Rapid Assessment of Sugars and Organic Acids in Tomato Paste Using a Portable Mid-Infrared Spectrometer and Multivariate Analysis

Thesis

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Congcong Zhang

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The Ohio State University

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Master's Dissertation Committee:

Dr. Luis E. Rodriguez-Saona, advisor

Dr. Lynn Knipe

Dr.Monica Giusti

Abstract

Tomatoes are the second highest grown and consumed vegetables in the U.S. The majority of tomatoes are thermally processed into tomato paste, then reconstituted into various products such as tomato sauce and ketchup. As an in-between product, high-quality and consistent paste is crucial for the tomato industry. Sugars and organic acids, which are responsible for the sweetness, sourness and influence on tomato flavor, are the major factors affecting consumer acceptability and are crucial for successful processing of tomato-based products. In addition, the Vitamin C content (L-ascorbic acid and L-dehydroascorbic acid) is an attractive index for the quality of tomato product both as a source of antioxidant and Vitamin. Current analytical techniques to determine sugars and organic acids of tomato paste rely on chromatography, which is time-consuming and labor-intensive. Cutting edge infrared sensor technologies can provide a valuable window into in-process food manufacturing to permit optimization of production rate, quality and safety of tomato products. The objective of this study was to develop a rapid and robust method for simultaneous determination of sugars (glucose, fructose and total reducing sugars) and organic acids (citric acid and total Vitamin C) in tomato paste using a portable mid-infrared spectrometer combined with multivariate analysis. Tomato paste samples (n=120) were kindly provided by a major tomato processing company in California. The spectra were directly collected by a portable mid-infrared spectrometer equipped with a triple reflection diamond ATR sampling device. High-performance liquid chromatography (HPLC) was used to determine the reference levels of reducing sugars (glucose, fructose and total reducing sugars) and organic acids (citric acid and Vitamin C). Partial least square regression (PLSR) was used to develop calibration and validation models. Paste compositional ranges were glucose (6.46-13.05 g/100g), fructose (6.82-14.29 g/100g), total reducing sugars (13.28-27.01 g/100g), citric acid (2.89-5.86 g/100g) and Vitamin C (74.30-106.77 mg/100g). PLSR models showed good correlation ($R_{CV}>0.91$, $R_{Val}>0.93$) between the mid-infrared spectrometer predicted values and reference values, and low standard errors of cross validation (SECV) of 0.57 g/100g for glucose and 0.69 g/100g for fructose, 1.15 g/100g for total reducing sugars, 0.29g/100g for citric and 2.44 mg/100g for total Vitamin C. Portable mid-infrared spectrometer could be a revolutionary tool for in-plant assessment of the quality of tomato-based products, which would provide the tomato industry with accurate results in less time and lower cost since no reagents nor sample preparation are required.

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Vita

2010...Zibo Experimental High School

2014...B.S. Food Science and Engineering, Beijing Forestry University

2014 to present ....Master Student, Department of Food Science and Technology, The Ohio State University

Fields of Study

Major Field: Food Science and Technology

Table of Content

List of Tables

List of Figures

Chapter 1: Introduction

Tomatoes (*Lycopersicon esculentum*) are one of the most produced vegetable crops worldwide, second only to potato. The United States is the world's third largest tomato producer behind China and India (FAO, 2013). Tomatoes are consumed either freshly or more frequently as processed tomato products such as tomato paste, tomato sauce, and pizza sauce. In the tomato season, the majority of tomatoes are processed into concentrated tomato paste with multiple steps including washing and sorting, hot or cold break, juice extraction, evaporation and sterilization (Koh, Charoenprasert & Mitchell, 2012). The concentrated tomato paste is then reconstituted into various different products such as tomato sauce, pizza sauce and ketchup (Zhang, Schultz, Cash, Barrett & McCarthy, 2014). As an intermediate product, the quality of tomato paste is crucial for the tomato processing industry. To maintain optimal and consistent quality, a variety of characteristics of the tomato paste are commonly tested on each batch of production, including color, soluble solids, consistency, titratable acidity, pH, sugars, organic acids and lycopene (Ayvaz et al., 2016; Zhang et al., 2014).

Sugars and organic acids are the two important quality parameters for tomato products that affect the consumers' perception and liking. The sweet-sour flavor of tomatoes and tomato products is contributed by the sugars and organic acids as well as their interaction with the volatiles in tomatoes (Baldwin et al., 2008). In addition, the levels of sugars (glucose and fructose) and organic acid (citric acid) are associated with other key quality parameters of tomato paste including soluble solids, pH and titratable acidity (TA), which enables them to provide rich information for the optimization of food processing. The total vitamin C content (L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA)) is considered to be a valuable index for tomato products.

Current methods for quantitative analysis of sugars and organic acids in food rely heavily on High Performance Liquid Chromatograph (HPLC), which is a reliable technique for chemical component separation and quantitation (Kamil, Mohamed & Shaheen, 2011). However, this approach is not well accommodated to in-line quality control of tomato paste, as it requires extensive sample preparations, use of hazard solvents and testers' professional skills. Therefore, there's a need for rapid, cost-effective and robust methods for the quality control of tomato products.

Mid-infrared spectroscopy has shown its potential in analyzing different chemical components in food and agricultural products as it is simple, fast and cost efficient (Wilkerson et al., 2013). The mid infrared (4000 cm^{-1} to 400 cm^{-1} wavenumber) is an important region in predicting specific chemical compounds of interest in the food matrix as the spectrum is formed due to the fundamental vibrations of the functional groups in different molecules. The development of portable FTIR instruments and versatile sampling accessories such as attenuated total reflectance (ATR) has made it possible for in-field analysis of food products. Previous researches have applied the portable mid-infrared spectrometer in analyzing quality parameters such as soluble solids, consistency, TA, pH, sugars, organic acids in tomato juice (Ayvaz et al., 2016; Wilkerson et al., 2013). However, no studies so far have been reported on the assessment of the sensory and nutrition related parameters—sugars and organic acids in tomato paste by using a portable spectrometer, which is valuable for tomato paste manufacturing.

This study focuses on the development of a rapid and robust method for simultaneous determination of sugars (glucose, fructose and total reducing sugars) and organic acids (citric acid and total Vitamin C) in tomato paste using a portable mid-infrared spectrometer combined with multivariate analysis. The thesis includes an overall review of the tomato industry, the quality control of processed tomato products, the mid-infrared spectroscopy techniques, and the explanation of my research.

Chapter 2: Literature Review

2.1 The Tomato Industry

Tomatoes are among the most cropped and consumed vegetables worldwide, with the total production of 161.3 million metric tons in 2013 (FAO, 2013). The United States is the third largest tomato producer behind China and India, producing around 12.7 million metric tons of tomatoes, which generates more than 2 billion dollars in annual farm cash receipts (FAO, 2013). The tomato industry in the United States can be divided into two types based on the market they target: fresh tomato industry and processing tomato industry. Fresh-market tomatoes are commercial-scaled produced in about 20 states of the nation, while California and Florida are the two leading states. Fresh-market tomatoes are harvested by handpicking and the retail prices of fresh tomatoes are higher than processing tomatoes with average price 1.40 to 2.47 U.S dollars per pound since 2000 (USDA, 2016).

Up to three fourths of the nation's tomato production was for further processing (USDA, 2016). California has been the primary source of processing tomatoes as well as processing tomato products, accounting for 96% of the total production. Indiana, Ohio, and Michigan account for most of the remaining production. Compared to fresh market tomatoes, tomatoes for processing require higher percentage of soluble solids (average 5 to 9 percent) and stronger peels, and they are machine-harvested (USDA, 2016). Given the significance of the processing tomato industry, the raw vegetables, final products, processing, quality control methods were reviewed as follows.

2.1.1 Tomato

The tomato (Lycopersicon esculentum), belonging to the Solanaceae family and Lycopersicon specie, is normally a self-pollinated, warm-season crop, while it can be successfully grown from the equator to as far north as 65 °N (Gould, 2013). There is some debate over the classification of the tomato either as a fruit or a vegetable because of its usages in different fields. Botanically, the tomato is the ripened ovary of the flower and retains the seeds of the host plant, which makes it under the category of fruit (Sterbenz, 2013). In consideration of cooking, the tomato is classified as a vegetable, because it is cooked for the main course of savoury rather than dessert (Dickinson, 2012). USDA classifies the tomato as a vegetable and reports the annual summaries of tomatoes within the vegetable lists in the Nation Agricultural Statistics Services database (Dickinson, 2012).

Tomato was believed to originate from the tropical America (Morrison, 1938). It was not known and cultivated in the United States until around 1830-1840, and even then there were a lot of bias on the edibility of the tomato fruit (Gould, 2013). The tomato industry got fast developed after the First World War with the appearance of hundreds of varieties of tomatoes and significant increase in the production of tomatoes (Gould, 2013). Now tomatoes are popular all over the world and have become the second largest produced vegetables in terms of dollar value (Thakur, Singh & Nelson, 1996).

Generally, the tomato contains 5%-10% dry matter, among which 1% is seed and skin (Thakur et al., 1996). Approximately 50% of the dry matter is reducing sugars, including mainly glucose, fructose and small quantities of other reducing sugars such as raffinose, arabinose, xylose, galactose as well as polyol such as myoniositol (Thakur et al., 1996). Sucrose normally accounts for less than 0.1% of the fresh weight (Davies & Kempton, 1975). Organic acids, which are primary citric acid and malic acid and minute amounts of amino acids, form around 10% of the dry matter in the tomato. The remaining 40% of the total dry matter is made up of minerals, pigments, Vitamins, lipids and alcohol-insoluble solids such as proteins, pectin, cellulose and hemicelluloses (Thakur et al., 1996). From the nutritional point of view, the tomato is considered as a good source of Vitamin C (19mg/100g fresh weight (FW)), Vitamin A (623IU/100g FW), flavonoids (mainly quercetin and kaempferol) and carotenoids (β-carotene and lycopene). Quercetin, the dominant flavonoids in the fresh tomato, usually ranges from 0.03 to 2.76mg/100g. The levels of β-carotene and lycopene have been reported as 0.28mg/100g and 5.68mg/100g respectively in the fresh tomato (Koh, Charoenprasert & Mitchell, 2012).

In addition to the nutrients, the fresh tomato contains potentially toxic glycoalkaloids—tomatine and solanine, which can protect the tomato plant from insects and microorganisms (Thakur et al., 1996). Tomatine is the major alkaloid in the tomato and the level of it decreases with the ripeness of tomato as well as storage. As a result, young green tomatoes have a level of tomatine as high as 3390mg/kg, while thoroughly ripened tomatoes only contain less than 5mg/kg tomatine. Solanine is usually in small amounts and not considered to cause health risks (Thakur et al., 1996).

2.1.2 Processed Tomato Products

Tomatoes are consumed more frequently in the processed form rather than the fresh form in the western diet. There are several different kinds of processed tomato products, either used as ingredient for other products or directly marketed to customers. Hayes, Smith and Morris (1998) summarized the useful definitions of the primary types of processed tomato products including tomato pulp, tomato juice, tomato puree and tomato paste.

Tomato pulp refers to the crushed tomatoes before or after removing the seeds or skins (Hayes et al., 1998). While, pulp is the suspended solid material in tomato products that can be removed by centrifugation or filter (Hayes et al., 1998). Tomato juice is the juice from whole crushed tomatoes with the removal of seeds and skins intended for consumption without concentration or dilution (Hayes et al., 1998). Tomato paste is the concentrated tomato product from tomato pulp with the removal of skins and seeds, which contains at least 24.0 percent of natural tomato soluble solids (NTSS) as sucrose (Hayes et al., 1998). Tomato puree is the concentrated tomato product containing 8.0 to 24.0 percent of NTSS (Hayes et al., 1998). Tomato puree in the U.S can be also called tomato pulp or concentrated tomato juice. Tomato serum refers to the centrifuged or filtered tomato juice without any suspended solid material. And tomato syrup is concentrated tomato serum (Hayes et al., 1998).

For different purposes of use, tomato paste and tomato puree are also graded based on either % total solids or NTSS by different countries or organizations. Different grades of tomato paste (USDA, 1977) and tomato puree (USDA, 1978) in the United States were shown in **Table 2.1**.

Table 2.1. Tomato paste and puree grades in the United States. Adapted from (USDA, 1977& 1978)

Grade	Tomato Puree (%NTSS)	Tomato Paste (%NTSS)
Light	8-10.2	24.0-28.0
Medium	10.2-11.3	28.0-32.0
Heavy	11.3-15.0	32.0-39.3
Extra Heavy	15.0-24.0	39.3 or more

2.1.3 Tomato Processing

During tomato season, large portions of tomatoes are first processed into concentrated tomato paste, which allows for long-term storage and preservation. Then concentrated tomato paste is reconstituted into other products such as tomato sauce, ketchup and other value-added products (Anthon, Diaz & Barrett, 2008). Tomato paste is basically processed in multiple steps that depend on an initial thermal treatment (hot or cold break) with the following pulping, filtering, evaporation and sterilization process (Koh et al., 2012). Each step of processing concentrated tomato paste is described below.

Washing and sorting: Before washing, fresh tomatoes are dry sorted to remove gross contamination. The washing process is a combination of soak operation and high-pressure spray rinse operation for the removal of soil, spray residues, dirt, mold, microorganisms, rodents, Drosophila eggs and larvae. Finally, the washed tomatoes are sorted and trimmed for off-color and defective fruit parts (Moresi & Liverotti, 1982).

Break: Washed and sorted tomatoes are chopped and pumped into a heat exchanger for the breaking process (Moresi & Liverotti, 1982). Tomatoes can be thermal treated by either hot break or cold break. During hot break, tomatoes are heated rapidly to

90 ℃～ 95 ℃ in order to inactivate pectin degrading enzymes especially, pectin methylesterase (PME) and polygalacturonase (PG) (Anthon et al., 2008). In this case, pectin can be protected from enzymatic breakdown, which contributes to tomato juice with high viscosity but the loss of color and flavor. During cold break, chopped tomatoes are heated to around 65℃, during which the PME and PG are still active. As a result, the 'cold break' juice is less viscous, more flavorful and gives greater serum separation (Shomer, Lindner & Vasiliver, 1984). The choice of break method depends on the intended use of the concentrated paste: hot break process is suitable for making concentrated tomato paste used in final products such as ketchup and pizza sauce, while cold break process is selected for paste that is supposed to be reconstituted into tomato juice or vegetable cocktails (Morris, 1991).

Juice extraction (pulping and filtering): Tomato juice is usually extracted by removing the skins and seeds (pomace) through pulper and finishers of different screen sizes. The screen size of the finishers plays an important role in the control of the texture of the final products (Hayes et al., 1998).

Evaporation: Tomato juice is concentrated using multi-stage evaporators ranging in time-temperature parameters based on the required viscosity of the final products (Koh et al., 2012). Evaporation under low pressure would lower the boil point of water and preserve most of the colors and flavor (Gould, 2013).

Sterilization, Cooling and Filling: The paste is sterilized at approximately 100℃ for 3-5min and is flash cooled to 35℃. The cooled paste is pumped into feed tank for aseptic filing (Koh et al., 2012).

2.2 Quality Control of Processed Tomato Products

Tomato products are made in multiple procedures during which the characteristics of the materials are changing. The quality of tomato products should be consistent when they reach the final stage of processing as the quality attributes have significant influences on customer perception and marketing. Monitoring the quality of tomato products during processing is crucial for the tomato industry. Attractive bright color, good aroma, high total acidity, high consistency and low serum separation, are some the favorable factors for customers (Thakur et al., 1996). Below is a review of commonly used parameters for the quality control of tomato product, including sugars, organic acids, Vitamin C, which are the focus of this thesis and other related key quality parameters.

2.2.1 Sugars and Organic Acids

Sugars and organic acids are important quality parameters as they are responsible for the flavor of the tomato products. One of the most typical sensory characteristics of tomatoes or tomato products is its sweet-sour flavor, which is determined by the sugar, acids as well as their interactions with the volatiles in tomatoes (Baldwin, Goodner & Plotto, 2008). It was reported that the flavor acceptability of the diced tomato was significantly improved by adding citric acid and reducing sugars (glucose/fructose) (Malundo, Shewfelt & Scott, 1995).

In addition, the levels of sugars and organic acids are highly correlated with the other quality parameters of the tomato product such as NTSS, titratable acidity (TA) and pH. As mentioned before, the main sugar contents in tomatoes are reducing sugars including glucose, fructose and small amount of raffinose, arabinose, xylose and galactose (Thakur et al., 1996). Glucose and fructose **(Figure 2.1)** are the largest contributors to the NTSS in tomato products. Titratable acidity and pH of the product are mostly contributed by citric acid **(Figure 2.2)**, which is the most abundant acid in tomatoes (Anthon, LeStrange & Barrett, 2011). Malic acid, only about one tenth of citric acid, is another acid contributing to TA of tomato products. The main amino acid glutamic acid is great flavor enhancer in tomatoes and tomato products (Anthon et al., 2011; Hayes et al., 1998).

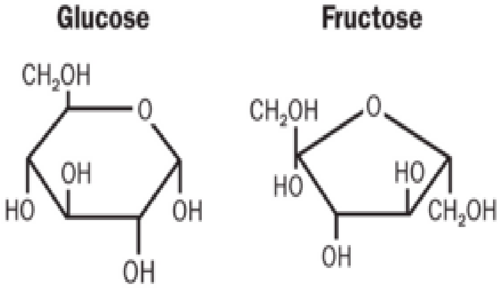

Figure 2.1. Molecular structure of glucose and fructose. Adapted from (Berger, 2013)

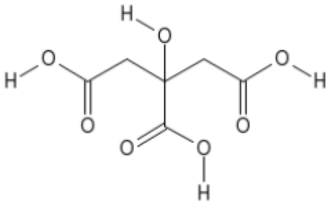

Figure 2.2. Molecular structure of citric acid. Adapted from (Wilkerson, 2012)

2.2.2 Vitamin C

Vitamin C is a valuable index for fruit and vegetables, both as an antioxidant and a vitamin. The recommended daily allowance (RDA) is 75mg/day and 90 mg/day respectively for men and women, which is sufficient to prevent scurvy and keep a stable body pool of 1500mg (Eitenmiller, Landen & Ye, 2007). Vitamin C content can be expressed as the sum of L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA). The most active form of Vitamin C is AA. It is labile to heavy metal ions, light, and oxygen and is easily oxidized to DHAA (Angberg, Nyström & Castensson, 1993; Bode, Cunningham & Rose, 1990) **(Figure 2.3)**. Even if DHAA does not show the bioactivity as AA does, it is considered to have similar biological behavior as AA, as it can be easily converted into AA in the human body (Wilson, 2002). DHAA can be further irreversibly oxidized to 2,3-diketo-gulonic acid, resulting in the loss of Vitamin C activity (Lewin, 1976).

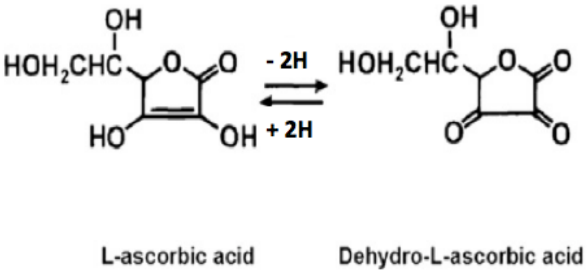

Figure 2.3. Degradation reaction of L-ascorbic acid to L-dehydroascorbic acid. Adapted from (Khalid, Kobayashi, Neves, Uemura & Nakajima, 2013)

The tomato is considered as a good source of Vitamin C, containing about 19mg/100g fresh weight, while the level of AA usually decreases during tomato processing (Koh et al., 2012). It was reported by Abushita, Daood and Biacs (2000) that half of the AA present in the raw fresh tomatoes was lost for tomato paste after processing. Koh and others (2012) found that hot break played an important role in the oxidation of AA and long time storage resulted in a significant loss of Vitamin C content in tomato paste, with only 19% remaining at 12 months. AA losses due to oxidation during thermal processing can be partially eliminated by deaerating tomatoes after crushing or keeping tomatoes subjected to breaking under vacuum (Gould, 1983; Morris et al., 1991).

Several different techniques have been used to determine the Vitamin C content including titrator (AOAC, 1990; Kabasakalis, Siopidou, & Moshatou, 2000), spectrometry (Arya, Mahajan & Jain, 1998) and amperometry (Arya, Mahajan & Jain, 2000). The most common method for quantitative analysis of Vitamin C is high performance liquid chromatography (HPLC), as it allows a good separation of different chemical components and provides highly accurate results (Nishiyama, Yamashita, Yamanaka, Shimohashi, Fukuda & Oota, 2004; Koh et al., 2012; Brause, Woollard & Indyk, 2003). In addition, there have been a number of studies for simultaneous determination of Vitamin C and other organic acids in fruit and vegetables (Kall & Andersen, 1999; Kacem, Marshall, Matthews & Gregory, 1986; Furusawa, 2001; Rodriguez, Oderiz, Hernandez & Lozano, 1992; Daood, Biacs, Dakar & Hajdu, 1994). The total Vitamin C content cannot be measured directly — dehydroascorbic acid should be reduced to ascorbic acid before HPLC analysis as dehyroascorbic acid doesn’t have any useful chromophore (Brause et al., 2003).

2.2.3 Other Key Quality Parameters

Soluble Solids

The soluble solid content is an important quality parameter for both fresh tomatoes and processed tomato products and is primarily contributed by reducing sugars in tomatoes. The soluble solid content is measured by refractometer and expressed as Natural Tomato Soluble Solids (NTSS) as % sucrose or as °Brix (AOAC, 1995; Hayes et al., 1998). The NTSS of fresh tomatoes has significant influences on the final yield, consistency and the overall quality of the tomato products (Thakur et al., 1996). Higher NTSS in fruit means that it requires smaller amount of fruit and less water evaporation to reach a certain quantity of final products (Thakur et al., 1996; Wilkerson, Anthon, Barrett, Sayajon, Santos & Rodriguez-Saona, 2013). The classification of different tomato products and different grades of the same products are made according to the NTSS (Zhang, Schultz, Cash, Barrett & McCarthy, 2014; Hayes et al., 1998).

Titratable Acidity and pH

Titratable acidity (TA) and pH are key processing characteristics of processed tomatoes, which are contributed by the organic acids in tomatoes. The titratable acidity is responsible for the flavor of the tomato products, usually measured using titration with NaOH and expressed in the form of % citric acid. (AOAC, 2000) The pH of tomato products plays an important role in microbial safety and food spoilage (Wilkerson et al., 2013). Tomatoes are classified as high-acid food (pH< 4.6) that requires more moderate sterilization process than low-acid food (pH> 4.6) for the disruption of thermophilic microorganisms to ensure food safety. Industrial tomato processors in California usually standardize their processed tomato products as a pH of 4.2 or 4.3 (Anthon et al., 2011). In general, the pH of tomatoes in standard cultivars ranges from 4.0 to 4.7, as a result it is allowed by the USDA standards to add organic acids to lower the pH of the final products if it is necessary during high–acid food processing (Barringer, 2004).

The acidity and pH of the processed tomato products are influenced by the processing conditions (Luh & Daoud, 1971). Tomato juice by hot break processing has higher pH and lower titratable acidity than cold-break juice due to the active pectolytic enzymes in the cold-break juice that produces acidic breakdown products (Stadtman, Buhlert & Marsh, 1977).

Consistency

Consistency, or gross viscosity, is a major quality parameter for tomato paste, sauce or puree that will affect the customer acceptability of the products (Thakur et al., 1996). Consistency refers to the flow property of non-Newtonian fluids with dissolved long chain molecules and undissolved particles (Barrett, Garcia, &Wayne, 1998). Some literatures report that the consistency of whole tomato juice is mainly determined by the insoluble content in the juice (Marsh, Buhlert & Leonard, 1980; Tanglertpaibul & Rao, 1987), while it is also reported that the soluble content in tomatoes can be another contributor to the gross viscosity (McColloch, Nielsen & Beavens, 1950; Luh et al., 1971). The empirical method to evaluate the consistency of tomato paste is using a Bostwick consistometer, which has limitations in validity for tomato products with more than 15% NTSS as the Bostwick consistency of tomato concentrates decreases exponentially with the increase of the product concentration (Tanglertpaibul & Rao, 1987). In this case, the Bostwick consistency of concentrated tomato products is usually measured after diluting the products to 12 °Brix at 20℃ (Zhang et al., 2014).

Serum Viscosity

Serum viscosity is the flow property of the dispersing medium of the tomato juice or sauce after removing the suspended particles (insoluble materials) by centrifugation. This serum viscosity of tomato products is contributed by the solutes present in tomatoes, especially the polymeric material-- pectin and is usually determined using a Canon-Fenske viscometer (Tanglertpaibul & Rao, 1987). Pectin contents in the final products are strongly influenced by tomato processing method. Hot-break process (>90°C) prevents the enzymatic breakdown of pectin and results in high pectin and high viscous products, while cold-break process (about 65°C) usually results in low viscous products with a better color and flavor (Anthon et al., 2008).

Color

Color is one of the most important quality parameters for processed tomato as it determines the customers' perception and liking (Barreiro, Milano & Sandoval, 1997). The red color of tomatoes is mainly due to the presence of lycopene, accounting for around 83% of the total pigments in tomatoes, and β-carotene, accounting for about 3% to 7% of the total pigments (Gould, 2013). The color of the tomato products is affected by many reactions during thermal processing. Lycopene degradation is the most common reaction resulting in color change, during which the trans form of the lycopene isomerizes to the cis structure (Barreiro et al., 1997). In the tomato industry, color is typically measured using a colorimeter and the 'L', 'a', 'b' scale is the most frequently used scale for tomato products (Hayes et al., 1998; Francis & Markakis, 1989).

2.3 Infrared Spectroscopy

In recent years, infrared spectroscopy has become popular in food and food product analysis, as it is rapid, robust, cost-effective, and is capable of analyzing most types of materials such as solids, liquids, gases, semi-solids, powders and polymers (Smith, 1999). Actually, it has been proven as an effective tool in analyzing food content such as proteins, oils, fats and sugars and acids (Wilkerson, 2012). Infrared radiation refers to the electromagnetic radiation with frequencies from 14,000 to 4 cm^{-1} and it can be divided into three parts: Near Infrared (NIR) from 14,000 to 4000 cm^{-1}, Mid Infrared (MIR) from 4000 to 400 cm^{-1}, Far Infrared (FIR) from 400 to 4 cm^{-1} (Guillen &Cabo, 1997). Infrared radiation is also know as heat that all objects in the universe at a temperature above absolute zero can give off. Infrared spectroscopy is a technique based on the interaction of infrared light with matter. When passing infrared radiation through the matter, it can be absorbed during their interaction, causing the change of molecular dipoles in the matter corresponding with vibrations or rotations (Stuart, 2004; Smith, 1999). Functional groups in the molecules tend to have infrared absorption in the same wavenumber range no matter what the structure of the rest is, which makes it possible to identify an unknown molecule from its infrared spectrum (Smith, 1999). Below is a review of the current techniques and instruments of infrared spectroscopy, which make it applicable to food analysis.

2.3.1 Mid-Infrared Spectroscopy

Both NIR and MIR are important techniques for different food analysis due to its moderate instrument cost, easy operation and high speed of measurement. MIR is mainly correlated with the transitions between vibrational states of molecules, involving a lot of the general stretching, bending and wagging motions of the chemical bonds in functional groups and thus, the overall MIR spectra can work as fingerprints for organic compounds (Sinelli, Spinardi, Di Egidio, Mignani, & Casiraghi, 2008). Meanwhile, the intensities of the MIR absorption bands are proportional to the concentrations of the related functional groups, making it a useful for quantitative analysis (Rodriguez-Saona & Allendorf, 2011). On the other hand, NIR reflects overtones and combination bands of fundamental transitions, resulting in more broad and less distinct spectra than MIR (Brás, Bernardino, Lopes & Menezes, 2005). Due to these differences, mid-infrared technique has the advantage of sensitive measurement of the specific chemical component in samples, while the near infrared region usually allows for the estimation of a class of compounds rather than an individual composition (Brás et al., 2005; Ścibisz, Reich, Bureau, Gouble, Causse, Bertrand & Renard, 2011). During the last decade, the application of MIR in quantitative analysis has increased by its powerful combination with chemometrics (Andrade, Gómez-Carracedo, Fernández, Elbergali, Kubista & Prada, 2003). The introduction of new sampling accessories—attenuated total reflection (ATR) has made MIR spectroscopy more accessible to food samples (Downey, Sheehan, Delahunty, O'Callaghan, Guinee & Howard, 2005).

2.3.2 Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is based on the idea of interference and the mathematical process of Fourier transform that decodes the interferogram to frequency spectrum (Stuart, 2004; Griffiths & De Haseth, 2007). Nowadays, FTIR spectrometer has become the predominant type of mid-infrared spectrometer used worldwide as it increased the speed and accuracy of measurement by replacing the prism and grating monochromators in the traditional dispersive machine with an interferometer (Rodriguez-Saona & Allendorf, 2011).

Michelson interferometer is the most common used interferometer, which comprises a semi-permeable beamsplitter, and two perpendicularly plane mirrors: one is fixed and the other is movable (Stuart, 2004). **Figure 2.4** shows the working mechanism of the Michelson interferometer.

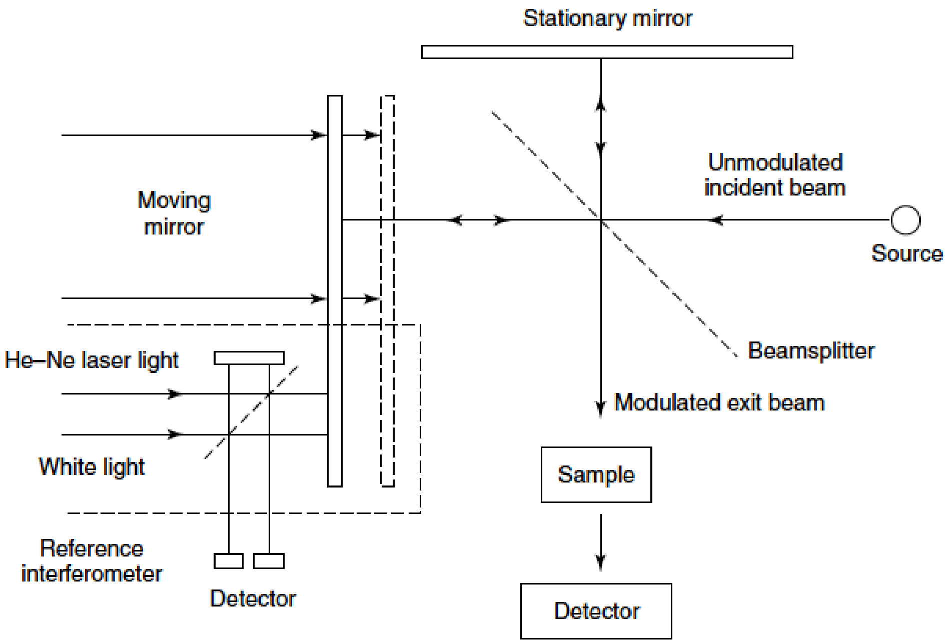

Figure 2.4. Diagram of the mechanism of a Michelson interferometer. Adapted from (Stuart, 2004)

When the collimated light from the light source impinges the ideal beamsplitter, half of it is transmitted to one mirror and half is reflected to the other mirror (Woodcock, Fagan, O'Donnell & Downey, 2008). As the travel path of one beam is fixed and the other is constantly changing due to the moving mirror, the two beams will interfere with each other when they are returned back by the mirrors and recombined at the beamsplitter (Woodcock et al., 2008). The modulated beam emerging from the interferometer at 90◦ to the unmodulated beam is called the transmitted beam, which will be detected in FTIR spectrometer (Stuart, 2004). The transmitted beam is then passed through the sample to the detector, turning out the interferogram containing the spectral information of the sample. Using Fourier transform algorithm, the interferogram is rapidly transformed from "Detector response vs Light path difference" to a typical "Absorption vs Wavelength" spectrum (Chen, Irudayaraj &

McMahon, 1998). Overall, FTIR has the advantages of improved signal to noise (S/N) ratio; increased light intensity through the sample, simultaneous acquisition of all wavenumbers, higher throughput, advanced wavelength resolution and accuracy, and reduced measurement time，making this technique an ideal tool for qualitative and quantitative analysis of food matrices (Rodriguez-Saona & Allendorf, 2011).

2.3.3 Attenuated Total Reflectance (ATR)

One of the key factors for successful application of FTIR in food analysis is the sample presentation method. A typical portion of the sample should be analyzed in order to obtain a relevant spectrum (Sinelli et al., 2008). The limitation of the traditional IR transmission spectroscopy is that the effective pathlength of the IR beam depends on the sample's thickness and orientation to the directional plane of the beam, hence the IR spectra for thick samples such as peanut butter, tomato paste or even solids will be too absorbing to precisely identify (Brain & Smith, 1996). This limitation can be overcome by diluting the sample with IR transparent salt and pressing into a pellet for analysis or pressing to a thin film before analysis (Pike Technologies, 2011).

Attenuated total reflectance (ATR) has become today's most widely used sampling accessory in FTIR as it allows spectral collection from solids, semisolids, liquids and thin films with little or no sample preparation, and has high sample-to sample reproducibility, low user-to-user spectral variation, which contribute to both qualitative and quantitative analysis with high accuracy and speed (Pike Technologies, 2011; Rodriguez-Saona & Allendorf, 2011). The primary benefits of the ATR result from its thin sampling pathlength and depth of penetration of IR light into the sample, that prevents total absorbing bands appearing in the IR spectrum (Pike Technologies, 2011)

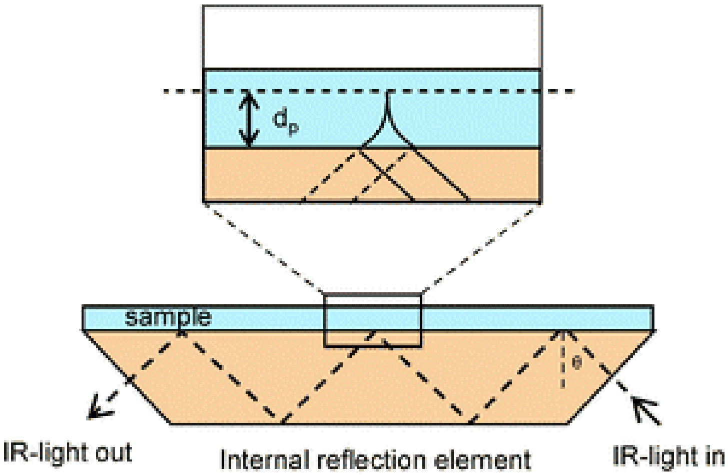

Figure 2.5. Schematic representation of ATR principle. Adapted from (Mojet, Ebbesen & Lefferts, 2010)

The design of ATR is based on the phenomenon of total internal reflection **(Figure 2.5)**. With ATR sampling, the IR light is directed into the internal reflection element, which is a crystal of relatively high refractive index made of diamond, zinc selenide, KRS-5 (thallium iodide/thallium bromide), or germanium (Rodriguez-Saona & Allendorf, 2011). The IR light travels inside the crystal and creates an evanescent wave that goes orthogonally into the sample intimately contacted with the crystal (Pike Technologies, 2011). IR spectrum is formed when the sample on the crystal interacts with the evanescent wave. Meanwhile, the evanescent wave is attenuated due to the sample's absorbance (Rodriguez-Saona & Allendorf, 2011).

The number of reflections within crystal may vary according to the length, thickness of the crystal and the angle of incidence (Pike Technologies, 2011). Since the absorbance of the sample is improved when increasing the number of reflections (Rodriguez-Saona & Allendorf, 2011), multi-reflection ATR is recommended for qualitative or quantitative analysis of minor component in the sample (Pike Technologies, 2011). **Figure 2.6** shows analysis of the carbohydrate content in a soft drink sample using a 10 reflection HATR accessory and a single reflection ATR. Apparently, the minor carbohydrate bands are more distinctive in multi-reflection ATR sampling accessory.

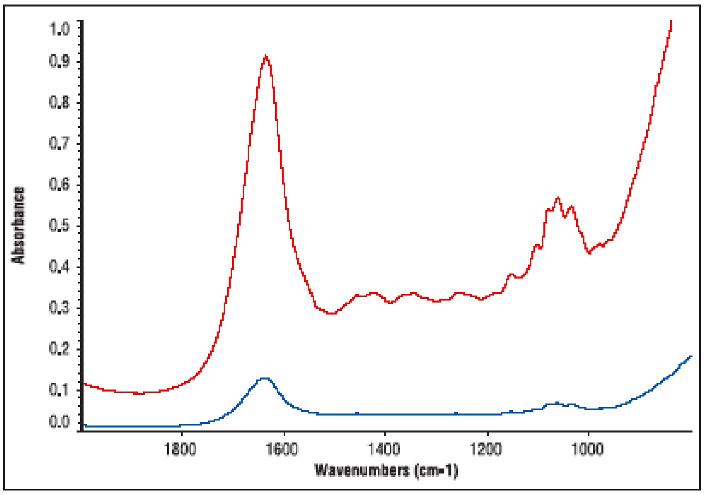

Figure 2.6. Spectra of a soft drink sample using ten reflection HATR (red) and single reflection ATR (blue). Adapted from (Pike Technologies, 2011)

For rigid, hard or irregular surface samples, high pressure is added to increase the sample contact with the crystal surface and thus increase the sample absorbance. In this case, the ATR crystal made of high hardness material is selected, such as diamond ATR, which is relatively easy to apply high pressure on (Pike Technologies, 2011).

2.3.4 Portable FTIR

In the latest five years, the new generation of FTIR-- portable FTIR systems, has gained a lot of popularity, as it is flexible, easy to use and convenient for on-site chemical analysis (Agilent Technologies, 2013). More importantly, these portable

FTIR systems are able to provide equivalently robust performance as the bench top units (Ayvaz, Sierra-Cadavid, Aykas, Mulqueeney, Sullivan & Rodriguez-Saona, 2016). These properties of the portable FTIR will make it an excellent quality control tool for the tomato processing industry.

The Agilent 4500 Series FTIR analyzers **(Figure 2.7)** are designed by Agilent Technologies® for the increasing needs of in-field analysis such as incoming materials and, processing or finished products in food, chemical and polymer industries (Agilent Technologies, 2013). Special for outside laboratory analysis, these series are compact, lightweight (only 6.8kg), battery-powered and enclosed in a weather resistant box (Agilent Technologies, 2013).

Figure 2.7. Agilent 4500 series portable FTIR analyzer. Adapted from (Agilent Technologies, 2013)

The 4500a FTIR units are available in different types of sampling accessories including Gas Cell, TumblIR, ATR to accommodate the analysis of different samples. Agilent 4500a FTIR ATR system is suitable for the analysis of pastes, gels, liquids and powders. For this system, diamond ATR sampling accessories of single bounce, three bounce or nine bounce are provided with the advantages of resistance and hardness, enabling the systems for quantitative and qualitative analysis for different types of food with no or little sample preparation.

Besides 4500 Series, Agilent Technologies® provides the world's smallest but most versatile and robust bench top FTIR units-- Cary 630 Laboratory FTIR. The Cary 630 FTIR has the advantage of interchangeable sampling modules **(Figure 2.8)** including standard transmission, DialPath, TumblIR, germanium ATR, diamond ATR, ZnSe multi-bounce ATR, specular reflectance and diffuse reflectance, which will fulfill the demands of multi-user environment. **Figure 2.9** shows the Cary 630 FTIR equipped with diamond ATR sampling accessory, which just slides in without any alignment.

Figure 2.8. Various sampling accessories for Cary 630 FTIR. From left to right are 10° specular reflectance accessory, diamond ATR, germanium ATR, ZnSe multi-bounce ATR, DialPath, TumblIR, diffuse reflectance accessory. Adapted from (Agilent Technologies, 2013)

Figure 2.9. Agilent Cary 630 FTIR equipped with diamond ATR sampling accessory. Adapted from (Agilent Technologies, 2013)

2.3.5 Chemometrics

The mid-infrared spectrum is able to show rich information about different chemical compounds in the samples according to the positions, intensities and shapes of the peaks. However, samples like foods are not pure component systems and as a result, will generate complicated spectra with overlapping peaks that makes routine analysis difficult. Multivariate analysis, containing a wide range of techniques such as data reduction, classification and regression, is considered as a powerful approach to deal with the complex spectrum data (Karoui, Downey& Blecker, 2010). The regression techniques can achieve quantitative analysis of the IR spectrum by comparing the spectral data with the corresponding values of the characteristics of interest measured by a reference method (Brás et al., 2005). Principal component regression (PCR) and partial least square regression (PLSR) are the two regression techniques that have been successfully applied to quantitative analysis of spectrum data as they are able to process a large number of variables even with high noise, collinear relation or incomplete information of the original data (Wold et al., 2001).

PCR and PLSR are similar as both of them compress a large number of variables to just a few orthogonal factors that are linear combined by the spectra (X), and use these factors to predict the levels of analyte (Y) in the samples. PCR decomposes the high dimensional spectrum data using only the spectra, while PLSR reduces the dimensions of the spectrum data, based on both the spectra and the reference concentration data (Hemmateenejad, Akhond & Samari, 2007). As a result, PLSR uses fewer orthogonal factors as predictors for the levels of analyte than PCR. Meanwhile, PLSR seems to be preferable for chemists than PCR. However, it was reported by Hemmateenejad and others (2007) that there was no significant difference in the predicting performances between PCR and PLSR with wavelength selection.

PLSR is used in developing calibration models for mid-infrared spectroscopy in analyzing sugars and organic acids. This technique has the characteristic that the precision of the model improves as the number of variables and observations increases (Wold et al., 2001). The development of a calibration with high precision can be time-consuming, as long as the model is build, analyses will be made within short time (Brás et al., 2005).

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Chapter 3: Rapid Assessment of Sugars and Organic Acids in Tomato Paste Using a Portable Mid-Infrared Spectrometer and Multivariate Analysis

Congcong Zhang, Didem P. Aykas and Luis E. Rodriguez-Saona

Department of Food Science and Technology

The Ohio State University

110 Parker Food Science and Technology Building,

2015 Fyffe Road

Columbus, Ohio 43210

3.1 Abstract

This study evaluated the performance of a portable and self-battery mid-infrared spectrometer for simultaneous determination of sugars and organic acids in tomato paste. A total of 120 tomato paste samples were used. The mid-infrared spectra were directly collected in duplicate using a portable mid-infrared spectrometer equipped with a triple reflection diamond ATR sampling device. High-performance liquid chromatography (HPLC) was used to determine the reference levels of simple sugars (glucose and fructose) and organic acids (citric acid and Vitamin C). Partial least square regression (PLSR) was used to develop calibration and validation models. Paste compositional ranges were glucose (6.46-13.05 g/100g), fructose (6.82-14.29 g/100g), total reducing sugars (13.28-27.01 g/100g), citric acid (2.89-5.86 g/100g) and Vitamin C (74.30-106.77 mg/100g). PLSR models showed good correlation (R_{CV}>0.91, R_{Val}>0.93) between the mid-infrared spectrometer predicted values and reference values, and low standard errors of cross validation (SECV) of 0.57 g/100g for glucose and 0.69 g/100g for fructose, 1.15 g/100g for total reducing sugars, 0.29g/100g for citric and 2.44 mg/100g for total Vitamin C. Portable mid-infrared spectrometer could be a revolutionary tool for in-field assessment of the quality of tomato-based products, which would provide the tomato industry with accurate results in less time and lower cost.

Key words: Tomato paste, mid-infrared spectrometer, PLSR, reducing sugars, organic acids

3.2 Introduction

Tomato (*Lycopersicon esculentum*) is one of the most consumed vegetables in the world, second only to potato. The United States is one of world's largest producers of tomato, where California ranks first for the production of processing tomatoes accounting for about ninety-six percent of the total production, followed by Indiana, Ohio and Michigan (Ayvaz et al., 2016; USDA, 2016). Every year, more than 75 percent of the tomatoes produced across the country are for further processing (USDA, 2016). The majority of these tomatoes are initially thermal processed into concentrated tomato paste in the tomato season, which is easy to store and distribute. Then as starting material, the concentrated tomato paste is reconstituted into various different products such as tomato sauce, pizza sauce and ketchup (Zhang et al., 2014). As an in-between product, the quality of tomato paste is of vital importance for the tomato processing industry, while it is always affected by factors like variation of the tomatoes and the processing conditions (Anthon et al., 2008). To maintain optimal and consistent quality, a variety of characteristics of the tomato paste are commonly tested on each batch of production, including color, soluble solids, consistency, titratable acidity, pH, sugars, organic acids and lycopene (Ayvaz et al., 2016; Zhang et al., 2014).

Sugars and organic acids are the two major quality parameters for tomato products that affect the consumers' perception and liking. The sweet-sour flavor, one of the most typical sensory characteristics of tomato and tomato products, is determined by the level of sugars and organic acids as well as their interaction with the volatiles in tomatoes (Baldwin et al., 2008). In addition, sugars and organic acids are highly associated with other key quality parameters of tomato paste including soluble solids, pH and titratable acidity (TA), which enables them to provide rich information for the optimization of food processing. The primary sugars in tomatoes are glucose and fructose, with small quantities of sucrose and other reducing sugars such as raffinose, arabinose, xylose and galactose (Thakur et al., 1996). The most abundant organic acid in tomatoes is citric acid, which is also the biggest contributor to the TA in the processed tomato products (Anthon et al., 2011). Another valuable organic acid content is Vitamin C. As a bioactive present in tomatoes, the total Vitamin C content (L-ascorbic acid (AA) and L-dehydroascorbic acid (DHAA)) is considered to be an attracting index for tomato products.

Current methods for the determination of sugars and organic acids in food rely heavily on High Performance Liquid Chromatograph (HPLC), which is a reliable method for chemical component separation and quantitation (Kamil, Mohamed & Shaheen, 2011). Despite its accuracy, this technique usually requires substantial sample preparation, use of hazardous solvents as well as testers' professional skills. More importantly, the traditional approach is not well accommodated to routinely in-line quality analysis as it is time consuming and relative expensive. Therefore, the development of rapid, cost-effective and robust methods is necessary for the quality control of tomato products.

Infrared spectroscopy has shown its potential for profiling chemical components in different food and agricultural products with simplicity and high time-cost efficiency (Wilkerson et al., 2013). Providing rich vibrational information about the functional groups in different organic components, mid-infrared (4000 cm^{-1} to 400 cm^{-1} wavenumber) is an important region in predicting specific chemical compounds of interest in the food matrix. Thanks to the development of the portable mid-infrared instruments and versatile sampling accessories such as attenuated total reflectance (ATR), mid-infrared spectroscopy can perform as a rapid and robust approach for in-field quantitative analysis by its powerful combination with chemometrics. In fact, there have been studies on the application of portable mid-infrared spectroscopy in the tomato industry, which showed good performance on predicting the quality attributes containing soluble solids, consistency, TA, pH, sugars, organic acids in tomato juice, with little or no sample preparations (Ayvaz et al., 2016; Wilkerson et al., 2013). However, no studies so far have been reported on the assessment of the sensory and nutrition related parameters—sugars and organic acids in tomato paste using a portable spectrometer, which is valuable for tomato paste manufacturing.

Our objective was to develop a rapid and robust method for simultaneous determination of sugars (glucose, fructose and total reducing sugars) and organic acids (citric acid and total Vitamin C) in tomato paste using a portable mid-infrared spectrometer combined with multivariate analysis.

3.3 Materials & Methods

3.3.1 Tomato Paste Samples

A total of 120 concentrated tomato paste samples with natural tomato soluble solids (NTSS) ranging from 25.9% to 36%, were kindly provided by a major tomato processor in California. All of these samples were thermally processed in July, August or September of the year 2015 without the addition of food additives.

3.3.2 HPLC Reference Analysis

3.3.2.1 Sugar Analysis

The levels of glucose and fructose were determined using the method described by Ayvaz and others (2016), with some slight alternation. Sugar analysis was done in duplicate. Approximately 0.2 g tomato paste sample of room temperature was exactly weighed and mixed with 1.6mL of HPLC grade water in a 2 mL centrifuge tube. The mixture was sonicated and vortexed until homogeneous, followed by the centrifugation at 10,000 rpm for 15 min at 25℃. Collected by a plastic syringe, the supernatant was then filtered through a 0.45μm pore Whatman nonsterile syringe filter into a HPLC vial. A 10μL of the filtered supernatant was injected into a Shimadzu reverse-phase HPLC (Shimazu, Columbuia, MD), equipped with dual LC-6AD pumps, a SIL-20AHT autosampler, a CTO-20A column oven and a RID-10A refractive index detector (Shimadzu, Columbia, MD). An Aminex HPX-87C carbohydrate column (300×7.8 mm i.d., 9μm particle size) with a Micro-Guard Carbo-C cartridge (30× 4.6mm) (Bio-Rad laboratories, Hercules, CA) was used for sugar separation at 80 ℃. HPLC grade water was used as mobile phase for isocratic elution at 0.6mL/min flow rate in a 30 minutes running time per sample. Chromatograms were integrated using LC Solutions software version 3.0 (Shimadzu, Columbia, MD). Quantitation of each sugar was performed using an external calibration curve developed by either glucose or fructose standard (Fisher Scientific, Fair Lawn, NJ). The concentration of total reducing sugars was the sum of the concentration of glucose and the concentration of fructose.

3.3.2.2 Organic Acid Analysis

Total Vitamin C content and citric acid were simultaneously determined using a method similar to the method which was reported by Vazquez and others (1993) with a few modifications. Analysis was done in duplicate. 0.3 g of tomato paste sample at room temperature was accurately weighed and mixed with 1.5mL of 4.5% meta-phosphoric acid (Fisher Scientific, Fair Lawn, NJ) into a 2 mL centrifuge tube, then vortexed and centrifuged at 10,000 rpm for 15 min at 4℃. The supernatant was treated with 100μL of 100Mm Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (Sigma Aldrich, St Louis, MO) and incubated in the refrigerator at 4 ℃ for 6 hours so that DHAA were totally reduced to AA. After filtering through a 0.45µm pore Whatman nonsterile syringe filter into a HPLC vial, 10 μL of the tomato sample was injected into an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA), equipped with a G1311A quaternary pump, a G1322A degasser, a G1313 ALS autosampler, a G1316A colcum and a G1315B DAD detector (Agilent Technologies, Santa Clara, CA). Prevail^{TM} Organic Acid column (150×4.6 mm i.d., 5μm particle size) (Waters Corporation, Milford, MA) was used for isocratic separation of acids at 20 ℃. The mobile phase was HPLC grade water acidified with sulfuric acid (Fisher Scientific, Fair Lawn, NJ) to pH 2.2. Chromatograms for organic acids were integrated using Agilent OpenLab CDS software (Agilent Technologies Inc., Santa Clara, CA), in which ascorbic acid was determined at 245nm and citric acid was at 220 nm. External calibration curves were prepared by commercial L-ascorbic acid standard (Sigma Aldrich, St Louis, MO) and citric acid standard (Fisher Scientific, Fair Lawn, NJ) for the quantitative analysis of total ascorbic acid and citric acid in tomato paste.

3.3.3 Mid-infrared Spectroscopy Analysis of Tomato Paste

The spectrum was collected on each sample at room temperature using an Agilent 4500a FTIR system coupled with a triple bounce diamond ATR sampling accessory (Agilent Technologies Inc., Santa Clara, CA), which was specifically designed for outside lab analysis. This unit was equipped with a ZnSe beam splitter and a thermoelectrically-cooled deuterated triglycine sulfate (dTGS) detector. On every spectrum collection, the system was set to collect information from 4000 to 650 cm^{-1} wavenumbers with a 4 cm^{-1} spectral resolution and to co-add interferograms of 64 scans to increase the signal to noise ratio. A background spectrum was collected for each sample to make up for the environment variations. Approximately 2 gram of tomato paste sample was directly applied on the ATR sampling device, where the sample should full cover and intimate contact with the crystal. The crystal was cleaned with 70% ethanol and dried with Kimwipe tissue (Kimberly-Clark Corp. LLC, Roswell, GA) between every measurement. Spectra were collected in duplicate for each sample and it took no more than 2 minutes per reading. Agilent MicroLab PC software (Agilent Technologies Inc., Danbury, CT, USA) was used to store and present the spectra data.

3.3.4 Multivariate Calibration: Partial Least Square Regression

Partial least square regression (PLSR) was used to calibrate the spectroscopic method by correlating the spectral data with the corresponding reference values of each characteristic of interest (Wold et al., 2001). This regression technique compresses the complex multivariate data (spectra) to just a few orthogonal factors that are linear combined by the spectra (X), which explain maximal covariance of the spectral matrix X and the reference vector (Y), and uses these factors to predict the levels of analyte (Wilkerson et al., 2013).

The original spectra were imported to the chemometrics software Pirouette version 4.0 (Infometrix, Inc., Bothell, WA), where they were preprocessed by smoothing and normalizing. The data set was randomly divided into two subsets: eighty percent of the data including the extreme values in each characteristic, for the calibration models and twenty percent for external validation. The PLSR calibration models were cross validated following the leave-one-out rule for the determination of the optimal latent factors kept for the models and for the detection of outliers. Samples with high values of leverage and studentized residual were removed from the calibration models as outliers. The correlation coefficient (R_{CV}) and standard error of cross validation (SECV) were used to evaluate the performance of the calibration models. Standard error of prediction (SEP), correlation coefficient of the validation set (R_{Val}) were calculated to determine the predictive capacity of the calibration models.

3.4 Results & Discussions

3.4.1 Reference Analysis for Sugars and Organic Acids

HPLC could achieve good separations of different sugars and organic acids in tomato paste samples. There were two major peaks in the chromatogram for sugar analysis **(Figure 3.1)**. By comparing with the chromatogram of standards, the first major peak at 6.58 min was determined as glucose and the second major peak at 8.16 min was fructose. There was low absorbance of sucrose, which was eluted at 5.54 min, shown as the broad, unresolved peak ahead of the peak of glucose.

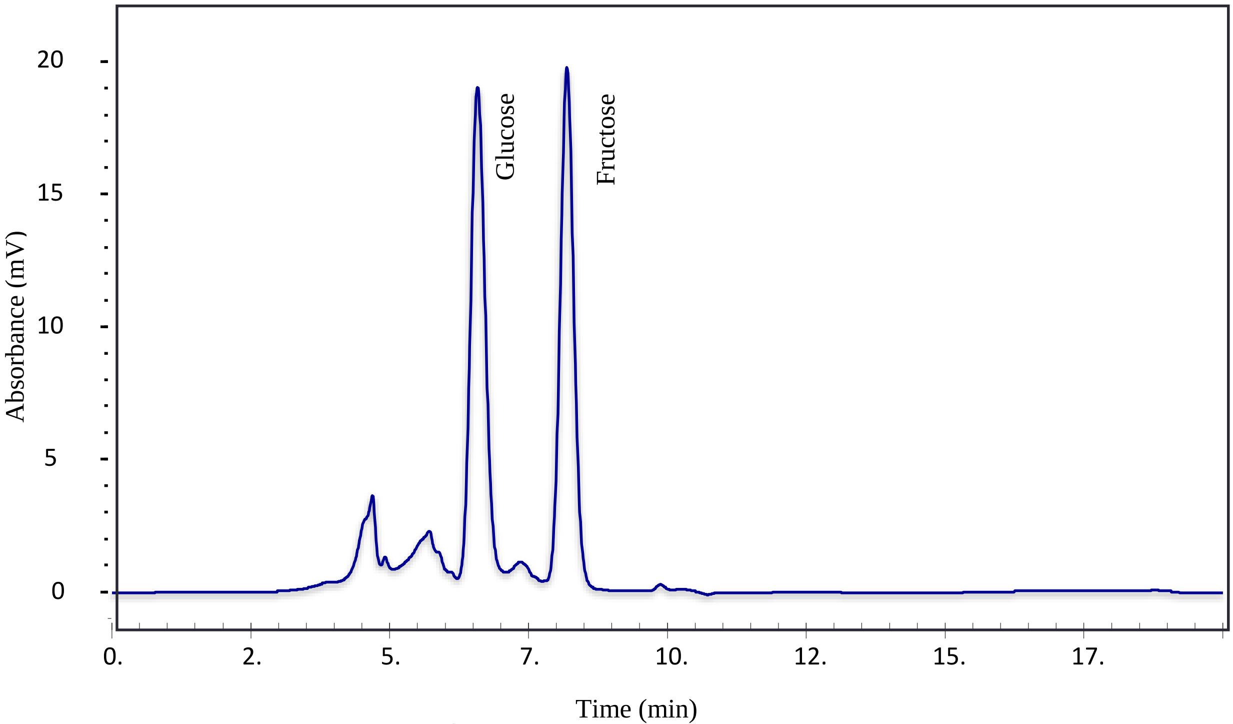

Figure 3.1. Chromatogram of sugars in the tomato paste.

Figure 3.2 shows a typical chromatogram of organic acids in the tomato paste sample. The blue and red lines respectively represented the compounds that have absorption at 245nm and 220nm wavelength. Accordingly, ascorbic acid had maximum absorbance at 245 nm and eluted in 4.17 min. Citric acid had maximum absorbance at 220nm and its retention time was 7.44 min. As DHAA was reduced to AA in the tomato paste, the total Vitamin C content was presented as ascorbic acid in the HPLC chromatogram.

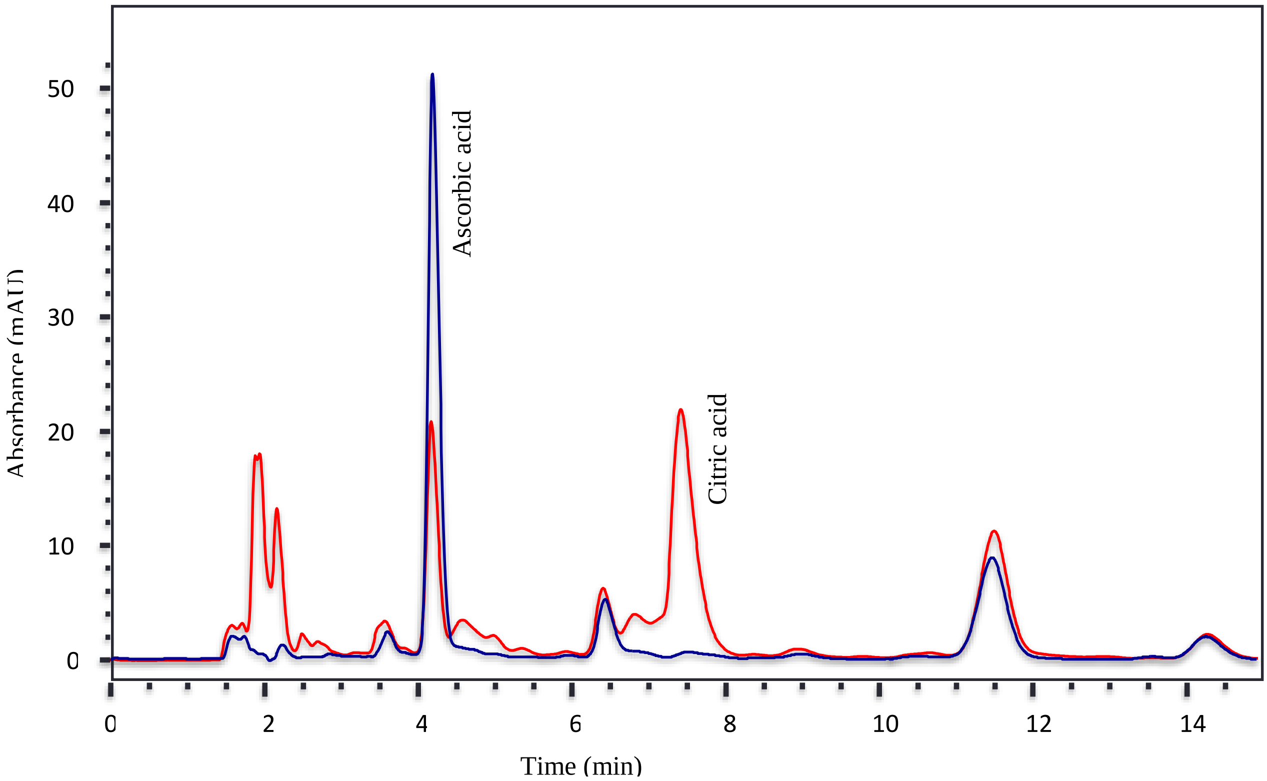

Figure 3.2. Chromatogram of organic acids at 245nm (blue) and 220 nm (red) in the tomato paste.

Based on HPLC chromatogram and external calibration curves, the concentration of sugars and organic acids were calculated. Reference compositional values are shown in **Table 3.1**. The levels of glucose and fructose in the tomato paste samples ranged from 6.46 to 13.05g/100g and 6.82 to 14.29g/100g respectively, which were higher than the values (5.75g/100g for glucose and 5.85g/100g for fructose) reported in the USDA nutrient database for canned tomato paste without salt added (USDA, 2015). Commercial canned tomato paste is a reconstituted product using the concentrated tomato paste as starting material. The tomato paste samples in current study were highly concentrated sample without any reformulation, which might result in higher values of reducing sugars.

The total reducing sugar levels in tomato paste were rarely reported, while the ratio of fructose to glucose with the range of 0.96 to 1.24 was comparable to the ratio 1.01-1.29 reported by Porretta, Sandei, Crucitti, Poli & Attolini (1992).

The concentration of citric acid in the tomato paste samples ranged from 2.89-5.86g/100g and the Vitamin C (total ascorbic acid) were from 74.30 to 106.77mg/100g. The levels of Vitamin C in our samples were much higher than the range 2.3 to 46 mg/100g reported by USDA (2015) for the canned tomato paste products, and slightly higher than the values reported by Koh and others (2012) as 67.54mg/100g. Vitamin C is labile and easy to degrade during tomato processing and long-time storage. In the tomato industry, concentrated tomato paste is usually stored for up to two years until distribution or remanufacturing (Koh et al., 2012). Our higher values are due to the higher concentrated samples and short- time storage.

Table 3.1. Reference analysis results of sugars and organic acids in the tomato paste.

	Glucose (g/100g)	Fructose (g/100g)	Total Sugars (g/100g)	Citric acid (g/100g)	Vitamin C (mg/100g)
Range	6.46-13.05	6.82-14.29	13.28-27.01	2.89-5.86	74.30-106.77
STD.	2.13	1.80	3.90	0.76	10.73

3.4.2 Spectral Analysis of Tomato Paste

Spectra **(Figure 3.3)** collected by the portable-FTIR unit were similar for all the tomato paste samples. The two broad bands around 3600-3000 cm^{-1} and 1700-1500 cm^{-1} were associated respectively with -OH stretching and -OH bending in water (Sinelli et al., 2000). The little bump around 2955- 2850 cm^{-1} was attributed to -CH$_3$

and -CH_2 stretching in a small amount of membrane lipids in crushed tomato products (Rohman &Man, 2011). Rich information was provided in the fingerprint region (1500 to 900 cm^{-1}) corresponding with sugars and organic acids. The bands with high intensities in 1200 to 900 cm^{-1} represented C-C and C-O stretching of carbohydrates, and bands around 1500 to 1200 cm^{-1} were associated with C-O-H, C-C-H and O-C-H bending (Wilkerson et al., 2013). Similar spectra on tomato paste were reported by Kamil and others (2011) using benchtop mid-infrared spectrometer, however, our bands were more distinctive and had higher absorption in the fingerprint region than theirs by taking advantage of the triple bounce ATR sampling device.

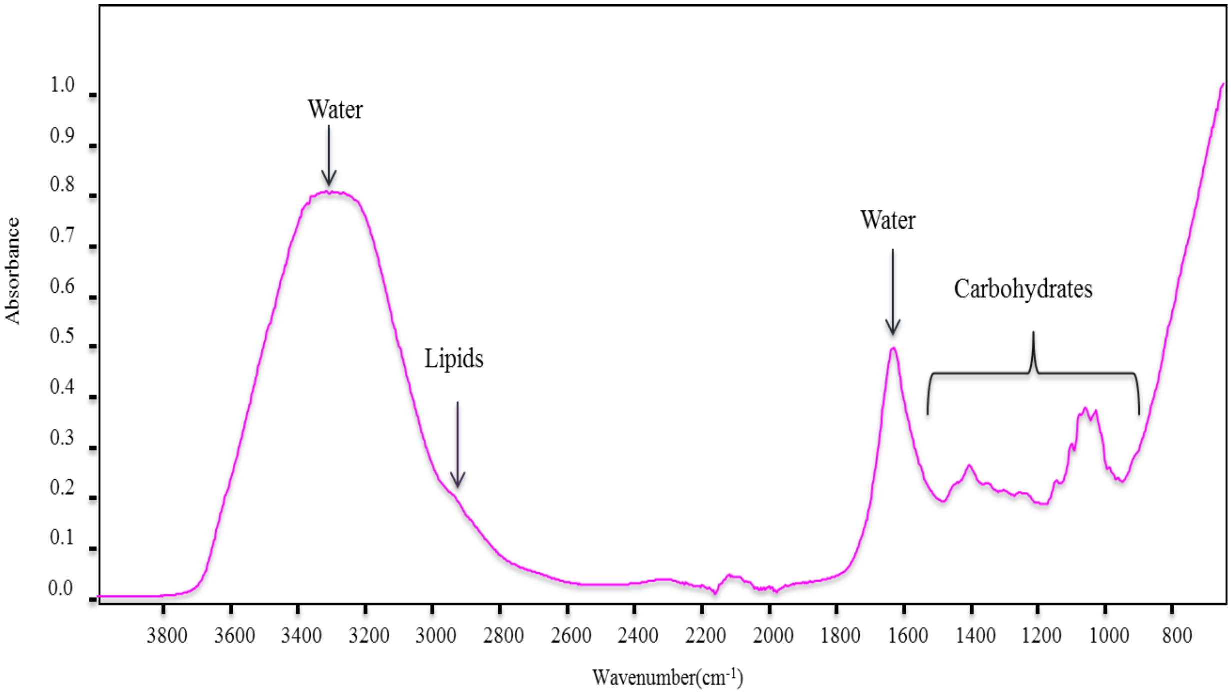

Figure 3.3. Mid-infrared spectrum of the tomato paste measured by a portable mid-infrared spectrometer equipped with triple bounce ATR sampling accessory.

3.4.3 PLSR Calibration Models for Sugars and Organic Acids

Tomato paste samples were randomly separated into two data sets: one was for calibration models and the other was the validation set used for external validation. The statistical parameters of the calibration and validation sets were shown in **Table 3.2**. Measurements that had high leverages and studentized residuals were not taken account into the PLSR models, resulting in the differences in the number of samples for different quality attributes. Performances of the calibration models were highly influenced by the selected relevant spectral range and preprocess of the spectra. The infrared region corresponding to high regression coefficient would be selected for calibration, while the wavenumbers dominated by noises such as the saturated water absorption, could be excluded from the models (Ścibisz et al., 2011). The calibration models generated for different parameters used the IR region within 1600 to 900 cm^{-1} **(Table 3.3)**. Spectra were pretreated by normalization and smooth to eliminate random noises. Statistical performances of the calibration models for sugars and organic acids are shown in **Table 3.3**. Accuracy of the calibration models increases with the increasing number of latent factors as more variation of the data set can be explained, while too many factors in the model result in overfitting of the data, making the models ineffective (Abdi, 2010). The calibration models for sugars and organic acids built on 3-4 latent factors, which could explain at least 90 percent of the total variance in each of these parameters. The models showed good performance with R_{CV} higher than 0.91 and low SECV compared with the ranges of each parameter. Our calibration models achieved similar performance with the literature that reported on the application of mid-infrared spectrometer to predict glucose, fructose, total reducing sugars and citric acid in tomato juice (Ayvaz et al., 2016;

Ścibisz et al., 2011), with fewer latent factors. This might due to higher detected infrared signal of sugars and organic acids in tomato paste than tomato juice.

Table 3.2. Statistical parameters of the sample sets used in developing calibration and validation models for sugars and organic acids in the tomato paste.

Parameter	Sample Set	Number of Samples	Range of Concentration	Mean	STD.
Glucose (g/100g)	Calibration	94	6.46-13.05	9.06	1.61
	Validation	23	6.78-12.42	8.92	1.63
Fructose (g/100g)	Calibration	90	6.82-14.29	9.90	2.00
	Validation	22	7.19-13.96	9.57	1.97
Total sugars (g/100g)	Calibration	90	13.28-27.01	18.99	3.56
	Validation	22	13.97-25.37	18.39	3.40
Citric acid (g/100g)	Calibration	90	2.89-5.86	4.06	0.71
	Validation	22	2.93-5.32	4.02	0.70
Vitamin C (mg/100g)	Calibration	58	74.30-106.14	87.96	9.36
	Validation	15	80.09-101.77	87.67	6.96

Table 3.3. Statistical performances of PLSR calibration and validation models for sugars and organic acids in the tomato paste.

Parameters	Region used[a]	Factors[b]	SECV[c]	R_{CV}[d]	SEP[e]	R_{Val}[f]
Glucose (g/100g)	1000-1200	4	0.57	0.94	0.50	0.94
Fructose (g/100g)	1000-1200	4	0.69	0.94	0.62	0.96
Total sugars (g/100g)	1000-1200	4	1.15	0.95	1.02	0.95
Citric acid (g/100g)	900-1600	4	0.29	0.91	0.26	0.93
Vitamin C (mg/100g)	900-1280	3	2.44	0.96	2.17	0.97

[a] Wavenumbers (cm^{-1}) used for the models were selected based on regression coefficient.
[b] Factors: optimal orthogonal factors used for the calibration models.
[c] SECV: standard error of leave-one-out cross validation.
[d] R_{CV}: standard error of leave-one-out cross validation.
[e] SEP: standard error of prediction using the validation set.
[f] R_{Val} : correlation coefficient of external validation.

The regression vector **(Figure 3.4)** was a weighted sum of loadings included in the PLSR calibration models, which was useful for detecting the most dominant part of spectrum in modeling the parameter of interest. Wavenumbers with high absolute values of regression coefficient were more related to the variation in the calibration set, thus were considered to play important roles in predicting the levels of the quality parameters. As the levels of glucose and fructose in the tomato paste samples were similar and more importantly, they were highly correlated, the regression spectra of glucose, fructose and total reducing sugars **(Figure 3.4)** generated by the PLSR calibration models were in similar shape. They showed common relevant peaks centered around 1126 cm^{-1}, 1081 cm^{-1}, 1042 cm^{-1} and 1018 cm^{-1}, that were associated with C-C and C-OH stretching in the reducing sugars. This was in accordance with Ayvaz and others (2016), who reported the important bands for glucose and fructose at 1105 cm^{-1}, 1080cm^{-1}, 1012cm^{-1} and 1047cm^{-1}. Similar band for reducing sugars in tomatoes was reported at 1082 cm^{-1} corresponding to C-O stretch vibration by Wilkerson and others (2013). The regression vector for citric acid **(Figure 3.4)** was most contributed by bands around 1545 cm^{-1}, 1176 cm^{-1}, 1076 cm^{-1} and 1010 cm^{-1}. It was mentioned in Wilkerson and others' study that 1020-1105 cm^{-1} was prominent region for citric acid (2013). Another study about the tomato spectrum also reported that bands in 1180-1460 cm^{-1} provided information for the carboxyl group COO^- stretching vibration. The discriminating bands for Vitamin C in tomatoes **(Figure 3.4)** were shown as the region 1022- 1097 cm^{-1} associated with C-O-C stretching and C-O-H bending according to Panicker, Varghese, & Philip (2006).

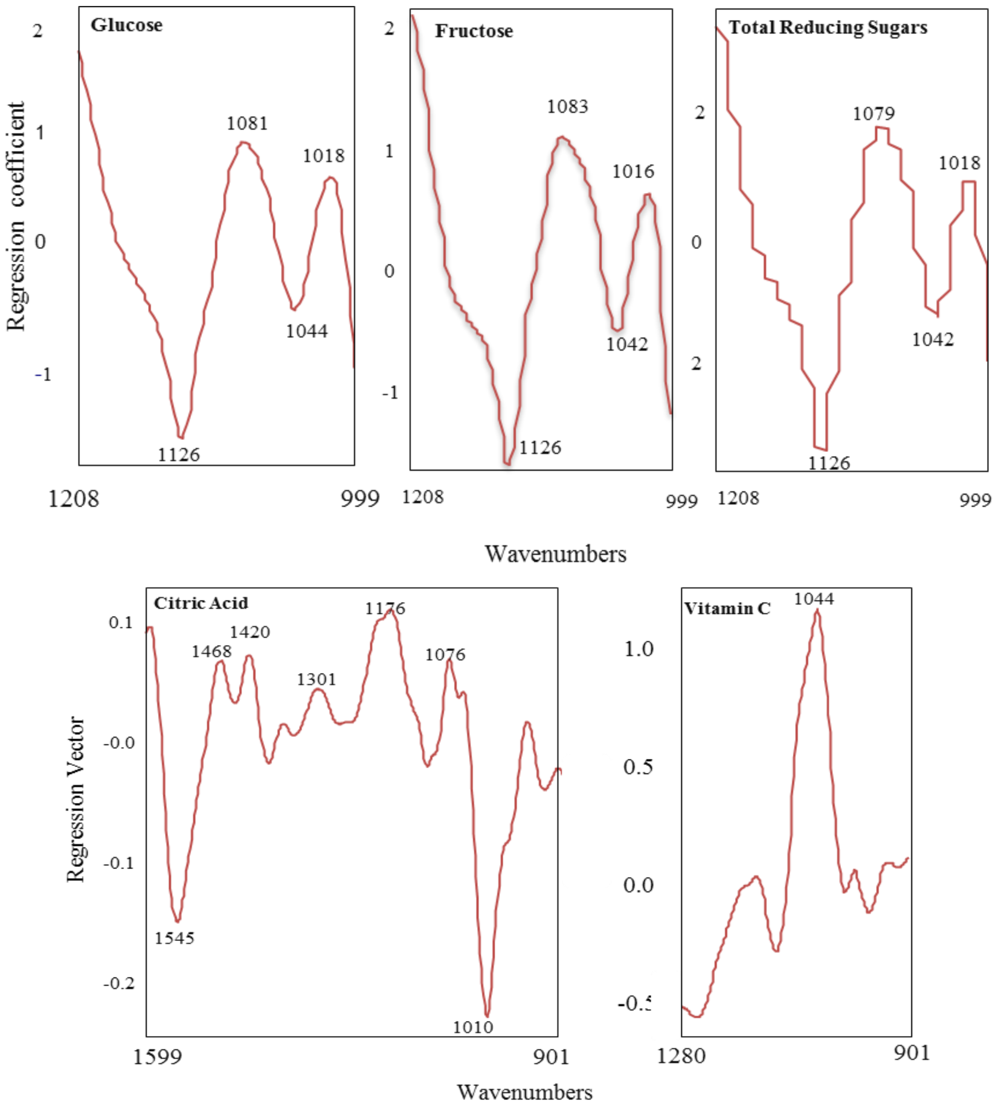

Figure 3.4. Regression coefficient of the PLSR calibration models for sugars and organic acids.

3.4.4 External Validation

The remaining twenty percent tomato paste samples were used to examine the predictive ability of the calibration models. Standard errors of prediction **(Table 3.3)** were similar to that of calibration models, meanwhile, the correlation coefficients of external validation were higher than 0.93, indicated that our calibration models had good ability in estimating glucose, fructose, total reducing sugars, citric acid and

Vitamin C. A direct view of the correlation between the PLSR models predicted values using a portable mid-infrared spectrometer and the measured values using reference methods for different characteristics were provided for both calibration and validation set in **Figure 3.5**. The correlation plot for citric acid was more scattered than others, leading to the lowest correlation coefficient (R_{cv} =0.91) among the five different models. In general, the model predicted values for sugars and acids were highly correlated with the measured values as most data points accumulated around the diagonal regression.

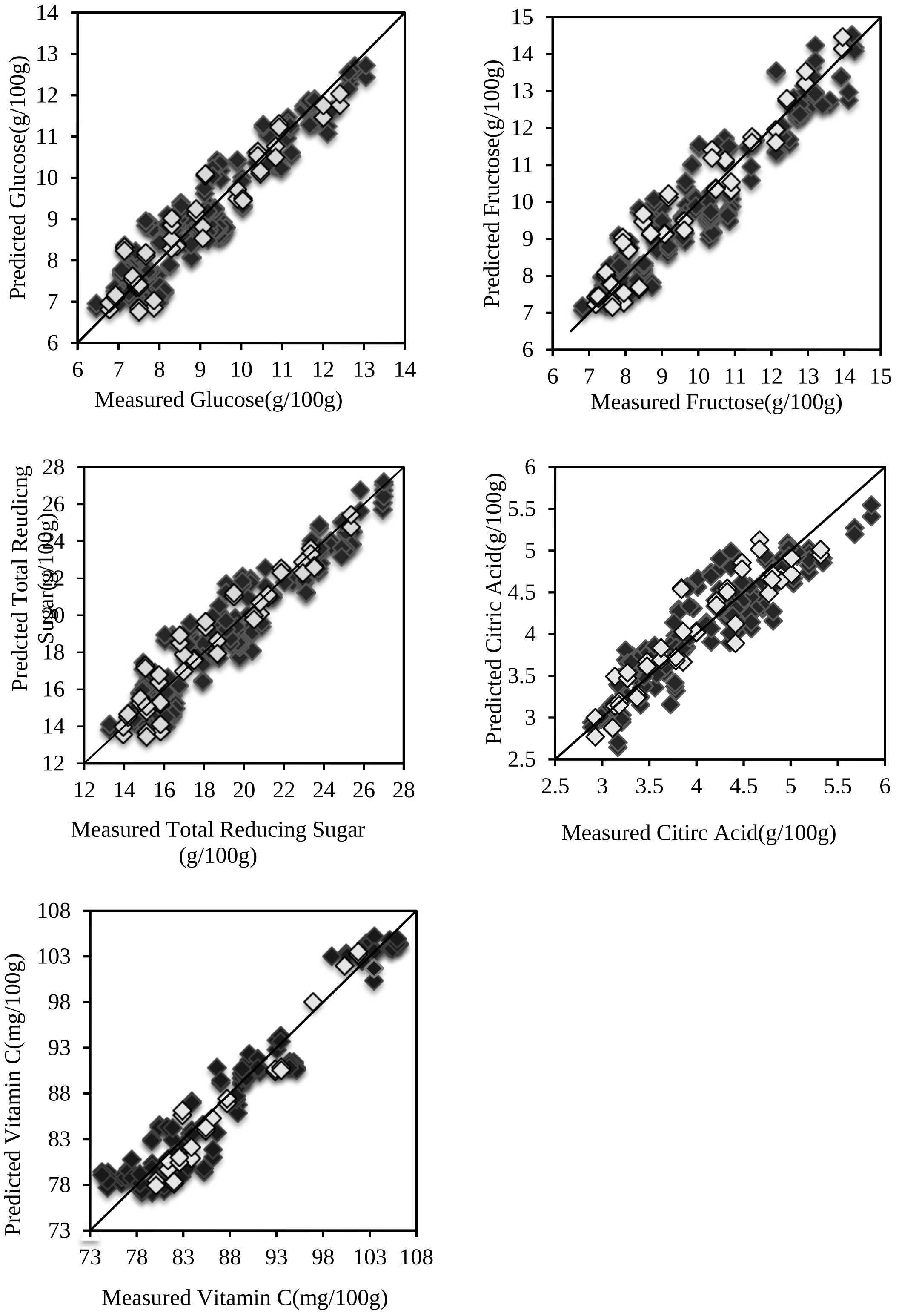

Figure 3.5. PLSR correlation plots between IR predicted values and measured values. Black and white squares represent calibration samples and validation samples respectively.

3.5 Conclusion

120 tomato paste samples were provided by a major tomato processor in California. The reference levels of glucose, fructose, total reducing sugars, citric acid and Vitamin C in the tomato paste were determined using HPLC methods. A rapid and reliable method was developed for simultaneous determination of glucose, fructose, total reducing sugar, citric acid and Vitamin C based on mid-infrared spectra and the reference values. The calibrated models based on the application of portable mid-infrared spectrometer could achieve accurate prediction of the levels of sugars and organic acids in tomato paste with high correlation coefficient (Rcv>0.91), low standard error of calibration and standard error of prediction. The time of analysis was considerably reduced using the portable mid-infrared spectrometer in comparison with the current HPLC methods. Portable mid-infrared spectrometer coupled with ATR could be an ideal tool for routinely in-plant assessment of the quality of tomato-based products, which would provide the tomato industry with accurate results in less time and lower cost.

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