld (blue), and Cary portable (green) spectrometer systems.....	52
Figure 2.3. Generated PLSR calibration models for citric acid on the (A) single-bounce ATR bench-top, (B) triple-bounce ATR bench-top, (C) portable Cary, and (D) handheld FlexScan.....	55

Chapter 1: Literature Review

1.1 The tomato crop industry

Tomato is the second most commonly grown vegetable in the world, with worldwide production totaling about 150 million tons in 2010 (FAO). The United States is the second largest producer of tomatoes which generate a significant \$2 billion in farm cash receipts annually (Economic Research Service 2012). Given the economic significance of the tomato and its global presence, it is necessary to give a brief overview of the worldwide and domestic tomato industry.

1.1.1 Taxonomy, origin, history, and uses of tomato

The tomato belongs to the *Solanaceae* family which also includes other vegetable crops such as chili and bell peppers (*Capsicum* spp.), potato (*Solanum tuberosum*), and tobacco (*Nicotiana tabacum*). Its genus (*Lycopersicon*) is believed to have originated in the coastal strip of western South America, a region that includes parts of Chile, Colombia, Ecuador, Bolivia, and Peru (Jones 1999; Sims 1980). The species was first domesticated in Mexico and was introduced into Europe in the mid-16th century but was not eaten often

due to the misconceived notion that it was poisonous like its relative, the deadly nightshade (Heiser 1969). The tomato was re-introduced back into America in the 18th century and by the end of the 19th century processed products such as soups, sauces, and ketchup were regularly consumed (Harvey and others 2002).

Today, tomatoes are one of the most widely eaten vegetables in the world, mostly stemming from the fact that they can be eaten fresh or in processed form. Some processed tomato products include tomato juice, tomato pulp, tomato paste, ketchup, and chili sauce (Economic Research Survey 2012). In addition to consumption, tomatoes serve as an excellent tool to improve the knowledge of horticulture crops as it's a 'model crop' for diverse physiological and biochemical studies because they are easily grown, have a short life cycle and are easy to manipulate (e.g. grafting, cuttings) (Kinet and Peet 1997). Similarly, the plant species is valued to study the physiology and biochemistry of seed development, germination, and dormancy (Suhartanto 2002).

1.1.2 Overview of worldwide tomato industry

The global production of fresh and processed tomatoes has increased by about 300% from 1965-2005 (Costa and Heuvelink 2005). In 2010, the annual worldwide production of tomatoes was estimated to be about 151 million tons with China being the major producer, followed by the U.S., India, and Turkey (FAO, **Table 1.1**). Notably, the majority of tomatoes produced from China (90%) are for fresh consumption while the bulk of tomatoes grown in the U.S. (95%) are for processing (Costa and Heuvelink 2005). It is reported that the ratio between productions for processing or fresh

consumption and the organization and structure of the industry and markets differ widely among countries (Costa and Heuvelink 2005).

Table 1.1. The top ten tomato-producing countries with production and area harvested data from 2010 (source: FAO).

Country	Production (million tons)	Area harvested (ha)
China	47.1	924,735
USA	12.9	158,590
India	12.4	634,400
Turkey	10.0	304,000
Egypt	8.5	216,385
Italy	6.0	118,822
Iran	5.3	146,985
Spain	4.3	58,300
Brazil	4.1	67,992
Russian Federation	2.0	115,200
World	151.7	4,412,757

1.1.3 Tomato industry in the U.S.A.

The U.S.A is the second highest producer of tomatoes and the world's leader in production of processing tomatoes. The state of California produces 95% of the nation's processing tomatoes and 30% of the fresh tomatoes (AMITOM 2003). California's long, warm, dry growing season meets the ideal conditions for growing tomatoes. Moreover, the use of hybrids, transplants, modern technology, and innovative research have equipped the state with increased efficiencies (Murray and others 2001).

The most fundamental differences among fresh-market and processing tomato industries are (Santos 2010):

- 1) Fresh-market tomatoes are handpicked, while processing tomatoes are machine harvested -All tomatoes were collected by hand before 1964 but, after labor shortages that year, mechanization was quickly adopted (Brandt and French 1983).
- 2) Processing tomatoes are harvested when red ripe; fresh-market tomatoes while still green (Saltveit 2005a).
- 3) Once out of the field, fresh-market tomatoes are allowed to mature over time, sometimes accelerating the process by addition of ethylene gas. Processing tomatoes are used immediately, usually within the same day Saltveit 2005a).
- 4) Growers establish business contracts with processing firms. Fresh tomatoes are sold on the open market (Economic Research Service 2012).
- 5) Processing cultivars have been selectively bred for over 50 years to develop characteristics that noticeably differentiate them from fresh-market varieties. They are bred for higher °Brix and stronger peels, which are necessary to resist the rigors of mechanized harvesting and produce high viscosity pastes (Garcia and others 2006).

As noted, there are striking differences among the industries, not only in the commerce chain, but also in those traits that are considered desirable for tomatoes in each market.

1.1.3.1 Fresh tomato industry

Fresh tomatoes are commercially produced in 20 states; primarily California and Florida which produce 65-75% of the nation's fresh tomato produce. States including Ohio,

Georgia, Virginia, and Tennessee are the next top four in terms of area planted (Economic Research Service 2012). Fresh market tomatoes are available year-round in the U.S. because imports supply during winter months. As expected, imports compete directly with U.S. field-grown fresh tomatoes and the greenhouse product (Cantliffe and Vansickle 2003). The economic research survey (2012) reports that imports account for about one-third of tomatoes consumed in the U.S.

The tomato is the fourth most consumed vegetable behind potatoes, lettuce, and onions (Economic Research Service 2012). Annual average fresh market consumption has risen steadily over the few couple decades. The rise has been attributed to constant popularity of salad bars and sandwiches such as BLT and submarines, improved tomato varieties, heightened consumer interest in these new varieties, more vegetable-rich diets from immigrants, and expanding national emphasis on health and wellness (Economic Research 2012). Surveys have concluded that about 70% of fresh tomatoes are consumed at home and the remaining 30% away from home (Economic Research 2012).

1.1.3.2 Processing tomato industry

As stated previously, growers contract with processors to process ripe tomatoes. A common initial processing step is the manufacture of tomato paste as a raw ingredient. Paste is packed in bulk containers and stored for use up to 18 months. This raw ingredient is sold for remanufacturing to make retail and food service packs of soups, sauces, catsup, and paste (Economic Research Service 2012).

Americans consume about 75% of their tomatoes in processed form which has been mostly attributed to the rise in pizza and pasta sauce intake over the last 30 years (Economic Research Service 2012). ERS estimates suggest that the largest use of processed tomatoes is in sauces (35%), followed by paste (18%), canned whole tomato (17%), and catsup and juice (each 15%). It is also thought that about one-third of all processed tomato products are purchased away from home at various foodservice restaurants (Economic Research Service 2012).

1.2 Tomato Physiology

The tomato is grown as an annual in temperate climates (Yamaguchi 1983). The growth habits between fresh and processing tomato varieties differ broadly as the major traits of processing tomatoes are determinate growth, dwarf habit, concentrated and uniform fruit set and ripening, tough skins, and a high soluble solids content (George 1999). Processing tomatoes are commonly transplants that do not require staking and are harvested mechanically. Comparatively, fresh market varieties are commonly staked to keep fruit away from the soil and the tomatoes are harvested by hand (Costa and Heuvelink 2005). Given the emphasis placed by this thesis on tomato products, it is necessary to start with a basic overview of the plant's physiology and to gain a general understanding of the fruit's characteristics and to become familiar with the components in the fruit that will be relevant in the study.

1.2.1 Fruit Characteristics

Botanically-speaking, the tomato is an annual shrubby plant with yellow clustered flowers on trailing stems (Yamaguchi 1983). The growth habit varies from indeterminate (shoot tips remain vegetative), to semi-determinate and determinate (shoot tips terminate in a flower cluster). Processing tomatoes are most commonly determinate which lends themselves to once-over mechanical harvesting when the fruit is ripe (Costa and Heuvelink 2005). Cultivars with indeterminate growth habit are used where multiple picks are economically justified, such as trellised fresh tomatoes (Saltveit 2005a). Determinate growth habits tend to be bushier and more compact (**Figure 1.1**). Tomato fruits are round, lobed, or pear-shaped varying in size from ½ to 5 inches in diameter. The ultimate size of the tomato fruit is correlated to a several parameters including (i) the number of carpels in the ovary; (ii) the number of seeds; (iii) the position of the fruit; (iv) the sequence of set in a truss; (v) the environmental conditions prevailing during the growth phase (Kinet and Peet 1997). Most tomato varieties' fruits are red when ripe but not all including *L. peruvianum*, *L. chilense*, and *L. hirsutum* which are green (Yamaguchi 1983).

The tomato plant is adapted to a wide range of climatic and soil conditions. Optimal temperature ranges is 70-75°F and chilling injury can occur if the temperature dips below 53°F for too long of a period of time. If the temperature exceeds 90°F during the storage of harvested mature green tomatoes, the formation of the red color, lycopene, is inhibited and the fruit is more yellowish when ripe (Yamaguchi 1983). There is a substantial range in fruit characteristics available today mainly due to disease-resistant hybrid

improvements that have led to higher yields and a larger range in fruit quality characteristics (fruit shape, color, acidity) (Jones 1999).

Figure 1.1. Growth characteristics of tomato types (Courtesy of Yamaguchi 1983).

1.2.1.1 Composition

The tomato fruit is classified botanically as a berry, with the size varying from small cherry types to larger ‘beefsteak’ types. Tomatoes contain 2-12 locules (divisions of the ovary) with many seeds (Jones 1999). This physical attribute is used to define the fruit type. For example, two locules are typically found in cherry and plum tomatoes which are commonly used for processing. Four to six locules are common commercial cultivars for fresh market and more than six locules are commonly home-grown as they are subject to cracking during shipping (Jones 1999).

Most tomato varieties are red in color due to the presence of the carotenoid, lycopene, an antioxidant. As seen in **Table 1.2**, water is the major component in ripe tomato followed by carbohydrates (4.7 g/100g) and protein (1.1 g/100g). Tomato varieties commonly

vary in soluble solids from 4.5-7.0%, mostly contributed from sugars and acids (Jones 1999).

Figure 1.2. Cross-section of two (A) and seven (B) locule tomato fruits. Two-locule fruits are more common in the processing market whereas larger locule-containing fruits are found in the fresh tomato market (Jones 1999).

The pH of tomato is normally around 4.5 and is carefully monitored during processing tomato manufacturing for the prevention of *Clostridium botulinum*. The ratio between the pH and solids content of the tomato fruit is a significant factor in its perceived flavor (Jones 1999).

Table 1.2. Composition of ripe tomato fruit (source: Jones 1999).

Component ^a	Ripe
Water (%)	93.5
Calories	22
Protein (g)	1.1
Fat (g)	0.2
Carbohydrates	
Total (g)	4.7
Fiber (g)	0.5
Ash (g)	0.5
Calcium (mg)	13
Phosphorous (mg)	27
Iron (mg)	0.5
Sodium (mg)	3
Potassium (mg)	244

^aPer 100 g

1.2.2 Nutrients

Nutrients in tomatoes offer a wide array of desirable characteristics beyond the provision of nourishment. Compounds present in the fruit determine its palatability, necessary processing requirements, and benefits to human health. As seen in **Table 1.3**, sugars and alcohol insoluble solids account for the majority of dry matter in tomato. The sections below will address the nutrients of greatest relevance to the tomato processing industry.

Table 1.3. Composition of dry matter in ripe tomato (Source: Davies and Hobson 1981).

Constituent	Dry Matter (%)
Sugars	
Glucose	22
Fructose	25
Sucrose	1
Alcohol insoluble solids	
Proteins	8
Pectic substances	7
Hemicelluloses	4
Celluloses	6
Organic acids	
Citric acid	9
Malic acid	4
Minerals	
(mainly K, Ca, Mg, P)	8
Others	
Lipids	2
Dicarboxylic amino acids	2
Pigments	0.4
Ascorbic acids	0.5
Volatiles	0.1
Other	1

1.2.2.1 Sugars

Evidence has shown that the flavor quality of tomatoes is largely dependent on combinations of different compounds. Volatile elements are mainly responsible for the aroma while sugars and acids determine the taste (Steven and others 1977; Petro-Turza and Teleky-Vamosy 1989). In addition to the organoleptic properties, glucose and fructose (Figure 3) constitute most of a tomato's soluble solids while sucrose contributes minimally (**Table 1.3**). The total sugar content in tomato varies from 2.5 to 3.5% (Gould 1974).

Figure 1.3. Molecular structure of predominant sugars in tomato fruits (Source: Santos 2010).

1.2.2.2 Organic and Amino Acids

As tomatoes develop, there is a general increase in sugars and a decrease in acidity. The acidity in mature fruits can be mainly attributed to organic acids such as citric acid (**Figure 1.4**), followed by malic acid (Thakur and others 1996). While organic acids only constitute 0.4% fresh weight (13% dry weight); they are important contributors for tart flavor (Saltveit 2005a).

Two percent of the tomato dry matter is contributed by dicarboxylic amino acids (**Table 1.3**). Several amino acids are found in fresh tomato juice with glutamic acid (**Figure 1.5**) comprising nearly 50% of the total weight of amino acids in fresh tomato juice (Miladi and Gould 1969).

Figure 1.4. Molecular structure of citric acid (C₆H₈O₇) (Source: Santos 2010).

Interestingly, processing of fresh tomato juice (220°F, 20 min) results in an increase of total acid. The increase in free amino acid content is due to the denaturation and partial hydrolysis of protein (Gould 1974). Upon heating, the formation of acetic acid among others has been reported due to the decomposition of sugars in the presence of acids (Crean 1969).

Figure 1.5. Molecular structure of glutamic acid (C₅H₉NO₄)

The pH of processing tomatoes is an important consideration as it influences processing conditions necessary for adequate thermal processing (Garcia and Barrett 2006). The final pH of the canned product must be below 4.5 to ensure microbiological safety (Galicia-Cabrera 2007). Although tomatoes are normally considered a high acid product

with a pH 4.6 or less (Gould 1974; Garcia and Barrett 2006), there is evidence that some tomatoes have a pH above this threshold.

1.2.2.3 Vitamins and Antioxidants

Ripe tomatoes are a good source of Vitamins A and C. Interestingly, both vitamins increase as the fruit ripens on the vine but does not increase if the mature green fruit ripens off the vine (Yamaguchi 1983). Consumption of tomato and tomato products has often been associated with a reduction in the risk of chronic diseases (Rao and Agarwal 1998; Giovannucci 1999; Arab and Steck 2000; Sesso and others 2003). Tomatoes have a remarkable combination of antioxidants, which includes lycopene, β -carotene, polyphenols and vitamin C (Tyssandier and others 2004). Tomato products are reported to be the major source of lycopene (O'Neill and others 2001), and merely 100 g of tomato can supply 24-48% of the recommended daily intake for vitamin C (Preedy and Watson 2008).

Lycopene (**Figure 1.6**) is the most abundant carotenoid in ripe tomatoes making it a significant factor in the commercial value of fresh tomatoes (Preedy and Watson 2008) since color is one of the first attributes assessed by consumers (Garcia and Barrett 2006). The lipophilic properties of lycopene and the hydrophilic properties of ascorbic acid from tomato have both demonstrated potent antioxidant activity (Lavelli and other 2000).

manufacture a tomato with higher solids content since that is less water to boil off (Saltveit 2005b). Viscosity is a complex physical property that is influenced by the amount and suspension of tomato solids, the solution of salts, proteins, sugars, and organic acids, as well as the size and linkage of pectins (Saltveit 2005b). Lycopene and beta-carotene are predominantly responsible for the color in tomato products. Breeders have focused on increasing the soluble solids, viscosity, and color of processing tomatoes over the past several years. Some difficulty has been found since the trait is polygenetic and greatly influenced by the environment. It has been suggested that the product of yield multiplied by the soluble solids content is more useful in estimating the productivity of processing tomatoes (Saltveit 2005b). In general, the tomato yields have increased but the soluble solids only slightly (Stevens 1994; Zamir and others 1999). Some success has been found, with high sugar and acid hybrids being rated as higher overall flavor intensity than standard cultivars (Ruiz and others 2006).

Soluble solids (SS) and titratable acids (TA) are important components of flavor as they impart sweet and tart notes. Interestingly, they provide an effect not only through their presence but also through their ratio. Fruit high in SS and high in TA impart 'good' flavor while those low in SS and high in TA were 'tart' (Table 4). 'Tasteless' fruit is noted when both sugars and acids are low in concentration. For fresh tomatoes, this may be due to the fact that harvested fruit that is stored at elevated temperatures hastens the respiratory loss of carbohydrates along with the acceleration of ripening (Saltveit 2005b). There is large variation among tomato cultivars for pH, total acidity, and sugar content. The pH of a ripe tomato typically ranges from 4.1 to 4.8. Storage at higher temperatures

causes the organic acids to metabolize and the pH to increase which is undesirable from a flavor and processing standpoint (Saltveit 2005b). The fresh tomato market also values the acid content of tomatoes from a flavor standpoint, but the firmness and color of the fruit are very important indicators of good quality to the consumers.

Table 1.4. Relationship between the level of sugars and titratable acids on the taste and flavor of fresh tomato fruit (Source: Kader and others 1978).

Titratable acidity	Sugars (soluble solids)	
	High	Low
High	Good	Tart
Low	Bland	Tasteless

1.3.2 Traditional quality control techniques

When processors receive tomatoes at the processing facility, very little is generally known about the raw fruit content. Parameters affecting the quality of tomato fruit are the cultivar, tomato maturity, growing location and climate, and processing conditions (Garcia and Barrett 2006). The decision to peel rather than send tomatoes for paste processing may be based on the historical knowledge of the cultivar, grower, or on data obtained from inspection stations (Garcia and Barrett 2006). These inspection stations sample tomatoes from each truck load that arrives at the processing plant to determine pH and soluble solids as these parameters are important considerations for processing conditions (Gordon and others 2011).

1.4 Infrared spectroscopy

Luong, Bouvrette, and Male report that “reliable and cost-effective analytical methods are increasingly needed in the food industry for the determination of specific chemical compounds in foods and food products” (1994). Various analytical methods have been developed, such as high performance liquid chromatography, and provide information concerning food components but are time-consuming, require sample pretreatment, expensive equipment, and require large volumes of solvents (both organic and aqueous) (Luong and others 1994). Given these disadvantages, there is a significant need for the development of a more rapid and equally accurate quality control technique for the food industry, specifically the processing tomato industry.

Infrared spectroscopy could serve as a viable candidate to rapidly assess food and food products. The advantages of IR spectroscopy include its speed, sensitivity, its ability to analyze samples in solid, liquid, and gas states, and its relatively low cost. Generally, a sample can be prepared, scanned and its results plotted in less than 5 minutes. Solids, liquids, powders, and even gases can be analyzed. Additionally, a unit can be purchased for a price that is lower in comparison to many other laboratory devices (Smith 1999). Near and mid-infrared spectroscopy have proven themselves as significant tools to analyze food constituents such as proteins, fats, oils, sugars, and water in food products (McClure 2007).

Infrared (IR) spectroscopy studies the interaction of infrared light with matter (Smith 1999). When IR radiation strikes a substance, the material containing chemical bonds absorbs the energy and causes vibrating, bending, and twisting from the bonds

themselves (Santos 2010). But what is very particular about this process is that the specific groups of atoms in the molecules (known as “functional groups”) always tend to absorb radiation from the same spectral area (Smith 1996). Since the absorption will be observed on the same range, regardless of the type of molecule the functional group is in, it is possible to correlate the range of absorption of any given sample with its chemical structure (Santos 2010). This way, the sensitivity of IR spectroscopy to the presence of functional groups in matter can be utilized for identifying unknowns, confirming identities and quantifying concentrations (Smith 1999). It is important to note, however, that different sample conditions such as aqueous samples or complex matrices prove challenging, as water bands and other factors can mask signal or hinder assignation of specific functional groups, respectively (Smith 1996).

The section below addresses more specifics to the principles and workings of today’s infrared spectrometer and its application in the food industry.

1.4.1 Equipment and Tools for Mid-Infrared Spectroscopy Analysis

Infrared radiation is divided into three parts: far-IR ($40\text{--}400\text{ cm}^{-1}$), mid-IR ($400\text{--}4,000\text{ cm}^{-1}$), and near-IR ($4,000\text{--}14,000\text{ cm}^{-1}$) (Guillen and Cabo 1997). Mid-infrared spectroscopy is an ideal analysis tool because of its capability to interpret the chemical functional groups of molecules in a food system. This region is very interesting to use in the study of organic compounds because the intensity of the absorption bands due to the vibrations of certain functional groups (Guillen and Cabo 1997) are proportional to concentration

(Santos 2010). This makes mid-IR spectroscopy ideal for qualitative and quantitative analysis of foods (Li-Chan and others 2010).

Fourier transform infrared spectroscopy (FT-IR) has been gaining acceptance as a good analytical tool for the last three decades (Ismail and others 1997). FTIR spectrometers are much more effective than the original dispersive devices because all the IR radiation passes through the sample and reaches the detector at the same time (Santos 2010). There is no reduction of the intensity of the IR radiation (the throughput advantage) and all the wavenumbers are detected at once (the multiplex advantage). The end result is a better spectrum collected at a faster time (Smith 1996).

Figure 1.7. General set-up of an infrared spectrometer and design of a Michelson interferometer (Courtesy of Baeton and Dardenne 2002).

Figure 1.7 shows the general set-up of a Fourier transform infrared spectrometer. In an FT-IR instrument, the monochromator of a conventional infrared spectrometer is replaced with a Michelson interferometer which splits the infrared beam using a semi-permeable beamsplitter (Gunzler and Gremlich 2002). The interferometer takes a beam of infrared light from a source to a beam splitter. The beam splitter is designed to transmit one-half of the incoming light towards a fixed mirror, and reflect the other half towards a moving mirror (Maurer 2012). Since the path traveled by one beam is fixed and the other is constantly changing (due to the mirror changing positions), the signal which exits the interferometer will be the result of these two beams “interfering” with each other (Thermo Nicolet 2001). When the two lights bounce back from the mirrors’ reflective surfaces, they return to the beam splitter where they are recombined, and directed to a sample holder (Maurer 2012). As the infrared light interacts with the sample, a fraction of the radiation is absorbed while the rest passes through towards a detector (Maurer 2012). The intensity of the signal from the detector, as a function of the change in optical pathlength corrected by a constant component, is called an interferogram (Ismail and others 1997). This interferogram showing intensity versus pathlength is converted by a mathematical algorithm, called a Fourier transform, into an IR spectrum giving absorbance versus frequency (Wehling 1998). **Figure 1.8** illustrates how an interferogram is Fourier transformed to generate a single beam infrared spectrum (Smith 1996). The absorption on each wavenumber can be quickly associated with the amount of specific functional groups present, making it an ideal technique for qualitative and quantitative analysis (Maurer 2012).

1.4.1.1 Transmission Techniques

Transmission techniques are a common way of obtaining infrared spectra by passing the infrared beam directly through the sample (**Figure 1.9**). Radiation of the right frequency will be absorbed while the rest will be transmitted. The detector placed directly behind the sample measures the resulting radiation (Tirpak and Young 2008). The main advantages of this technique are that transmission spectra have high signal-to-noise ratios, require relatively inexpensive tools to prepare samples for analysis, and are a universal technique that works on solids, gases, liquids, and polymers (Smith 1996).

Figure 1.8. An illustration of how an interferogram is Fourier transformed to generate a single beam infrared spectrum (Source: Smith 1996).

Figure 1.9. Basic illustration of transmission FTIR sampling, where the infrared beam passes through the sample then strikes the detector (Modified from Tirpak and Young 2008).

1.4.1.2 Attenuated Total Reflectance (ATR)

The ATR technique is popularly used to obtain spectra of solids, liquids, semisolids, and thin films by mounting an accessory to the sample compartment of the FT-IR (Smith 1996). The heart of the accessory is a crystal of infrared transparent material of high refractive index such as zinc selenide, diamond, or germanium. Due to the crystal's proper refractive index and the light's proper angle of incidence, the infrared energy reflects off the crystal and the radiation undergoes total internal reflection (Smith 1996). The standing wave of radiation, or evanescent wave (**Figure 1.10**), is slightly larger than the crystal and just penetrates beyond the crystal surface into space (Stuart 2004). If a sample is brought into contact with the crystal, it can interact with the evanescent wave, absorb infrared radiation, and produce a spectrum (Smith 1996). Key advantages of ATR include that it is an easy-to-use, non-destructive sampling technique and is more useful for soft or semisolid samples (such as peanut butter or tomato sauce) that would be too

absorbing if obtained from transmission (Smith 1996). For quantitative analysis, the condition, position, and cleanliness of the ATR crystal must be consistent to obtain reproducible data as these factors affect depth of penetration (Smith 1996). Zinc selenide is the most common ATR crystal material as it is hard, impervious to everything but strong acids and bases, and has a high depth of penetration. The only notable disadvantage is that it cuts off a small part of the mid-infrared region ($600\text{--}400\text{ cm}^{-1}$) (Maurer 2012).

Figure 1.10. Schematic of a typical attenuated total reflectance crystal (Source: Stuart 2004).

1.4.1.2 Multivariate analysis and chemometrics

Multivariate analysis is defined as a “generic term for any statistical technique used to analyze data from more than one variable.” Chemometrics, a type of multivariate analysis, can be defined as a “sub-discipline of chemistry that uses mathematical and

statistical methods for the interrogation of chemical data” (Santos 2010). It provides information about the chemical structure, composition and physic-chemical properties of a given sample (Von and others 1988). These techniques have many applications, including the creation of process control charts, classification patterns, data visualization and multivariate calibrations (Santos 2012). When used in calibration modeling, they are able to produce functions that relate simple measurements (e.g. IR spectra) to more difficult, absolute or complex ones (e.g. lengthy chromatographic analysis of a substance) (Walmsley 2000; Santos 2010).

When a spectral fingerprint is obtained, the output is not a single number (Santos 2010). Instead, thousands of wavenumbers are collected at the same time. If ordinary linear statistical analysis was performed to model the data, there would be a serious risk of over-fitting. A powerful method, known as Partial Least Squares (PLS), has been successfully used to avoid over-fitting during the quantitative analyses of numerous spectroscopic techniques (Haaland and Thomas 1988) and is currently available to be used in conjunction with FTIR (Santos 2010). PLS regression is based on the extraction of “latent variables” (Moseholm 1988); only those components that are most important in explaining a model’s variation are used. By working this way, the PLS regression can reduce the information contained in thousands of wavenumbers to just a few latent variables, often less than 10 (Santos 2010).

PLS is applied to the simultaneous analysis of two data sets (e.g., the measured spectra and the analyzed samples) with the purpose of creating a linear model able to predict a desired characteristic (y) from a measured spectrum (x) (Santos 2010). PLS is now

routinely used by both academia and industry to rapidly correlate spectroscopic fingerprints with a desired chemical/physical characteristic (Norgaard and others 2000).

1.4.2 Current Technologies

New technologies have heightened the sensitivity and versatility of FTIR transmission and reflection practices. Since the turn of the 21st century, the technological design of FT-IR spectrometers have become more robust and “provide unrivalled analytical performance under real-world conditions” (Agilent Technologies 2011b). State-of-the-art bench-top spectrometer systems with specific sampling accessories are being manufactured to provide food, pharmaceutical, and other analytical industries with rapid and sensitive equipment. While these high-performing spectrometers are ideal for routine, in-lab research analysis, there is limited flexibility with out-of-lab interests. The sample of importance must always come to the lab for analysis. Scientific companies have sought to fill this gap by designing portable and handheld FT-IR systems that provide the same level of reliability and sensitivity as bench-top systems but allow for more flexibility since the unit can be easily carried and transferred. The compact size and equal sensitivity of the portable systems to the bench-top make it an ideal technique for the food industry as it can provide accurate analytical results with the benefit of easy transport. The material listed below highlights some of the recent technology from Agilent Technologies[®] (Connecticut, USA).

1.4.2.1 Portable spectrometers

Portable or handheld spectrometers operate using the same components and principles as a bench-top FTIR but offer unique properties in terms of flexibility and equal sensitivity. Some of the first systems (Thermo Scientific's TruDefender) was originally developed by the government for identification of weapons materials and hazardous or illegal substances (Rein 2008). Current systems reflect use in multiple industries. One such example is Agilent's Cary 630 spectrometer (**Figure 1.11**). It is a robust, easy to use, superior performing FTIR in a compact design that offers a wide range of sample interfaces that are fully interchangeable (Agilent Technologies 2011a). This machine's advantages include innovative and intuitive design, reliable and versatile, compliant, compact, and affordable (Agilent Technologies 2011a).

Figure 1.11. Cary 630 FTIR from Agilent Technologies (2011a).

1.4.2.2 Transmission accessories

Agilent Technologies[®] has developed a transmission module (**Figure 1.12a**) that allows for the measurement of solids, liquids, and gases after proper sample preparation. Solids are analyzed in a pressed KBr pellet or a nujol mull, liquids are measured with a traditional sealed or demountable cell, and gases can be measured using the Agilent Cary 630 FTIR gas cell (**Figure 1.12b**). The transmission module can be used for quantitative and qualitative analyses, transmission measurements, and analysis of solids, liquids and gases (with appropriate sample preparation) (Agilent Technologies 2011). Unique DialPath technology facilitates long pathlength FTIR transmission measurement of liquids, without the inconvenience of cumbersome infrared transmission cells (Agilent Technologies 2011).

A. Transmission module[®]

B. Cary 630 FTIR gas cell[®]

C. DialPath[®] multipath liquid transmission

Figure 1.12. Transmission tools from Agilent Technologies (Connecticut, USA) (Source: Agilent Technologies 2011a).

The DialPath interface (**Figure 1.12c**) provides high sensitivity, easy to use features and repeatability in a design that enables you to instantaneously select any of three factory-set pathlengths (Agilent Technologies 2011). The three pathlengths deliver the versatility to handle both qualitative library matching, as well as different levels of quantitative analysis (Agilent Technologies 2011). DialPath specifications include:

- Quantitative analysis
- Qualitative analysis, library search
- Transmission measurements
- Liquids and thin polymer films (< 50 μm thick)
- Fixed pathlengths—set of three factory-calibrated
 - 30, 50, 100 μm
 - 50, 100, 250 μm
- Repeatability = $\pm 0.25 \mu\text{m}$
- ZnSe windows
- Spectral range: 5,100–600 cm^{-1}

1.4.2.2 Reflectance accessories

Agilent's diamond ATR accessory (**Figure 1.13**) is the most common sample interface used in infrared spectroscopy, because it is easy to use and provides high-quality spectra with no sample preparation (Agilent Technologies 2011). It is frequently used with solid samples since the diamond's strength can withstand the applied pressure for good contact (in contrast to the zinc selenide crystal that would likely break under excessive pressure).

It is widely used in food and beverage analysis, chemicals, pharmaceuticals, fuel and oil analysis (Agilent Technologies 2011).

Figure 1.13. Diamond ATR accessory used with Agilent Cary 630 FTIR.

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Chapter 2: Rapid Assessment of Quality Parameters in Processing Tomatoes Using Handheld and Bench-top Infrared Spectrometers and Multivariate Analysis

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2.1 Abstract

Two portable infrared sensors were evaluated for the rapid determination of quality parameters in processing tomatoes. A total of 370 hot-break juices were prepared from ~40 processing tomato varieties grown in 5 California counties. The levels of sugars (glucose and fructose), acids (citric, malic, and glutamic), soluble solids, titratable acidity, and pH in these juices were determined using standard reference methods. For FT-IR analysis, juices were centrifuged, filtered through Whatman® filter (0.45µm), and directly applied to the FT-IR crystal (15-40µL) to obtain spectra. Partial least squares regression (PLSR) was used to generate correlation models, both calibration and validation, between the component concentration and the sample spectra. The PLS validation models showed good ability in estimating the sugars, acids and especially soluble solids in tomato for both the transmission DialPath portable system and bench-top unit using a triple-bounce ATR, judging by the high correlation coefficient of validation ($R_{\text{val}} > 0.80$) and low standard error of predictions (SEP). Prediction errors of 1.3 g/L for estimation of sugars (fructose, glucose), 0.22 °Brix, and 0.22 g/L for glutamic and citric acids were determined using the 1800-900cm⁻¹ infrared region. The IR portable unit may provide the tomato processing industry with an efficient method for in-field, high throughput quantification of quality parameters in tomatoes.

2.2 Introduction

Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop next to potato with production occurring in 144 countries (FAOSTAT 2004). Fresh and processed tomatoes are one of the mostly widely produced and consumed vegetables in the U.S. and generate annual revenue of approximately \$2 billion in farm cash receipts (Economic Research Service 2009).

In the U.S., the state of California currently produces about 12 million tons of processing tomatoes annually, accounting for more than 90% of the U.S. crop and about 40% of the processing tomato production worldwide (Anthon and others 2011). Processing tomatoes are used to produce a variety of products, including whole peeled fruit and diced tomatoes, as well as various juices and purees. The largest portion of this crop is thermally processed and concentrated into tomato paste. The most important quality attributes in processing tomatoes are soluble solids, pH, titratable acidity, viscosity and color (Saltveit 2005). Tomato fruit composition is approximately 93% water and 7% solids. Total solids are further classified according to their water solubility as soluble and insoluble solids (Nielsen 1998). Approximately half of the total solids are reducing sugars, with slightly more fructose than glucose. The remaining solids consist of organic acids (citric and malic), amino acids, proteins, lipids, minerals, pectic substances, cellulose and hemicellulose (Barringer 2004).

Soluble solids are a key parameter in tomato paste production. Tomato paste is produced and sold based on its soluble solids content thus soluble solids dictate the factory yield. Higher soluble solids in the incoming fruit means that fewer tons of tomatoes will be

needed to produce a given amount of paste (Gould 1992). Furthermore, water removal during evaporation of juice to paste is an energy intensive process. Producing paste from fruit with high levels of soluble solids is less expensive since less water needs to be removed to obtain the desired soluble solids content (Nichols 2006).

A second key parameter in paste production is pH, which plays a vital part in microbiological safety and food spoilage. Tomatoes are high-acid foods, thus, require less drastic thermal treatments than foods classified as low-acid ($\text{pH} > 4.6$) for the destruction of spoilage microorganisms to ensure food safety (Anthon and others 2011). Generally, the pH of tomatoes has been reported to range from 3.9 to 4.9, or in standard cultivars, 4.0 to 4.7 (Sapers and others 1977), hence, the USDA standards of identity allow organic acids to be added to lower the pH when needed during processing of high acid foods (Barringer 2004). The pH of the tomato is determined by its organic acid content with citric acid being the most abundant.

Sugars and organic acids are responsible for the sweetness and tartness, and are major factors affecting flavor acceptability (Baldwin and others 2008; De Bruyn and others 1971; Stevens and others 1977; Petró-Turza and Teleky-Vámosy 1989). The development of processing tomato varieties with altered compositions requires the efficient and accurate evaluation of thousands of tomato breeding lines (De Nardo and others 2009). Traditional methods for sugar and acid determination include the use of enzymatic kits and chromatography (Horowitz 2000). Chromatographic methods are typically accurate and allow for the determination of multiple components juice from a single sample. Limitations include time-consuming sample preparation, use and disposal

of hazardous solvents, low sample through-put, and a high skill-set for the testing personnel. Enzyme based methods are rapid, require little sample preparation, and allow for a high sample throughput (Vermeir and others 2007). However, by their nature these tests can only determine a single juice component at a time. If a complete profile of the juice is desired, multiple tests will need to be run, greatly increasing the time and expense involved with these methods. Infrared (IR) spectroscopy presents an ideal alternative in assessing processing tomatoes as it is a simple, time and cost-efficient technique that can potentially provide information on many juice components from a single measurement. It has already shown promise in analyzing other food and agricultural products (Chen and Sun 1991). The mid-IR region ($4000\text{-}400\text{ cm}^{-1}$) produces absorption bands for most functional groups which allows for a direct correlation between specific chemical parameters of interest (Ellis and Goodacre 2006). Fourier-transform infrared (FT-IR) techniques combined with chemometrics offer tomato processors and breeders powerful tools for the rapid assessment of tomato quality attributes. Portable IR units would enable the food manufacturer to quickly assess the quality of the incoming raw material, the quality of their product, and allow for timely corrective measures during manufacture. Portable systems are simple to use and require minimal or no sample preparation, thus reducing assay time and helping to streamline the analytical procedure so that it is more applicable to higher sample throughput.

The objective of the present research was to develop a simple, quick and reliable methodology for the determination of processing tomato quality parameters ($^{\circ}\text{Brix}$, pH, titratable acidity, fructose and glucose, citric and glutamic acids). Additionally, we

evaluated the performance of two novel portable infrared units against bench-top IR spectrometers.

2.3 Materials and Methods

2.3.1 Tomato plant material

A total of 40 processing tomato varieties were obtained from five counties (Fresno, Kern, Merced, San Joaquin, and Yolo) located in central California during the 2010 and 2011 growing seasons (**Figure 2.1, Table 2.1**). Counties provided one to three replicates of each variety totaling 370 samples. In each California county, tomatoes were planted by cooperating commercial growers as part of a long-term program of evaluation of new tomato cultivars under the coordination of the Department of Food Science and Technology and the Cooperative Extension Program at the University of California-Davis (Murray and others 1999). All tomatoes were manually harvested at the commercial red-ripe stage during the mid-season (mid-August through early October). Tomatoes were selected from the middle of the plant, avoiding the top and bottom set of tomatoes.

Figure 2.1. Five California counties where the processing tomato samples were grown.

2.3.2 Sample preparation

All tomatoes were washed, towel dried, and sorted for defects. A “microwave break” method, developed by the Department of Food Science and Technology at University of California, Davis (Leonard and others 1980) to simulate a hot break process, was used to prepare tomato juice samples as described previously (Garcia and Barrett 2006). The juice samples were immediately evaluated for soluble solids, pH, and titratable acidity (% citric). The remaining portion of the blended tomato juices were stored in 15 mL Fisherbrand® plastic centrifuge tubes (Waltham, MA) at -40°F for later chemical and FTIR analysis. During all analytical stages, special care was taken to protect samples from unnecessary light and heat exposure.

Table 2.1. Selected tomato varieties from 5 California counties for 2010 and 2011 growing seasons.

<i>Kern</i>		<i>Merced</i>		<i>Fresno</i>		<i>Yolo</i>		<i>San Joaquin</i>	
<i>2010</i>	<i>2011</i>	<i>2010</i>	<i>2011</i>	<i>2010</i>	<i>2011</i>	<i>2010</i>	<i>2011</i>	<i>2010</i>	<i>2011</i>
BQ 163	HM 9905	N 6400	H7709	AB 2	SUN 6366	H 5508	HMX 1884	N 6400	H5508
H 4007	N6385	HMX 7885	C299	BQ 163	AB 0311	N 6400	H5608	HMX 7885	N6402
AB 2	H9780	AB 3	HMX 1890	Nun 6385	H9780	BQ 205	HM 9905	AB 3	N6385
N 6400	C298	H 9780	HM 9905	H 9780	N6398	H 5608	H5508	H 5508	H5608
HMX 9903	N6398	Nun 6385	HMX 1884	UG 19406	DRI 0319	H 8504	HMX 1885	CXD 282	HMX 1885
H 9780	UG 19406		HMX 1885	H 7709	AB3	H 4007	BQ186	HMX 9903	SUN 6366
CXD 255	BQ265	UG 19406	H5608	BQ 205	UG 19006	CXD 255	UG 19406	Nun 6385	BQ 163
HMX 9906	N6394	CXD 280	H5508	Nun 6394	N6394	SUN 6366	HMX 9903	AB 2	BQ 205
H 7709	N6402	BQ 205	N6394	HMX 7885	BQ265	Nun 6385	N6385	HMX 9905	H3402
CXD 280	HMX 9903	CXD 255	BQ 163	BQ 198	HMX 1890	BQ 163	SUN 6366	UG 19406	H9780
H 5508	HMX 1884	Mariana	AB2	CXD 255	BQ 163	HMX 7885	N6394	H 9780	N6398
BQ 205	C299	H 4007	DRI 0319	SUN 6366	AB2	HyPeel 849	N6402	BOS 7210246	AB3
UG 19406	H5608	SUN 6366	BQ265	H 5608	N6385	H 6809	N6398	CXD 255	BQ265
BQ 187	HMX 1890	UG 19006	H9780	H 4007	UG 19406	H 9780	AB 2	FV (H 9663)	N6394
N 6398	HMX 1885	N 6398	H3402	N 6398	H3402	CXD 280	HMX 1890	H 4007	HM 9905
HMX 9905	BQ186	AB 2	N6385	CXD 280	UG 19306	HMX 9906	H3402	BQ 198	BQ186
H 5608	AB 0311	H 5608	N6404	AB 3	H5508	UG 19406	UG 19006	BQ 163	UG 19006
H 6809	BQ 163	BOS 7210246	BQ 205	CXD 282	H5608	AB 3	BQ 205	Nun 6394	AB2
UG 19006	H3402	H 7709	UG 19406	H 6809	HM 9905	CXD 282	AB 0311	UG 19006	DRI 0319
BOS 7210246	AB3	H 8504	UG 19306	N 6400	HMX 1884	H 7709	BQ 163	SUN 6366	C299
Nun 6394	DR I0319	CXD 282	AB3	UG 19006	C299	UG 19306	H9780	BQ 205	UG 19406
HMX 7885	BQ 205	BQ 163	HMX 9903	HMX 9906	N6404	N 6398	UG 19306	CXD 280	H7709
Nun 6385	H5508	BQ 187	UG 19006	HMX 9903	H7709	AB 2	C298	UG 19306	HMX 1890
UG 19306	AB2	H 5508	N6398	H 5508	BQ186	HMX 9905	AB3	H 5608	UG 19306
CXD 282	H7709	HMX 9905	C298	H 8504	HMX 1885	UG 19006		H 8504	N6404
AB 3	N6404	HMX 9906	AB 0311	UG 19306		BQ 187		HMX 9906	
SUN 6366	SUN 6366	BQ 198	N6402	BOS 7210246		Nun 6394		H 7709	
BQ 198		Nun 6394	SUN 6366	BQ 187		BQ 198			
H 8504		H 6809		HMX 9905		HMX 9903			
		HMX 9903				BOS 7210246			

2.3.3 Reference Analysis: Sugars, Acids, Soluble Solids

2.3.3.1 Enzymatic Determination of Sugars and Acids

Sugars and acids were quantified using an enzymatic procedure as previously described (Vermeir and others 2007). Analysis was done using enzyme reagent kits (R-Biopharm, Marshall, MI), modified for use in 96-well microplates with a final assay volume of 200 μ L. The procedure followed kit instructions except that the volumes of water used to prepare the reagents were modified so that the final reagent concentrations were the same for the 200 μ L volume used here as for the 3 mL procedure described in the kit instructions. Tomato juice samples were clarified by centrifuging for 5 minutes at 16,100 xg. Aliquots of the supernatants were diluted 100-fold with water for acid analysis, and 1000-fold with water for sugar analysis. To perform the assay 100 μ L aliquots of the diluted supernatants were mixed with 100 μ L of the modified kit reagents in the microplate wells. Absorbance at 340 nm was measured then 4 μ L of the appropriate enzyme suspension was added and the absorbance at 340 nm monitored until a stable new reading was obtained. Concentrations of sugars and acids were calculated from the absorbance differences at 340 nm and the extinction coefficient for NADPH of 6,300 $M^{-1}cm^{-1}$. Standard solutions provided with the kits were used to verify the accuracy of the method.

2.3.3.2 pH and Titratable Acidity

Pulped juice tomato samples were evaluated for titratable acidity using titration with NaOH (AOAC 2000). The remaining juice was then deaerated and the temperature was adjusted to 25°C before pH determination.

2.3.3.3 Soluble Solids (°Brix) Determination

For estimation of soluble solids content, 1.5mL of tomato puree was centrifuged at 10,000 rpm (15 minutes, 25°C) and the supernatant was filtered through Whatman® non-sterile syringe filters (0.45µm). The filtered tomato serum (40µL) was measured using the Leica Mark II Plus Abbe Refractometer Model 10494 (Leica, Buffalo, NY). Measurements were taken once for each sample and 70% ethanol was used to clean in between samples. Refractive index was expressed as % soluble solids in °Brix.

2.3.4 Infrared Spectroscopy Analysis

Samples were divided into calibration and validation sets to compare the performance of the regression models developed by collecting mid-infrared (MIR) spectra with different systems. A total of 210 samples were analyzed from the 2010 growing season and an additional 160 samples from the 2011 growing season. Two thirds of the samples (245 tomato juices) were used for calibration models and the remaining one third (125 tomato juices) were used for prediction models. Samples were randomly selected from each county for inclusion in calibration and validation models.

Aliquots (1.5 mL) from each thawed, blended tomato juice sample were centrifuged at 10,000 rpm for 15 min at 25°C. The supernatant was filtered through Whatman® non-

sterile syringe filters (0.45 μ m) and collected in 1.5 mL Fisherbrand[®] centrifuge tubes. Spectral data from all tomato juice samples were collected using 3 spectrometers: a bench-top system equipped with a single-bounce and triple-bounce zinc selenide (ZnSe) attenuated total reflectance (ATR) accessories, a handheld (FlexScan[™], Agilent, Santa Clara, CA) unit equipped with a single-bounce diamond ATR and a Cary 630 portable IR spectrometer using a ZnSe dial-path transmittance accessory.

The filtered juice (15-50 μ L) was applied directly at the surface of the crystals (ATR or ZnSe transmittance) for spectral acquisition in less than 2 minutes. Duplicate, independent measurements were taken on each sample and background spectra were acquired every sample for the handheld and portable units or every 5 samples for the bench-top model to account for environmental variations. In-between measurements, the crystal was carefully cleaned with 70% ethanol and dried with Kimwipe tissue (Kimberly-Clark Corp. LLC, Roswell, GA).

The bench-top model used was an Excalibur Series 3100 Fourier-Transform infrared spectrometer (Agilent Technologies Inc., Santa Clara, CA) with a potassium bromide beam splitter and a deuterated triglycine sulfate (DTGS) detector, operating at 4 cm^{-1} resolution. A horizontal ATR sampling accessory, coupled with a ZnSe crystal plate with a refractive index of 2.5, allowed a triple reflection within the sample at an incidence angle of 45⁰ for highest infrared sample throughput (Pike Technologies, Madison, WI). An additional ZnSe crystal was obtained for single-bounce bench-top analysis. The samples were scanned at room temperature and mid-infrared spectra were collected at wavenumbers from 4000 to 700 cm^{-1} . Subsequently, the sample spectra were corrected

against the background spectrum of air. Interferograms of 32 scans were co-added followed by Beer-Norton apodization. Spectra were displayed in terms of absorbance and viewed with Win-IR Pro[®] Software (Agilent Technologies Inc., Santa Clara, CA). The instrument was constantly purged with CO₂-free dry air from a CO₂RP140 dryer (Dominick Hunter, Charlotte, NC).

The portable Cary 630 FTIR unit (Agilent Technologies Inc., Santa Clara, CA) used a 30µm transmission sampling accessory with a temperature-stabilized DTGS detector, ZnSe beam splitter, operated at a spectral resolution of 4 cm⁻¹ and the mid-infrared spectral range of 4000 – 700 cm⁻¹ was collected. A total of 64 scans were co-added to improve signal-to-noise ratio.

The handheld FlexScan FTIR unit (Agilent Technologies Inc., Santa Clara, CA) used a single-bounce diamond ATR crystal with a ZnSe beam splitter and temperature-stabilized DTGS detector operating at 4 cm⁻¹ resolution. The samples were scanned at room temperature using the 4000-700 cm⁻¹ region. Interferograms of 64 scans were co-added to improve signal-to-noise ratio.

2.3.5 Multivariate Analysis: Partial Least Squares Regression (PLSR)

PLSR, a pattern recognition technique, was used to analyze the complex data sets and to generate predictive models to estimate the different tomato juice quality parameters. PLSR is a bilinear regression method that compresses a huge number of variables into few latent factors that are linear combinations of the spectral frequencies (X) and use these factors to ascertain for the analyte's concentration (Y) explaining much of the covariance of X and Y (Martens and Martens 2001). This technique has the potential to

estimate the component concentration, as well as the chemical properties of the spectra (Haaland and Thomas 1998). Precisely, PLSR succeeds in the advancement of creating multivariate calibration models in the field of spectroscopy because it keenly utilizes the concentration information (Y variable) in establishing the manner in which the regression factors are calculated from the spectral data matrix (X), thus, reducing the effect of extraneous X variations in the calibration model (Martens and Martens 2001). Accordingly, the degree of competence and versatility of this analytical approach offer a more information-rich data set of reduced dimensionality and eliminates data noise, which results in a more accurate and reproducible calibration models (Wold and others 2001).

Spectral data were exported from the spectrometers as GRAMS.spc files and imported into the multivariate statistical program Pirouette® for Windows Chemometrics Modeling Software, version 4.0 (Infometrix, Inc., Bothell, WA). These were then analyzed by partial least squares regression (PLSR) that was cross-validated using a leave-one-out approach to generate calibration models and subsequently transformed using the multiple specular component (MSC) function. The calibration models correlated the spectra against the concentration of each tomato analyte (Brix, pH, titratable acids, glucose, fructose, citric and glutamic acids). Performance of these models was evaluated in terms of outlier diagnostics, standard error of cross-validation (SECV), correlation coefficient (R^2), and number of factors.

Graphic evaluation of the calibration models were done to guarantee an arbitrary distribution of residuals. Residuals and leverage were used for the evaluation of outliers.

An outlier was indicated either by large residual or unusual residual pattern. Conversely, leverage was used to determine its potential contribution to the estimated calibration model. Hence, any observation with atypical and large residual or leverage was re-analyzed and eliminated if it was considered a substantial outlier, thereafter, the model was recalculated. Standard error of cross-validation (SECV) is an approximation of the standard error of prediction, that is, the weight of an anticipated error when independent samples are predicted using the model (Mark and Workman 1991). External validation was performed on the prediction sample set to assess the ability of the calibration model to withstand unknown variability. The correlation coefficient (R^2) is a statistical measure that allows us to determine the amount of variation in the data that is adequately modeled by the calibration equation as a fraction of the total variation. An R^2 of 1.0 indicates that the calibration model represents 100% of the variance within the data and is illustrated by a regression line perfectly fitting the data (Mark and Workman 1991). The calibration model that generated an excellent combination of the minimum SECV, higher R^2 and optimized numbers of latent factors was selected the best for spectral data set on each tomato parameter.

2.4 Results and Discussion

2.4.1 Quality parameters of tomato fruit

The large number of tomato varieties (~40) grown in 5 different California counties resulted in wide ranges of compositional levels (**Table 2.2**). Overall, values ranged from

10.0-21.4 g/L (glucose), 11.0-20.6 g/L (fructose), 1.29-3.86 g/L (citric acid), 1.00-3.21 g/L (glutamic acid), 3.2-6.7 (°Brix), 4.22-4.87 (pH), and 0.152-0.348 (% citric) (**Table 2.2**). Similar contents were reported in other processing tomato juice studies (Anthon and others (2011); Barrett and others (2007); Barrett and Garcia (2006)). The data also is consistent with findings from Daood (1994) where 0.90-1.62 g/100g for glucose, 1.25-1.70 g/100g for fructose, and 6.04 mg/g citric acid nutrient levels in tomato fruits were reported. In general, the values found in this study are within those reported in the literature taking into account that nutrient levels may be affected by variety, maturity, temperature and soil nutrients among others (Gould 1974).

Table 2.2. Reference method results of quality parameters in tomato samples from the 2010 and 2011 growing seasons.

County	Number of Varieties		Glucose (g/L)	Fructose (g/L)	Citric Acid (g/L)	Glutamic Acid (g/L)	pH*	Soluble Solids (°Brix)	Titrateable acidity* (% citric)
Kern	42	Range	13.2 - 21.0	13.8 - 20.6	2.20 - 3.86	1.41 - 2.57	4.31 - 4.76	5.1 - 6.7	0.171 - 0.302
		Average $\pm$ SD	15.0 $\pm$ 2.20	15.4 $\pm$ 2.26	3.03 $\pm$ 0.40	2.19 $\pm$ 0.49	4.59 $\pm$ 0.12	5.41 $\pm$ 0.73	0.253 $\pm$ 0.034
Yolo	40	Range	13.2 - 21.4	13.3 - 20.1	1.97 - 3.76	1.00 - 2.50	4.22 - 4.77	3.5 - 5.4	0.185 - 0.348
		Average $\pm$ SD	16.3 $\pm$ 2.54	16.6 $\pm$ 2.29	2.64 $\pm$ 0.51	1.84 $\pm$ 0.45	4.51 $\pm$ 0.10	4.65 $\pm$ 0.46	0.253 $\pm$ 0.038
Merced	42	Range	12.1 - 19.4	12.6 - 20.0	1.52 - 3.59	1.92 - 3.08	4.34 - 4.73	5.0 - 6.4	0.179 - 0.335
		Average $\pm$ SD	16 $\pm$ 2.16	16.6 $\pm$ 2.37	2.34 $\pm$ 0.38	2.26 $\pm$ 0.35	4.55 $\pm$ 0.10	5.58 $\pm$ 0.34	0.232 $\pm$ 0.032
San Joaquin	38	Range	10.0 - 18.5	11.0 - 18.1	1.29 - 2.53	1.54 - 2.61	4.39 - 4.87	3.2 - 5.4	0.152 - 0.283
		Average $\pm$ SD	13.7 $\pm$ 1.67	14.6 $\pm$ 1.66	1.98 $\pm$ 0.30	2.19 $\pm$ 0.33	4.57 $\pm$ 0.10	4.35 $\pm$ 0.49	0.218 $\pm$ 0.031
Fresno	39	Range	12.3 - 18.6	12.8 - 20.0	1.98 - 3.39	1.68 - 3.21	4.30 - 4.66	5.0 - 6.2	0.184 - 0.338
		Average $\pm$ SD	17.3 $\pm$ 2.38	17.7 $\pm$ 2.39	2.68 $\pm$ 0.36	2.21 $\pm$ 0.43	4.57 $\pm$ 0.14	5.59 $\pm$ 0.34	0.265 $\pm$ 0.037

* TA and pH results were unreliable for samples from 2011 growing season; therefore, results display 2010 samples only.

2.4.2 Calibration model development using tomato juice samples from the 2010 growing season

Tomato samples from the 2010 growing season were screened on the bench-top (single-bounce and triple-bounce ATR accessories), FlexScan handheld, and Cary portable

spectrometer systems. Typical ATR and transmission spectra obtained from tomato juice supernatants (no pulp) showed strong water absorption bands (1582-1692 and 2971-3627 cm^{-1}). As seen in **Figure 2.2**, the ATR triple-bounce accessory used on the bench-top spectrometer provided increased absorbance intensity through multiple interactions between the incident IR radiation and the sample which improved the signal resolution of low concentration components (Pike Technologies 2004). Important bands in the fingerprint region (1500-900 cm^{-1}) were associated with C-O and C-C stretching modes (900-1153 cm^{-1}) and O-C-H, C-C-H, and C-O-H bending vibrational modes (1474-1199 cm^{-1}) (Stuart 2004; Irudayaraj and Tewari 2003). The Cary portable system generated higher absorption intensity compared to the single- and triple-bounce ZnSe ATR bench-top system (**Figure 2.2**). The effective pathlength (EPL) obtained by using the multi-reflection ATR accessory was $\sim 13.08 \mu\text{m}$ (Pike Technologies 2004), a 3-fold increase from a single-bounce ATR accessory (EPL=4.6 μm), while the DialPath transmittance accessory for the Cary 630 system provided an EPL of 30 μm for data acquisition. The saturated signals between 3800-2800 cm^{-1} are due to the strong infrared absorptivity of water (104.4 $\text{M}^{-1} \text{cm}^{-1}$) and are unfortunately void of any information (Grdadolnik 2002).

Figure 2.2. Infrared absorption spectrum of the tomato samples on bench-top (single-bounce ATR in pink, triple-bounce ATR in purple), FlexScan handheld (blue), and Cary portable (green) spectrometer systems.

The cross-validated leave-one-out PLSR model performance statistics for the 2010 tomato samples on the bench-top, portable, and handheld FTIR systems are displayed in **Table 2.3**. By selecting the optimum number of latent variables (factors) that minimized the standard error of cross validation (SECV) of the model, ~95-99% of the cumulative variance for the systems was explained. The number of factors (3-10) used in the models reduced spectral noise and potential over-fitting which could impair its ability to estimate composition in unknown samples.

In general, the single- and triple-bounce ATR accessories used in the bench-top and portable Cary systems performed similarly to each other in terms of correlation coefficient of cross validation (R_{cv}) and SECV (**Table 2.3**). The SECV for the quality parameters on the bench-top and Cary portable systems were ~1.1 g/L (sugars), 0.23 g/L (citric acid), 0.20 g/L (glutamic acid), 0.016 % citric (TA), 0.05 pH, and 0.18 °Brix (soluble solids) (**Table 2.3**). The FlexScan handheld system showed slightly inferior

performance to the other systems as seen by the lower R-value and higher SECV for nearly all quality parameters (**Table 2.3**).

Table 2.3. Performance statistics for PLS regression models generated for quality parameters in processing tomatoes from the 2010 growing season on FT-IR systems.

Analyte	Technique	N	Range of Concentration	Factors	SECV ^a	R _{CV} ^b
Glucose (g/L)	Benchtop - 3 bounce ^c	205	10.0-21.4	4	1.13	0.76
	Benchtop - 1 bounce ^c	200		4	1.16	0.77
	Portable - Cary ^d	195		4	1.12	0.80
	Handheld - FlexScan ^d	180		5	1.20	0.76
Fructose (g/L)	Benchtop - 3 bounce ^c	205	11.0-20.6	3	1.11	0.73
	Benchtop - 1 bounce ^c	190		4	1.08	0.73
	Portable - Cary ^d	200		4	1.11	0.73
	Handheld - FlexScan ^d	190		5	1.17	0.73
Citric Acid (g/L)	Benchtop - 3 bounce ^c	200	1.29-3.86	9	0.21	0.88
	Benchtop - 1 bounce ^c	190		8	0.23	0.89
	Portable - Cary ^d	210		7	0.25	0.88
	Handheld - FlexScan ^d	190		5	0.32	0.80
Glutamic Acid (g/L)	Benchtop - 3 bounce ^d	205	1.00-3.21	8	0.19	0.90
	Benchtop - 1 bounce ^c	190		9	0.19	0.88
	Portable - Cary ^d	195		9	0.15	0.93
	Handheld - FlexScan ^d	170		5	0.21	0.84
Titratable Acidity (% Citric)	Benchtop - 3 bounce ^c	205	0.152 - 0.348	9	0.014	0.92
	Benchtop - 1 bounce ^d	190		10	0.015	0.93
	Portable - Cary ^d	205		9	0.018	0.88
	Handheld - FlexScan ^d	180		6	0.025	0.74
pH	Benchtop - 3 bounce ^c	200	4.22-4.87	8	0.04	0.82
	Benchtop - 1 bounce ^d	190		9	0.04	0.83
	Portable - Cary ^d	195		9	0.05	0.81
	Handheld - FlexScan ^d	160		6	0.05	0.61
°Brix	Benchtop - 3 bounce ^d	200	4.2-6.7	8	0.17	0.96
	Benchtop - 1 bounce ^c	190		8	0.20	0.96
	Portable - Cary ^d	200		7	0.19	0.96
	Handheld - FlexScan ^d	170		5	0.25	0.93

Note: Models were generated using multiple specular component transformation. Smoothing was used with FlexScan and Bench-top (1 bounce) systems to reduce noise interferences.

^aSECV: standard error of cross-validation

^bR_{CV}: correlation coefficient of cross-validation

^cSpectral region 1800-900 cm⁻¹ included

^dSpectral region 1500-900 cm⁻¹ included

Overall, a high number of outliers were identified from the FlexScan unit based on leverage and studentized residual analysis (**Table 2.3**). Interferences from the water regions' strong absorption bands ($1582\text{--}1692\text{ cm}^{-1}$ and $2971\text{--}1395\text{ cm}^{-1}$) are likely reducing the signal-to-noise ratio in the FlexScan, thus masking the signal for the bending (O-C-H, C-C-H, and C-O-H) vibrational modes in the $1500\text{--}1200\text{ cm}^{-1}$ region. The estimated quality parameter contents measured by the ATR-IR or transmission spectroscopy showed coefficient of determination (R_{cv}) ranging from 0.61-0.96 with reference values (**Table 2.3**).

Figure 2.3 shows the PLSR models generated for citric acid on the single and triple-bounce ATR bench-top, the portable Cary 630 system with DialPath transmission accessory, and the handheld FlexScan diamond ATR system. **Figure 2.3D** illustrates that higher data scattering, or a higher SECV (**Table 2.3**), was observed from the FlexScan handheld as the data points were more scattered along the regression line. The Cary 630 portable system provided much stronger correlation as seen in the tighter predictions or lower SECV (**Figure 2.3B**). These models are helpful visualizations of the relationship between measured quality parameters and predictive capability of the infrared systems.

2.4.3 Re-calibration model development using tomato juice samples from the 2010 and 2011-growing season by selected FT-IR systems

Based on the performance of PLSR models to estimate quality parameters in tomato juices from the 2010 growing season, the triple-bounce ATR-containing bench-top and portable Cary FT-IR systems were selected to develop a validated model to rapidly assess

processing tomatoes. The bench-top acted as a “control technology” and the portable Cary 630 unit was compared to the performance of the bench-top.

Figure 2.3. Generated PLSR calibration models for citric acid on the (A) single-bounce ATR bench-top, (B) triple-bounce ATR bench-top, (C) portable Cary, and (D) handheld FlexScan.

PLSR calibration models were built using two-thirds of the processing tomato samples from the 2010 and 2011 growing seasons. The included spectral range for the Cary portable FT-IR was 1500-900 cm^{-1} to exclude saturated water absorption signals. The 1800-1500 cm^{-1} signal was not as overwhelming for the bench-top system, therefore

some or all parts of this region were included when developing the models (**Table 2.4**). Multiplicative scatter correction (MSC) transformation was applied to the data as a pre-processing treatment. Calibration model summary statistics for five quality parameters (glucose, fructose, citric acid, glutamic acid and °Brix) are presented in **Table 2.4**. Reference data for titratable acidity and pH was not reliable for tomato juices from the 2011 growing season, therefore was not included in calibration model development (**Table 2.2**).

Overall, PLSR calibration models generated by tomato juices from the 2010-2011 seasons yielded higher correlation coefficients ($R_{cv} = 0.81-0.95$) than previously found with 2010 samples only ($R_{cv} = 0.73-0.96$, **Table 2.3**) and similar SECV levels. This indicated that by increasing the number and diversity of the samples, it allowed for the reduction of impact from irrelevant spectral-variations (noise) in the calibration model. This capability provided a more information-rich data set of reduced dimensionality and eliminated data noise that resulted in more accurate and reproducible calibration models (Martens 1989; Warnock and Peck 2010; De Maesschalck and others 1999). Both instruments displayed very similar, and adequate, estimating capacity for all sugar and acid quality parameters ($R_{cv}=0.82-0.94$ bench-top, $0.81-0.95$ portable), which indicated that the selected principal components were modeling about 81-95% of the variance within the samples (Mark and Workman 1991).

Table 2.4. Calibration and validation performance statistics for PLS regression models generated for quality parameters in processing tomatoes from the 2010-2011 growing season on bench-top (triple-bounce ATR) and Cary portable systems.

		Range of						
Analyte	Technique	N	Concentration	Factors	SECV ^a	R _{CV} ^b	SEP ^c	R _{val} ^d
Glucose (g/L)	Benchtop - 3 bounce ^e	285	10.0 - 21.4	8	1.20	0.85	1.47	0.83
	Portable - Cary ^g	345		4	1.22	0.85	1.14	0.88
Fructose (g/L)	Benchtop - 3 bounce ^e	285	11.0 - 20.6	3	1.19	0.82	1.23	0.80
	Portable - Cary ^g	325		4	1.14	0.81	1.21	0.80
Citric Acid (g/L)	Benchtop - 3 bounce ^f	270	1.29 - 3.86	8	0.21	0.86	0.21	0.85
	Portable - Cary ^g	360		8	0.24	0.87	0.24	0.88
Glutamic Acid (g/L)	Benchtop - 3 bounce	270	1.00 - 3.21	7	0.22	0.84	0.25	0.79
	Portable - Cary ^g	330		9	0.18	0.88	0.19	0.86
°Brix	Benchtop - 3 bounce	300	4.2 - 6.7	8	0.20	0.94	0.23	0.92
	Portable - Cary ^g	370		6	0.21	0.95	0.21	0.96
^a SECV: standard error of cross-validation								
^b R _{CV} : correlation coefficient of cross-validation								
^c SEP: standard error of prediction								
^d R _{val} : correlation coefficient of validation								
^e Spectral region included 1800-900 cm ⁻¹								
^f Spectral region included 1800-1600, 1500-900 cm ⁻¹								
^g Spectral region included 1500-900 cm ⁻¹								

The use of loadings plots enabled identification of portions of the original spectra that were important for discrimination. Loadings identified which areas of the IR spectra were more related to sample variation; when they were particularly large (either below or above zero) they denote areas of the original spectra that were important for discrimination. The most relevant spectral region for sugars was 1200-1000 cm⁻¹, which includes the C-O and C-C stretching modes of carbohydrates (Scibisz and others 2011). The most intense peaks for sugars were 1062 cm⁻¹ on the bench-top system and 1082 cm⁻¹, characteristic of the C-O stretch vibration. The band at 1062 cm⁻¹ is in accordance to results reported by Sivakesava and Irudayaraj (2000). Prominent peaks for citric acid discrimination included the 1020-1105 cm⁻¹ region for both systems and, additionally,

1716 cm^{-1} on the bench-top system, which reflected the carbonyl stretch ($\text{C}=\text{O}$) vibration (Stuart 2004). Interestingly, relevant spectral regions for glutamic acid were in the 1020-1105 cm^{-1} region but also included a prominent band at 1405 cm^{-1} for symmetric stretching of the carboxylic group (Barth 2000). Discrimination for soluble solids utilized attributes of both acids and sugars' spectral regions, relying on prominent peaks in the 1000-1130 cm^{-1} region and a small peak at 1406 cm^{-1} .

2.4.4 External validation using an independent set of tomato juices

External validation of the PLSR models, obtained with the calibration samples, was performed using the remaining third of tomato juice samples from 2010-2011 growing seasons. Although several authors have reported that SECV gives a realistic estimate of the error of prediction of samples not included in the calibration (Meuret and others 1993; Shenk and Westerhaus 1996; Martens and Dardenne 1998), this step is necessary to obtain an independent measurement of the equation's accuracy, expressed as SEP i.e. standard error of prediction (Windham and others 1989). Examination of the validation statistics shown in **Table 2.4** reveal similar SECV and SEP values for MIR predictions of all quality parameters examined, indicating that the application of these MIR models of both systems gave robust predictions under practical conditions (Fontaine and others 2001). It is a normal finding that the standard errors obtained by validation were slightly higher than those of cross-validation (Mark and Workman 1991).

The PLS regression models showed good ability in estimating the sugars, acids and especially soluble solids in tomato for both triple-bounce ATR bench-top and Cary 630 portable FT-IR systems judging by the high R_{val} and low SEP results (**Table 2.4**).

Comparatively, a study completed by Schibisz and others (2011) obtained similar mid-IR modeling results in quantifying sugars, citric acid, and soluble solids in tomatoes using a bench-top system, with reported $R_{\text{val}} > 0.92$ for glucose, fructose, citric acid, and soluble solids in tomatoes and standard error of predictions of 0.87 g/L, 1.04 g/L, 0.39 g/L, and 1.84 °Brix, respectively. Other works have been reported by Pedro and Ferreira (2007) supporting the use of bench-top FT-IR systems for assessing tomato quality; however, no research has yet been reported on the use of portable systems. Interestingly, the Cary 630 unit equipped with a transmission DialPath accessory provided superior performance in estimating sugars, acids, and soluble acids in processing tomatoes compared to the single-bounce ZnSe ATR bench-top and FlexScan handheld that utilized a single-bounce diamond ATR, and similar performance to the triple-bounce ZnSe ATR bench-top (**Table 2.4**). This demonstrates that some novel portable FT-IR systems may provide the tomato processing industry with a rapid method to evaluate processing tomatoes with equivalent levels of reliability and sensitivity as bench-top systems but allow for more flexibility since the unit can be easily carried and transferred. Our findings support the use of a portable FTIR with transmission sampling accessory for rapid assessment of quality parameters in processing tomatoes.

2.5 Conclusion

Reliable infrared spectroscopy methods were developed for the rapid determination of quality parameters in processing tomatoes using the triple-bounce ZnSe ATR-containing

bench-top and the Cary 630 portable IR spectrometer with ZnSe dial-path transmittance accessory. Calibration and validation PLSR model performance statistics ($R_{cv} = 0.81-0.95$, $R_{val} = 0.79-0.96$) were consistently strong between both systems. The disparity from the FlexScan handheld FT-IR comes from the reduced signal-to-noise ratio due to strong water absorption and low overall signal intensity. The Cary 630 portable IR spectrometer with ZnSe dial-path transmittance accessory may provide the tomato processing industry with an efficient method for in-field, high throughput quantification of quality parameters in tomatoes while also allowing for increased flexibility.

2.6 References

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