PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ESCUELA DE INGENIERIA

ASSESSMENT OF EXTERNAL AND INTERNAL QUALITY OF BLUEBERRIES USING IMAGES

GABRIEL A. LEIVA VALENZUELA

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences

Advisor:

JOSÉ MIGUEL AGUILERA

Santiago of Chile, August 2013

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*Para **Marcela**, mis **padres** y para mis **hermanos**, con amor les dedico las siguientes páginas que representan un montón de rabias, esfuerzo y enormes alegrías.*

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Inside Universities, in each laboratory or offices there are invaluable people, not only in their knowledge, but also in human quality. To all of them, I would like to thank: Dr. José Miguel Aguilera, my supervisor, who always has the correct weekly statement to reinforce our work. Dr. Renfu Lu, because despite the distance, always has been close to support, correct and guide my work. In similar ways, my committee members deserve a huge acknowledgement: Dr. Mery, Dr. Pedreschi, Dr. Quevedo and Dr. Vial always present in this process and always willing to share their knowledge and ideas. Also all my gratitude for my contemporary or former laboratory / department mates/teachers/friends: Loreto, Rommy, Javiera R, María Paz, Ely T, Ross, Marcela Q, Cata V, Cata C, Lisette, Wence, Guillermo, Salomé, Mane, Debbie, Germán M, Cristobal A, Sra. Hilda, Karina, and Gladys. Ops!!!!! And I would like to thanks the always present Profe Abel Arrieta, who encouraged me to study and study and study. Finally, very far in distance (USDA-MSU): but very close in estimation Fernando, Irwin, Ahmed, Stacy, Miranda, Hallie, Lyo, Javi O, Sebastian and Yira.

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Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Doctor in Engineering Sciences by

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ABSTRACT

During storage and shipping of blueberries visible and internal fruit damages can lead to rejection at the destination. High maturity variability of blueberry, make it valuable to study non-destructive methodologies for their internal and external assessment during the postharvest. This would improve the quality of blueberries shipped overseas. This thesis has two principal themes: the external quality evaluation of blueberries using pattern recognition techniques with visible images and, the assessment of internal quality using hyperspectral images.

For external quality evaluation, the automatic detection of distinctive blueberry orientations (Stem end and Calyx end) and defective fruit - fungally decayed, shriveled and mechanically damaged by impacts or compressed-, were achieved with statistical pattern recognition methods. First, the four-classes (including control berries without damage) of harvested blueberries were imaged to extract color and geometrical features. Features were then selected using sequential forward selection for use by classifiers. Finally, results were validated with external 10-fold cross validation. Using linear discriminant analysis, support vector machine and a probabilistic neural network was able to distinguish the blueberries' orientation in 96.5 % of the cases. The classifiers achieved average performances of 98.3 %, 96.7 %, and 93.3 % for fungally decayed, shriveled, and mechanically damaged blueberries.

Two sensing modes (i.e. reflectance and transmittance) of hyperspectral imaging technique were studied for assessing the internal quality of postharvest blueberries (i.e. soluble solids content and softening). This technique combines image processing and spectroscopic analysis to build predictive models. For reflectance, better firmness correlation coefficient for prediction (Rp = 0.87) were obtained with a push broom system, compared to soluble solids content (SSC) predictions (Rp = 0.79). When a higher resolution hyperspectral imaging system was employed, better SSC predictions (above Rp=0.90) were obtained in comparison with those from firmness (Rp=0.78). Transmittance predictions showed lower correlations than reflectance in most cases. Fruit orientation was also evaluated, and it was found that slightly better predictions for stem end in comparison with equator and calyx. Finally, when wavelengths selection was performed, the prediction errors, on average, increased by only 5% when the number of wavelengths was reduced from 478 and 295 for reflectance and transmittance ,respectively, to 90 wavelengths of 10 intervals and 9 wavelengths each. This research has demonstrated the feasibility of implementing inspection systems for automatic sorting of blueberries for external quality using visible color imaging and for internal quality attributes using hyperspectral imaging to enhance product quality and marketability.

Members of the Doctoral Thesis Committe:
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Santiago, August 7th, 2013

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

EVALUACIÓN DE LA CALIDAD EXTERNA E INTERNA DE ARANDANOS CON IMAGENES

Tesis enviada a la Dirección de Investigación y Postgrado en cumplimiento parcial de los requisitos para el grado de Doctor en Ciencias de la Ingeniería.

GABRIEL A LEIVA VALENZUELA

Resumen

Durante el almacenamiento y el transporte de los arándanos, algunos defectos de la fruta pueden provocar rechazos en algún momento de su comercialización haciendo valiosos los estudios sobre metodologías no destructivas para su evaluación externa e interna durante su poscosecha. De este modo, al ser implementadas, la calidad podría mejorar. La presente tesis tuvo dos ejes principales: la evaluación externa de la calidad de arándanos poscosecha con técnicas de reconocimiento de patrones de imágenes a color, y la evaluación de su calidad interna utilizando imágenes híper espectrales para la construcción de modelos predictivos.

Para la evaluación externa de la calidad en arándanos, se implementó un reconocimiento estadístico de patrones para detectar automáticamente las dos principales orientaciones (pedicelo y cáliz) y tres de los defectos más comunes: arándanos con desarrollo de hongos, encogimiento por deshidratación y arándanos con impactos mecánicos o comprimidos. En primer lugar, se adquirieron imágenes de grupos de arándanos clasificados en las dos orientaciones y en las cuatro clases de defectos de arándanos poscosecha (incluida la clase control). Luego de la adquisición, se extrajeron características cromáticas y geométricas para posteriormente seleccionarlas utilizando el algoritmo de “búsqueda secuencial hacia adelante”. Estas características seleccionadas fueron utilizadas para entrenar distintos tipos de clasificadores y determinar cuál de ellos entregaba la mejor clasificación. El entrenamiento y prueba del reconocimiento de

patrones fue realizado con 10-validación cruzada lo que permitió su validación estadística. Como resultado se encontró que los clasificadores que arrojaron mejores desempeños fueron el discriminante de análisis lineal, la máquina de soporte vectorial y una red neuronal probabilística. Estos clasificadores posibilitaron la distinción de la orientación de los arándanos en un 96.8% de los casos. El reconocimiento automático de daños fue de un 98,3 %, 96,7 %, and 93,3 % para arándanos con desarrollo de hongos, arándanos con encogimiento por deshidratación y arándanos con daño mecánico respectivamente en el mejor de los casos.

Para evaluar la calidad interna de los arándanos (contenido de sólidos solubles y ablandamiento), se construyeron modelos predictivos a partir de la información espectral de imágenes híper espectrales en dos modos de detección (reflectancia y la transmitancia) y tres orientaciones de frutas (pedicelo, cáliz y ecuador). Esta técnica se basa en una combinación de procesamiento de imágenes y de análisis espectroscópico. De este modo, se obtienen múltiples imágenes con información espectral adecuada para construir los modelos. Para las imágenes adquiridas en reflectancia mediante un equipo de barrido, se obtuvieron mejores predicciones que firmeza (R_P = 0,87) en comparación con SSC (R_P = 0,79). En otro estudio realizado, cuando se aumentó la resolución de la imagen en un sistema estático, se obtuvo una mejor predicción de SSC (por encima de R_p = 0,90) de firmeza (R_p = 0,78). Las predicciones obtenidas con imágenes de transmitancia muestran una correlación inferior a las obtenidas por reflectancia en la mayoría de los casos. Adicionalmente, se evaluó el efecto de la orientación de los arándanos, encontrando una mejor, pero muy leve, predicción para las imágenes de pedicelo en comparación con las obtenidas para ecuador y cáliz.

Finalmente, se implementó una selección de longitudes de onda desde las 478 originales para las imágenes en reflectancia y desde 295 para transmitancia hasta sólo 90 imágenes distribuidas en 10 intervalos de grupos de 9 longitudes de onda cada uno para ambos tipos de imágenes. De esta manera, los errores de predicción en promedio aumentaron sólo el 5%.

Esta investigación ha demostrado la factibilidad del uso de imágenes tanto la clasificación automática de calidad externa usando visión por computador así como la evaluación de la calidad interna mediante imágenes híper espectrales para mejorar la calidad del producto y su comercialización.

Miembros del comité de tesis doctoral:

José Miguel Aguilera
Franco Pedreschi
Domingo Mery
Roberto Quevedo
Renfu Lu
Cristian Vial

Santiago, 7 de agosto de 2013

LIST OF CONTENT

LIST OF PAPERS

ISI Papers

This thesis is based on the following manuscripts:

Chapter 3: Leiva-Valenzuela, G.A., Aguilera, J.M., 2013. Automatic detection of orientation and diseases in blueberries using image analysis to improve their postharvest storage quality. Food Control 33, 166-173.

Chapter 4: Leiva-Valenzuela, G.A., Lu, R., Aguilera, J.M., 2013. Prediction of firmness and soluble solids content of blueberries using hyperspectral reflectance imaging. Journal of Food Engineering 115, 91-98.

Chapter 5: Leiva-Valenzuela, G.A., Lu, R., Aguilera, J.M., 2013. Assessment of internal quality of blueberries using hyperspectral transmittance and reflectance images with whole spectra or selected wavelengths. Submitted to Food and Bioprocess Technology.

Proceedings

Parts of this work have also been presented at international congresses:

Leiva-Valenzuela GA, Lu, R., Aguilera J. 2013. Assessment of internal quality of blueberries using hyperspectral images with selected wavelenghts. Proceedings of InsideFood Symposium, 9-12 April 2013, Leuven, Belgium.

Leiva G, Mondragón G, Mery D., Aguilera J. 2011. The automatic sorting using image processing improves postharvest blueberries storage quality. Proceedings of 11th International Congress on Engineering and Food. Athens, Greece.

Leiva-Valenzuela G, Lu, R., Aguilera J. 2012. Assessment of internal quality of blueberry using hyperspectral imaging. Proceedings of 2012 ASABE Annual Meeting Paper, Dallas, USA, July, 2012

1. INTRODUCTION

1.1. Why blueberries?

Blueberry is an important fruit worldwide whose consumption has increased in recent years due to its good flavor and high antioxidant capacity, which is good for anti-aging.

The United States of America is the leading blueberry exporter, followed by Canada (Faoestat 2009). In recent years, countries in the southern hemisphere (e.g., Argentina, Chile, New Zealand and South Africa) have increased fruit export to the northern hemisphere by taking advantage of seasonal differences in production. But long-distance transoceanic shipment requires delivering higher quality and more consistent fresh blueberries at the origin country in order to meet the quality standards upon arrival at the destination.

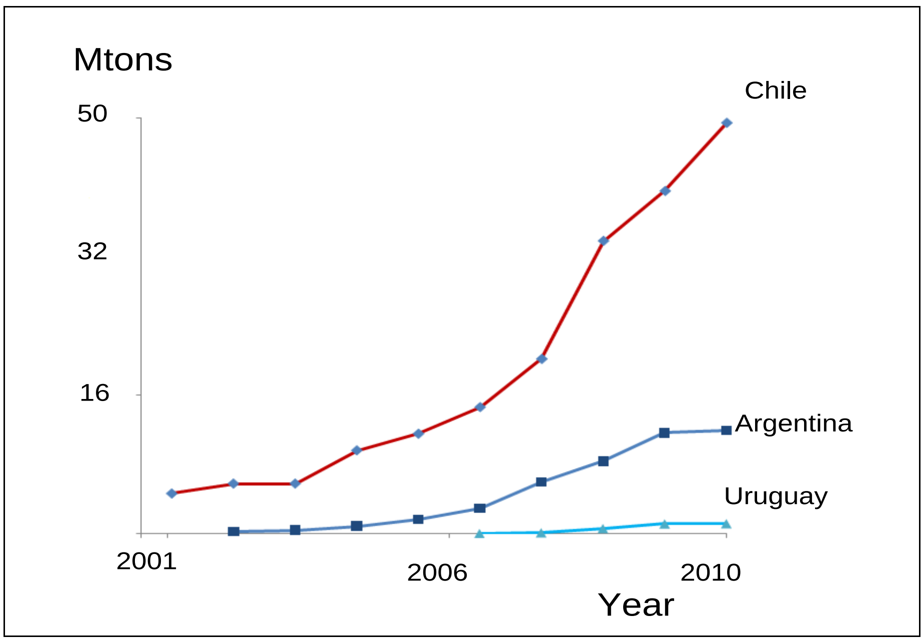

Figure 1.1 Evolution of blueberry exportation for the principal producing countries in South America (Kong 2009)

Fruit south hemisphere overall export has been fruit have doubled in recent years. In Chile, for example, fruit exportations grew from USD $ 1.4 million in 1999, to USD 2,7 million in 2010 (Figure, 1.1). Moreover, the blueberry exports lead this improvement; between 2001 and 2010, it has increased almost 10 times (Kong 2009).

1.2. Automation technology to enhance the quality of blueberries

When fruits are exposed to light, a small part of the light is reflected at the surface causing specular reflectance or gloss, while most of the light energy penetrates into the fruit tissue for only a very short distance and then exits from the fruit. The remaining part of the light penetrates deeper into the tissue, which is then partially absorbed by fruit tissue or passes a section of the fruit before exiting, generating diffuse reflectance and/or body reflectance or interactance. Finally, a minimal portion of the light passes the whole fruit, producing so called diffuse transmittance or body transmittance (Abbott 1999). Transmittance measurement is thus strongly affected by the optical properties size and shape of fruit, and it requires a high intensity light source (Figure 1.2). Those fruit optical properties can be measured by computer vision or hyperspectral sensors and in the most of cases is the basis of determination of fruit quality.

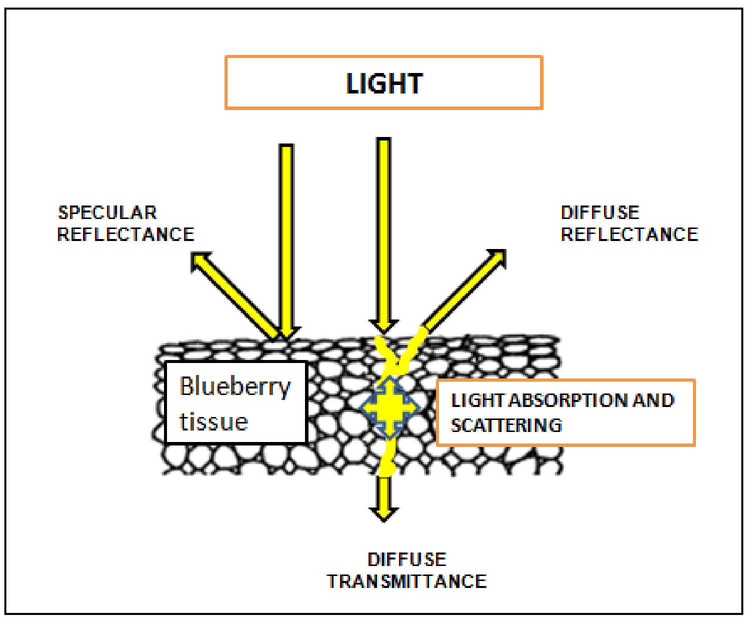

Figure 1.2 The optical properties of fruit tissue can help to improve technology using image techniques to acquire reflected or transmitted light.

Accurate determination of blueberry quality is challenging because individual fruits are small, dark in color, and vary greatly in external and internal quality characteristics. Traditional manual inspection is still widely used; it is slow, unreliable and dependent on workers availability. For these reasons, it is increasingly important to study the quality improvement of blueberries in the agricultural production and marketing chain using automation technologies. Previous studies have shown that applications of computer vision in quality control in food are more accurate, safe and quicker than human sight (Aguilera and Briones 2005). Computing vision technology which is based on image processing, as an alternative to visual inspection, is now being used in various foods and agricultural commodities sorting systems; it is objective, consistent, rapid, and economical (Brosnan and Sun 2002; Kumar-Patel et al. 2012). Images have been effectively used to classify or recognize quality in agricultural and food commodities including apple (*Malus domestica*) (Paulus and Schrevens 1999), strawberry (*Fragaria* spp.) (Bato et al. 2000), pistachio (*Pistacia vera*) (Pearson and Toyofuku 2000), fungal decayed chestnut (Donis-González et al. 2013; Wang et al. 2011b), potato chip (Pedreschi et al. 2006), tortilla (Mery et al. 2010a), pizza (Sun and Brosnan 2003b, a), chocolate chip biscuits (Davidson et al. 2001), cheese (Wang and Sun 2002a, b, 2001), pork meat (Lu et al. 2000; Faucitano et al. 2005), between others.

Currently, commercial sorting systems are available. For high-speed sorting of agricultural products (up of 2 tons h^{-1}) and are able to reject up to 95% of out-of-range blueberries. However, these sorters sort blueberries based on the detection of surface color, and they are limited or not are able to recognize specific defects such as fungal decay and shriveling. Beside color, shape and texture, there are other quality parameters that define the blueberries internal quality. Two of the most important ones are softness or firmness and sweetness.

Considerable research has been reported on the development of automatic sorting and grading techniques for firmness, a measurement of softening, for blueberries. Currently, several commercial sorting systems are available, which are based on detection of the impact response of blueberries when they hit a pressure sensor. While commercial systems allow high speed sorting, they are only able to reject up to 80% of soft fruit. Soluble solids content (SSC), an accepted measure of sweetness, is another important quality attribute for blueberries. It is usually determined from the juice extracted from fruit flesh using the refractometric method (Noh and Lu 2007).

Over the past decade, hyperspectral imaging has emerged as a powerful inspection technique for food and agricultural products. The technique allows acquisition of both spectral and spatial information about an object simultaneously (ElMasry and Sun 2010). Since each hyperspectral image is represented by a 3-D spectral data cube or hypercube (Geladi et al. 2004; Nicolaï et al. 2006), it is thus advantageous over conventional imaging or spectroscopy technique in quality and safety inspection of food and agricultural products (Noh and Lu 2007). The technique was applied to small fruits like strawberry, a fruit relatively close to blueberry, for detection of bruises (Tallada et al. 2006) and prediction of dry matter, SSC, acidity or firmness (ElMasry et al. 2007; Nagata et al. 2005). These studies have shown the feasibility of hyperspectral imaging for measuring the appearance through image processing and physicochemical properties using spectral information.

The purpose of this thesis is to evaluate the suitability of non-destructive measurement of blueberries using image techniques. Computer vision and hyperspectral imaging both are image-based techniques suitable to assess the quality of agricultural commodities. While computer vision is commonly used to evaluate the external fruit appearance, hyperspectral imaging allows evaluation of both internal and external characteristics.

1.3. Hypothesis and objectives

The hypothesis of this thesis is that computer vision and hyperspectral imaging techniques are reliable technologies to assess automatically the external and internal quality of blueberries. They can thus be used for simultaneous inspection of multiple quality aspects of blueberries, such as external defects, soluble solids content, firmness, color, size, presence of insects, bruise, mold, and shriveling. Therefore, the general objective of this thesis is to evaluate the two important image techniques for improvement of the postharvest blueberries quality.

This general objective is achieved with the following specific objectives:

- Develop automatic color image processing algorithms for detection of fruit orientation to facilitate fruit defect detection.
- Create segregations plans by detection of visible fruit diseases.
- Evaluate the feasibility of hyperspectral imaging techniques utilization to assess the internal quality of blueberries.
- Improve the prediction of internal quality attributes by studying and combining different sensing modes and fruit orientation Effect.
- Enhance image processing efficiency by selection of appropriate wavelengths in hyperspectral imaging techniques.

1.4. Outline

This thesis consists of one review chapter, three experimental chapters and a conclusion.

Chapter 2 (review) explains why non-destructive evaluation of berries is valuable considering market and consumer requirements and how image-based non-destructive technologies afforded in this thesis work. It finally reviews the major scientific advances in this field of knowledge.

In Chapter 3, the external quality of blueberries was evaluated using statistical pattern recognition with color images. Algorithms were implemented for identification of three classes of defects caused by diseases commonly found in blueberries during postharvest storage. Additionally, algorithms were implemented for whether the fruit orientation detection.

Chapter 4 positively answer the question of whether hyperspectral reflectance imaging technique is suitable to predict soluble solids content and softening in blueberries, two parameters that are most of important o internal quality.

Chapter 5 reached an important improvement in the assessment of internal quality of blueberries using two sensing modes (transmittance and reflectance) in a high resolution hyperspectral imaging system. Moreover, the effect of orientations was further evaluated by inclusion of a third orientation (equator). Finally, a methodology to select the most appropriate wavelengths was proposed, since the speed of image acquisition and processing by a hyperspectral imaging system is not enough to meet the requirement for sorting lines in the packinghouse. Chapter 6 concludes this thesis. A thesis overview can be found in Figure 1.3

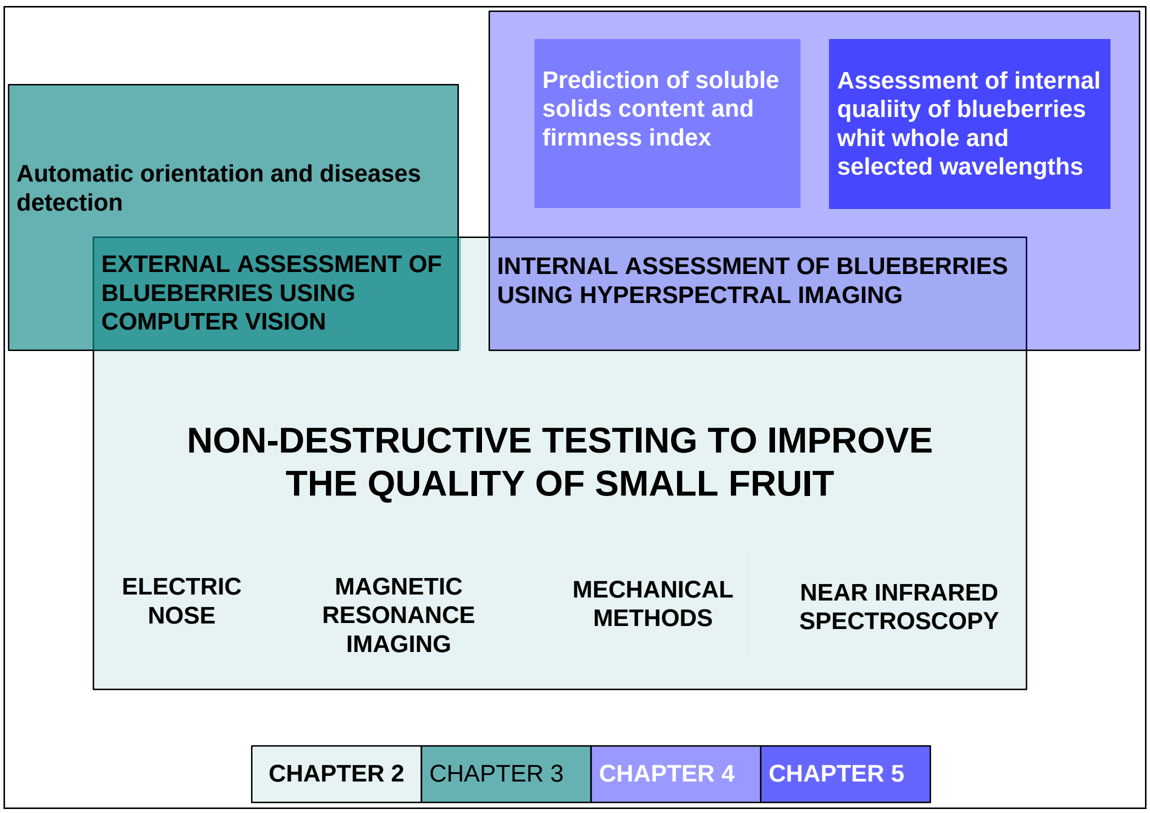

Figure 1.3 Overview of the studies comprising this thesis.

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2. NON-DESTRUCTIVE TESTING TO IMPROVE THE QUALITY OF SMALL FRUIT

Abstract

Small fruit such as berries, grapes and cherries are susceptible to postharvest damages which need to be assessed at the time of packing. Despite being a common practice, quality selection by hand is slow and unreliable, and its cost has increased over time. Therefore, the development of quick and reliable new technologies to assess the quality of small fruit on-line and without direct intervention is now necessary in compliance with more demanding markets. The most important visible quality attributes during postharvest include color, shape, and homogeneity, absence of defect fruit such as shriveling, fungal decay, and mechanical damage. Internal quality descriptors include sweetness, firmness and absence of insects.

This chapter reviews the principal non-destructive techniques applied to small fruits including, impact and vibration, computer vision, hyperspectral imaging, near infrared spectroscopy, electric nose, and magnetic resonance.

2.1. Introduction

Small fruit is a non-botanical group of fruits, commonly including berries, grape, cherry, cherry tomato and others, which are small in size and have elastic properties. These fruits command high prices because consumers now associate their consumption with beneficial health effects associated to anti-aging properties.

Commercially, grape, strawberry, blueberry and raspberry are the most important small fruits around the world, whose quality depends on physiological and environmental factors (FAO 2011). Fresh market requirements exert a pressure to improve sorting practices by assessing the quality of individual fruits, which normally is carried out by hand; the increment in labor costs has led to the implementation of non-destructive, mechanical sorting systems to improve the classification of fruits by firmness.

Automated systems replace the tasks that used to be performed by skillful operators with eyes and hands (Aguilera and Briones 2005). Image processing is intended to evaluate the external fruit quality in replacement of human vision which is the basis of non-destructive computer vision technology (Brosnan and Sun 2004; Bull 1993; Moreda et al. 2009; Rocha et al. 2010). Image analysis of a real scene by computer allows studying attributes of large number of products and forming the base of scaled-up processes (Zitová and Flusser 2003; Rosenfeld 1988; Golnabi and Asadpour 2007; Finlayson et al. 2001).

Near infrared spectroscopy and multi or hyperspectral imaging techniques offer the capability of internal quality assessment by measuring internal quality attributes (i.e. sugars, firmness, acidity etc.). These techniques are non-destructive technique and require little or no sample preparation.

Although automatic sorters based on these technologies are being used for major large fruits such as apple, oranges and pears, their implementation for small fruits has been limited up to now.

2.2. Small fruit

Small fruit is a non-botanical group of fruits, commonly including berries, grape, cherry, cherry tomato and others which are small in size and have elastic properties for texture measurement. Their consumption has increased in recent years because of good flavor and high antioxidant capacity, which is supposedly good as a anti-aging therapy and has positive protective effects against several pathological conditions (i.e. cancer, stroke, heart attack and Alzheimer's disease (Piljac-Zegarac and Samec 2011).

Grape production is led by China (9,174,280 MT), Italy (7,115,500 MT) and United States of America (6,692,950 MT). Strawberry production is important in United States of America (1,782,053 MT), Spain (514,027 MT) and Turkey (302,416 MT); Turkey is the main cherry producer (438,550 MT) followed by United States of America and Iran with 303,363 MT and 241,117 MT respectively. For blueberry, the trade is commanded by United States of America (196,905 MT), Canada (112,363 MT) and Poland (8,595 MT). Others small fruits of importance are raspberry, cranberry, blackberry, boysenberry, gooseberry, currants and elderberry (FAO 2011).

Quality of small fruits is determined by physiological and environmental factors such as variety, nutriments and humidity of soil, presence and control of pathogens and cultivation practice. The principal negative vectors during fruit development and maturity are gray mold, causing fungal decay, anthracnose, alternaria, rhizopus and other caused by environmental factors (Table 2.1). Postharvest storage of these fruits is often restricted to only a few weeks even under optimal temperature and humidity conditions. Moreover, they suffer color change, softening, pitting, peduncle browning and dehydration during retail operations (Linke et al. 2010).

Quality standards of commercial fresh berry fruits are defined by the law of supply and demand. However, the *Agricultural Marketing Service* from the United States Department of Agriculture defines standards for grading fresh fruit. These standards are based on "*measurable attributes such as color, caliber and defects absence that describe the value and utility of the products*". In general, the best grade quality standard (U.S. No.1) for berries, cherry and grape includes the following factors: similar varietal characteristic, mature, cleanness, well colored, not overripe, absence of crushes, split or leaking, free from attached stems, molds, decay, insect or evidence, mummified berries, clusters. Moreover, the classification in grades implies the fulfillment of size requirements (AMS-USDA 2011). Since most of those attributes are visible, the implementation of sensing automatic technologies based on computer vision is quite adequate and reliable.

Accurate determination of quality of small fruit is challenging because individual fruits are small and vary greatly in external and internal quality characteristics. In packing operations small fruit such blueberry, is often graded manually which is slow and unreliable and depends on workers' availability. For these reasons there is currently major interest in automation technology in order to improve fruit quality determination during packaging operations. This chapter reviews several optic technologies in non-destructive assessment of internal and external quality of small fruits.

Table 2.1. Appearance attributes and defects and diseases of principal small fruits (Gross 2004); (Antonelli et al. 2004); (Walter et al. 1997); (J.P. Kidd 2003); (Perry et al. 2002)

Species	**Diameter (mm)**	**External appearance**	**Principal diseases and defect causes**
Blueberry (*Vaccinium sp*)	12	Red to black, round to oblong	Fungal decay caused by gray mold (*Botrytis cinnerea),* irregular maturity in clusters, shriveling, mummy berry, anthracnose, *Alternaria sp*., red ringspot virus, russets, anthracnose, Rhizopus (*Rhizopus stolonifer*).
Strawberry (F*regaria sp.)*	20	White, red, pink, conical	Soft fruit, gray mold, Rhizopus, leather rot, winter injury. Tarnished plant bug, spittle bug, wet stem scars, attached stems, green berries.
Cranberry (*Vaccinum erthrocarpum*)	10	Yellow, round	Sunscald, berry speckle, cottonball, ringspot, rots: bitter, early, blotch, viscid yellow and black rot.
Blackberry (R*ubus ursinus, Rubus argutus*)	25	Red to black. Fruit is an aggregate of small drupelets. Round to conical.	Non-uniformity maturity, whitespots, dorsophila suzukii. Fungal decay caused by *Botrytis cinerea* and *Colletotrichum spp.*
Raspberry (*Rubus idaeobatus*)	20	Yellow to red. Fruit is an aggregate fruit composed of more or less 100 small drupelets forming a conical structure.	Fungal decay caused by gray mold, ringspot virus, shriveling, leakers, UV damage (white drupelets). Insect injury ascane maggot (*Pegomya rubiura*), redberry mite (acalitus essigi), anthracnose
Boysenberry (*Rubus ursinus x idaeus*)	25	Red to Dark purple. Fruit is an aggregate fruit composed of small drupelets. Cylindrical.	Fungal decay caused by gray mold, yellow rust, downly mildew (dry berry disease)
Blackcurrant (*Ribes nigrum)*	10	Black round	Irregular maturity in clusters, insect injury; small brown spots and fungal decay caused by gray mold.
Gooseberry (R*ibes uva-crispa*)	19	Green, yellow or reddish, round	Irregular maturity in clusters, insect injury fly (*Epochra canadiensis*), Gosseberry fruit worm (*Zophodia convolutella*), slugs and snails (*Helix aspersa* and *Cepaea spp*.) Small brown spots caused by gray mold.
Elderberry (*Sumbucus nigra*)	7	Black, round	Fungal decay caused by gray mold, irregular maturity in clusters, insect injury
Redcurrant (*Ribes rubrum*)	10	Red, round	Fungal decay caused by gray mold, irregular maturity in clusters, insect injury
Loganberry (R*ubus x Loganobaccus)*	20	Red to deep purple, conical to cylindrical	Dryberry disease (*Phyllocoptes gracifis*), insect injury ascane maggot (*Pegomya rubiura*), redberry mite (*Acalitus essigi*), gray mold, anthacnose.
Cherry (*Prunus sp, Subgenus cerasus*)	15	Yellow, red or purple; round	Fungal decay caused by gray mold, pitting, bruising, shriveling, Fungal decay caused by blue mold (*Penicillium expansum*), *Alternaria sp*., *Monilinia fructicola* brown rot, *Cladosporium sp*., and *Aspergillus niger.*
Grape (*Vitis sp)*	25	Green, yellow, red, purple or black, round to oblong	Fungal decay caused by gray mold, waterberry, sunburn, almeria spot, bunched, shriveling, Black rot *(Aspergillus niger)*, blue rot *(Penicilliun spp.*, Rhizopus rot *(Rhizopus stolonifer* or *R. oryzae)*; sun scald.

2.3. Computer vision systems

Computer vision is one of the most useful technologies to evaluate external fruit quality. It is objective, consistent, rapid, and economical. The technology offers an automated alternative to manual inspection and is currently being used in various food and agricultural sorting systems. While computer vision may be applied using different acquisition techniques such as color, magnetic resonance (MRI) and X-ray, visible imaging is the most widespread in evaluate food and agriculture products.

2.3.1. Image acquisition

An image is formed when waves from electromagnetic radiation are partially reflected, transferred or emitted by an object from a real scene and these waves are captured by sensors and transduced in electrical signals as a 2-dimensional array of numbers called a matrix. The matrix consists of columns (M) and rows (N) that define small square regions called picture elements or pixels (Seeram and Seeram 2008). In other words, a digital image is the interpretation of one or more numerical matrix, where the object of interest has numeric affinities that represent colors or gray intensity zones inside the image. Thus, computational techniques operated with algebraic principles can detect specific fruit features (Leiva-Valenzuela and Aguilera 2013).

In computer vision, images of external attributes of samples are acquired with charge-coupled device (CCD) or Complementary metal–oxide–semiconductor (CMOS) cameras that capture wavelengths of the visible spectrum (Du and Sun 2004). The extracted information depends on both the scene illumination and external elements such as the optic system, sensors and signal processing. In a typical image acquisition system, high-quality illumination is designed to acquire homogeneous information about the sample under testing. Choosing a right illuminant source often is a difficult problem because the sample reflectance may induce specular reflectance and saturations forcing adjustment to ensure an appropriate processing (Valous et al. 2009). Therefore, the characteristics of the sample surface may increase levels of reflectance producing distortion that changes correct fruit measurements. The object to be measured should maintain an appropriate distance to the image device. Moreover, occlusion and shadows should be avoided in order to allow correct segmentation and measurement.

Depending on the application, computer vision configurations vary from in-room to outdoor systems. In most in-room applications, image acquisition, light, distance and angle of acquisition have to be standardized and a laboratory colorimeter is needed. Laboratory colorimeters are extensively used in science to understand color, shape and texture changes induced by reactions, time, or chemical treatments of samples, allowing the first approach to design a classification process. Commercial vision-based sorters are add product value by physically segregation of food and agricultural products into quality grades . They require faster sensors and image processing systems able to reduce image blur and classify products in real time. Generally, outdoor system implementation requires light and geometric correction. Outdoor systems are generally referred to those used in precision agriculture.

2.3.2. Preprocessing and segmentation

The role of preprocessing is to segment the pattern of interest from the background, remove noise, normalize the pattern, and perform any other operations which will contribute in defining a compact representation of patterns (Jain et al. 2000).

Specifically after acquisition, images are transformed with algebraic operations to improve signal to noise ratio; this stage, known as image pre-processing, includes filtering, histogram correction, registration, channel transformations (Ibrahim et al. 2008). Therefore, subsequent segmentation can be performed.

Segmentation is the stage where the area of interest is recognized from the background region in order to discard most non-relevant elements of the image, or accent important regions of interest to be analyzed subsequently. For food application, robust algorithms are required for isolating regions of interest in food color images using threshold and morphological operations (Mery and Pedreschi 2005). However, historically, the most used algorithms is the Otsu method (Otsu 1979). Other segmentation algorithms include adaptive image segmentation (Jiang et al. 2008), texture segmentation using Gabor filters (Zhang 2002), and classification segmentation (Mery et al. 2011).

After segmentation, color images could be transformed from RGB color channels to others (i.e. grayscale; L*,a*, b*; H, S, I coordinates) by applying mathematical relations and transformations in order to improve the image processing (León et al. 2006).

2.3.3. Statistical pattern recognition

Pattern recognition studies how machines may "watch" the environment in order to take decisions about how an object wedges in a category. A pattern is represented for a group of geometric and chromatic features able to define two or more classes. A pattern is represented by a set of features or quantitative attributes able to be segregated by decision boundaries between pattern classes establish by concepts of statistical decision theory operated in two modes: training or learning and classification or testing. In training operation, the features extraction / selection module finds the appropriate features for representing the input patterns and the classifier is trained to partition the feature space while in the classification mode, the trained classifier assigns the input pattern to one of the pattern classes under consideration based on the measured features (Figure 2.1) (Jain et al. 2000). Pattern recognition techniques can be applied over a set of images to segregate or sort groups of images by setting one or more differentiation criteria. Those criteria are defined for extracting and selecting image features, training and testing classifiers and validating the methodology.

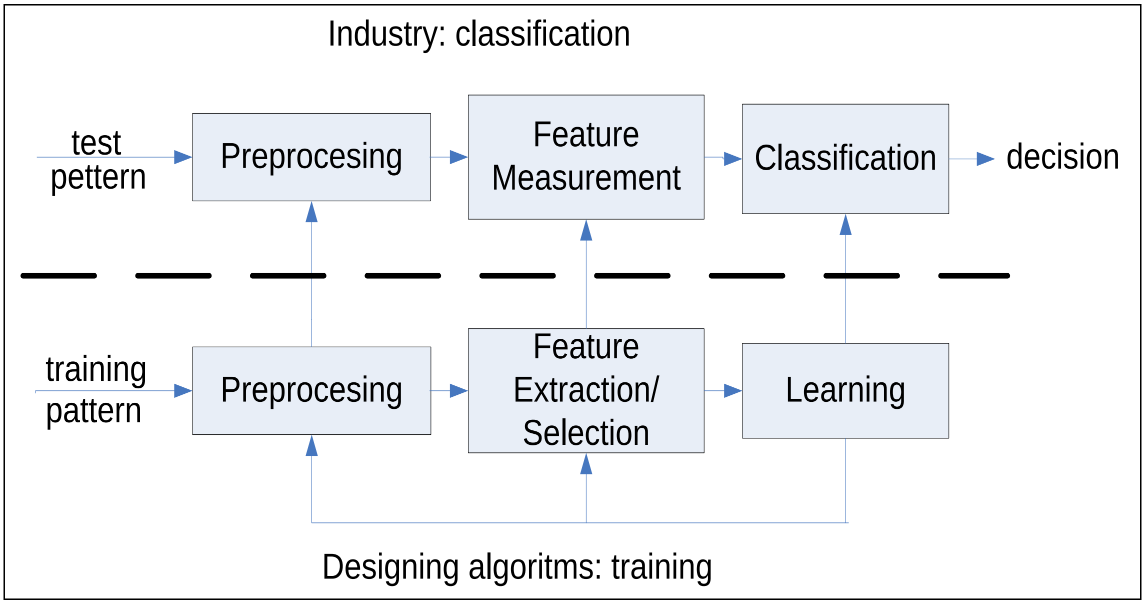

Figure 2.1: The recognition system is operated in two modes: training (learning) and classification (testing) (Jain et al. 2000). The most common industrial applications of computer vision involve the classification stage. Nevertheless, training is closely related during the process. It allows a continuous improvement of the system.

2.3.3. 1. Feature extraction and selection

After segmentation, the sample´s quantitative attributes from the region(s) of interest (is) are measured. When a system is trained, the first step consists of extraction of features using algorithms (the most features from known category images as possible), After that, features must be selected by their capacity of correctly separating the classes (Mery and Soto 2008). Attributes or features include: *Standard geometrical features* such as area, orientation, Euler number, and solidity; invariant features like Hu and Flusser and Suk moments (Hu 1962a; Flusser and Suk 2005) (Table 2.2.); Fourier descriptors which facilitates the shape determination of a region and its neighborhood (Persoon and Fu 1977). Thus, invariant moments are calculated to obtain information about the patterns of the shape regardless the overall size of the regions of interest.

A second group is *Intensity features*, Haralick and Gupta texture and Local Binary Pattern (LBP) features take into account the distribution of the intensity values in the region. Haralick features are often extracted in order to obtain information about the intensity values distribution (Table 2.3). Basically, to obtain these textural features a co-occurrence matrix is computed per intensity image, which represents the joint probability distribution of intensity pairs of neighboring pixels. Thereafter, the mean and range of a mask containing five different neighboring pixels generated 14 basic *Tx* features summarizing information regarding the overall appearance quality of blueberries in each of the different color channels, where darker colors present in healthy, ripe fruit have a lower intensity and variability in comparison with lighter colors present in shriveled and fungally decayed fruit (Donis-González et al. 2012; Haralick 1979; Haralick and Shapiro 1993). Standard intensity features (Table 2.4) are related to mean, standard deviation of intensity in region, mean first-order derivative in the boundary and second order derivative in the region; contrast features which provide information of the intensity between a region and its neighborhood (Mery and Soto 2008).

LBP uses both statistical and structural characteristics of texture; it is a powerful tool for texture analysis. In the LBP operator, local texture patterns such as means, variances, etc. are extracted by comparing the value of neighborhood pixels with the value of the central pixel and are represented using binary codes (Kumar and Pang 2002; Pietikäinen et al. 2000).

$$LBP_{(d,h)} = \sum_{i=1}^{8} s(g_0 \cdot g_i) 2^{i-1} \qquad (2.27)$$

Where

$$s(g_0 \cdot g_i) = \begin{cases} 1, if \ g_i \geq g_0 \\ 0, if \ g_i \leq g_0 \end{cases} \qquad (2.28)$$

In Which g_0 is the gray value of the central pixel in the circularly symmetric neighbourhood, g_i takes the different eight pixels values from the neighborhood.

Table 2.2. Invariant moments

Hu moments

$$HU\_I_1 = \eta_{20} + \eta_{02} \tag{2.1}$$

$$HU\_I_2 = (\eta_{20} + \eta_{02})^2 + (2\eta_{11})^2 \tag{2.2}$$

$$HU\_I_3 = (\eta_{30} + 3\eta_{12})^2 + (3\eta_{21} - \eta_{03})^2 \tag{2.3}$$

$$HU\_I_4 = (\eta_{30} + \eta_{12})^2 + (\eta_{21} - \eta_{03})^2 \tag{2.4}$$

$$HU\_I_5 = (\eta_{30} - 3\eta_{12})(\eta_{30} + \eta_{12})[(\eta_{30} + 3\eta_{12})^2 - 3(\eta_{21} - \eta_{03})^2] + (3\eta_{21} - \eta_{03})(\eta_{21} + \eta_{03})[3(\eta_{30} + 3\eta_{12})^2 - (\eta_{21} - \eta_{03})^2] \tag{2.5}$$

$$HU\_I_6 = (\eta_{20} - \eta_{02})[(\eta_{30} + \eta_{12})^2 - (\eta_{21} - \eta_{03})^2] + 4\eta_{11}(\eta_{30} - \eta_{12})(\eta_{21} + \eta_{03}) \tag{2.6}$$

$$HU_{I_7} = (3\eta_{21} - \eta_{03})(\eta_{30} + \eta_{12})[(\eta_{30} + \eta_{12})^2 - 3(\eta_{21} + \eta_{03})^2] - (\eta_{30} - 3\eta_{12})(\eta_{21} + \eta_{03})[3(\eta_{30} + \eta_{12})^2 - (\eta_{21} - \eta_{03})^2] \tag{2.7}$$

Where,

$$\eta_{ij} = \frac{\mu_{ij}}{\mu_{oo}^{(1+\frac{i+j}{2})}} \tag{2.8}$$

and μ_{oo} is the central moment, and i and j describe pixel position of the segmented image (I).

Flusser and Suk moments

Moments are derived from complex moments of the image:

$$c_{pq} = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty}(x + iy)^p(x + iy)^q f(x, y)dxdy \tag{2.9}$$

While the segmented object is rotated by an angle(α), each of the complex moments of the image preserves its magnitude, as its phase is shifted by $(p - q)\alpha$

$$c'_{pq} = e^{-1(p-q)\alpha}c_{pq} \tag{2.10}$$

Table 2.3. Haralick textural features (Donis-González et al. 2012; Haralick 1979)

Texture	**Formula**
1. Angular second moment	$\sum_n \sum_m C(n,m)^2$ (2.11)
2. Contrast	$\sum_{i=0}^{N_g-1} n^2 \left\{ \sum_{i=1}^{N_g} \sum_{j=1}^{N_g} C(n,m) \right\}, \lvert n-m \rvert = i$ (2.12)
3. Correlation	$\frac{\sum_n \sum_m (nm)p(n,m) - \mu_z \mu_y}{\sigma_z \sigma_y}$ (2.13) Where μ_z, μ_y, σ_y and σ_z are the means and standard deviation of p_z and p_y, the partial probability density functions.
4. Sum of Squares: Variance	$\sum_i \sum_j (n-\mu)^2 C(n,m)$ (2.14)
5. Inverse difference moment	$\sum_i \sum_j \frac{1}{1+(n-m)^2} C(n,m)$ (2.15)
6. Average sum	$\sum_{i=2}^{2N_g} i p_{n+m}(i)$ (2.16) Where, N_g is the number of gray tones of the image (I). x and y are the coordinates (row and column) of an entry in the coocurrence matrix coordinates.
7. Variance sum	$\sum_{i=2}^{2N_g} (i-f_8)^2 C_{n+m}(i)$ (2.17)
8. Entropy sum	$-\sum_{i=2}^{2N_g} C_{n+m}(i) \log\{C_{n+m}(i)\} = f_8$ (2.18)
9. Entropy (HXY)	$-\sum_n \sum_m C(n,m) \log(C(n,m))$ (2.19)
10. Variance difference	$\sum_{i=0}^{N_g-1} i^2 \, C_{z-y}(i)$ (2.20)
11. Entropy difference	$-\sum_{i=0}^{N_{g-1}} p_{n-m}(i) \log\{p_{n-m}(i)\}$ (2.21)
12. Correlation measurement - 1	$\frac{HXY - HXY_1}{\max\{HX, HY\}}$ (2.22)
13. Correlation measurement - 2	$(1-\exp[-2(HXY_2 - HXY)])^{\frac{3}{2}}$ (2.23) HX and HY are the entropies of C_z and C_y. $HXY_1 = -\sum_n \sum_m C(n,m) \log\{C_z(n)C_y(m)\}$ (2.24) $HXY_2 = -\sum_n \sum_m C_z(n)p_y(m) \log\{C_z(n)C_y(m)\}$ (2.25)
14. Maximum correlation coefficient	Square root of the second largest eigenvalue of: $Q(n,m) = \sum_t \frac{C(n,t)C(m,t)}{C_x(n)C_y(t)}$ (2.26)

Fourier and discrete cosine transform coefficients where images are analyzed in the frequency domain. In Fourier domain image, each pixel represents a particular frequency contained in the spatial domain image; Gabor features are based on 2D Gaussian-shaped band-pass filter, with dyadic treatment of the radial spatial frequency range and multiple orientations representing measurement in both space and frequency domains (Mery et al. 2012).

$$F_{\mathrm{n}} = \sum_{k=0}^{L-1}(i_k + j * j_k)e^{-j\left(\frac{2\pi kn}{L}\right)} \quad (2.29)$$

for $n = 0, \dots, L-1$.

where (ik, jk) are the border pixel coordinates for $k = 0, \dots, L-1$, L is the number of pixels that are part of the perimeter border of a complex number region denoted by $(i_k + j * j_k)$ with $j = \sqrt{-1}$.

Gabor filters have been applied to image recognition problems because of its optimal localization properties in both the spatial and frequency domains (Huang et al. 2005). In this sense, Gabor features provide information concerning the development of any singularity of sample and is a complex exponential modulated by a Gaussian function:

$$f(x,y) = \frac{1}{(2\pi\sigma_x\sigma_y)}\exp\left(-\frac{1}{2}\left(\frac{x^2}{\sigma_x^2} + \frac{y^2}{\sigma_y^2}\right)\right)\exp(2\pi j u_0 x) \quad (2.30)$$

σ_x and σ_y denote the Gaussian envelope along the x and y axes, and u_0 defines the radial frequency. In the frequency domain, the Gabor function acts as a band pass filter, using the Gaussian function.

The self-similar filter banks can be obtained by dilations and rotation of $f(x,y)$ through the generating function:

$$f_{rs}(i,j) = \propto^{-P} f(i',j') \quad (2.31)$$

where,

$$i' = \propto^{-P} (i(cos\theta_r) + j(sin\theta_s)) \quad (2.32)$$

and

$$\propto^{-P} > 1; r = 1,2, \dots, S; \;\; s = 1,2 \dots L. \quad (2.33)$$

r and s represent the index for dilation and orientation respectively. S is the total number of dilatations and L is the total number of orientations, θ_s is the angle for each orientation s. For a given segmented image $I(i, j)$, the magnitude of filtered image $I_{rs}(i, j)$ is obtained by Gabor filter f_{rs}:

$$I_{rs}(i,j) = \{[f_{rs}(i,j)_e * I(i,j)]^2 + [f_{rs}(i,j)_0 * I(i,j)]^2\}^{\frac{1}{2}} \quad (2.34)$$

Where "*" denotes a 2 dimension convolution operation, and $f_{rs}(i,j)_e$ and $f_{rs}(i,j)_0$ represent the real (even), and imaginary (odd) parts of the Gabor filter (Eq. 10) respectively.

Table 2.4. Standard intensity features

Mean:		**Standard Deviation:**	
$\mu = \frac{1}{n}\sum_{i=1}^{n} x_{ij}$	(2.35)	$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij} - \bar{x}\right)^2}$	(2.36)
Kurtosis:		**Skewness:**	
$k = \frac{\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij}-\bar{x}\right)^4}{\left(\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij}-\bar{x}\right)^2\right)^2}$	(2.37)	$s = \frac{\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij}-\bar{x}\right)^3}{\left(\frac{1}{n}\sum_{i=1}^{n}\left(x_{ij}-\bar{x}\right)^2\right)^3}$	(2.38)
Mean gradient first-order derivative:		**Mean Laplacian Second-order Derivative:**	
$f'\left(x_{ij}\right) = \frac{x_{ij+1} - x_{ij}}{(ij+1) - ij}$	(2.39)	$f''(x_{ij}) = \frac{f'(x_{ij}) - f'(x_{ij-1})}{(ij+1) - ij}$	(2.40)

Where, x_{ij} represents the gray-scale value of pixels in each of the segmented intensity images, and *n* the total number of evaluated pixels (*ij*) in the segmented image.

After the features extraction, normalization is a requirement to process data to facilitate the classification; in this way, the extracted features are arranged and transformed in order to attain a group of features in the same order of magnitude (Mery and Soto 2008).

To conclude with features management, it is necessary to select the best features before classification. Thus, the principal idea is selecting a minor subset of features from total features that leads to the smallest classification error (Jain et al. 2000; Zhang 2002). In order to reduce the number of features, some strategies are employed. The best strategy should optimize a reduced number of features with the maximal classification hits. Among the useful selection strategies are sequential forward selection (SFS), nearest neighbor (KNN), linear and quadratic discriminant analysis" (LDA /QDA) and "Rank key features by class sorting criteria" (RANKFS) based on the "Relative Operating Characteristic curve" (R.O.C) and the "Student test" (Jain et al. 2000; Bishop 2007).

2.3.3. 3. Classification and validation

Once objects of interest are selected and measured, it would be desirable to classify or assign them into classes according to selected features. A simple statistical approach is the so-called supervised classification in which training sets of previously defined classes are selected by the operator (Aguilera and Briones 2005). Useful supervised classification algorithms to build decision lines, planes or hyper planes of the selected features are: linear and quadratic discriminant analysis (LDA or QDA), Mahalanobis distance (MD), support vector machine (SVM), and "Probablistic Neural Network" (PNN) (Ren et al. 2006; Leiva et al. 2011; Mery et al. 2010b). Differently, unsupervised classification does not require human to have the prior knowledge of the classes, and it mainly uses some clustering algorithms to classify an image data (Richards and Jia 1994). Classification is done using statistic or clustering algorithms such as K nearest neighbors (kNN), k-means, hierarchical clustering and mixture models, to determine which objects correspond to one or other class.

Performance of the classifier is measured as the ratio of correctly classified images in reference to its supervised categorical class to the total number of tested images. Detailed information about statistical pattern recognition can be found in Jain (2000), Duda (Duda et al. 2000), and Bishop (Bishop 2007) . More extensive information about computer vision and image processing can also be found in book and reviews (Sun 2008; Aguilera and Briones 2005; Russ 1998; Gonzalez et al. 2009).

2.3.4. Applications

The use of color images offers an alternative to visual inspection, and has been broadly used in various foods commodities because it is objective, reliable, rapid, and economical (Brosnan and Sun 2002; Kumar-Patel et al. 2012). Images have been effectively used to classify or recognize quality in several foods including potato chips (Pedreschi et al. 2006; Pedreschi et al. 2007), tortillas (Mery et al. 2010a), pizza (Sun and Brosnan 2003b, a), chocolate chip biscuits (Davidson et al. 2001), cheese (Wang and Sun 2002a, b, 2001), and pork meat (Lu et al. 2000; Faucitano et al. 2005), salmon bones (Mery et al. 2011)

In detection or classification of postharvest fruit defects, image processing allows the segregation of different quality degrees for fruits such as olive (Riquelme et al. 2008; Wang et al. 2011a), apple (Xing et al. 2005; De Belie et al. 1999; Unay and Gosselin 2006; Throop et al. 2005,Paulus and Schrevens 1999), bayberry (Zhang et al. 2005, Lu et al. 2010), pistachio (Pearson and Toyofuku 2000) , grape (Zoffoli et al. 2008), blueberry (Leiva et al. 2011; Leiva-Valenzuela and Aguilera 2013), fungally decayed sliced chestnut (Donis-González et al. 2013) , strawberry (*Fragaria* spp.) (Bato et al. 2000).

Comprehensive reviews of non-destructive imaging technology in fruit postharvest have been done by Studman (2001), Brosnan and Sun (2004), Kondo (2010), Wu and Sun (2013b) and Jackman and Sun (2013).

However, image processing applications for small fruits are still limited in scope. First studies in sweet cherry, tried to recognize basic fruit parts such as stems (Wolfe and Sandler 1985). Later studies have focused on shape characterization; a comprehensive determination of 3D cherry shapes would permit the understanding of fruit geometry; change of fruit shape with development, effects of environmental factors and differences in fruit shape among cultivars (Beyer et al. 2002). Also, freshness of cherry has been explained by detection of changes in fluorescence images of peduncles correlating chlorophyll properties with color (Linke et al. 2010). This technique not only helped verify quality of cherries, but also better understands the physiology of cherries. Simple applications of computer vision have also allowed the rating of cherries by color since the color of the skin is an important indicator of ripeness and quality in orchard (Rosenberger et al. 2004; Wang et al. 2012a).

In-line computer vision systems completed with image processing to segregate or classify fruit are main commercial applications. For strawberries, a traditional mechanical system with sorting algorithms to determine color and size was developed for grading fruit (Liming and Yanchao 2010a).

In recent years, some research has been focused on non-destructive recognition of images by extraction of patterns (Figure 2.2) to segregate defective berries such as those decayed, shriveled or mechanically damaged, and distinguish orientation (Leiva-Valenzuela and Aguilera 2013). Despite the possibilities offered by computational capabilities and new sensors, only a few commercial blueberry sorting systems based on images are available. These commercial systems allow for high-speed sorting (up of 2 tons h^{-1})for overall detection of berry colors and are able to reject up to 95% of bad fruit in appearance. However, they cannot recognize defects on fruit surfaces such as fungal decay and shriveling.

A mobile, visible computer vision system was developed to estimate the yields of wild blueberry (Zaman et al. 2008; Zaman et al. 2010; Zhang et al. 2010). The system implemented algorithms based on a “blue index” which weighted the blue color on the green and red of RGB images, allowing a better detection of mature blueberries in order to design models to mapping the yield of orchards

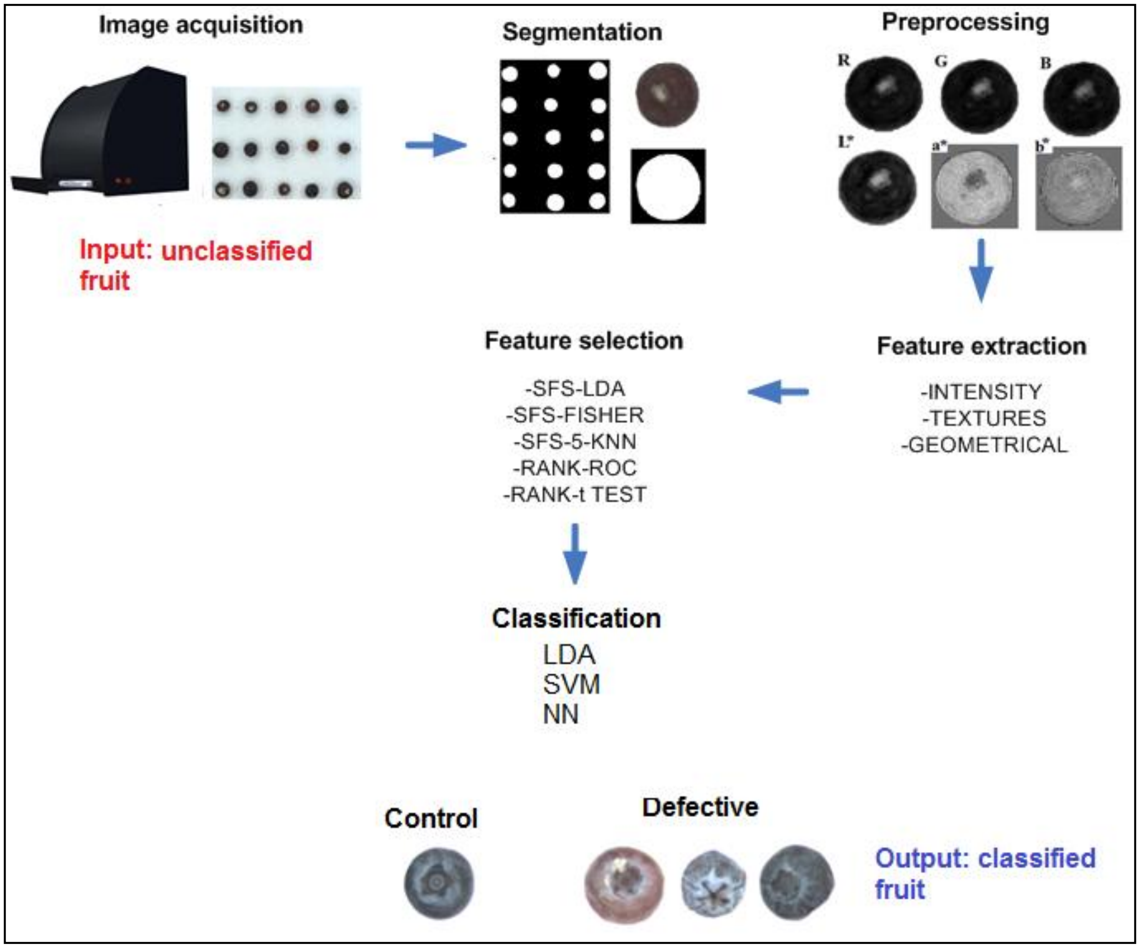

Figure 2.2. Automatic detection of defective blueberries using a computer vision system (Leiva-Valenzuela and Aguilera 2013).

2.4. Near infrared spectroscopy and hyperspectral imaging

Conventional color or grayscale imaging techniques are inadequate for measuring chemical constituents or internal quality attributes of fresh fruit because they only record the spatial distribution of light intensities over a broadband spectrum without detailed information for individual wavelengths considering that many chemical constituents are only sensitive to specific wavelengths. Differently, visible and near-infrared spectroscopy (NIRS) has become an important non-destructive technique for chemical analysis and quality assessment of agricultural and food products covering the spectral region where many components interact with light in the form of absorption, reflection, transmission at specific wavelengths (Lu 2008). Hyperspectral imaging (HSI) is a more advanced technology that integrates visible and near-infrared spectroscopy and imaging techniques by generation of a spatial map of spectral data.

2.4.1. Hyperspectral imaging equipment

A basic hardware hyperspectral imaging system consists of a sample positioning unit, a light source and an imaging unit.

Sample positioning unit can consisted of a mobile or static unit depending on the requirement of application. Mobile system is composed of a line belt conveyor, where each sample is placed and moved to the imaging area underneath the imaging unit..

Because a hyperspectral image is composed of multiple spectra, its acquisition of is slower than NIRS and its equipment requires the adaptation of the hardware to operate under the conditions imposed by sensors. There are basically two types of HIS: tunable filter based equipment, which generally acquire hyperspectral images of static samples (Ariana and Lu 2010) push broom or line-scan system, where samples are carefully placed over a sample holding try which is attached to a motorized linear stage. With this system is able to scan samples simultaneously maintaining the control of the sample position, the illumination and the acquisition conditions in order to improve the performance of experimentation (Leiva-Valenzuela et al. 2013).

Tungsten halogen lamps usually are used as light source because they are cheap, offer high intensity in the near infrared region and the spectral output is continuous. While in NIR spectroscopy light is focused on a specific zone of fruit, in hyperspectral reflectance imaging light should irradiate the whole surface of sample causing the disadvantage of large amounts of heat generation. Additionally, tungsten halogen lamp has a short operational lifetime. Finally, the spectral characteristic of the output can drift with time, and the radiant energy is not equal in different wavelengths (Cen and He 2007).

The imaging unit consisted of camera with imaging sensor, an imaging spectrograph usually attached to the camera to acquire hyperspectral images. This spectrograph is composed for a monochromator and optical components such as collimators, beam splitters, lenses integrating spheres and optical fibres. Monochromator is an optical device that transmits a selectable band of wavelengths selected from the wider range of wavelengths, thus, can produce monochromatic signals. Spectrophotometers are classified according to the type of monochromator: a limited spectral resolution *filter instrument* (wheel monochromator), a scanning *instrument* (prism or grating monochromator) is commonly used to select individual frequencies. *Fourier transform spectrophotometers* generate modulated light using interferometers which convert the time domain signal of the light into a spectrum with Fourier transform. Laser system has multiple specific wavelength laser light sources, hence acts as monochromator, *Acoustic optic tunable filter* uses optical-band-pass (constituted by an anisotropic crystal which change its optic properties when an acoustic wave is applied) to diffract the light in specific wavelengths by varying the frequency of acoustic signals (Tang et al. 1998). *Liquid crystal tunable filter* use a birefringent liquid crystal filter to retard light rays which pass through in phase. Therefore, wavelengths can be separated obtaining high spectral resolution when electronically tunable stages in series are combined. Most common by their features photodiode array PDA is being more used. Their high acquisition speed and the absence of moving parts allow online fruit grading implementation. This system is based in the dispersion of radiation by fixed grater which focuses the light onto an array of silicon or Indium Gallium Arsenide photodiode detectors (Nicolaï et al. 2007). Detectors quantify the intensity of reflected or transmitted and selected light by converting photons into electrons. There are two major types of solid state area detector: CCD and CMOS. In both types of cameras, photodiodes are made of light sensitive materials such as Silicon (Si), Indium gallium arsenide (InGaAs), and mercury cadmium tellurium (MCT or HgCdTe) capable to convert radiation energy to electrical signal. The main differences between their, is that both photodetector and read out amplifier in each pixel are included within the CMOS image sensor improving the imaging speed for online industrial inspection. However CMOS cameras acquire images with higher noise and dark current than the CCDs because of the on-chip circuits used to transfer and amplify signals, and as a result of lower dynamic range and sensitivity than CCDs. (Wu and Sun 2013a). Order-blocking filter can be installed onto the sensor of the camera to prevent wavelengths below or above the range of measurement; a fixed focal length lens are attached to the front of the spectrograph.

2.4.2. Sensing modes

In NIRS as well in hyperspectral imaging, products are subjected to NIR radiation in order to measure their spectra The spectral characteristics of the incident ray are modified while it passes through the product due to wavelength dependent absorption and scattering processes (Ruiz-Altisent et al. 2010a). This change depends on the chemical composition of the product, its light scattering properties which are related to the microstructure as well as on the lightning configuration or sensing mode.

In hyperspectral imaging generation, light interaction with sample can be classified in three sensing modes: *reflectance*, where light source and detector are mounted over the sample under a specific angle, thus specular reflection is avoid, *transmittance* where the light source is positioned opposite to the detector in consequence, the penetration of NIR radiation into fruit tissue decreases exponentially with the depth (Figure 2.4). In the third sensing mode, *interactance* (not suitable to hyperspectral imaging), the light source and detector are positioned parallel to each other in such a way that light due to specular reflection cannot directly enter the detector by means of a bifurcated cable in which fibers leading to the source and detector are parallel to each other and in contact with the product, or by means of a special optical arrangement (Nicolaï et al. 2007). Generally, reflectance and interactance are related to chemiometric and food microstructure while transmittance has been used in detection of foreign internal materials such as worms or pits (Figure 2.3).

2.4.3. Hypercube acquisition and spectral image processing

Hyperspectral images are building of hundreds of contiguous wavebands for each spatial position of a product. The resulting spectrum acts like a fingerprint which can be used to characterize the composition of that particular pixel Accordingly, each pixel in a hyperspectral image contains the spectrum of that specific position (Gowen et al. 2007).

In consequence, each hyperspectral imaging is a conjunct of grayscale images which represents a single band of spectral wavelength. Therefore, hyperspectral image is a conjunct of multiple images acquired at the same time where rows and columns define a spatial position for variables which localized a continuous range of wavelengths (Figure 2.3).

Hyperspectral imaging is similar to color imaging in the spatial information, but add spectral information which increase the capacity of sense minor components such chemicals (Wu and Sun 2013a). Additionally, due to NIR light properties, the light penetrates objects in higher degree than in color images allowing the interpretation of internal phenomena.

Visible and near infrared (Vis-NIR) covers the spectral region between 400 and 2500 nm providing more structural information about vibration behavior of combinations of bonds.

After irradiation of food or agricultural commodities by NIR radiation, the absorption of energy of occurs when organic molecular groups O–H, C–H, C–O and N–H start vibrating (stretching or bending) and specific wavelengths NIR radiation are transformed in heat. In consequence, reflected or transmitted light generates overtones in spectrogram signal allowing the interpretation of differences between compositions of samples. The NIR region is divided into short-wave NIR (SW-NIR) and medium and long wave NIR from 1300 nm. The SW-NIR absorb high overtones band while medium and long wave NIR, first or second overtone. Considering the intensity of absorption decreases when the overtone increases, SW-NIR is usually applied in transmission analysis with long path length and common NIR is used in diffuse reflection analysis (Cen and He 2007).

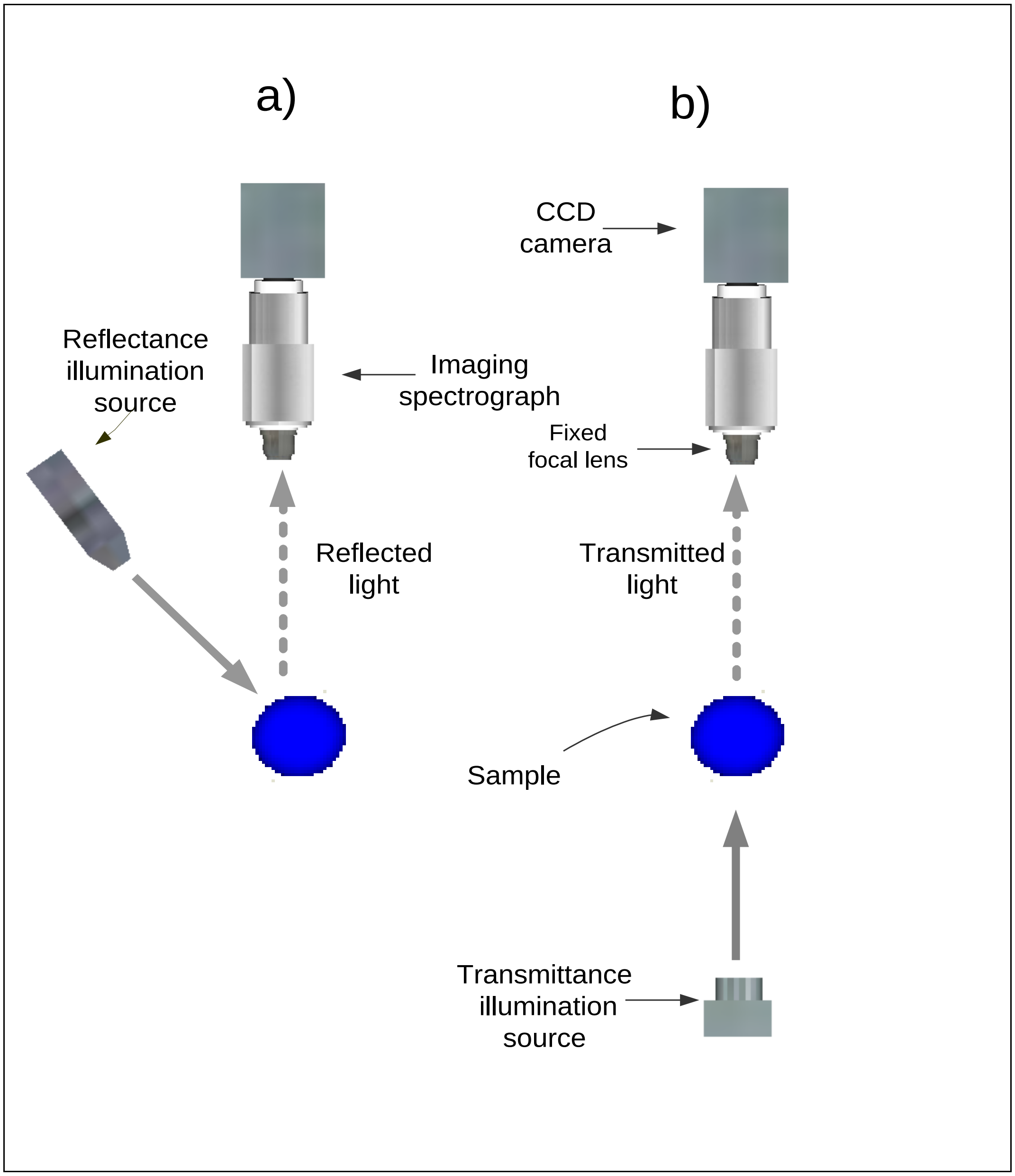

Figure 2.3. Sensing modes in hyperspectral image acquisition a) reflectance, b) transmittance.

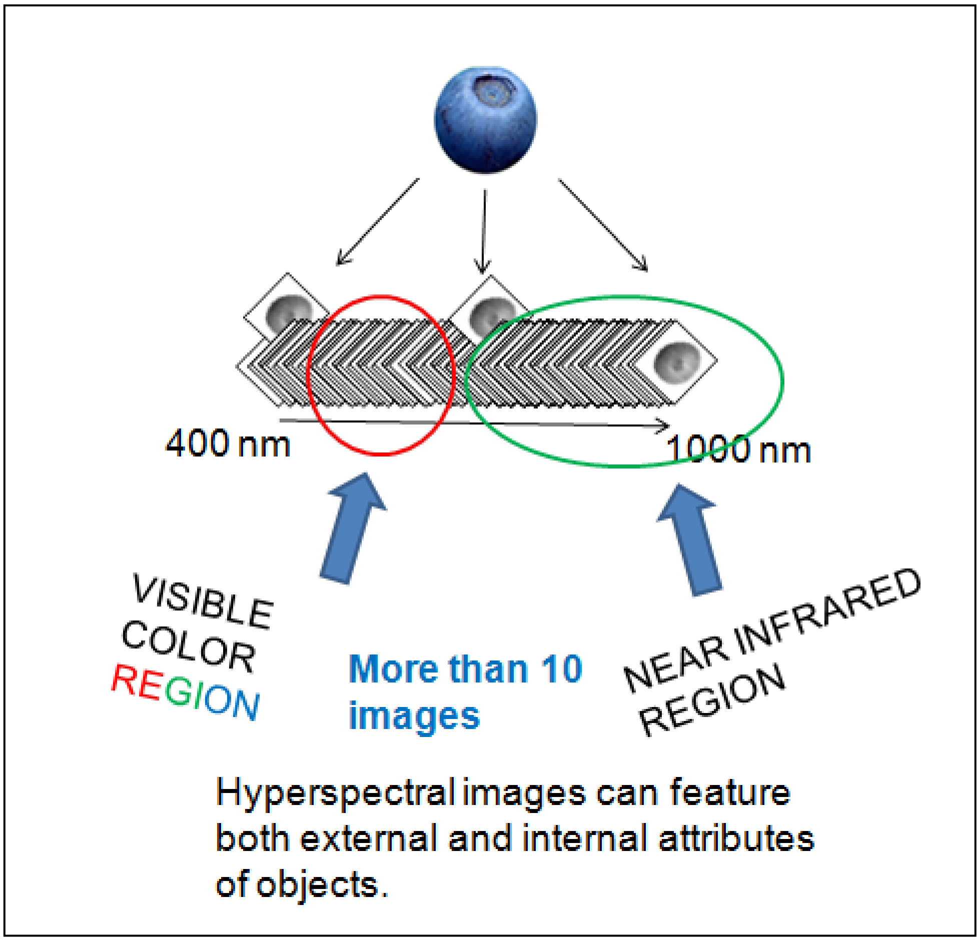

Figure 2.4. Understanding hyperspectral imaging acquisition

2.4.3.1 Pre-processing

For correction of the light source effect, hyperspectral reflectance images are also acquired from diffuse reflectance standard using the same imaging parameters as that for samples. For transmittance images corrections, generally a cylindrical reference disc made from white Teflon is mounted next to the frame of the sample holding unit (Lu and Ariana 2013). Dark current images are acquired with the camera shutter being closed. Therefore, the intensity values for each hyperspectral image are corrected by the reference and transmittance image pixels by pixels in both spatial and spectral dimensions (Ariana and Lu 2010; Tallada et al. 2006):

$$RI = \frac{IS(x,y) - ID(x,y)}{IR(x,y) - ID(x,y)} \qquad (2.41)$$

where *RI* is the relative reflectance or transmittance at pixel location (x,y), *IS* is the sample image value, *ID* is the dark frame image value, and *IR* is the reference image pixel value.

After the relative values of reflectance or transmittance are obtained, each spectrum is frequently smoothed by smothering algorithm. The most used is the Savitzky-Golay derivative (Savitzky and Golay 1964). Since the relative reflectance and transmittance spectra may vary among the fruit and within each image area, maximum normalization is suggested by (Xing and Guyer 2008) . In this way, normalized images result of dividing the relative transmittance value at each wavelength by their peak intensity value. Once preprocessing was carried out, segmentation is implemented to segregate region of interest from the background for each hypercube image. Generally, images at specific wavelengths are choose in order to apply common image processing algorithms such threshold selection method from gray-level histogram (Otsu 1979).

2.4.3.2. Multivariate analysis

From region of interest, features such mean intensity or others presented in 2.3.3. 1, are extracted. Therefore a matrix of features serves as basis of relation between spectroscopy and attributes of samples. Multivariate analysis can be classified into qualitative classification and quantitative regression (Wu and Sun 2013a). Qualitative regression uses techniques of pattern recognition (see chapter 2.3.3) and quantitative regression or prediction performed with algorithms such Partial Least Square regression (PLS) or interval partial least square which is suitable for select specific zones from the spectra.

PLS approach was originated around 1975 by Herman Wold. It can analyze data with strongly correlated, noisy, and numerous X-variables, and also simultaneously model several response variables (Wold et al. 2001). In spectroscopy, PLS are applied to extract the required information from the convoluted spectra and is required to decompose substantial quantity of features into useful information establishing simple and comprehensible relationship between NIR or spectral imaging data and chemical tested samples attributes (Nicolaï et al. 2007). For these reasons, PLS is one of the most popular methods that have been used to build models with orthogonal latent variables that are oriented along directions of maximal covariance between the spectral matrix and the response vector (Nicolaï et al. 2007). The latent variables come from a fusion of image features obtained using such a method as principal component analysis (PCA). Although non-linear techniques are becoming increasingly used, PLS allows simpler interpretation and comparison of results, avoiding the overtraining problem that is commonly encountered with complex non-linear models.

Interval partial least squares (iPLS) is one of the commonly used algorithms for selecting the most efficient wavelength regions and developing an optimized local PLS model built with fewer variables, allowing a reliable spectral data reduction (Nørgaard et al. 2005). Basically, in the iPLS method, the entire spectra are first split into smaller equidistant regions and PLS regression models are then developed for each of the sub-intervals, using the same number of latent variables for the selected region with lower error. An optimized region can be found by subtracting or adding new variables (Zou et al. 2007; Nørgaard et al. 2000a). Hence, iPLS could yield similar prediction results without using complete spectra information, while having the advantages of decreased computational time and knowing specific wavelength regions useful for predict attributes. A flow diagram of an hyperspectral image processing is presented in Figure 2.5.

2.4.5. Applications

Most applications of NIRS and Hyperspectral imaging in foods include determination of sugars, firmness and acidity (Bobelyn et al. 2010; Wu and Sun 2013a) . Therefore, NIRS is an attractive non-destructive technique to measure quality in food requiring little or no sample preparation; it is flexible and versatile, applicable to multiproduct and multi component analysis, thus enabling testing of both the raw material and the end product, as well as allowing simultaneous measurement of several analytical parameters; it generates no waste, is less expensive to run than destructive analytical methods (González-Caballero et al. 2010b).

NIRS in non-destructive measurement has been extensively used to evaluate internal quality in fruits, particularly in apples (Mendoza et al. 2012; Mendoza et al. 2011; Kavdir et al. 2009; Alamar et al. 2007; Peirs et al. 2005; Bobelyn et al. 2010; Peirs et al. 2001). However considerable less information about applications to small fruit can be found.

In order to assess internal quality of grapes, NIRS, has been used for soluble solid content (SSC) and acidity in grapes (Baiano et al. 2012; González-Caballero et al. 2010a; Parpinello et al. 2013; Fernández-Novales et al. 2009; Cao et al. 2010) and strawberry (Sánchez et al. 2012).

Hyperspectral imaging system was used to classify different varieties of bayberry employing principal component analysis to reduce data and artificial neural networks (Li et al. 2007). Also, (Sugiyama et al. 2010) presented a study with NIRS to detect leaves and stems as foreign materials in freeze blueberries, this study is part of a major idea that evolve and automatic sorter to be implemented in industry.

New tendencies in NIRS research point out in the implementation of handle mobile systems able to predict SSC directly in bunches in orchards (González-Caballero et al. 2010b). Furthermore to measure grape acidity, a portable NIRS equipment was developed (Chauchard et al. 2004).

Since small fruit is difficult to orientate to carry out a reliable measurement, near infrared signals are affected to superficial fruit. Therefore, Multispectral and hyperspectral image techniques allow the whole inspection of the fruit surface improving predictions.

Despite the promising results obtained by studies with many wavelengths, the speed of image acquisition and processing by a hyperspectral imaging system is not enough to meet the present requirement of the sorting lines in the packinghouse. For this reason, reduction of spectral dimensionality was considered in several studies, which would allow hyperspectral imaging technique to be implemented online as a multispectral imaging system with fewer wavelengths. For example, to predict solid soluble content in strawberries, (Nagata et al. 2005) selected wavelengths of 915, 765, 870, 695 and, 860 nm, obtaining 89% correlation coefficients for prediction. In another other experiment to detect bruises caused by exerting different pressures with a universal testing machine they acquired images from 650 to 1000 nm finally selecting two wavelengths (825 and 980 nm) using stepwise linear discriminant analysis. Finally to classify bruised areas, they applied different methodologies including discriminant analysis, a normalized difference between pixels values and artificial neural networks, achieving similar classification accuracies (70-80%) (Tallada et al. 2006). Also HSI has been used to detection of bruises in strawberries (Tallada et al. 2006), internal quality factors as acidity and solid soluble content (Nagata et al. 2005), quality attributes as moisture content, total soluble solids, and acidity (ElMasry et al. 2007). An extensive review of HIS applications in strawberries was done by (Nagata and Tallada 2008). In those researches, selection of optimal wavelengths was also a primary objective.

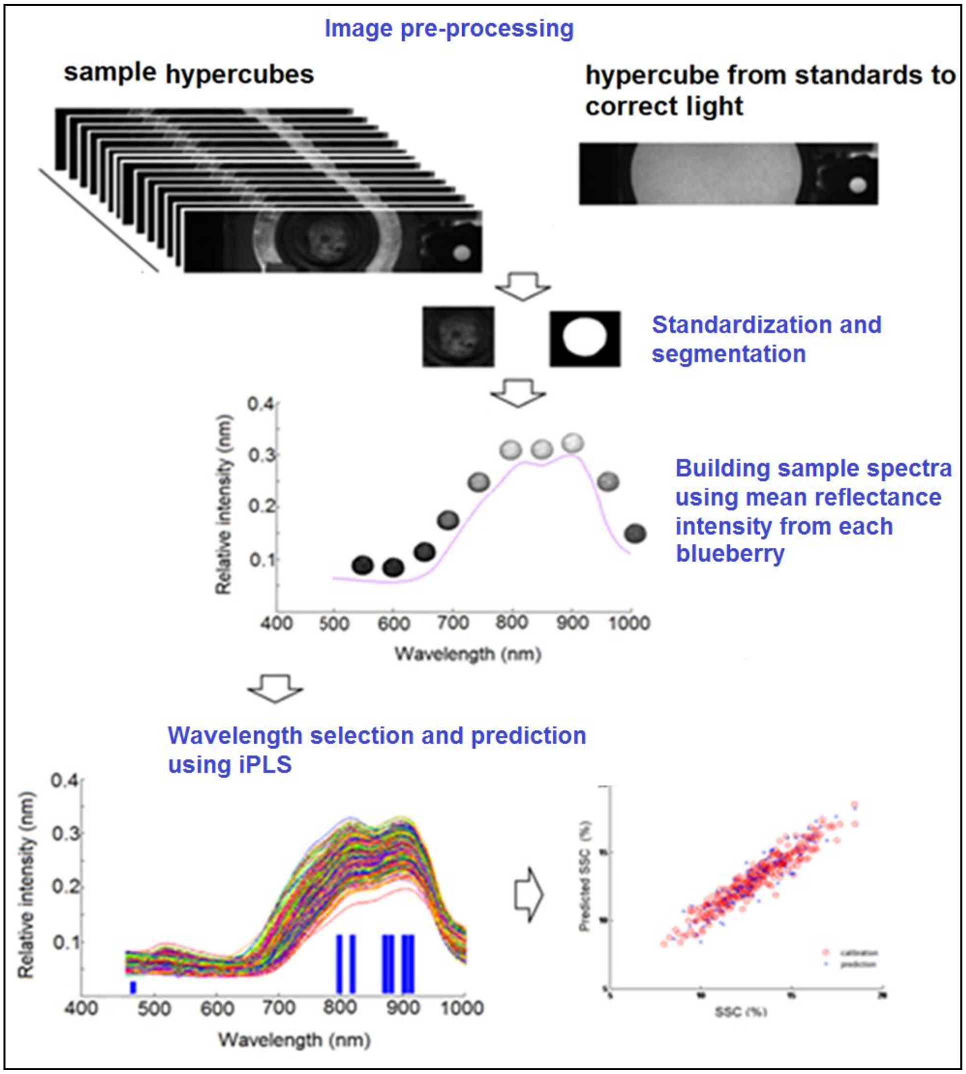

Figure 2.5. Process of building models to predict internal attributes of blueberries with hyperspectral images.

Table 2.5 shows different applications to measure fruit attributes using hyperspectral imaging systems. For strawberries, hyperspectral imaging systems have been used to evaluate their internal quality, as performed by (Nagata et al. 2005) used a multiple linear regression stepwise analysis to determine wavelength and develop calibration models to predict firmness and solid soluble content. They estimated firmness of 70% full-ripe strawberries using images acquired at a wavelength of 680 nm with a discrete correlation coefficient for prediction of 64.5%.

Table 2.5. Hyperspectral imaging systems used in different applications to measure fruit attributes

Technique	Wavelength (10^3 nm)	Fruit, objective, modeling	Quality descriptor	Reference
HIS	0.4 – 1.0	Injured Apple classification with neural network.	Chilling-injured apples and normal apples	(ElMasry et al. 2009)
HIS	0.320 – 1.1	Rotted Mandarin classification with LDA and decision trees	*Penicillium digitatum* rotten mandarins and normal mandarins	(Gómez-Sanchis et al. 2008)
HIS-Scattering	0.4 – 1.0	Apple Mealiness prediction with PLS-discriminant analysis .	Juiciness, hardness and mealiness	(Huang and Lu 2010)
HIS-Scattering	0.64-0.73	Peaches, prediction and wavelength selection	Ripeness	(Lleó et al. 2011)
HIS	1.0-1.6	Apple, discriminant PLS, classification	Bitter pit and corky tissue identification	(Nicolaï et al. 2006)
HSI laser-induced fluorescence	0.41	apples, prediction, NN-principal components	Skin hue and Chroma, flesh chroma, SSC, acidity, firmness	(Noh and Lu 2007)
In-orchard mobile HSI	0.4–1.0	Green citrus fruit, classify cation with Multidimensional Scatter - LDA	Detection of green fruit in a citrus tree	(Okamoto and Lee 2009)
HIS-reflectance	0.5 -1.0	Blueberries, prediction using PLS- principal components	Firmness, SSC	(Leiva-Valenzuela et al. 2013)
HIS scattering	0.5-1.0	Peaches, prediction using MLR	Firmness	(Lu and Peng 2006)
HIS-scattering	0.45-1.0	Apple, prediction using MLR	Firmness, SSC	(Peng and Lu 2007) (Peng and Lu 2008)
HIS-REF/TRANS	0.45 – 0.1	Cucumbers, detection using PLS Discriminant analysis	Internal fly infestation	(Lu and Ariana 2013)
HIS-scattering	0.5 -1.0	Apple, prediction using PLS	Firmness, SSC	(Mendoza et al. 2011)
HIS	0.45-0.93	Red grapefruits , classify, thresholding method based in Spectral information divergence	Differentiation of canker from normal fruit peels.	(Qin et al. 2009)
HIS Spatially resolved steady-state	0.5-1.0	Apples, pears, peaches, kiwifruits, plum, cucumber zucchini squash, tomatoes. Optical properties were determinate using the steady-state diffusion theory model	Optical properties proposing a methodology to evaluate maturity.	(Qin and Lu 2008)
HIS-reflectance	0.4-0.7	Jujubes fruits, classification, stepwise discriminant analysis.	Condition of the surface condition (insect damage), stem-end/calyx-end/cheek	(Wang et al. 2011c)
HIS- airborne	0.41 - 0.90	Citrus orchard, classification, Neural network	Yield of citrus orchard	(Ye et al. 2006)
HIS- airborne	0.41 - 0.90	Citrus orchard, classification, Neural network, MLR	Yield of citrus orchard	(Ye et al. 2008)

In grapes, (Chauchard et al. 2004) gave other methodology to select the wavelengths to predict acidity. This study suggested the necessity of testing other methodologies to select wavelengths to improve prediction. Least-squared support vector machine are compared to partial least square and multivariate linear regressions. LS-SVM evaluated with "least one left cross validation", produces more accurate prediction with performances in the test group of images of 86% in comparison with 77 and 68% of traditional least square and multivariate linear regressions.

Recently, prediction of soluble solids content and firmness has been studied using a hyperspectral push broom imaging system (Leiva-Valenzuela et al. 2013). This study suggested the two classes sorting by firmness and sweetness.

2.5. Other techniques

2.5.1. Mechanical methods

Requirements imposed by the fresh market and processing enforce assessing a threshold for berries firmness to sort fruit as soft or hard (Prussia et al. 2006). As a result, numerous studies have been carried out for nondestructive measurement of small fruit firmness. Generally, the measurement systems have simulated the effect of fingers squeezing the fruit to detect soft juicy fruits like tomato and berries when sample are measured by the creep test, that defines the deformation-time behavior under constant load (Abbott 1999). Accordingly, (Slaughter and Rohrbach 1985) (Prussia et al. 2006) suggest a standardized firmness measurement of blueberries by measuring the slope of force/deformation curves when fruit were subjected to compression by two parallel plates under a constant loading velocity. Consequently two commercial instruments based on this principle, called FirmTech I and FirmTech II (Bio-Work Inc., Kansas, USA), are currently available for measuring blueberry firmness. Nevertheless, these instruments are not suitable for in-line sorting use and can induce superficial bruises.

Less destructive and more automatic methods include vibration (Bower and Rohrbach 1976), roll-bounce separation (Wolfe et al. 1980), and relaxation modulus measured from the amount of impact force during rebound over a rigid piezoelectric transducer

(Rohrbach et al. 1982; Lee and Rohrbach 1983), (Delwiche 1987). More recently, an air-puff rebouncing tester was used to assess the firmness of blueberries, which measures the deflection or deformation of the fruit under a puff of pressurized air and in order to be implemented in sorting lines (Li et al. 2010).

Currently, several types of commercial sorting systems are available, which are based on detection of the impact response of blueberries when they hit a pressure sensor (e.g., the "*Berrytek*" sorting system from Woodside Electronics Corp., CA, USA and "*Soft Sorta*" from BBC Technologies Ltd., Ohaupo, New Zealand). While these commercial systems allow a high sorting speed (up of 2 tons h^{-1}), they are only able to reject up to 80% of the actual soft fruit (http://bbctechnologies.com/en_US/softsorta.htm). Moreover, for countries which require long term shipping from origin to destination, mechanical selection could slightly affect the external quality by induction of bruises.

2.5.2. Methods based in aroma detection

An electronic nose or electronic sniffer is a non-specific sensor array able to describe the atmosphere that surrounds a fruit. In this technology, the sensor detects the reaction between volatiles released from a fruit with semi-conductor materials (Simon et al. 1996). Therefore, sensors respond when are exposed to volatile compounds generating a sort of chromatogram with different peaks, then, a characteristic 'fingerprint' (Demir et al. 2011).

Using an electronic nose, packaged blueberry aroma detection for ripeness measuring was studied by (Simon et al. 1996). They detected a low emission of butyl acetate, 2-butoxyethanol and CO_2 when blueberries were in the latest stages of maturity. Also this study combined aroma with color information in order to improve the accuracy. Mechanical damage also correlated well with specific volatile compounds product of tissues destruction during storage using multivariate analysis, consequently, electronic nose was used to classify induced damaged berries (Demir et al. 2011).

As other non-destructive techniques presented before, the velocity of aroma characterization by these sensors is still too slow. For this reason characterization of strawberry aroma with headspace fingerprint - mass spectrometry (HFMS) was compared with traditional Gas chromatography - mass spectrometry (GC-MS). In specific super oxygen and elevated CO_2 modified atmosphere, HFMS can feature fruit ripeness faster than GC-MS transferring aroma into the ionization chamber of a mass spectrometer avoiding prior chromatographic separation. Hence, HFMS can be considered a good tool for rapid screening of strawberry flavor (Berna et al. 2007). In the same way, a detection of volatile organic compounds in real time for strawberries, blackberries, raspberries, blueberries, white and red currants was used to monitor their postharvest aging by proton transfer reaction-mass spectrometry (PTR-MS). This technique consists in the ionization of volatile organic compounds by proton transference, then aromas can be efficiently measured allowing their storage monitoring through the increment of concentration of methanol, acetaldehyde and ethanol (Boschetti et al. 1999).

2.5.3. Magnetic resonance imaging

Magnetic resonance imaging is based on the principles of nuclear magnetic resonance and has achieved general acceptance as a powerful tool for the diagnosis of clinical condition and also in plant science (Clark et al. 1997). MRI is based in quantum mechanical properties of certain sample atomic nuclei Nuclei with an odd number of protons or neutrons (e.g. ^{1}H, ^{19}F, ^{31}P, and ^{13}C) have random spin and precession movements. When sample is subjected to energy from specific electromagnetic pulses, the low energy protons pass to high energy state making harmonic their precession movement. When energy descends, protons pass to relaxed state emitting energy, thus, the precessing magnetization induces a small voltage in a surrounding tuned coil by the process of electromagnetic induction and it is this voltage which forms the NMR signal which is transformed in images (Richardson et al. 2005). Two mean time parameters are involved in the volume element of MRI (voxel) formation: t_1 and t_2. While t_1 is referred to spin longitudinal relaxation time, t_2 spin-spin relaxation time referred to interaction between atoms.

Magnetic resonance imaging has been widely used to probe into the interior of fruits (Clark et al. 1997; Abbott 1999; Abbott et al. 2010). MRI, for example, has been used in fruits like apple to characterize tissue (Defraeye et al. 2013), to detect internal browning development (Gonzalez et al. 2001), mealiness (Barreiro et al. 1999), core breakdown (Lammertyn et al. 2003) and for evaluation of ripening and storage changes (Létal et al. 2003). In tomato MRI has been used to investigate structural aspects (Musse et al. 2009a) and monitoring ripening (Musse et al. 2009b). Also table olives (Brescia et al. 2010) and palm fresh fruit bunches ripening (Saeed et al. 2012) was evaluated with MRI. Table 2.3 shows the different techniques to non-destructively measurement of small fruit.

In small fruit, several studies of fruit tissues used magnetic resonance to understand structural changes during maturity or postharvest. In black currants (Glidewell et al. 1998) the structure changes during development were studied observing differences in tissues, as in the space around the ovules in the ovary, or consequent on variations in the size of cells and vascular architecture of blackcurrants throughout development. More specific studies were achieved in order to visualize the damage caused by infection by *Botrytis cinerea*, ripening development and the determination of seeds in strawberry when MRI parameters were adjusted (Goodman et al. 1996).

Table 2.6. Techniques used to detect or measure small fruit attributes.

Small fruit	Target	Technique	Reference
Cherry	Shapes	CV	(Beyer et al. 2002)
Blueberry	Orchard yield	CV	(Swain et al. 2010) (Zaman et al. 2008; Zaman et al. 2010; Zhang et al. 2010)
Blueberry	Mold, shriveling, bruises, fruit orientation	CV	(Leiva-Valenzuela and Aguilera 2013)
Strawberry	Grading	CV	(Liming and Yanchao 2010b)
Cherry	in-orchard color rating	CV	(Wang et al. 2012a)
Cherry	Maturity	CV	(Linke et al. 2010)
Grape, Blueberry	SSC, Acidity	NIRS	(González-Caballero et al. 2010b)
Grape		NIRS	(Chauchard et al. 2004)
Bayberry	Varieties classification	NIRS	(Li et al. 2007)
Strawberry,	SSC, pH, bruises, moisture content	HSI	(ElMasry et al. 2007)
Cherry	Defect	MSI	(Guyer and Yang 2000)
Cherry tomatoes	Defect	HIS	(Cho et al. 2013)
Strawberry	SSC, firmness	HSI	(Nagata et al. 2005)
Blueberry	SSC, firmness	HSI	(Leiva-Valenzuela et al. 2013)
Strawberry	Bruises	HSI	(Tallada et al. 2006)
Blueberry	Foreign material detection	HSI	(Sugiyama et al. 2010)
Strawberry	Maturity	EN	(Berna et al. 2007)
Blueberry	Firmness	EN	(Simon et al. 1996)
Blueberry	Bruises	EN	(Demir et al. 2011)
Blueberry	Mold, yeast	EN	(Li et al. 2010)
Strawberry Table grape Cherry	Maturity	MRI	(Clark et al. 1997)
Strawberry Blueberry Raspberry Blackberry Currant	Maturity	PTR-MS	(Boschetti et al. 1999)

Abbreviations: PTR-MS proton transfer reaction-mass spectrometry; NIRS: Near infrared spectroscopy, MRI: magnetic resonance imaging, EN: electronic nose, Hyperspectral imaging system, CV: computer vision system

The most of those studies are focused in the adjustment of MRI parameters and in the simple observation of sample. MRI was used to evaluated changes in water status and mobility of sugar on cherry tomato during maturity (Ishida et al. 1994).

Also red raspberry fruits were subjected to micro MRI to study changes in fruit tissue determining the movement of hydrogen protons during ripening (Williamson et al. 1992). Authors empathizes the non-destructive advantages of MRI in comparison with scanning electron microscopy.

(Zion et al. 1994) presented magnetic resonance images that detected hidden pits en real-time in processed cherries. Evaluating their data, an 88% of performance in pitted cherries. Since magnetic resonance imaging (MRI) is still expensive for practical implementation requiring complex equipment and detailed data processing (Jackman et al. 2011), literature referred to small fruit postharvest applications is limited. Moreover, sorting applications are restricted by its processing velocity and its low resolution accented in small fruit. Table 2.6 presented a summary of non-.destructive techniques applied to small fruit.

2.6. Conclusion

Since small fruits are fragile, high value commodities highly susceptible to postharvest damages during transportation or storage, their quality assessment is critical to ensure an optimal commercialization. Hence, developing non-destructive technologies is not only a challenge, but also a necessity.

In recent years, non-destructive methods, fundamentally those based in image processing, have increasingly been used as a method to segregate fruit such as apple, orange and kiwi during postharvest. In small fruit this tendency is slightly more slowly. However, due to their many advantages (i.e. non-invasive, absence of sample preparation, velocity of results delivery, minimal cost of processing, versatility in types of analysis), an increment of research and commercial implementation is projected in short time.

There are several techniques based in computer vision, NIR spectroscopy, mechanical firmness detection, aromas and nuclear resonance, reliable to be implemented in in-line sorting systems in packing houses. Mechanical methods to assess firmness use are widespread in sorting lines, however may induce slightly superficial bruises which might trigger fruit deterioration when commercialization requires long shipping. Still slowly and expensive to be implemented in commercial lines, aroma based systems allow the whole fruit evaluation determining the overall condition. High cost of magnetic resonance imaging systems impedes their implementation. Differently, computer vision (CV) and NIR spectroscopy applications are fast, have an affordable cost of implementation and presented high performances to detect external attributes or are suitable to detect both internal and external attributes correlating with chemical and textural information (NIR). However NIR signal spectrometers require a careful previous positioning of sample to enhance the signal and make it representative. Differently, hyperspectral imaging systems are slower and expensive than computer vision and NIR spectrophotometers, however allow both external and internal whole characterization of fruit. Therefore, computer vision, NIR spectrometry and hyperspectral imaging systems allow better performances in external and internal quality descriptors detection or classifications. Therefore, further studies should active explore in their implementation.

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3. AUTOMATIC DETECTION OF ORIENTATION AND DISEASES IN BLUEBERRIES USING IMAGE ANALYSIS TO IMPROVE THEIR POSTHARVEST STORAGE QUALITY

Abstract

The production of the South American blueberry has increased by over 40% in the last decade. However, during storage and shipping, several problems can lead to rejections. This work proposes a pattern recognition method to automatically distinguish stem and calyx ends and detect damaged berries. First, blueberries were imaged under standard conditions to extract color and geometrical features. Second, five algorithms were tested to select the best features to be used in the subsequent evaluation of classification algorithms and cross-validation. The blueberries classes were control, fungally decayed, shriveled, and mechanically damaged. The original 225 features extracted were reduced to 20 or fewer with sequential forward selection. The best classifiers were Support Vector Machine and Linear Discriminant Analysis. Using these classifiers made it possible to successfully distinguish the blueberries' orientation in 96.5 % of the cases. For classifying blueberries into the fungally decayed, shriveled, and mechanically damaged classes, the average performances of the classifiers were above 98 %, 93.3 %, and 90 % respectively. All of the experiments were evaluated using external images with 95 % confidence – 10-fold cross-validation. These results are promising because they will allow for the increase in export quality when implemented in production lines.

3.1. Introduction

Blueberry is an important fruit worldwide whose consumption has increased in recent years due to its good flavor and high antioxidant capacity, which is good for anti-aging. The United States of America is the leading blueberry exporter and consumer (FAO 2009).In recent years, countries in the southern hemisphere (e.g., Argentina, Chile, New Zealand and South Africa) have increased fruit export to the northern hemisphere. However, long-distance shipping requires delivering higher-quality and more consistently fresh blueberries to meet quality standards upon arrival.

Accurate determination of blueberry quality is challenging because individual fruits are small, dark in color, and vary greatly in external and internal quality characteristics. Traditionally, blueberry quality was inspected by hand in sorting lines at the origin of fruitproduction for color, size, and the absence of defects and foreign materials. However, in blueberry quality assessment, sorting by hand is inefficient and unreliable, making the implementation of automatic systems necessary. Previous studies have shown that applications of computer vision in quality control in food are more accurate, safe and quicker than human inspection (Aguilera and Briones 2005).

Detailed information about statistical pattern recognition can be found in the work of Jain (Jain et al. 2000), Duda (Duda et al. 2000), and Bishop (Bishop 2007) . Previously, (Leiva-Valenzuela et al. 2013) incorporated image processing techniques into a push broom hyperspectral reflectance system to select areas and extract the mean intensity of individual blueberries to predict internal attributes (i.e., soluble solids content and firmness). Leiva et al. (2011) used visible and external features to sort fungally diseased blueberries and were able to recognize more than 95% diseased singles using linear discriminant analysis and 10-fold cross validation.

Computer vision, as an objective, consistent, rapid, and economical technology, offers an automated alternative to visual inspection and is currently being used in various food and agricultural sorting systems (Brosnan and Sun 2002; Kumar-Patel et al. 2012). Specifically, color computer vision has been used to effectively assess quality in strawberries (*Fragaria* spp.) (Bato et al. 2000), pistachios (*Pistacia vera*) (Pearson and Toyofuku 2000), fungally decayed sliced chestnuts (Donis-González et al. 2013), olives (Riquelme et al. 2008; Wang et al. 2011a), apple (De Belie et al. 1999; Xing et al. 2005) and bayberries (Lu et al. 2010). Reviews of non-destructive inspection technologies for postharvest fruit were reported in Studman (2001) and Brosnan and Sun (2004).

Currently, several commercial sorting systems are available for sorting blueberry (e.g., the "*Berrytek*" sorting system from Woodside Electronics Corp., CA, USA, and "*Color Sorta*" from BBC Technologies Ltd., Ohaupo, New Zealand). These commercial systems allow for high-speed sorting (up of 2 tons h^{-1}) and are able to reject up to 95% of low fruit (http://bbctechnologies.com/en_US/colorsorta.htm). However, these sorters are based on the overall detection of berry color, and have limited ability to recognize specific defects, such us fungal decay and shriveling. Therefore, using pattern recognition algorithms is necessary to improve the ability to separate diseased fruits. This chapter proposes a pattern recognition methodology to extract and select visible features from images to sort blueberries into four classes—control (good blueberries), shriveled, fungally decayed, and mechanically damaged blueberries—and two fruit orientations—stem end and calyx end. This research will facilitate the development of automatic sorters that can separate fruits exhibiting visible disease damages.

3.2. Materials and methods

Experiments were carried out in the Biomaterials Laboratory of the Department of Chemistry and Bioprocess Engineering at Pontificia Universidad Católica de Chile (PUC) in Santiago, Chile between August 2009 and February 2010. Image processing was carried out using Matlab R2007a and its image processing toolbox (The Mathworks, Inc., Natick, MA, USA).

3.2.1. Samples

“Highbush” blueberries, (*Vaccinium corymbosum* var. ‘Star’) were acquired from the central region of Chile. Before experimentation, blueberries were stored at 0° C for 8 weeks to induce different levels of postharvest damage. Before the image acquisition, samples were visually inspected and divided into four classes based on appearance and surface defects (Figure 3.1). *Control blueberries* were defined as well-colored, spherical fruits free of irregularities such as spots, hairlines, impacts, compression, wrinkles, and the present of mold.

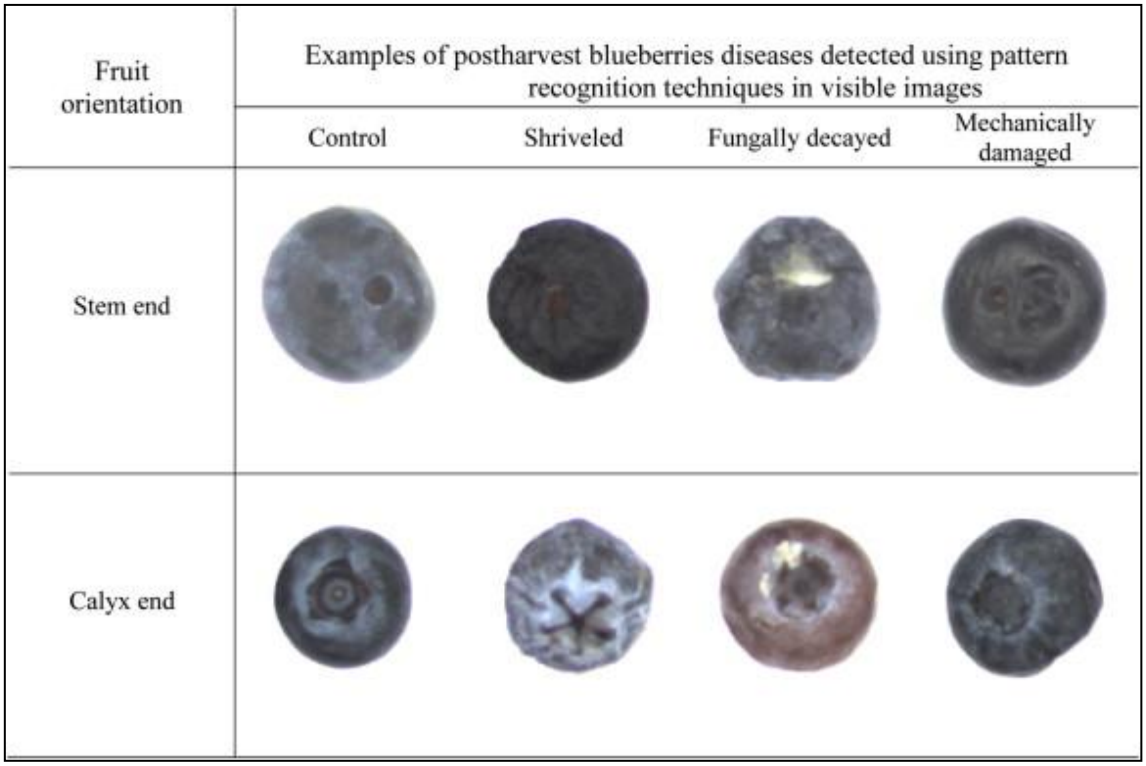

Figure 3.1. Examples of dual-orientation blueberry classes after segmentation. Control blueberries were defined as well-colored, spherical fruits free of irregularities. Fruits with wrinkles over their surfaces were defined as shriveled blueberries. Decayed blueberries were fruits with at least one mold spot over their surfaces or a characteristic purple color and a relatively softer texture around the pedicel than that of the rest of the fruit. Finally, mechanically damaged blueberries were defined as berries with visible changes in their external geometry as a result of compression or impact.

Fruits with wrinkles over their surfaces were defined as *shriveled blueberries*. In most of the cases, wrinkles appeared as concentric curves close to the stem end. Fungally decayed *blueberries* were defined as fruits with at least one isolated mold spot over their surfaces or a characteristic purple color and a relatively softer texture around the pedicel than that of the rest of the fruit. The threshold area used to distinguish decayed from non-decayed fruits was approximately 1/50 of the overall image area. Finally, *mechanically damaged* blueberries were defined as berries with visible changes in their external geometry as a result of compression or impact caused by transportation or storage.

3.2.2. Image acquisition, segmentation and color transformation

Images were acquired under standard conditions using a DVS-lab colorimeter (Digital Vision Solutions, www.divisol.cl). Each fruit was imaged along two orientations, the calyx end and stem end, for each class in groups of 15 berries (480 x 640 pixels color image). Images were stored in the bitmap picture (BMP) format.

Image segmentation was implemented in two steps. The first step consisted of the recognition of single berries by cropping the original image in pre-defined regions. The second step consisted of building a binary mask to recognize the fruit from the background using threshold segmentation and morphologic color image operations. Those morphologic operations included the elimination of small, isolated groups of pixels, the slight erosion of blueberry borders to avoid confusion with shadow pixels, and the filling of holes inside the binary segmented images (Otsu 1979; Mery and Pedreschi 2005). After segmentation, color images were decomposed into red (R), green (G) and blue (B) channels and then converted to grayscale and L*,a*, b* color space images using the method of Leon and others (2006), producing seven intensity images. Therefore, 1640 images each of 100 x 100 pixels (25 kb for each) were analyzed using pattern recognition algorithms.

3.2.3. Feature extraction and selection

The patterns of the different blueberry classes were recognized using “Balu”, a free-use toolbox for pattern recognition (http://dmery.ing.puc.cl/index.php/balu/) developed in the Department of Computer Science, Pontifical Catholic University of Chile, Santiago, Chile.

From the seven intensity images, 225 features were extracted. Six standard features describing simple intensity information regarding the mean, standard deviation, and mean first and second derivative along the boundaries of the region of interest (Mery et al. 2011; Nixon and Aguado 2008), 16 Fourier descriptors (Persoon and Fu 1977), and 176 rotation invariant local binary pattern features were extracted to compute the texture through the relationship between the intensity of each pixel and eight neighbors to produce occurrence histograms of local binary patterns (Pietikäinen et al. 2000). Finally, invariant moments were calculated to obtain information about pattern shape regardless of the size of the regions of interest. Overall, 27 features were extracted from the grayscale images, including 7 Hu moments (Hu 1962b) and 4 Flusser and Suk moments (Flusser and Suk 2005). The mathematical expressions used to extract all features are presented in sub-chapter 2.3.3. After feature extraction, normalization was performed (Mery and Soto 2008). The k extracted features were arranged in a k –vector, $w = [w_1 \dots w_K]^T$, resulting in the normalized features $\widetilde{w}_{(a,b)}$:

$$\widetilde{w}_{(a,b)} = \frac{w_{(a,b)} - \overline{w}_b}{\sigma_b} \tag{3.1}$$

a takes values from 1 to the number of samples, and b takes values from 1 to the number of features. $\overline{w}_b$ and σ_b are the mean and standard deviation of the b-th feature .

After extraction and normalization, it was necessary to select the best features before classification. Thus, the principal objective is to select a small subset of features from the total features that leads to the smallest classification error (Jain et al. 2000; Zhang 2002). To reduce the number of features, five strategies were tested. The best strategy should optimize a reduced number of features with the maximal classification hits. The selection strategies used were sequential forward selection (SFS), with the objective functions Fisher discriminant, 5-nearest neighbors (5-KNN), and linear discriminant analysis (LDA), and Rank key features by class sorting criteria (RANKFS), which does not feature an objective function, based on the relative operating characteristic curve (ROC) and the Student test (Jain et al. 2000; Bishop 2007). The appropriate number of selected features for these strategies was defined as a maximum of 10% of the initially extracted features or the number reached when the performance plateau was reached.

3.2.4. Classification

To train the pattern recognition algorithms, images were sorted into the four classes described in 3.2.1. With the selected features, decision lines, planes or hyper planes were implemented using LDA, Quadratic discriminant analysis (QDA), Mahalanobis distance (MD), K nearest neighbors (kNN) (k is the number of neighbors and was fixed to 5), Support Vector Machine (SVM), and Probabilistic Neural Network (PNN) (Ren et al. 2006; Leiva et al. 2011; Mery et al. 2010b).

The performance of the classifiers was measured as the ratio of correctly classified images with respect to their supervised categorical class to the total number of tested images.

3.2.5. Experimentation

Sixteen experiments were performed to determine the performance of six classification arrays and the differences between fruit image orientations: (i) differentiation of stem end from calyx end, (ii) differentiation of control blueberries from shriveled blueberries, (iii) differentiation of control blueberries from fungally decayed blueberries, (iv) differentiation of control blueberries from mechanically damaged blueberries, (v) differentiation of control blueberries from all other damaged blueberries, and (vi) the classification of the four classes separately. For classification arrays ii, iii, iv, v, and vi, the performances of the classifiers were evaluated using images of the two orientations separately and both orientations simultaneously.

To define which strategies of features selection and which classifiers were the best, the results of classification array (v) with both orientations was used as a reference because it is the most general classification for detecting diseased blueberries.

3.2.6. Validation

The classifiers were validated using a 10-fold stratified cross-validation technique, yielding an average estimate of classifier performance with 95% confidence intervals for the classification pool (Jain et al. 2000). In the cross-validation, 90% of the samples were used for training and 10% were used for 10 validation replications.

A diagram of the pattern recognition technique applied in this study is shown in Figure 3.2.

3.3 Results and discussion

3.3.1 Extracting and selecting the best features

From each blueberry image, 225 features were extracted and then reduced to only 4-13, depending on the number of images involved in each experiment. To reduce the number of features, 5 combined strategies were used. Designed to have one extractor and one classifier, the strategies were able to select the most important features to accurately detect blueberry class based on quality and fruit orientation. The best strategy was SFS-Fisher, which gave the best features to achieve performances close to 95 %. With this approach, it was not necessary to use *Principal Component Analysis* (PCA) or other feature fusion techniques that are accompanied by a loss of information. In this study, SFS-Fisher selected original features, allowing for shorter processing times in production sorting lines. This matter is relevant to industrial applications, in which a short processing time is one of the most important requirements. Moreover, the high level of features reduction with respect to the number of images used for classification helped avoid the problem of overtraining the algorithms.

Table 3.2 shows the best selected features from the three most relevant experiments: differentiation of control from all other damaged blueberries, differentiation of stem end from calyx end, and differentiation of control blueberries from fungally decayed blueberries. These experiments provide information about the overall appearance of blueberries, their orientation, and one of the most serious diseases, fungal decay, which can easily spread from one berry to entire clamshells or trays during transportation.

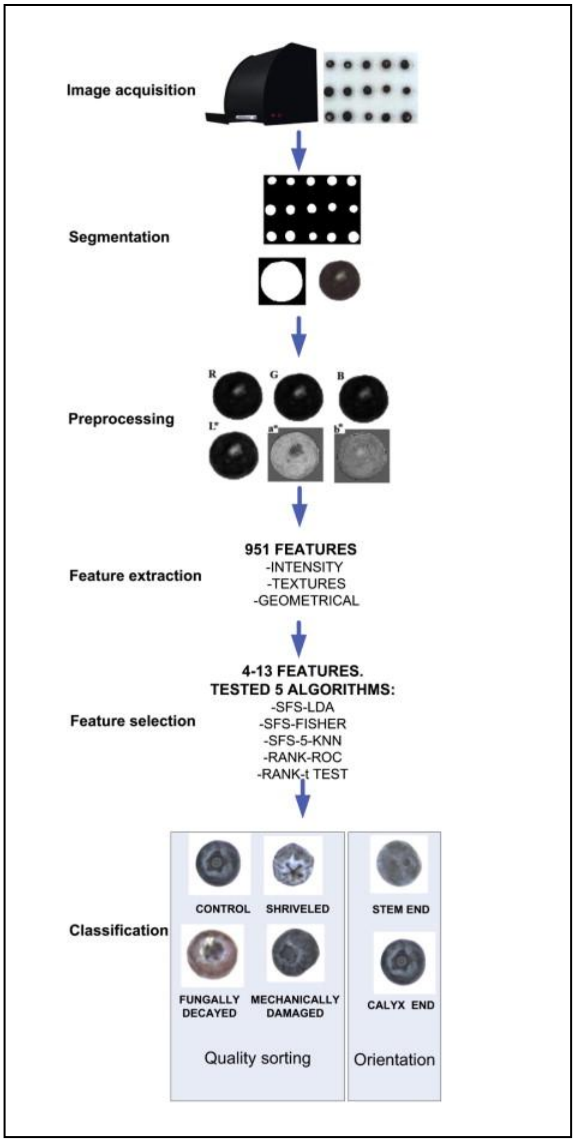

Figure 3.2. Recognition of damaged blueberries color images using image patterns.

Classification improved with the increasing number of features (Figure 3.3). In pattern recognition studies, mores features imply better results (Mery and Soto 2008); when new features are added, the performance of a classifier improves until a certain limit where performance remains stable is reached.

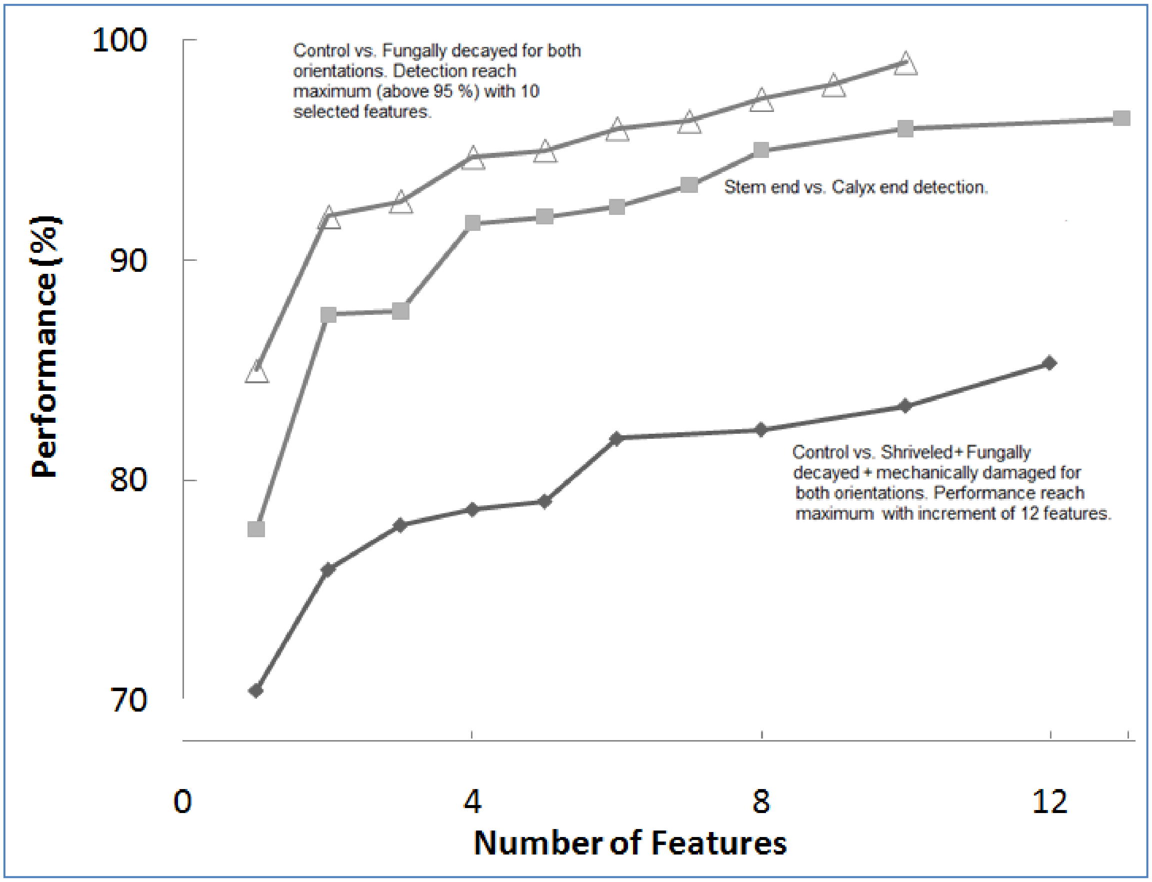

Figure 3.3. The classification performance improves with the increment in the number of features until reaching a plateau. The average performances for three specific situations are exposed: fruit orientation detection (calyx end vs. stem end), single disease detection (Fungally decayed vs. control) and control vs. unspecific disease classification (Control vs. Shriveled + fungally decayed + mechanically damaged for both orientations.

The best features selected for the detection of diseased blueberries were linear binary pattern (LBP), and intensity information. LBP uses both statistical and structural characteristics of texture; it is a powerful tool for texture analysis. In the LBP operator, local texture patterns such as means, variances, etc. are extracted by comparing the value of neighborhood pixels with the value of the central pixel and are represented using binary codes. Additionally, the LBP utilized in this chapter were rotational invariant in order to avoid false correlation since fruit attributes do not depend on the image rotation degree in the acquisition (Kumar and Pang 2002; Pietikäinen et al. 2000). Simple intensity information summarizes the overall appearance quality of blueberries in each of the different color channels, where darker colors present in healthy, ripe fruit have a lower intensity and variability in comparison with lighter colors present in shriveled and fungally decayed fruit.

Table 3.2. Best features for detection of diseased blueberries for three experiments.

n	Control vs. Shriveled +. Fungally decayed + Mechanically damaged for Both orientations	Stem end vs. Calyx end	Control vs. Fungally decayed for both orientations
1	LBP(1,26) [R]	LBP(1,32)[B]	Intensity Kurtosis[a]
2	Intensity Mean[a*]	LBP(1,5)[G]	LBP(1,33) [R]
3	LBP(1,23) [G]	Intensity-Mean	Intensity-Mean [b]
4	Intensity StdDev [b*]	[b*]	Hu-moment2
5	Intensity Skewness [b*]	LBP(1,28)[B]	LBP(1,19)[L*]
6	LBP(1,19) [b*]	Intensity-	LBP(1,10)[L*]
7	LBP(1,7) [B]	Skewness [a*]	LBP(1,3)[R]
8	LBP(1,14) [L*]	Mean boundary	LBP(1,13)[R]
9	Hu-moment-1	gradient[a*]	LBP(1,17)[b*]
10	Fourier-des 1	LBP(1,22)[b*]	LBP(1,3)[b*]
11	LBP(1,7)[G]	LBP(1,23)[b*]	
12	Fourier-des 5	LBP(1,14)[g]	
13		LBP(1,19)[b]	
		LBP(1,20)[g*]	
		LBP(1,24)[b*]	
		LBP(1,8)[B]	

LBP(*o*,*q*): Local binary patterns, where *o* is the number of pixels compared with *q* – neighboring pixels. LBP features were rotation invariant by consideration of eight orientation values.
Between the brackets [] are the color channels used to extract features (L* = lightness, a* and b* = color opponents, R = red, G = green, B = blue, gray. For references, see Section 2.3.

3.3.2. Selecting the classifier

Eight classifier algorithms were tested to define the most suitable algorithm to sort defect classes of blueberries. To this end, one classification experiment was used as a reference. This experiment consisted in segregating bad blueberries (generally damaged berries: shriveled + fungally decayed + mechanically damaged) from good blueberries (control), and images were acquired in both orientations. For the classification algorithms LDA, SVM and PNN, the yields were close to 84 %, with a confidence interval between 77 and 90%, higher than the yields of QDA, MAH, and KNN, which ranged from 71 to 81 %. The worst classifier was MDI, with a performance of 70.7 % (Figure 3.4). Thus, LDA, SVM, or PNN could have been chosen as suitable classifiers. However, because LDA is simpler than SVM and PNN, it was ultimately chosen.

3.3.3. Detecting defects and orientations of blueberries

The classification performance results for the defect and orientation detection of blueberries (according to section 3.2.5) are summarized in Table 3.3.

Table 3.3. Performance for different experimental classifiers of blueberries with visible damages.

Experiment	**Performance (C.I.)***		
	Stem end	Calyx end	Both orientations
Two classes detection:			
-Control vs. Shriveled	93.33 (86.05-100)[a]	96.67 (91.42-100)[a]	93.3 (88.18- 98.48)[d]
-Control vs. Fungally decayed	98.33 (94.59-100)[a]	100 (100-100)[b]	99.00 (96.80, 100)[e]
-Control vs. Mechanically damaged	93.33 (86.05-100)[a]	90.00 (81.24-98.76)[a]	87.50 (80.67-94.33)[d]
-Control vs. Shriveled +. Fungally decayed + Mechanically damaged	96.67 (92.96, 100.00)[d]	90.67 (84.24-97.07)[d]	85.34 (80.00-90.67)[c]
-Stem end vs. Calyx end	---	---	96.45 (93.66-99.24)[c]
Four classes detection:			
-Control vs. Shriveled vs. Fungally decayed vs. Mechanically damaged	78.33 (69.83- 86.84)[d]	74.67 (65.07-84.27)[d]	63.65 (56.40-70.91)[c]

*performances and confidence interval were calculated using 10 fold cross validation with 95% of confidence. [a] 60 images, [b] 45 images, [c]225 images, [d]120 images, [e]105 images.

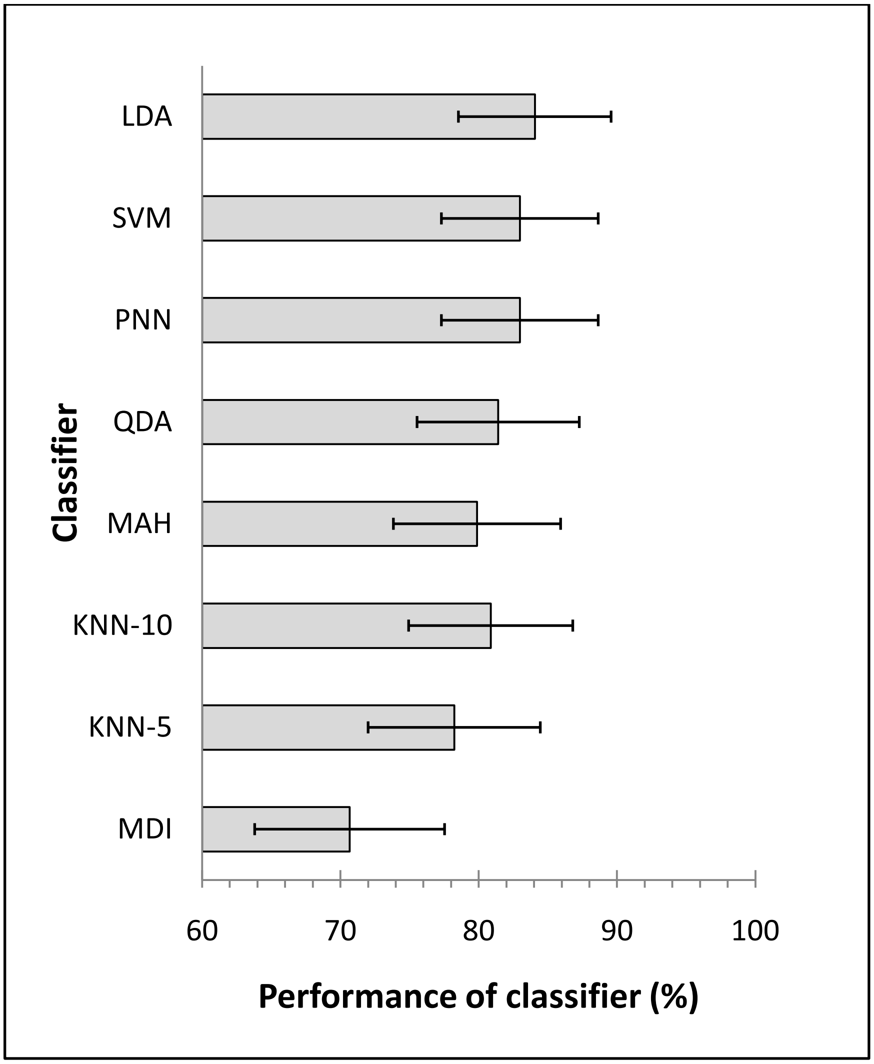

Figure 3.4. Performance of classifiers used to distinguish "control" from diseased blueberries. LDA, SVM, and PNN show the best results (over 82.9. %). Error bars represent the confidence interval calculated with 10-fold cross-validation (95% confidence).

In differentiating shriveled blueberries from the control, the performance values ranged between 93.3 and 96.7 %. The detection of mechanically damaged blueberries exhibited lower performance values between 87.5 and 93.3, while the best results were obtained for the detection of fungally decayed blueberries, with performance value ranging between 98.3 and 100 %. These specific detections were performed using three different fruit orientation groups of images: the first group of images was obtained along the stem end, the second along the calyx end, and the third group along both orientations at the same time. In general, the performances obtained for the stem end images were better than those for the calyx end images. The images showing both orientations simultaneously yielded the worst results for shriveled and fungally damaged but not mechanically damaged blueberries. These results reflect the influence of the calyx end structures (shaped like a crown of petals), which may adversely affect the image texture features and thus the classification. In this study, the detection of blueberry orientation was successfully achieved. A preliminary orientation recognition performance of 96.45 % with a confidence interval of 93.7 and 99.2% was obtained. In a commercial environment, it may be impractical to position each fruit into a specific orientation for automatic measurement. Moreover, manual orientation may lead to the excessive manipulation of each fruit, which could induce mechanical tissue damage.

Therefore, defect detection methodologies were separately constructed for each of two orientations, stem end and calyx end. Only these two orientations were studied because they are the predominant orientations when blueberries, which are ellipsoidal in shape, with the short axis aligned along the stem-calyx axis, are transported on a conveyor in a single layer.

Today, though manual handling is the most widespread to assess blueberry quality throughout the world, machine vision systems are gradually gaining ground. However, computer vision methods do not recognize specific defects in blueberries such as drying, fungal decay and mechanical damage. This specificity depends on the objective and tolerances of the segregation algorithm used. In this sense, the work presented has the goal of improving the ability of computer vision methods to detect common visible defects and fruit orientation in post-harvest blueberries. Further work should include the evaluation of the algorithms in real-time implementation and the development of complementary of sensors to simultaneously evaluate the internal quality of blueberries.

3.4 Conclusion

In this study, a computer vision system was used to acquire color images of different diseased blueberries along stem and calyx-end orientations. Of the 225 original features, only 4-13 were incorporated using SFS-Fisher. The best classifiers were LDA, SVM and PNN. Good orientation detection was obtained, with an average classifier performance of 96.45 (10-fold cross-validation). Also, specific visible damages were detected, with the best average classifier performances of 96.7, 100.0, and 93.33 % for shriveled blueberries, fungally decayed blueberries, and mechanically damaged blueberries, respectively. This study shows that the proposed statistical pattern recognition methodology is promising for the online sorting and grading of blueberries with respect to different defects and orientations. Further improvements in blueberry classification may be achieved by studying other non-visible damages and evaluating maturity states using an integrated computer vision system equipped with others sensor such as hyperspectral cameras.

3.5 Abbreviations

Bit Mapped Picture	BMP
Charge-coupled-device	CCD
Forward orthogonal search algorithm maximizing the overall dependency	FOSMOD
K-neareast neighbor with n neighbors	n-KNN
Local binary patterns	LBP(o,q)
Linear discriminant analysis	LDA
Mahalanobis distance	MAH
Minimal distance	MDI
Probabilistic neural network	PNN
Quadratic discriminant analysis	QDA
Rank key features by class sorting criteria	RANKFS
Relative operating characteristic curve	ROC
Sequential forward selection	SFS
Support vector machine	SVM

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4. PREDICTION OF FIRMNESS AND SOLUBLE SOLIDS CONTENT OF BLUEBERRIES USING HYPERSPECTRAL REFLECTANCE IMAGING

Abstract

Currently, blueberries are inspected and sorted by color, size and/or firmness (or softness) in packinghouses, using different inspection techniques like machine vision and mechanical vibration or impact. A new inspection technique is needed for effectively assessing both external features and internal quality attributes of individual blueberries. This paper reports on the use of hyperspectral imaging technique for predicting the firmness and soluble solids content (SSC) of blueberries. A pushbroom hyperspectral imaging system was used to acquire hyperspectral reflectance images from 302 blueberries in two fruit orientations (i.e., stem and calyx ends) for the spectral region of 500-1,000 nm. Mean spectra were extracted from the regions of interest for the hyperspectral images of each blueberry. Prediction models were developed based on partial least squares method using cross validation and were externally tested with 25% of the samples. Better firmness predictions ($R = 0.87$) were obtained, compared to SSC predictions ($R = 0.79$). Fruit orientation had no or insignificant effect on the firmness and SSC predictions. Further analysis showed that blueberries could be sorted into two classes of firmness. This research has demonstrated the feasibility of implementing hyperspectral imaging technique for sorting blueberries for firmness and possibly SSC to enhance the product quality and marketability.

4.1. Introduction

Blueberry is an important fruit in the world, and its consumption has increased significantly in recent years because consumers like its flavor and antioxidant capacity for anti-aging. The United States of America is the leading blueberry exporter, followed by Canada (Faoestat 2009). In recent years, countries in the southern hemisphere (e.g., Argentina, Chile, New Zealand and South Africa) have increased fruit export to the northern hemisphere by taking advantage of seasonal differences in production. But long-distance transoceanic shipment requires delivering higher quality and more consistent fresh blueberries at the origin country in order to meet the quality standards upon arrival at the destination.

Accurate determination of blueberry quality is challenging since individual fruit are small and dark in color, and vary greatly in external and internal quality characteristics. Traditionally, blueberry quality was inspected by humans *in situ* at the sorting line for color, size, and absence of defects and foreign materials. Human sorting is inefficient and unreliable. Moreover, humans are not capable of sorting fruit based on soluble solids content and firmness, two quality attributes that are not only important to the consumer, but also directly impact the shelf life of blueberries.

Numerous studies have been carried out for nondestructive measurement of blueberry firmness. For soft juicy fruits like tomato and berries, firmness measurement based on the creep test, which defines the deformation-time behavior under constant load, is preferred (Abbott 1999). However, there is no accepted standard method for creep measurement. (Slaughter and Rohrbach 1985) assessed the firmness of blueberries by measuring the slope of force/deformation curves when fruit were subjected to compression by two parallel plates under a constant loading velocity. A commercial instrument based on this method, called FirmTech (Bio-Work Inc., Kansas, USA), is currently available for measuring blueberry firmness (Prussia et al. 2006). The instrument is, however, only suitable for laboratory use. More recently, an air-puff rebouncing tester was used to assess the firmness of blueberries, which measures the deflection or deformation of the fruit under a puff of pressurized air (Li et al. 2010).

Considerable research has been reported on the development of automatic firmness sorting and grading techniques for blueberries. Earlier studies from the late 1970s until the mid-1980s were primarily focused on mechanical sorting techniques. They include vibration (Bower and Rohrbach 1976), roll-bounce separation (Wolfe et al. 1980), and relaxation modulus measured from the amount of impact force during rebound over a rigid piezoelectric transducer (Rohrbach et al. 1982; Lee and Rohrbach 1983). Currently, several types of commercial sorting systems are available, which are based on detection of the impact response of blueberries when they hit a pressure sensor (e.g., the "*Berrytek*" sorting system from Woodside Electronics Corp., CA, USA and "*Soft Sorta*" from BBC Technologies Ltd., Ohaupo, New Zealand). While these commercial systems allow high speed sorting (up of 2 tons h^{-1}), they are only able to reject up to 80% of soft fruit (http://bbctechnologies.com/en_US/softsorta.htm).

Soluble solids content (SSC), an accepted measure of sweetness, is another important quality attribute for blueberries. It is usually determined from the juice extracted from fruit flesh using the refractometric method (Noh and Lu 2007). In the past 20 years, many studies have been reported on predicting SSC in fruits using near-infrared spectroscopic technique. The near-infrared (NIR) region, covering approximately 780-2,500 nm, is related to vibration and combination overtones of the fundamental O–H, C–H and N–H bonds, which are the primary structural components of organic molecules (Sinelli et al. 2011a). Most NIR studies were reported for large size fruits (e.g., apple and pear), while only a few studies were focused on small fruits like berries. NIR technique is now being used in packinghouses for sorting such fruits as apple for SSC and/or internal defects (Nicolaï et al. 2007).

Over the past decade, hyperspectral imaging has emerged as a powerful inspection technique for food and agricultural products. The technique allows acquisition of both spectral and spatial information about an object simultaneously (ElMasry and Sun 2010). Since each hyperspectral image is represented by a 3-D spectral data cube or hypercube (Geladi et al. 2004; Nicolaï et al. 2006), it is thus advantageous over conventional imaging or spectroscopy technique in quality and safety inspection of food and agricultural products (Noh and Lu 2007). Many studies were reported for quality evaluation of fruits and vegetables, such as firmness and SSC of apples (Lu 2004), detection of surface defects in apples (Lu 2003; Nicolaï et al. 2006), and internal defects in cucumbers (Ariana and Lu 2010). The technique was also applied to small fruits like strawberry, a fruit relatively close to blueberry, for detection of bruises (Tallada et al.

2006) and prediction of dry matter, SSC, acidity or firmness (ElMasry et al. 2007; Nagata et al. 2005). These studies have shown the feasibility of hyperspectral imaging for measuring the appearance through image processing and physicochemical properties using spectral information. However, no study has been reported on using hyperspectral imaging for blueberry quality detection. Because of its capability for providing both spectral and spatial information, the technique would be suitable for simultaneous inspection of multiple quality attributes and defects of blueberries, such as SSC, firmness, color, size, presence of insects, bruises, molds, and shriveling.

This paper reports on the application of hyperspectral imaging technique for predicting the SSC and firmness of blueberries in the visible and short-wave near-infrared region of 500-1,000 nm. Hyperspectral reflectance images were acquired for blueberries in two different orientations (i.e., stem-end and calyx-end facing toward the imaging device). Calibration models using partial least squares method were developed to predict the firmness and SSC, and the effect of fruit orientation on the model performance was evaluated.

4.2. Materials and methods

Experiments were carried out in the postharvest engineering laboratory of the U. S. Department of Agriculture (USDA) Agriculture Research Service (ARS) at Michigan State University (MSU) in East Lansing, Michigan, USA between September 2011 and February 2012.

4.2.1. Samples

Commercial highbush blueberries (*Vaccinium corymbosum*) were purchased from a local grocery store in East Lansing, Michigan on October 10, 2011 (Driscoll Strawberry Associated, Inc. Watsonville, CA 95077). The fruit were visually inspected for appearance and surface defects. Only those fruit free of visual defects (such as scars, cuts, shrivel, etc.) were selected for the experiment. They were stored in a refrigerated air storage room at 4 °C for various time periods from three to 14 days before experiments were performed. Blueberries were removed from cold storage three hours before the experiment was started to allow the fruit to reach room temperature (~22 ºC).

4.2.2. Hyperspectral image acquisition

Hyperspectral images were acquired for 302 blueberry samples using an in-house built "pushbroom" or "line-scan" hyperspectral reflectance imaging system (Cen et al. 2011a; Cen et al. 2011b).

The system consisted of a mobile sample positioning unit, an imaging unit, a line light source, and an in-house developed Windows-based software program to control the system and acquire hyperspectral images (Figure 4.1). The sample positioning unit was composed of a motorized linear horizontal stage (Twintrac, TSZ8020, US23T22104-8LS, US Automation, Laguna Hills, CA, USA) and a sample holding tray (11 x 18 x 0.8 cm) with five rows of 8 mm diameter holes, allowing to place 40 blueberries each time.
The imaging unit consisted of a high performance 14-bit electron-magnifying charge-coupled detector (EMCCD) camera (Luca^{EM} R604, ANDOR^{TM} Technology, South Windsor, Connecticut, USA), an imaging spectrograph attached to the camera (ImSpector V10E, Spectral Imaging Ltd., Oulu, Finland), and an optical lens. The line light source consisted of a 20 W tungsten halogen light bulb (HL-2000-HP, Ocean Optics, Dunedin, FL, USA) and a spot-to-line converter to provide line light covering a spectral range of 360-2,000 nm (FTISL16854-6, Fiberoptics Technology Inc., Pomfret, CT, USA). The software allowed saving image files in BIL format (band interleave by line) to be processed later.

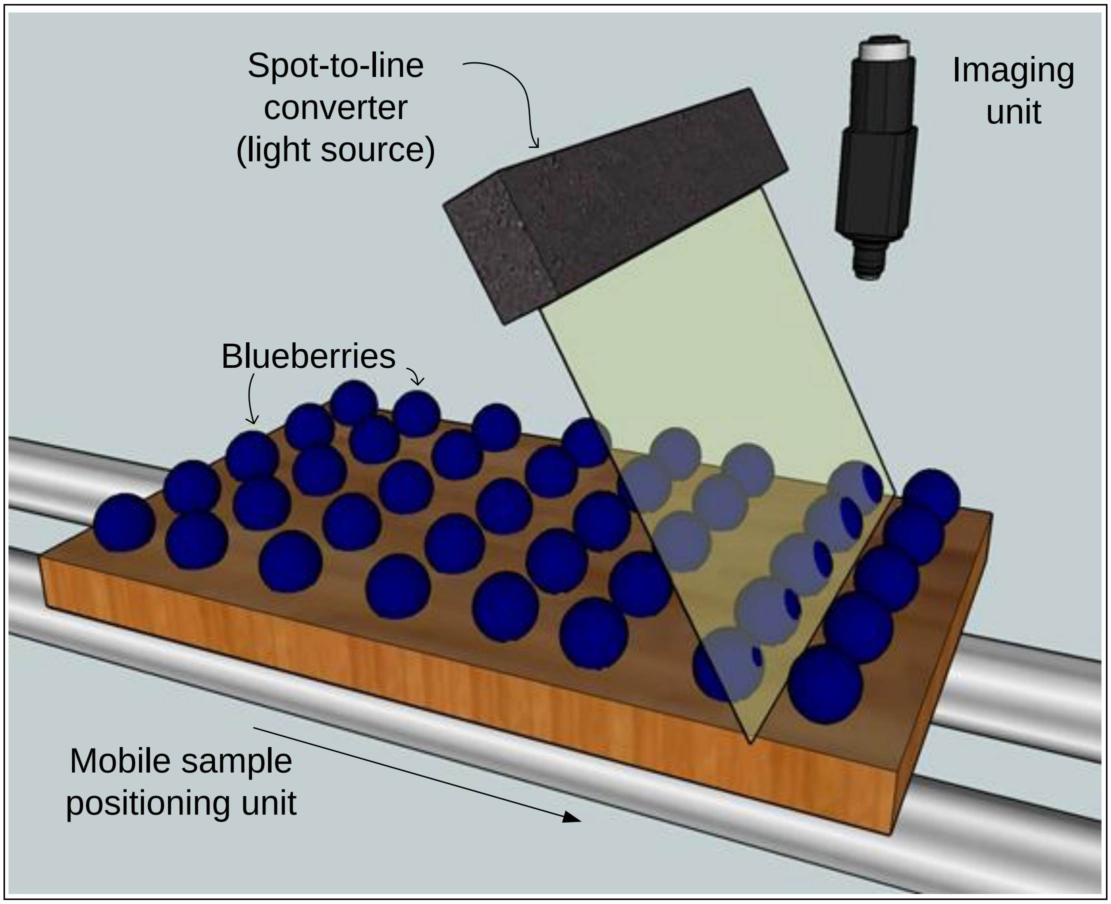

Figure 4.1. Schematic of a pushbroom hyperspectral imaging system for quality assessment of blueberries.

Forty blueberries were placed on the sample holding tray which was moving at a speed of 1.7 mm s^{-1}. Each blueberry was imaged for each of the two orientations, i.e., with the calyx end and the stem end facing vertically toward the imaging system. The exposure time was adjusted to 200 ms in order to obtain good quality images without saturation. Each time, the system was able to scan five blueberries simultaneously for a total of 40 fruit, resulting in a hypercube of 502 scanning lines × 498 spatial pixels × 125 wavelengths between 360 to 1070 nm, with binning operations of 2 × 8 for the spatial and spectral directions, respectively. Each hypercube had a spectral resolution of 6.13 nm/pixel and spatial resolutions of 0.4 mm/pixel along the scanning line direction and 0.4 mm/pixel between the scan lines.

For correction of light source effects, hyperspectral images were also acquired from a 99% reflectance panel (Spectralon Target SRT 99-120, Labsphere, Inc., North Sutton, NH, USA), using the same imaging parameters as that for blueberries, except that the exposure time was set at 5 ms to avoid saturation. Dark current images were also acquired with the camera shutter closed.

4.2.3. Reference Measurements

Destructive measurements of firmness index (FI), SSC and skin and flesh color were carried out after the image acquisition. A digital colorimeter (Model CR-400, Minolta-Konica Sensing Inc., Osaka, Japan) was used to measure the skin color of the blueberries immediately after the imaging. Surface color was measured from the stem end and the calyx end zone of each berry (on the same two sides where hyperspectral images were acquired).

After the color measurements, firmness measurements were performed with a Texture Analyzer (model TA.XT2i, Stable Micro Systems, Inc., Surrey, U.K.), following the method of (Slaughter and Rohrbach 1985). Each berry fruit was compressed between two parallel plates at a constant velocity of 0.5 mm/s for a total deformation of 3 mm. The berry was oriented with its stem-calyx axis approximately parallel to the compression plates. FI was calculated as the slope of force/deformation curves between 0.5 mm and 2.5 mm of displacement showing a straight line in most of the cases.

After FI measurements, each berry was equatorially cut in half. The color of fruit flesh was again measured using the digital colorimeter. Thereafter, juice was extracted from the two berry halves and SSC (%) was measured using a digital refractometer (model PR-101, Atago Co., Tokyo, Japan).

4.2.4. Image processing

Image processing was carried out using Matlab R2007a and its image processing toolbox (The Mathworks, Inc., Natick, MA, USA). A sequence of image processing steps for the multi-sample hyperspectral images were taken to generate the data needed for prediction models. They included sample image corrections using the reference image to obtain relative reflectance images, segmentation and identification of berries, and mean spectra calculations.

The intensity values for each hyperspectral image were corrected by the reference image pixels by pixels in both spatial and spectral dimensions (Ariana and Lu 2010; Tallada et al. 2006):

$$RI = \frac{IS(x,y) - ID(x,y)}{IR(x,y) - ID(x,y)} \cdot 10^4 \qquad (4.1)$$

Where *RI* is the relative reflectance at pixel location (x,y), *IS* is the sample image value, *ID* is the dark frame image value, and *IR* is the reference image pixel value. The dimensions of each hypercube was reduced from 502 × 498 × 125 pixels to 502 × 498 × 82 pixels, after removal of the noisy regions of the image beyond the spectral region of 500-1,000 nm.

After the preprocessing, an automatic three-step algorithm was implemented to segregate each blueberry from the background for each hypercube of 40 blueberries and calculate their mean spectra (Figure 4.2). The first step consisted of building a binary mask to recognize the fruit from the background using threshold segmentation (Otsu 1979) in the hypercube. This was accomplished on the spectral image at 672 nm which gave the maximum contrast between the blueberries and the background. The second step was the recognition of single berries by combining tilting and labeling operations on the masked image. Tilting is a partial rotation of images by any angle degrees in the counterclockwise direction around its center point and labeling refers to as assigning different numbers to different segmented regions going from left to right and up to down. After tilting the mask in a specific angle (15º), each sample was identified through correlating the spatial position between the mask and each hypercube wavelength. The third step consisted of segmenting each one of the 302 blueberries at each of the 82 wavelengths for each of the two orientations (calyx and stem ends), which resulted in 49,528 normalized gray scale images of 61 x 61 pixels (10 kb for each image). From the regions of interest of each segmented blueberry image, mean reflectance was computed by averaging over all pixels.

4.2.5. Prediction models for fruit orientation evaluation

Hyperspectral images or spectroscopic data contain a large amount of information, much of which is redundant (Moltó et al. 2010). Hence, a reduction of features was performed on the mean reflectance spectra using principal component analysis to obtain no more than top 20 principal components. Calibration models for predicting FI and SSC were then developed using partial least squares regression (PLSR) for the obtained principal components. PLSR generates a set of score vectors by maximizing the covariance between the features set and membership matrix (Li et al. 2012).

To ensure the models were not over fitted and the prediction results truly represent the model performance, the samples were first divided into four separate folds randomly (Mery et al. 2011; Mendoza et al. 2011). Three folds (or 75% of all samples) were used for calibration and the remaining fold was used for independent test or prediction.

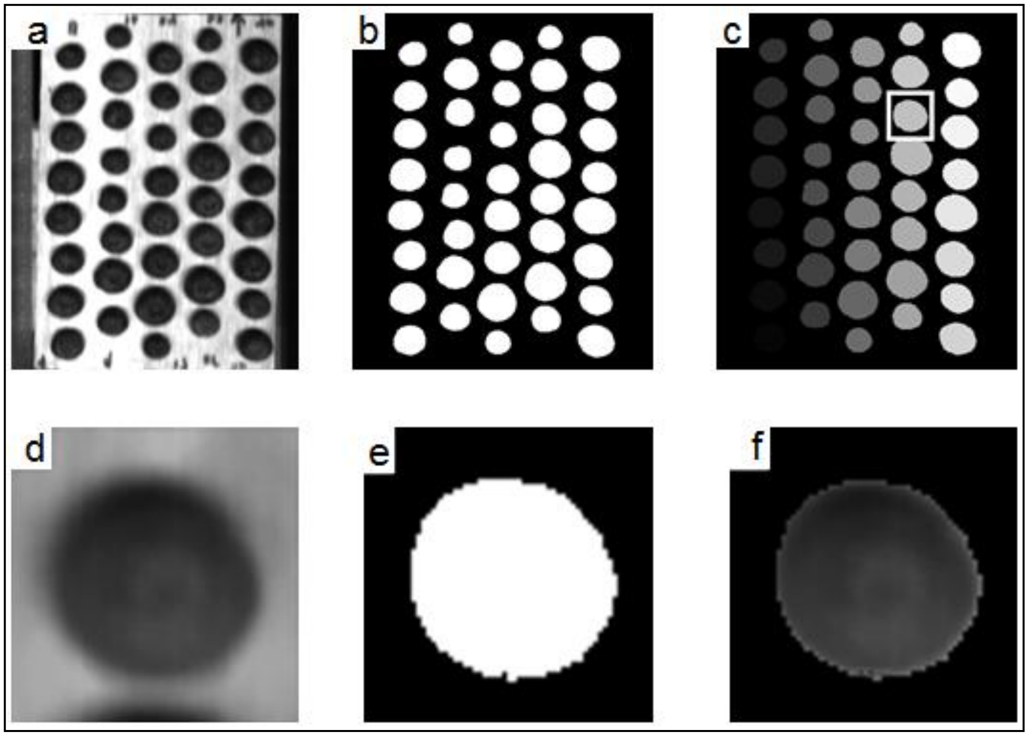

Figure 4.2 a) Images of 672 nm used to find regions of interest; b) regions of interest obtained using a Otsu threshold and morphological operations; c) labeling of a blueberry; d) close-up image of a blueberry at 672 nm; e) the region of interest for the blueberry using Otsu threshold and morphological operations to remove false regions; f) final image of the blueberry.

Calibration models for FI and SSC were developed using PLSR from the calibration samples. Cross validation was used to determine the number of latent variables for the calibration models. Thereafter, the calibration models were used to predict the independent fold of samples. The models were evaluated using root mean squares errors for cross calibration (RMSECV) and prediction (RMSEP).

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2}{n_p}} \qquad (4.2)$$

In addition, correlation coefficients for calibration (R_c) and prediction (R_p) and RPD, defined as the ratio between the sample standard deviation and RMSEP, were also calculated. RPD values measure the ability of a model for classification (Nicolaï et al.

2007). An RPD value between 1.5 and 2 means that the model is able to discriminate two classes. Values between 2 and 2.5 indicate that coarse quantitative predictions are possible and a value above 2.5 means good to excellent prediction accuracy.

The above procedure was repeated four times. For each new run, one fold of samples in the calibration set was rotated out and replaced with the test samples from the previous run. The rotated out samples were then used as the new test samples. Finally, the results (i.e., R_c, R_p, RMSECV and RMSEP) from the four runs were averaged to estimate the final performance of the models.

In a commercial operating environment, it may be impractical or impossible to orientate each fruit to a specific orientation for optical measurement. Moreover, orientating may cause excessive manipulation of each fruit, which could induce tissue damage. Therefore, it is necessary to evaluate the effect of fruit orientation on hyperspectral imaging prediction of the firmness and SSC of blueberries, so that an appropriate approach can be developed for implementation of the technique for automatic sorting. For this reason, prediction models for firmness and SSC were built separately from the mean spectra data for each of the two orientations, which were designated as T1 for the stem end and as T2 for the calyx end. Only these two orientations were studied because they are the predominant orientations when blueberries, which are of ellipsoid shape with the short axis being aligned along the stem-calyx axis, are transported on a conveyor in single layer. Furthermore, two more data arrays were created from the mean spectra for the two fruit orientations in order to enhance firmness and SSC prediction.

For the first data set, designated as T3, the data acquired for the stem and calyx regions for each sample were treated as if they came from two different samples. This would allow evaluating the models without considering the orientation of samples. For the second data set, or T4, two mean spectra for the stem and calyx end regions were averaged to obtain one spectrum. This would simulate the possible implementation of two sensors for measuring each blueberry for the two orientations at the same time.

Finally, the prediction results from the sets of data (i.e. T1, T2, T3 and T4) were compared using Duncan tests with 95% confidence (Statgraphics Centurion XV, Statpoint Technologies Inc., Warrenton, VA, USA).

4.3. Results and discussion

The distributions of FI and SSC for the blueberry samples are presented in Figure 4.3. The variability of FI, as measured by the ratio between standard deviation and mean of the samples, was at least twice that of SSC for the same samples Figure 4.3a.

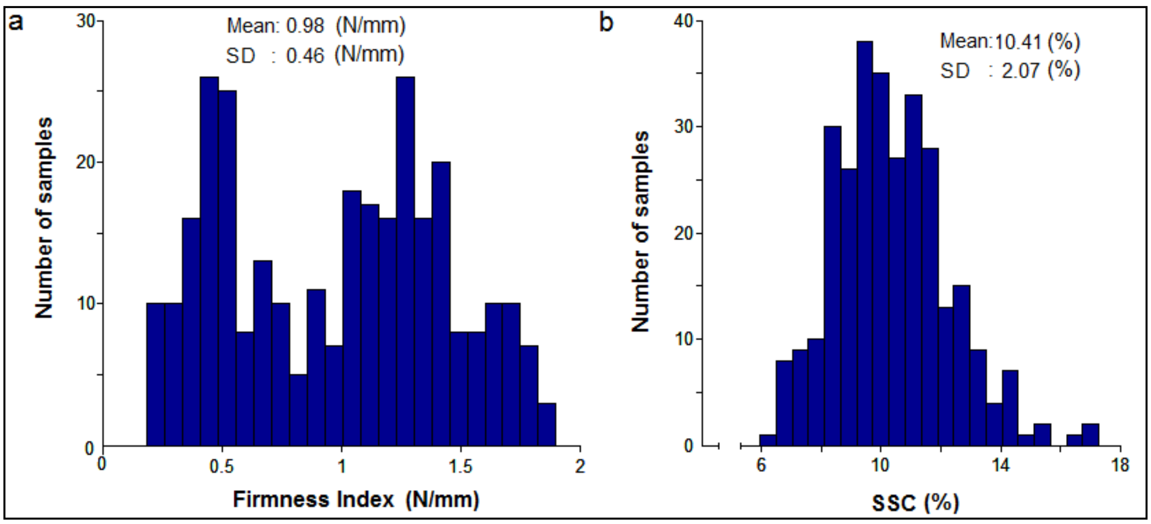

Figure 4.3. Distribution of (a) firmness index and (b) soluble solids content for the test samples.

FI measurements for the samples varied between 0.19 and 2.42 N/mm with the mean of 0.98 N/mm and the standard deviation of 0.46 N/mm. Lower values of FI indicate softer fruit. SSC measurements for the samples varied between 6.0% and 18.5% with the mean value 10.4% and the standard deviation of 2.1% in Brix (Figure 4.3b). The FI distribution showed two modes centered at 0.5 N/mm and 1.4 N/mm, respectively, with fewer samples near the mean value. This was not observed for SSC.

4.3.1. Image and spectral features of blueberries

A quick visual examination of the hyperspectral image data showed large differences between the reflectance images for different blueberries in the NIR region, whereas it was difficult to ascertain the reflectance differences for the test samples in the visible region due to their low reflectance (Figure 4). Differences in reflectance between the two orientations for the visible and NIR region were not apparent (Figure 4.4).

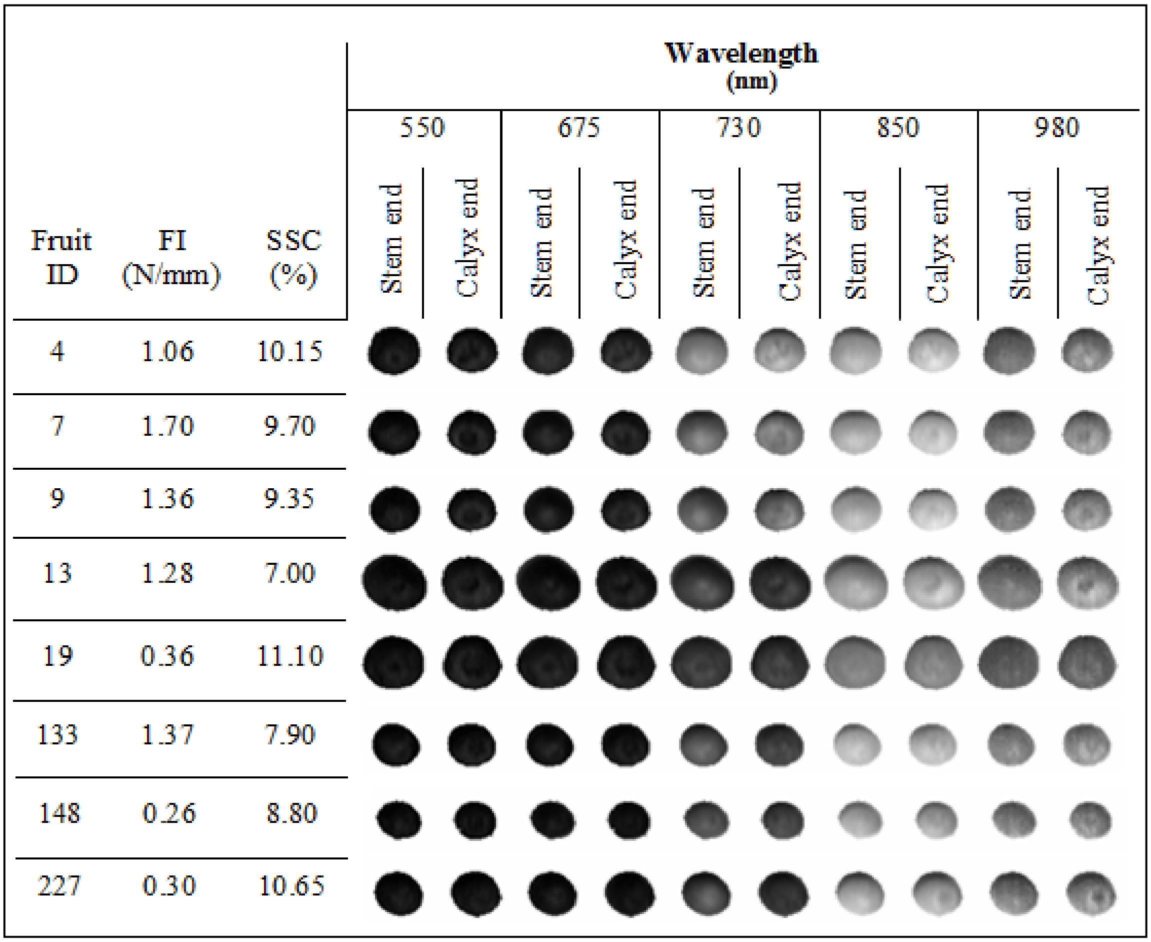

Figure 4.4. Relative reflectance images for eight blueberries at different wavelengths, with different levels of firmness index (FI) and soluble solids content (SSC).

Generally, the images for samples with higher SSC and low FI had lower reflectance in the NIR region, compared with those samples with low SSC and higher firmness. In contrast, blueberries with higher FI and low SSC showed higher reflectance levels in the NIR region.

Figure 4.5 shows the mean relative reflectance spectra of all blueberry samples in the stem-end orientation for 500-1,000 nm. Considerably lower and more consistent reflectance was observed for all blueberry samples for the visible region of 500-675 nm.

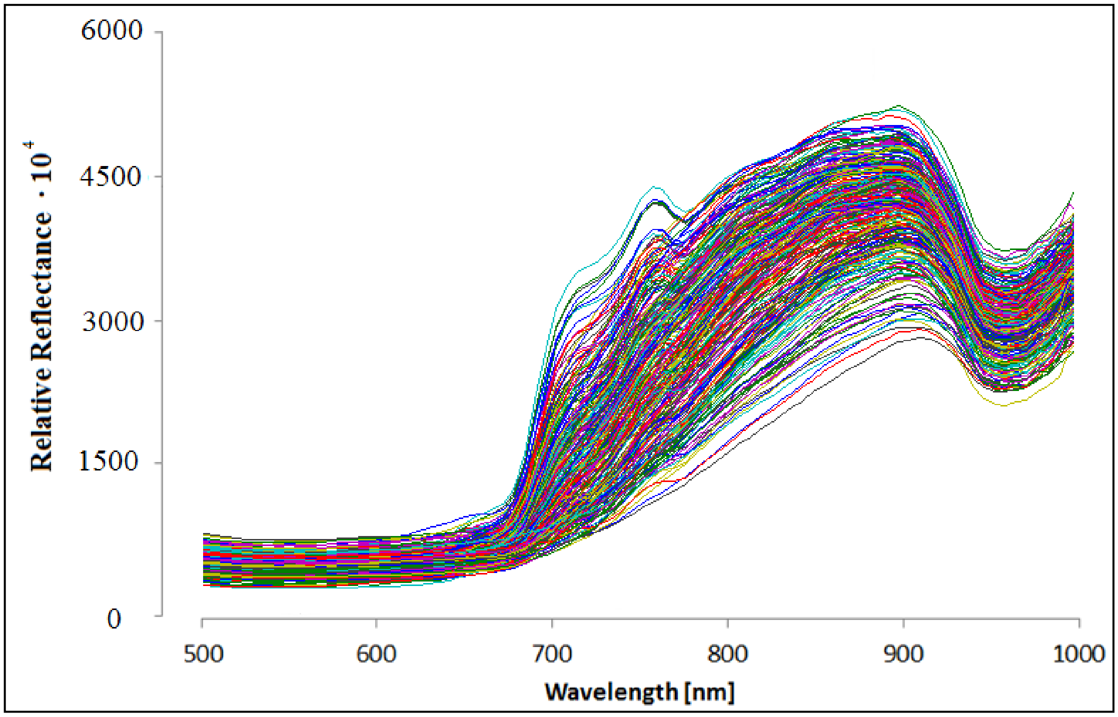

Figure 4.5. Mean relative reflectance spectra for 302 blueberry samples measured from the stem-end orientation.

Beyond 675 nm, reflectance for all samples started to increase dramatically and a peak occurred at 765 nm for most of the samples. This range reflects the maturity of blueberries accompanied with the change of fruit color caused by anthocyanins and chlorophyll (Peshlov et al. 2009). On average, reflectance around 850 nm was about six times that of the visible region. In other words, blueberries had much stronger absorption in the visible region of up to 675 nm due to the presence of deep dark pigments in the skin, compared to that for the rest region of 675-1,000 nm. An absorption peak was observed for the samples at 970-980 nm, which was likely attributed to the combination effect of OH groups from carbohydrates (970 nm) and water (980 nm), since blueberries had a SSC of 6-15% and an estimated water content of 80–90%. Blueberries are high in anthocyanin and covered with a layer of wax. Reflectance spectra from the stem-end images in the visible region were highly variable in relative term, because less ripe fruit had a dominant purple color, compared with the dark color of ripe berries. Likewise, greater absolute variability in reflectance was observed for the spectral region of 700-1,000 nm.

4.3.2. Correlation between the color and quality of blueberries

Since most fruit tested were in a mature or ripe state, variations in the color of the test fruit were relatively small and difficult to observe with the naked eye. However, local differences in the visible reflectance over the surface of some blueberries were observed due to the presence of wax. These differences were not observed in the NIR range. The instrumental measurements of fruit skin color showed small changes between the fruit, with the mean values of x = 7.07 ± 1.21, y = 7.12 ±1.22 and z =9.71 ± 1.82. The average color measurements for the flesh of all samples were x = 7.39 ± 2.25, y = 7.14 ± 2.40 and z =5.06 ± 1.30.

Table 4.1. Correlation of fruit skin and flesh color measurements with firmness index (FI) and soluble solids content (SSC) of blueberries.

Color parameter	Correlation Coefficient			
	Skin		Flesh	
	FI	SSC	FI	SSC
x	0.18± 0.10	-0.04±0.02	0.69±0.04	-0.35±0.01
y	0.18±0.10	-0.04±0.02	0.73±0.03	-0.38+0.00
z	0.14±0.10	-0.02±0.03	0.54±0.08	-0.24±0.05

Using simple regression analysis, low correlations were obtained of the three skin color parameters (i.e. x, y, and z) with FI (R=0.14 - 0.18) and SSC (R=-0.02 - -0.04) (Table 1). However, considerably higher correlations were obtained between flesh color and firmness (R=0.54 - 0.69 for the three color parameters) (Table 4.1).

These results showed that skin color is inappropriate for evaluating the firmness and SSC of blueberries.

4.3.3. Prediction of firmness and SSC for fruit orientations

Calibration and prediction results for the FI of blueberries for the four sets of data are summarized in Table 4.2. R_c values ranged between 0.88 and 0.92, while R_p values were between 0.83 and 0.87, with the RMSEPs of 0.23-0.26 N/mm. The RPD values were between 1.8 and 2.0. The number of latent variables used to predict FI was between 12 and 16. These results suggested that improvements in the measurement technique and data analysis methods are still needed in order to achieve more accurate measurement of the firmness of individual blueberries. However, in postharvest quality sorting and grading, we normally do not need to measure the exact value of firmness for each fruit; instead, we are only interested in sorting blueberries into different firmness classes. Based on the obtained RPD values, it would be feasible to sort blueberries into two firmness classes: soft vs. firm (Nicolaï et al. 2007). Classification of the fruit into two firmness classes is also in agreement with the firmness distribution of the samples, which had two relatively distinctive firmness distribution groups (Figure 3a), and the mean value of firmness would be a good criterion to remove soft (less firm) fruit in the packing line.

No statistical differences in the firmness predictions were found ($p<0.05$, Duncan test) for the four data sets (Table 2). This finding is significant because it showed that there is no need to orient individual fruit when hyperspectral imaging technique is implemented for sorting blueberries. This would, thus, simplify the design of a hyperspectral imaging-based sorting system.

The SSC prediction results using the spectral region of 500-1,000 nm were not as good as those for firmness, as measured by correlation coefficient, with the R_c values ranging between 0.78 and 0.82 and the R_p of 0.69-0.79 (Table 4.2). The models had higher RMSEP values between 1.30% and 1.55%, and the values of RPD were only between 1.3 and 1.6 (Table 4.2).

Table 4.2 Average prediction results of firmness index and soluble solids content for blueberries using partial least squares regression for four different data sets of two fruit orientations.

	Prediction results of Firmness Index, using images in the range of 500 – 1000 nm					
Treatment	Latent Variables	R_c	RMSECV	R_p	RMSEP	RPD
T1	12 ± 3	0.883 ± 0.025	0.216 ± 0.022	0.829 ± 0.053	0.26 ± 0.04 ^{A}	1.8 ± 0.2
T2	15 ± 3	0.911± 0.022	0.191 ± 0.022	0.854 ± 0.022	0.24 ± 0.02^{A}	1.9± 0.1
T3	16 ± 1	0.889 ± 0.007	0.211 ± 0.006	0.851 ± 0.028	0.25 ± 0.01^{A}	1.9 ± 0.1
T4	16 ± 1	0.918 ± 0.005	0.183 ± 0.006	0.869 ± 0.015	0.23 ± 0.01^{A}	2.0 ± 0.1
Prediction results of Soluble Solids Content using images in the range of 500 – 1000 nm						
Treatment	Latent Variables	R_c	RMSECV	R_p	RMSEP	RPD

	Prediction results of Firmness Index, using images in the range of 500 – 1000 nm					
Treatment	Latent Variables	R_c	RMSECV	R_p	RMSEP	RPD
T1	15 ± 3	0.816 ± 0.019	1.196 ± 0.048	0.690 ± 0.012	1.55 ± 0.13 ^{B}	1.3 ± 0.1
T2	13 ± 1	0.818± 0.010	1.143 ± 0.014	0.761 ± 0.036	1.36 ± 0.03 ^{C}	1.5 ± 0.1
T3	18 ± 2	0.780 ± 0.017	1.295 ± 0.038	0.682 ± 0.024	1.53 ± 0.08 ^{B}	1.4 ± 0.1
T4	18 ± 1	0.882 ± 0.016	0.973 ± 0.059	0.788 ± 0.035	1.30 ± 0.10 ^{C}	1.6 ± 0.1
	Prediction results of Soluble Solids Content, using images in the range of 750 – 1000 nm					
Treatment	Latent Variables	R_c	RMSECV	R_p	RMSEP	RPD
T1	9 ± 1	0.741 ± 0.003	1.390 ± 0.048	0.711 ± 0.017	1.48 ± 0.13^{C}	1.4 ± 0.1
T2	16 ± 5	0.797± 0.039	1.242 ± 0.057	0.728 ± 0.027	1.45 ± 0.12^{C}	1.4 ± 0.1
T3	18 ± 1	0.737 ± 0.012	1.400 ± 0.036	0.685 ± 0.038	1.50 ± 0.10 ^{C}	1.4 ± 0.1
T4	12 ± 1	0.815 ± 0.014	1.197 ± 0.033	0.764 ± 0.018	1.37 ± 0.08 ^{C}	1.5 ± 0.1

T1: stem end orientation.

T2: calyx end orientation.

T3: each orientation (stem end and calyx end) measurement was treated as if it came from a different sample.

T4: mean spectra for the stem and calyx ends for each berry were averaged.

R_c: Average correlation coefficient of calibration over four calculations.

R_p: Average correlation coefficient of prediction over four calculations.

RMSECV: root mean squares error of cross validation over four calculations.

RMSEP: root mean squares error of prediction over four calculations.

RPD: ratio of standard deviation to RMSEP.

RMSEPs with the same letters are not significantly different at p<0.05 using Duncan test.

There was a statistically significant difference in the prediction results between the two orientations; better results were obtained for the stem end (T1) (R_p= 0.76) than for the calyx end (T2) (R_p=0.69). Among the four data sets, T4 (i.e., the average of two mean reflectance spectra for each fruit) gave better results than T3 (p>0.05, Duncan test) (Figure 4.6a).

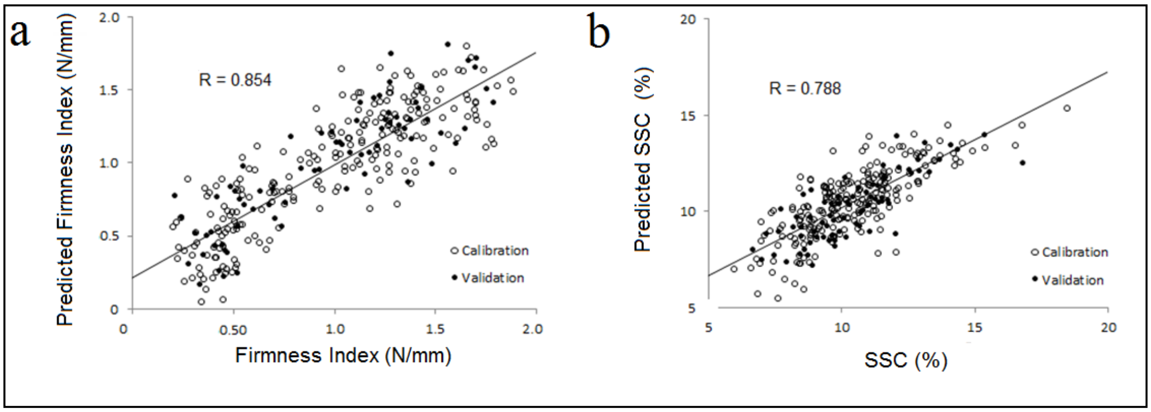

Figure 4.6. Partial least squares prediction of a) firmness for the stem end region (T2) and b) soluble solids content (SSC) using the fused spectra from both the stem and calyx end regions (T4).

Previous studies (Lu 2004; Ruiz-Altisent et al. 2010b) suggested that the visible spectral region is not useful for SSC prediction. Hence, further analyses were performed for the NIR region of 750-1,000 nm. Compared to the spectral region of 500-1,000 nm, the use of 750-1,000 nm did not result in significant improvements ($p<0.05$, Duncan test) for the prediction of SSC (the second table vs. third table of Table 4.2). The R_p and RMSEP for the stem end for 500-1,000 nm were 0.76 and 1.36%, respectively, in comparison with 0.73 and 1.45% for 750-1,000 nm. Despite this, the SSC models for 750-1,000 nm had fewer latent variables (9-18) for three out of the four data sets, compared with those (13-18) for 500-1,000 nm.

4.5. Conclusion

In this research, a pushbroom hyperspectral imaging system was used to acquire reflectance images for blueberries in the stem and calyx end orientations. Relatively good predictions of firmness were obtained, with the best R_p of 0.87 and an RPD of 2, which suggested the feasibility of sorting blueberries into two firmness classes. Fruit orientation did not have a significant effect on firmness prediction, and hence there is no need to orient fruit for hyperspectral imaging. Lower correlations (R_p=0.69-0.79) were obtained for SSC prediction, compared with those reported for large fruits such as apple and citrus. This study showed that hyperspectral imaging is promising for online sorting and grading of blueberries for firmness and perhaps SSC as well. Further improvements in firmness and SSC prediction may be achieved through the use of higher spatial and spectral resolutions in the acquisition of hyperspectral images and an improved lighting design. More research is also needed to assess other blueberry quality attributes or properties (e.g., antioxidant compounds) and common defects such as bruise, fungal decay, and shriveling.

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5. ASSESSMENT OF INTERNAL QUALITY OF BLUEBERRIES USING HYPERSPECTRAL TRANSMITTANCE AND REFLECTANCE IMAGES WITH WHOLE SPECTRA OR SELECTED WAVELENGTHS

Abstract

Hyperspectral imaging has been used in previous studies for assessing firmness and soluble solids content of fresh fruit. To apply this technique for automatic sorting and grading of blueberries, it is necessary to investigate different sensing modes (i.e., reflectance and transmittance), evaluate the effect of fruit orientation on fruit quality prediction, and develop robust prediction models with fewer wavelengths. In this study, a hyperspectral imaging system was used to acquire reflectance and transmittance images from 420 blueberries in three fruit orientations (i.e., stem end, calyx end and equator) for the spectral region of 400-1,000 nm. Mean spectra were extracted from the regions of interest for the hyperspectral images of each blueberry. Calibration models for firmness index (FI) and soluble solids content (SSC) were developed using partial least squares regression for the reflectance and transmittance spectra as well as their combined data. Further, interval partial least squares (iPLS) regression with 10 different intervals of nine wavelengths was used to reduce the spectral dimensionality. Overall, reflectance gave better results (the best correlation for prediction (R_p) of 0.90 for SSC and 0.78 for FI) than transmittance (R_p of 0.76 for SSC and 0.64 for FI). For reflectance, FI and SSC predictions for the stem-end orientation were better than for the other two orientations, while fruit orientation had little or insignificant effect on transmittance predictions. Combination of reflectance and transmittance spectra did not yield improved prediction results for both FI and SSC. The prediction errors for iPLS, on average, increased by only 5%, compared to PLS for the whole spectra. The research demonstrated that it is feasible to implement hyperspectral imaging technique for sorting blueberries for SSC and possibly firmness, using appropriate wavelengths.

5.1. Introduction

In recent years, countries in the southern hemisphere have increased blueberry export to the northern hemisphere. Because of the long distance shipment, it is especially important to perform internal and external quality determination for individual fresh blueberries to ensure their quality upon arrival at the destination. Soluble solids content (SSC) is an important parameter in evaluating quality of fruit (Rodriguez-Saona et al. 2001); it is usually determined from the juice extracted from the fruit flesh using the refractometric method (Noh and Lu 2007). Softening of blueberries is a major quality concern because it promotes mold development and enzymatic browning, resulting in inferior products that are not suitable for marketing. Hence individual blueberries should be inspected for firmness to avoid spoilage and possible rejection by the consumer.

Considerable research has been reported on automatic detection and segregation of defective or inferior blueberries. Earlier research from the late 1970s till the mid-1980s was mainly focused on segregating soft berries using mechanical techniques such as vibration (Bower and Rohrbach 1976), roll bounce (Wolfe et al. 1980), and impact (Rohrbach et al. 1982; Lee and Rohrbach 1983). In recent years, more research has been focused on non-destructive optical techniques, such as image analysis, to segregate defective berries (Leiva-Valenzuela and Aguilera 2013). Reflectance near-infrared spectroscopy was used to evaluate the blueberry's nutraceutical content (Sinelli et al. 2008), monitor dehydration (Sinelli et al. 2011b) and build quality grading models (Yang et al. 2012). Optical techniques have wide acceptance in agriculture because they are efficient and fast in acquiring a large quantity of information and have the potential for simultaneous assessment of multiple quality attributes in the case of spectroscopy and for simulating human vision in the case of image analysis.

More recently, hyperspectral imaging technique has emerged as a new technique for quality and safety inspection of food and agricultural products(ElMasry and Sun 2010). A typical hyperspectral image consists of hundreds or even thousands of narrow-band or spectral images, with each pixel in the image being associated with a spectrum which may cover both visible and near-infrared wavelengths. Many studies have been reported on using hyperspectral imaging for prediction of firmness and SSC, two important quality attributes for large fruits like apple (Lu 2004; Mendoza et al. 2011; Mendoza et al. 2012; Romano et al. 2011; Vanoli et al. 2011; Wang et al. 2012b; Peng and Lu 2007, 2008). Several studies also demonstrated the feasibility of the technique for assessing small fruits like strawberry (Nagata et al., (2005) and blueberry (Leiva-Valenzuela et al., (2013). Hyperspectral imaging and near-infrared spectroscopy may be implemented in different sensing modes (i.e., reflectance, interactance and transmittance). While reflectance is the most used, transmittance was also used to detect internal defect such as bruises in apple (Clark et al. 2003), pear (Han et al. 2006) and pickling cucumbers (Ariana et al. 2006; Ariana and Lu 2010) and predict SSC in cantaloupes (Dull et al. 1989) and honeydew melons (Dull et al. 1992), and the SSC and firmness of apples (Fan et al., (2009). Transmittance measurements are, however, influenced by such factors as fruit geometry and size. Fruit's internal structures such as calyx, pit or pedicel can change the light transfer pattern by blocking, absorbing or deviating the light passing through the fruit. Moreover, spectral profiles could also be affected by irregularities in the geometry of samples. In implementing the transmittance mode, it is important that sufficient light penetrates the entire fruit without causing damage to the fruit (Fraser et al. 2003; Nicolaï et al. 2007; Clark et al. 2003). Blueberries are small in size, round in shape and relatively homogeneous in internal structure (i.e., with no hard pit or core). Hence it is technically feasible to use hyperspectral transmittance imaging for assessing internal quality of blueberries. However for the blueberry industry, neither spectroscopy nor hyperspectral imaging is now being used for quality grading or sorting.

Image processing is a critical step in the application of hyperspectral imaging technology. Hyperspectral images are usually processed using such techniques as filtering, correction and segmentation, so as to extract specific features such as mean of intensity in the region of interest. These features are then used to build linear or nonlinear prediction or discriminant models. Partial least squares (PLS) regression is one of the most popular methods that have been used to build models with orthogonal latent variables that are oriented along directions of maximal covariance between the spectral matrix and the response vector (Nicolaï et al. 2007). The latent variables come from a fusion of image features obtained using such a method as principal component analysis (PCA). Although non-linear techniques have been increasingly used recently, PLS allows simpler interpretation and comparison of results, avoiding the overtraining problem that is commonly encountered with complex non-linear models. However, the need for processing a large number of spectral images has been the main obstacle in the application of hyperspectral imaging for high-speed online sorting and grading of agricultural products. Consequently, considerable studies have been focused on the development of spectral data reduction algorithms. Interval partial least squares (iPLS) is one of the commonly used algorithms in selecting the most efficient wavelength regions for developing an optimized local PLS model built with fewer variables (Nørgaard et al. 2005). Basically, with the iPLS method, the entire spectra are first split into smaller equidistant regions and PLS regression models are then developed for each of the sub-intervals, using the same number of latent variables for the selected region with lower error. An optimized region can be found by subtracting or adding new variables (Zou et al. 2007; Nørgaard et al. 2000a). Hence, iPLS could yield similar prediction results without using complete spectra information, while having the advantages of decreased computational time and knowing specific wavelength regions from which the most useful information is obtained.

This article reports on the use of hyperspectral imaging technique for predicting internal quality attributes of blueberries using either whole spectra or selected wavelengths in the visible and short-wave near-infrared region of 400–1,000 nm. Hyperspectral reflectance and transmittance images were acquired for blueberries in three different orientations (i.e., stem end, equator and calyx end facing toward the imaging device). Calibration models using PLS, coupled with iPLS for selection of wavelengths, were developed to predict firmness and SSC, and the effect of fruit orientation on the performance of the prediction models was evaluated.

5.2. Materials and methods

Experiments were carried out in the postharvest engineering laboratory of the U. S. Department of Agriculture (USDA) Agricultural Research Service (ARS) at Michigan State University (MSU) in East Lansing, Michigan, USA between March and May of 2012.

5.2.1. Samples

Commercial "Rabbit edge" blueberries (*Vaccinium corymbosum. Var Tifblue*), originally produced in Chile, were purchased from a local grocery store in East Lansing, Michigan, United States of America, on March 22, 2012 (North Bay Produce, Inc. Traverse City, MI 49685-0988). The fruit were visually inspected for appearance and surface defects. Only those fruit free of visual defects (such as scars, cuts, shrivel, etc.) were selected for the experiment. They were stored in refrigerated air at 4 °C for various time periods from three to 14 days before experiments were performed. Blueberries were removed from cold storage three hours before the experiment was begun to allow the fruit to reach room temperature (~22 ºC).

5.2.2. Hyperspectral image acquisition

Hyperspectral images were acquired for 420 blueberry samples using a prototype hyperspectral imaging system developed in the USDA/ARS postharvest engineering laboratory at Michigan State University, East Lansing, Michigan (Ariana and Lu 2010).

The hyperspectral imaging system (Figure 5.1) consisted of imaging, illumination and conveying units. The imaging unit consisted of a 1376 × 1040 pixel charge-coupled device (CCD) camera (Sensicam QE, The Cooke Corp., Romulus, MI, USA), an imaging spectrograph (ImSpector V10E OEM, Spectral Imaging Ltd., Oulu, Finland) covering the spectral range of 400–1,000 nm, and a 23 mm fixed focal lens (Xenoplan, Schneider Optics, Hauppauge, NY, USA). Two illumination sources were used in the prototype; one was for reflectance and the other for transmittance.

The reflectance illuminant was a 150 W tungsten halogen lamp with a DC-regulated power supply (Fiberlite A240P, Dolan-Jenner Industries, Lawrence, MA, USA) connected to a dual fiber optic line light. The transmittance light source consisted of a 410 W tungsten halogen lamp with a built-in reflector (FXL/5, Eiko Ltd., Shawnee, KS, USA). This lamp was housed in a custom-made enclosure containing mirror and lens to direct the light to the sample. The light enclosure was installed underneath the sample holding unit coupled with an adjustable diaphragm whose opening was set at 9.15 mm in diameter to ensure that only the light that has transmitted the whole blueberry would be detected by the hyperspectral imaging unit. The lamp for transmittance measurements was controlled by a programmable DC power supply (model VSP-12010, Yorba Linda, CA, USA). The hyperspectral imaging system was originally designed for simultaneous acquisition of reflectance (400-750 nm) and transmittance (750-1,000 nm) image. For this particular study, reflectance and transmittance images were acquired separately, both covering the spectral region of 400-1,000 nm, so that we could better compare the performance of the two sensing modes for blueberry quality assessment.

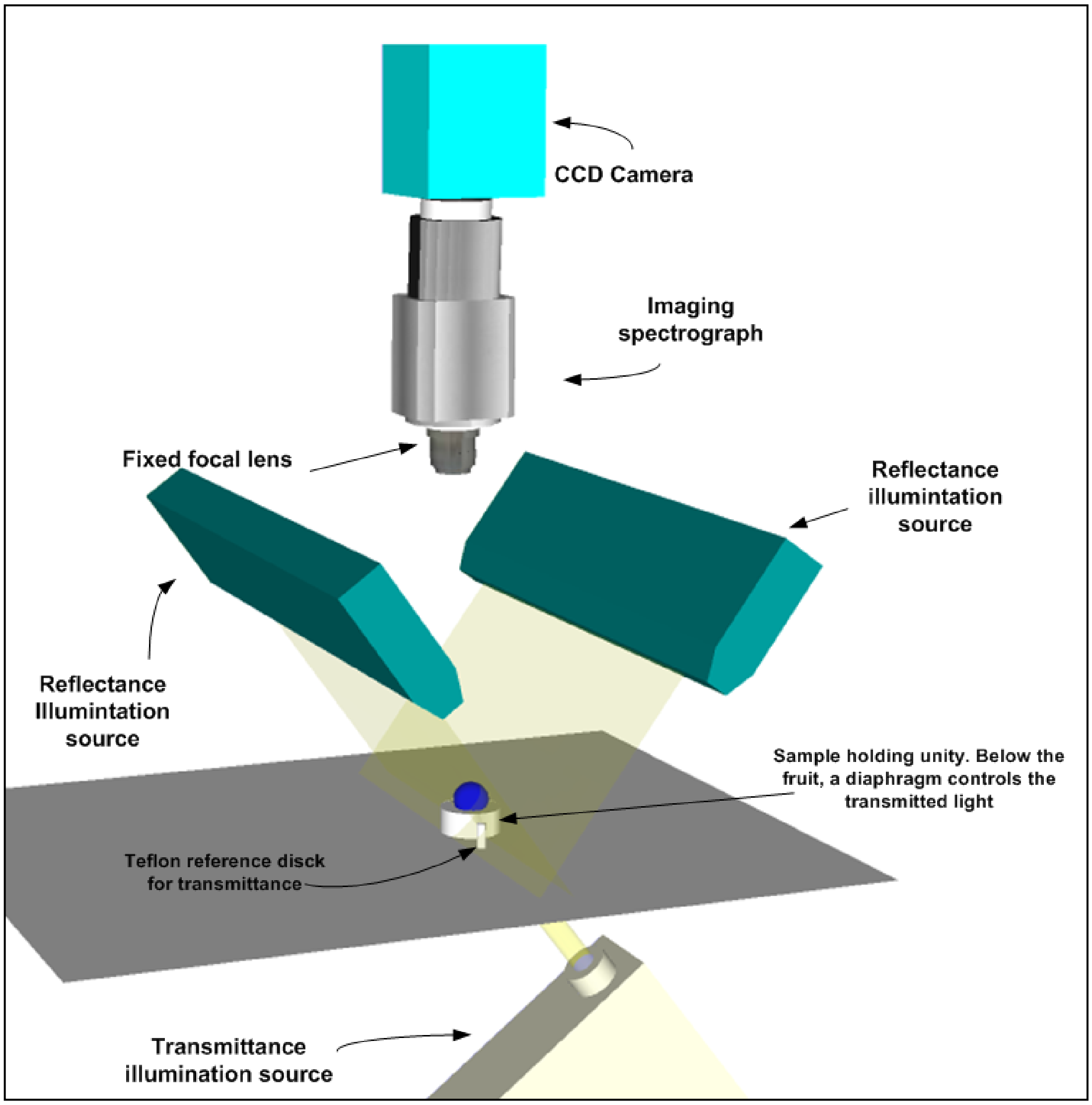

Figure 5.1 Schematic of the hyperspectral reflectance and transmittance imaging system for blueberry quality assessment.

Each blueberry was placed on the sample holding unity and then imaged for each of the three orientations (i.e., with the calyx end, the stem end and equator facing vertically toward the imaging system). The exposure time was set at 150 ms and 35 ms for reflectance and transmittance, respectively, for obtaining good quality images without saturation. Each hypercube consisted of 150 scanning lines × 688 spatial pixels × 520 wavelengths covering the spectral region of 400-1,000 nm, after 2 × 2 binning operations for the spatial and spectral directions. Consequently, the hypercubes had a spectral resolution of 1.30 nm/pixel and the spatial resolution of 0.167 mm/pixel along the scanning line direction and between the scanning lines.

For correction of the light source effect, hyperspectral reflectance images were also acquired from a 20% diffuse reflectance standard (Spectralon® Reflectance Standard SRS-20-010, Labsphere, Inc., North Sutton, NH, USA) using the same imaging parameters as that for blueberries. For transmittance images corrections, a cylindrical reference disc made from white Teflon (6 mm in diameter and 6 mm in thickness) was mounted next to the frame of the sample holding unit, and it was connected with a fiber optic line from the front of the lens in the light enclosure toward the disc (Ariana and Lu, 2008). Dark current images were acquired with the camera shutter being closed.

5.2.3. Blueberry quality measurements

Destructive measurements of firmness index (FI) and SSC were carried out after the image acquisition. Firmness measurements were performed with a Texture Analyzer (model TA.XT2i, Stable Micro Systems, Inc., Surrey, U.K.), following the procedure used by (Leiva-Valenzuela et al. 2013; Slaughter and Rohrbach 1985). Each berry fruit was compressed between two parallel plates at the constant velocity of 0.5 mm/s for a total deformation of 3 mm. The berry was oriented with its stem-calyx axis approximately parallel to the compression plates. FI was calculated from the slope of the force/deformation curve between 0.5 mm and 2.5 mm displacement, which was relatively straight in most of the cases.

After the FI measurements, each berry was equatorially cut in half. Juice was then extracted from the two berry halves and SSC (%) was measured using a digital refractometer (model PR-101, Atago Co., Tokyo, Japan).

5.2.4. Image processing

Image processing was carried out using Matlab R2008a and its image processing toolbox (The Mathworks, Inc., Natick, MA, USA). The image processing steps included sample image corrections using the reference image to obtain relative reflectance and transmittance images, segmentation of berries, and mean spectra calculations.

The intensity values for each hyperspectral image were corrected by the reference image pixels by pixels in both spatial and spectral dimensions (Ariana and Lu 2010; Tallada et al. 2006):

$$RI = \frac{IS(x,y) - ID(x,y)}{IR(x,y) - ID(x,y)} \qquad (5.1)$$

where *RI* is the relative reflectance or transmittance at pixel location (x,y), *IS* is the sample image value, *ID* is the dark frame image value, and *IR* is the reference image pixel value (Figure 5.2a and 5.2b).

After the relative values of reflectance or transmittance were obtained, each spectrum was smoothed by the Savitzky-Golay derivative algorithm of order zero (Savitzky and Golay 1964). The same procedure was implemented for reflectance and transmittance.Since the relative reflectance and transmittance spectra varied greatly among the fruit and within each image area, maximum normalization (Xing and Guyer 2008) was applied by dividing the relative transmittance value at each wavelength by the peak intensity value in each image. These peaks usually occurred at ~840 nm for transmittance and at ~880 nm for reflectance. After further removal of border regions in each hypercube layer (Figure 2c) and noisy spectral region reduction, the reflectance images were reduced from 400 × 688 × 478 pixels to 150 × 181 × 520 pixels covering the spectral region of 400-1,000 nm, whereas the transmittance images were reduced to 150 x 181 x 295 for the spectral region of 563-939 nm.

After the preprocessing, segmentation was implemented to segregate each blueberry from the background for each hypercube and calculate their mean spectra (Figure 2d). The first step consisted of building a binary mask to recognize each berry from the background using the threshold segmentation (Otsu 1979) in the hypercube. This was accomplished on the spectral image at 657 nm, which gave the maximum contrast between the blueberries and the background for both reflectance and transmittance images. As a result, a total of 602,280 grayscale images for reflectance and 371,700 images for transmittance, 150 x 181 pixels each and free of background, were obtained for the three orientations' images (calyx and stem ends and equator) from the 420 blueberries. Mean reflectance and transmittance were computed by averaging over all pixels from the regions of interest for each segmented blueberry image.

5.2.5. Prediction models for soluble solids content and firmness

Calibration models for predicting SSC and FI were developed using PLS (Figure 5.2e). To ensure the models were not over fitted and the prediction results truly represented the model performance, the samples were first divided into four separate folds randomly (Donis-González et al. 2013; Mery et al. 2012). Three folds (or 75% of all samples) were used for calibration and the remaining fold was used for independent test or prediction. Leave-one-out cross validation was used to determine the number of latent variables for the calibration models. Thereafter, the calibration models were used to predict the independent samples. The models were evaluated using root mean squares errors for cross validation (RMSECV) and prediction (RMSEP), referred to as calibration variance and imprecision of prediction model, respectively.

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2}{n_p}} \qquad (5.2)$$

In addition, correlation coefficients for calibration (R_c) and prediction (R_p) and residual predictive deviation (RPD), defined as the ratio between the sample standard deviation and RMSEP, were also calculated. RPD values measure the ability of a model for classification (Nicolaï et al. 2007).

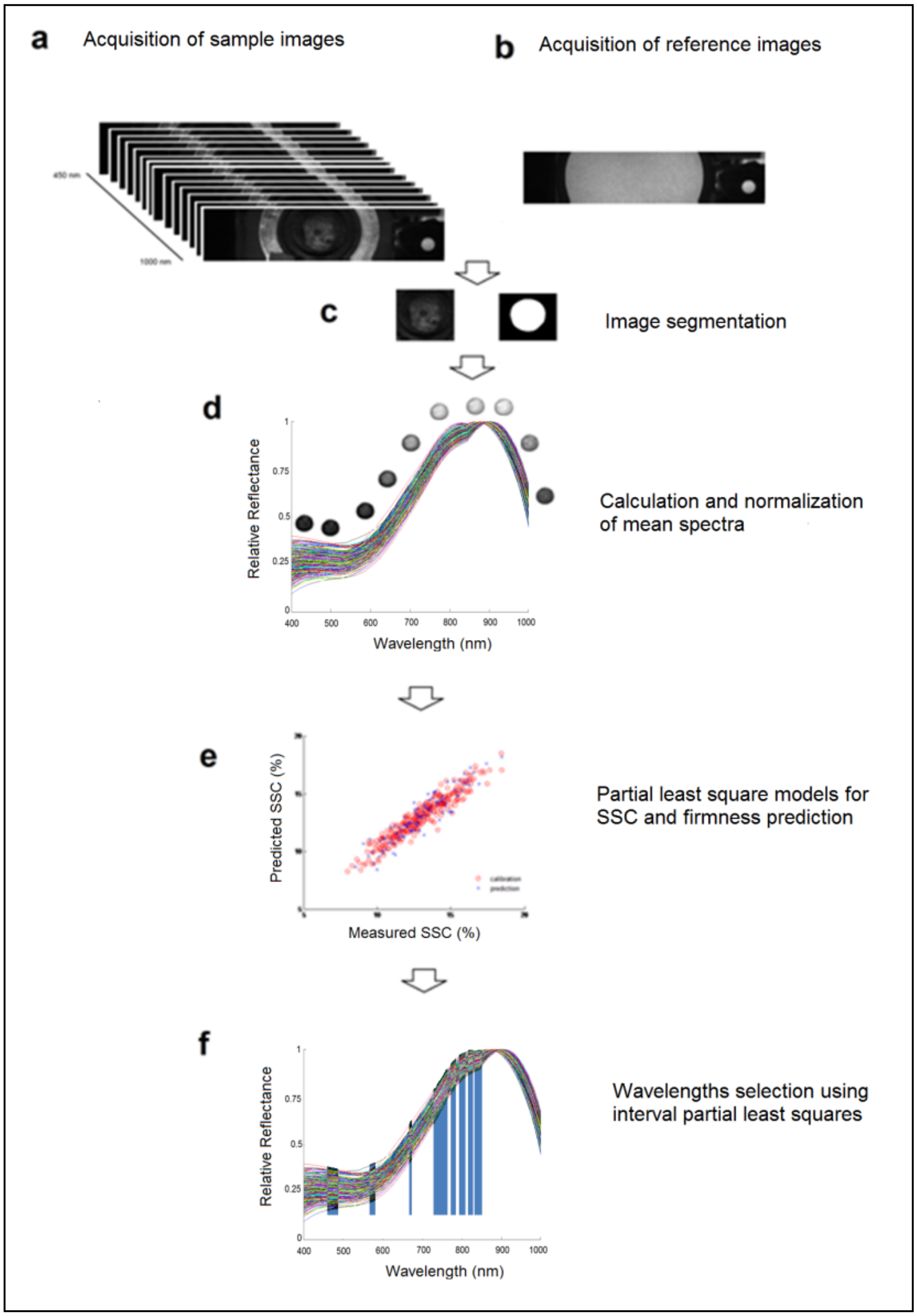

Figure 5.2 Flow diagram for the assessment of internal quality of blueberries.

The above procedure was repeated four times. For each new run, one fold of samples in the calibration set was rotated out and replaced with the test samples from the previous run. The rotated out samples were then used as the new test samples. Finally, the results (i.e., R_c, R_p, RMSECV, RMSEP and RPD) from the four runs were averaged to estimate the performance of the models.

The prediction results from different sets of data were compared using Duncan tests with 95% confidence (Statgraphics Centurion XV, Statpoint Technologies Inc., Warrenton, VA, USA).

5.2.6. Wavelengths selection

Hyperspectral images contain a large amount of information, much of which is redundant (Moltó et al. 2010). Moreover, processing of hyperspectral images in real time is still too slow and impractical for industrial applications. For this reason, it is necessary to select the best known wavelengths from the entire spectra data.

To select the best wavelengths, interval PLS (iPLS) was used (Figure 5.2f). This method builds a series of sub PLS models in adjacent but non-overlapping intervals with the same dimensions as that for the conventional PLS to minimize the RMSECV with cross validation (Xu et al. 2012; Nørgaard et al. 2000b). In the present study, 90 wavelengths were selected for each of 12 cases (i.e., two quality attributes × two sensing modes × three fruit orientations), which were evenly distributed in 10 intervals of nine adjacent wavelengths each (Figure 5.2e). The intervals could be adjacent but not overlapped. The intervals that provided the lowest RMSECVs were selected with the same criterion used in whole spectra PLS prediction.

5.3. Results and discussion

The SSC and FI distributions for blueberry samples are presented in Figure 5.3. The variability for SSC, as measured by the ratio of standard deviation to the mean of samples, was 31.1%, more than doubling 15.0% for FI; it was also quite large compared with other fruits such as apple. For instance, the apple samples tested by Mendoza et al. (2011) had the variability of 10.7% and 28.2% for SSC and firmness, respectively, for three varieties of apple.

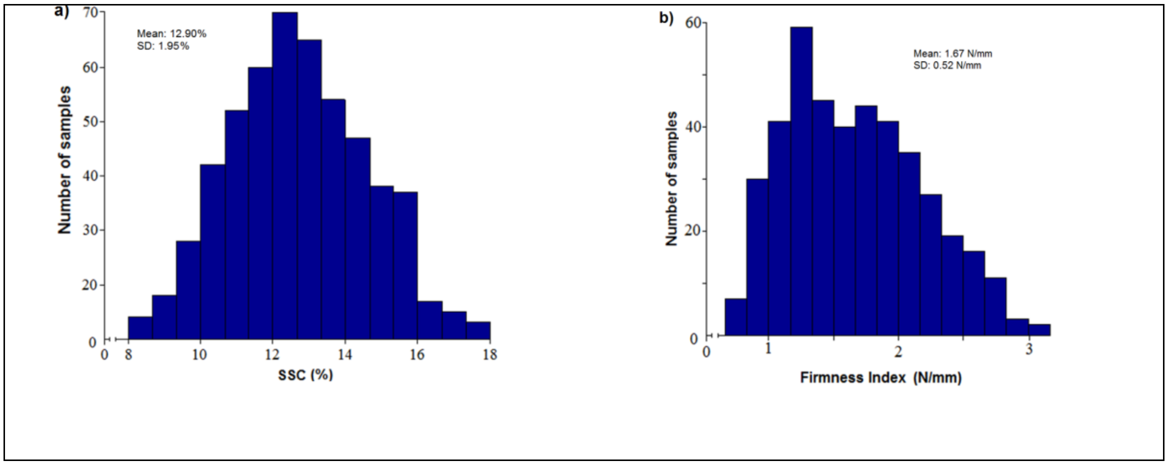

Figure 5.3. Distribution of (a) soluble solids content (SSC) and (b) firmness index (FI) for 420 blueberry samples.

Large SSC and FI variations among the blueberries are good for building robust calibration models; they also suggest the need of having an effective quality sorting technique in order to assure consistent quality.

5.3.1. Spectral characters of blueberries

Figure 5.4 shows the normalized mean relative reflectance and transmittance spectra for the 420 blueberry samples. Noisy signals beyond 400-1,000 nm for reflectance and beyond 563-939 nm for transmittance were discarded. Two distinctive regions were observed from the reflectance spectra (Figure 5.4a). The first region was in the visible region between 400 nm and 650 nm. In this region, the blueberries had low relative reflectance (<40%). Prior to the normalization, the variation of spectra among the samples for the visible region was smaller (~20%). However, after the normalization, the spectra's variation in the visible region was greater than that for the NIR infrared. Lower values of reflectance in the visible region were attributed to the relatively homogeneous, dark color of the fruit and the absorption of light by anthocyanins in mature blueberries at 490-550 nm (Yang et al. 2012; Peshlov et al. 2009). For the second region starting around 650 nm, reflectance increased rapidly and reached a peak at a wavelength close to 800 nm and another peak at 900 nm. Reflectance decreased rapidly at the wavelengths of 900-1,000 nm, which was likely attributed to the combination effect of OH groups from carbohydrates and water at 970 nm since blueberries had a SSC of 8-18% and an estimated water content of 80-90%. Since the hyperspectral imaging system used in this study had a higher spectral resolution than the one used in our previous study (Leiva-Valenzuela et al. 2013), it allowed to better distinguish the two reflectance peaks.

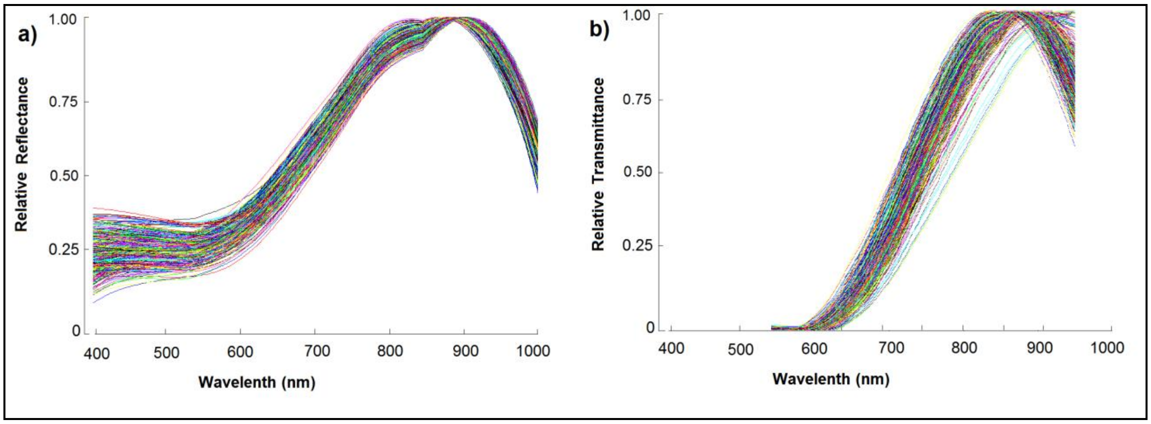

Figure 5.4. Relative reflectance (a) and transmittance (b) spectra, after normalization by the maximum value of each spectrum, for 420 blueberry samples measured from the stem-end orientation.

The transmittance spectra were smoother with fewer features, compared with the reflectance spectra. Below the visible wavelength at 600 nm, no or little light was transmitted through the fruit. Transmittance increased steadily from 600 nm and reached peak around 840 nm. Blueberries had good transmittance in the near-infrared region of 750-939 nm. It should be pointed out that the ranges of values for the original reflectance and transmittance spectra prior to the normalization were quite different because they were corrected using two different reference materials and because of the different lighting setups for the two sensing modes. The normalization procedure minimized the effect of fruit size (5.5% variation among the samples) on the transmittance spectra. The procedure, when combined with the Savitzky-Golay smoothing algorithm, improved firmness and SSC prediction results by about 10% (the results are not further discussed below).

5.3.2. Prediction of soluble solids content and firmness

Table 5.1 shows the SSC and FI prediction results from the PLS models with whole spectra for the three fruit orientations plus their combination that was obtained by averaging the spectra of three orientations for both reflectance and transmittance modes.

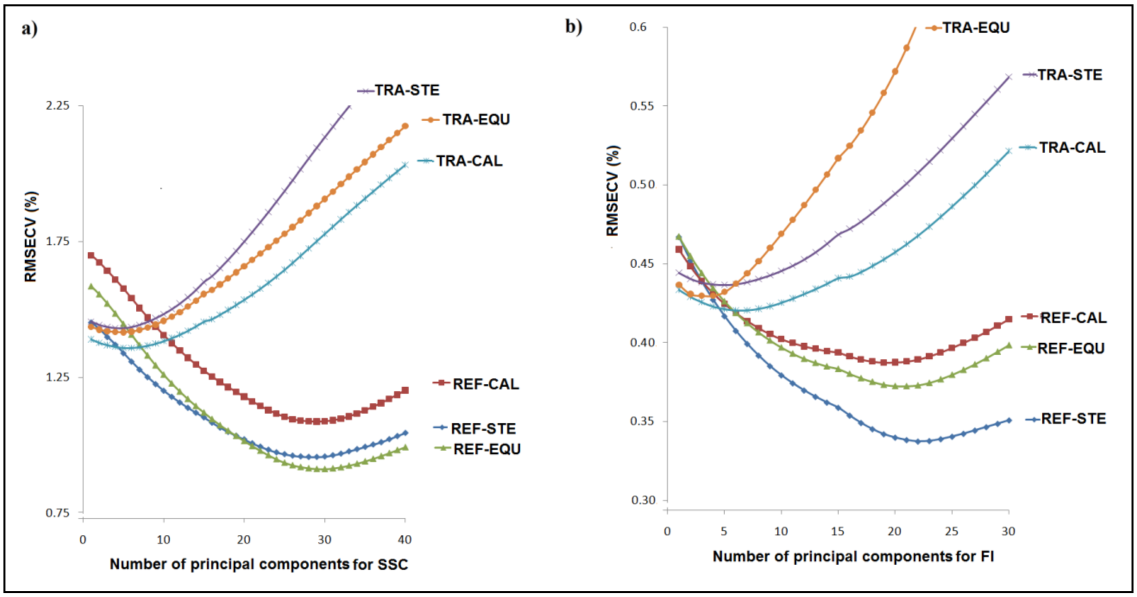

Figure 5.5. Curves of the root mean squares error for cross validation **(**RMSECV) for (a) soluble solids content (SSC) and (b) firmness index (FI) prediction, where symbols REF and TRA refer to reflectance and transmittance, respectively, followed with a specific fruit orientation (i.e., EQU for equator, STE for stem end and CAL for calyx end).

Table 5.1. Calibration and prediction results for soluble solids content (SSC) and firmness index (FI) of blueberries using partial least squares regression for three fruit orientations and their average spectra.

Orientation	PC	R_c	RMSECV	R_p	RMSEP	RPD
SSC prediction, using reflectance images						
Stem end	29 ±3	0.952 ± 0.007	0.61 ± 0.04	0.888 ± 0.022	0.94 ± 0.11 ^{AB}	2.1 ± 0.2
Calyx end	28 ± 3	0.936± 0.013	0.70 ± 0.07	0.869 ± 0.009	1.01 ± 0.07 ^{B}	2.0 ± 0.1
Equator	28 ± 2	0.952 ± 0.005	0.61 ± 0.03	0.900 ± 0.020	0.88 ± 0.02 ^{A}	2.3 ± 0.0
Average	30 ± 0	0.960 ± 0.003	0.56 ± 0.02	0.926± 0.014	0.76± 0.07 ^{C}	2.6 ± 0.2
SSC prediction, using transmission images						
Stem end	8 ± 2	0.733 ± 0.015	1.35 ± 0.03	0.719 ± 0.046	1.39 ± 0.05 ^{D}	1.4 ± 0.0
Calyx end	9 ± 1	0.774± 0.014	1.26 ± 0.03	0.760 ± 0.031	1.31 ± 0.06 ^{D}	1.5± 0.1
Equator	8 ± 2	0.741 ± 0.017	1.33 ± 0.04	0.725 ± 0.024	1.40 ± 0.09 ^{D}	1.4 ± 0.1
Average	10 ± 1	0.769 ± 0.009	1.27 ± 0.02	0.753 ± 0.029	1.35 ± 0.06 ^{D}	1.5 ± 0.1
FI prediction, using reflectance images						
Stem end	22 ± 7	0.855 ± 0.034	0.27 ± 0.03	0.770 ± 0.022	0.33 ± 0.02 ^{E}	1.5 ± 0.1
Calyx end	16 ± 1	0.753 ± 0.007	0.34 ± 0.00	0.692 ± 0.017	0.38 ± 0.02 ^{FG}	1.4 ± 0.1
Equator	17 ± 1	0.781 ± 0.014	0.32± 0.01	0.732 ± 0.015	0.35 ± 0.02^{EF}	1.5 ± 0.1
Average	25 ± 5	0.865 ± 0.026	0.26 ± 0.02	0.782 ± 0.020	0.33 ± 0.03 ^{E}	1.6 ± 01
FI prediction, using transmission images						
Stem end	9 ± 2	0.620 ± 0.019	0.41 ± 0.01	0.589 ± 0.002	0.42 ± 0.02 ^{H}	1.2 ± 0.1
Calyx end	10 ± 1	0.672± 0.007	0.38 ± 0.00	0.636 ± 0.024	0.40 ± 0.01 ^{GH}	1.3 ± 0.0
Equator	10 ± 2	0.672 ± 0.054	0.38 ± 0.02	0.636 ± 0.038	0.40± 0.02 ^{H}	1.3 ± 0.1
Average	10 ± 3	0.660 ± 0.035	0.39 ± 0.02	0.634 ± 0.011	0.40 ± 0.02 ^{GH}	1.3 ± 0.1

PC: Number of principal components; R_c: Average correlation coefficient of calibration over four calculations; R_p: Average correlation coefficient of prediction over four calculations; RMSECV: Average root mean squares error of cross validation over four calculations; RMSEP: Average root mean squares error of prediction over four calculations. RPD: Ratio of standard deviation to RMSEP; RMSEPs with the same letter(s) are not significantly different at $p<0.05$ using Duncan test.

The optimal number of principal components (PCs) for each PLS model was determined based on the minimization of RMSECV. This statistical parameter indicates the modeling error or calibration variance, thus the imprecision (quality) of the calibration model when tested internally. The RMSECV curves for different PCs were plotted (Figure 5), and the optimal number of PCs was chosen when the global minimum in RMSECV was reached. As shown in Figure 5.5 and Table 5.1, better calibration performances were generally obtained with 8-13 PCs for transmittance and 16-30 for reflectance. Fewer numbers of PCs for both SSC and FI models for transmittance are in agreement with the fact that the transmittance spectra had fewer features compared to the reflectance spectra (Figure 5.4). The number of PCs for each of the three orientations was quite consistent for SSC prediction with reflectance and for both SSC and FI prediction with transmittance.

Blueberry orientation had small or no significant effect on SSC and FI prediction (Table 5.1). For reflectance, SSC predictions for the calyx-end direction were not as good as that for the stem-end and equatorial directions (R_p of 0.87 versus 0.88 and 0.90). Likewise, FI predictions for the stem end were better than for the calyx end and equator (R_p of 0.77 versus 0.69 and 0.73). For transmittance, the differences in SSC and FI prediction for the three orientations were either insignificant or quite small (R_p of 0.72-0.76 for SSC and R_p of 0.59-0.64 for FI). The different reflectance prediction results for the three orientations may be explained from the external structures of blueberries. The surface of the calyx end is more irregular compared to the stem end region, and these irregularities would have affected light reflectance measurements, which may explain why the predictions of SSC and FI for the calyx end for reflectance were lower than that for the stem end and equator. In transmittance mode, light passes the whole fruit, and it is thus reasonable to expect that fruit orientation would have less effect on transmittance measurements, compared to reflectance mode.

When the two sensing modes were compared ($p<0.05$, Duncan test), significantly better results for SSC and FI prediction for the three fruit orientations were obtained with reflectance than with transmittance (Table 5.1). For SSC predictions with reflectance, R_c values ranged between 0.94 and 0.96, with R_p of 0.87-0.90, RMSEPs of 0.88-1.01% and RPDs of 2.1-2.3. In comparison, lower SSC prediction results for transmittance were obtained, with R_c of 0.73-0.77 and R_p of 0.72-0.76, RMSEP of 1.31-1.40% and RPD of 1.4-1.5. The same tendency for FI predictions was observed; reflectance gave better results with R_p of 0.69-0.78, RMSEP of 0.33-0.38 N/mm and RPD of 1.4-1.6, compared to transmittance with R_p of 0.59-0.64, RMSEP of 0.40 -0.42 N/mm and RPD 1.2-1.3. These results demonstrated that transmittance mode was not as suitable as reflectance mode for prediction or classification of blueberries for firmness and SSC, as further shown in Figure 5.6.

FI predictions using either reflectance or transmittance were not as good as those for SSC. The differences between SSC and FI results from this study are in general opposite to the results reported previously (Leiva-Valenzuela et al. 2013). Factors like variety and the different condition of blueberries for the two studies may have contributed to these differences. The blueberries tested in this study were conspicuously different from those in the previous study; the mean FI value and the coefficient of variation for this study were 1.67 N/mm and 15.0% respectively, versus 0.98 N/mm and 46.9% in the previous study. Larger FI variations in the samples are conducive to achieving a higher correlation coefficient.

No or little improvement was found when the two sensing modes were combined, i.e., the normalized mean reflectance and transmittance spectra were combined into one single spectrum for each sample, compared with reflectance alone (Table 5.2 versus Table 5.1). For SSC, the RMSEP for the reflectance data averaged over the three fruit orientations was 0.76% versus 0.81% for the combined sensing modes, with the corresponding RPD of 2.6 versus 2.5. Moreover, no improvement in FI prediction was also obtained when reflectance and transmittance were combined; the RPD of 1.6 was the same as that for reflectance alone. Furthermore, SSC or FI predictions for the three orientations were not statistically different ($p<0.05$, Duncan test). This suggests that there is no need to orient individual fruit when hyperspectral reflectance imaging technique is implemented for sorting SSC blueberries, which would simplify the sorting system design. In conclusion, reflectance alone is sufficient for assessing blueberry quality and there is no need to integrate reflectance with transmittance.

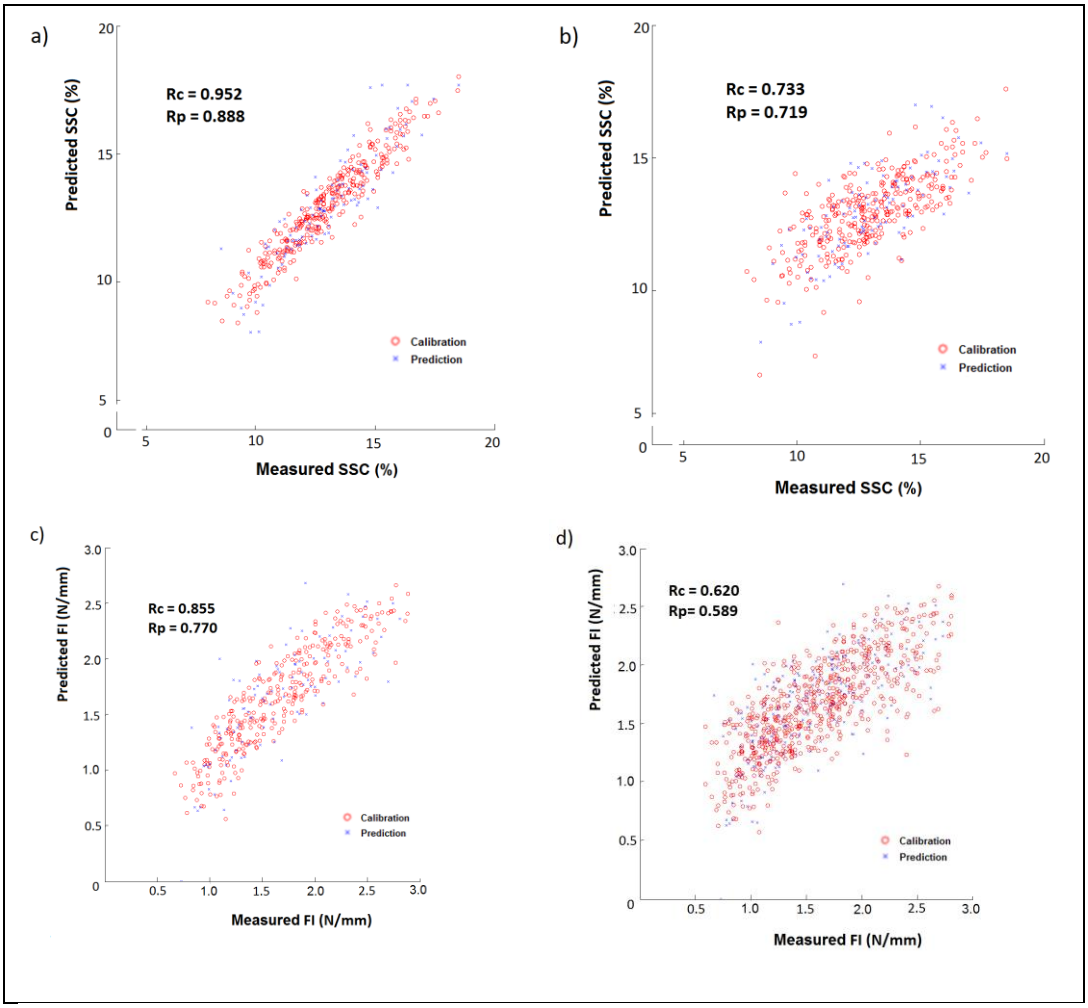

Figure 5.6. Partial least squares predictions of: a) soluble solids content (SSC) by reflectance, b) SSC by transmittance, c) FI by reflectance and d) FI prediction by transmittance, for both calibration and testing sets of samples in the stem end orientation.

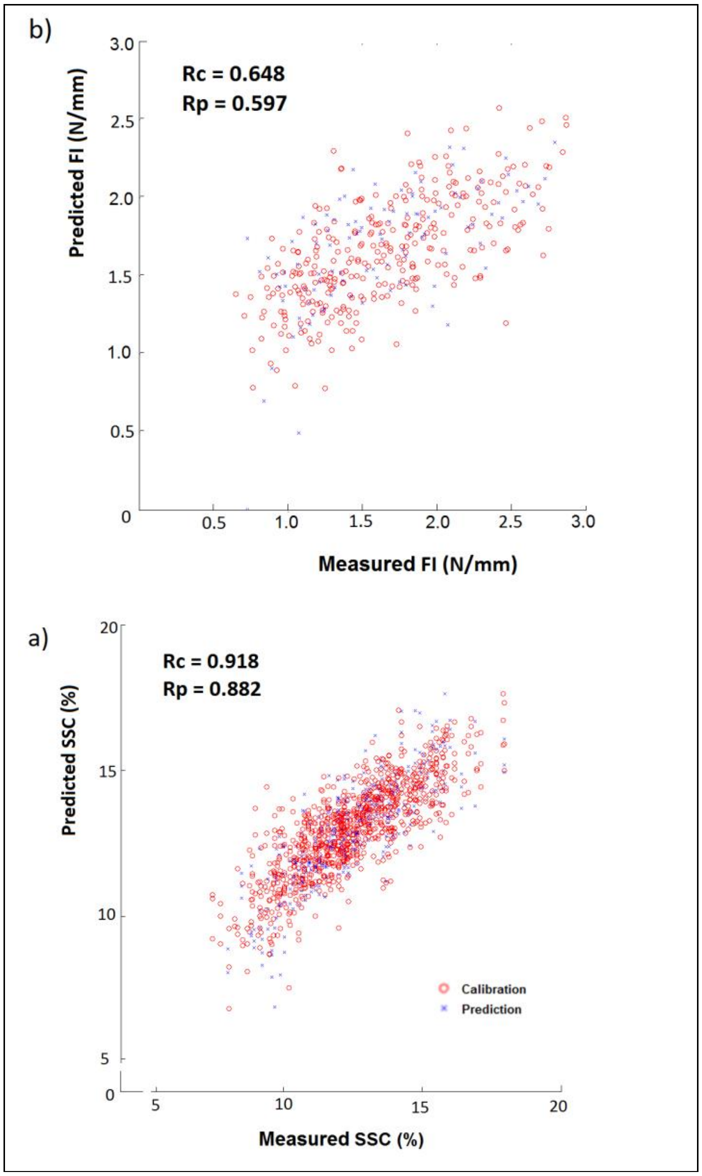

Figure 5.7. Calibration and prediction results from using selected wavelength intervals for the stem end spectra for: a) soluble solids content (SSC) in reflectance and b) firmness index (FI) in transmittance.

Table 5.2. Calibration and prediction results for soluble solids content (SSC) and firmness index (FI) of blueberries in three fruit orientations and their average spectra, using partial least squares regression for the combined reflectance and transmittance images.

Orientation	PC	R_c	RMSECV	R_p	RMSEP	RPD
SSC prediction						
Stem end	30± 0	0.943 ± 0.003	0.66 ± 0.02	0.878 ± 0.008	0.97 ± 0.03 ^{AB}	2.1± 0.1
Calyx end	30± 1	0.933± 0.003	0.72 ± 0.02	0.873 ± 0.028	1.01 ± 0.08 ^{A}	2.0± 0.2
Equator	29 ± 1	0.941 ± 0.003	0.67 ± 0.02	0.896 ± 0.019	0.89 ± 0.07 ^{AC}	2.2 ± 0.2
Average	30 ±1	0.948 ± 0.002	0.63 ± 0.01	0.915 ± 0.008	0.81 ± 0.03 ^{C}	2.5 ± 0.1
FI prediction						
Stem end	21±1	0.832 ± 0.013	0.29 ± 0.01	0.780 ± 0.041	0.33 ± 0.03 ^{DE}	1.6 ± 0.1
Calyx end	23±4	0.831± 0.029	0.29 ± 0.02	0.750 ± 0.025	0.35 ± 0.02^{E}	1.5 ± 0.1
Equator	20 ± 1	0.810 ± 0.009	0.30 ± 0.01	0.767 ± 0.021	0.34 ± 0.02 ^{DE}	1.5 ± 0.1
Average	21 ± 1	0.830 ± 0.003	0.28 ± 0.00	0.793 ± 0.016	0.32 ± 0.01 ^{D}	1.6 ± 0.0

PC: Number of principal components; R_c: Average correlation coefficient of calibration over four calculations; R_p: Average correlation coefficient of prediction over four calculations; RMSECV: Average root mean squares error of cross validation over four calculations; RMSEP: Average root mean squares error of prediction over four calculations. RPD: Ratio of standard deviation to RMSEP; RMSEPs with the same letter(s) are not significantly different at $p<0.05$ using Duncan test.

5.3.3 Spectral dimension reduction

Despite the encouraging results obtained using all wavelengths, the speed of image acquisition and processing by a hyperspectral imaging system is not enough to meet the requirement for the sorting lines in the packinghouse. For this reason, reduction of spectral dimensionality was further considered in this study, which would allow hyperspectral imaging technique to be implemented online as a multispectral imaging system with a few wavelengths. Wavelengths selection was accomplished using iPLS with 10 intervals of nine wavelengths each.

Figure 5.7 shows the selected wavelength intervals in reflectance and transmittance for prediction of SSC and FI for the three fruit orientations. Mean spectra for the three orientations were reduced to 90 selected wavelengths distributed in 10 groups each of nine wavelengths. The majority of intervals that were selected for SSC and also for FI prediction were in the NIR region (Figure 5.7). This finding is in agreement with previous studies (Lu 2004; Ruiz-Altisent et al. 2010b) that the visible spectral region is not useful for SSC prediction. For SSC prediction, the most repeatedly selected wavelengths were from 870 nm to 980 nm. For FI prediction, wavelengths in both visible and NIR regions were needed.

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Prediction results for SSC and FI using the two sensing modes for the selected wavelengths by iPLS are summarized in Table 5.3. Figure 5.8 shows predictions of SSC and FI using reflectance and transmittance images for the stem end with reduced wavelengths for one of the four model runs.

Generally, prediction results for the whole spectra and selected wavelengths are quite close. Only in SSC prediction using reflectance for the equator orientation, was statistical difference between the whole spectra and wavelength intervals observed ($p<0.05$ using Duncan test). The RMSEPs for SSC prediction using reflectance spectra were between 0.76-1.01% for the entire spectra, compared with 0.95-1.11% for the reduced data set and an average decrease of 10.3%. For FI prediction using whole spectra or selected wavelength intervals, no significant changes were observed for both sensing modes. Hence it is possible to substantially reduce the number of images needed for the model building.

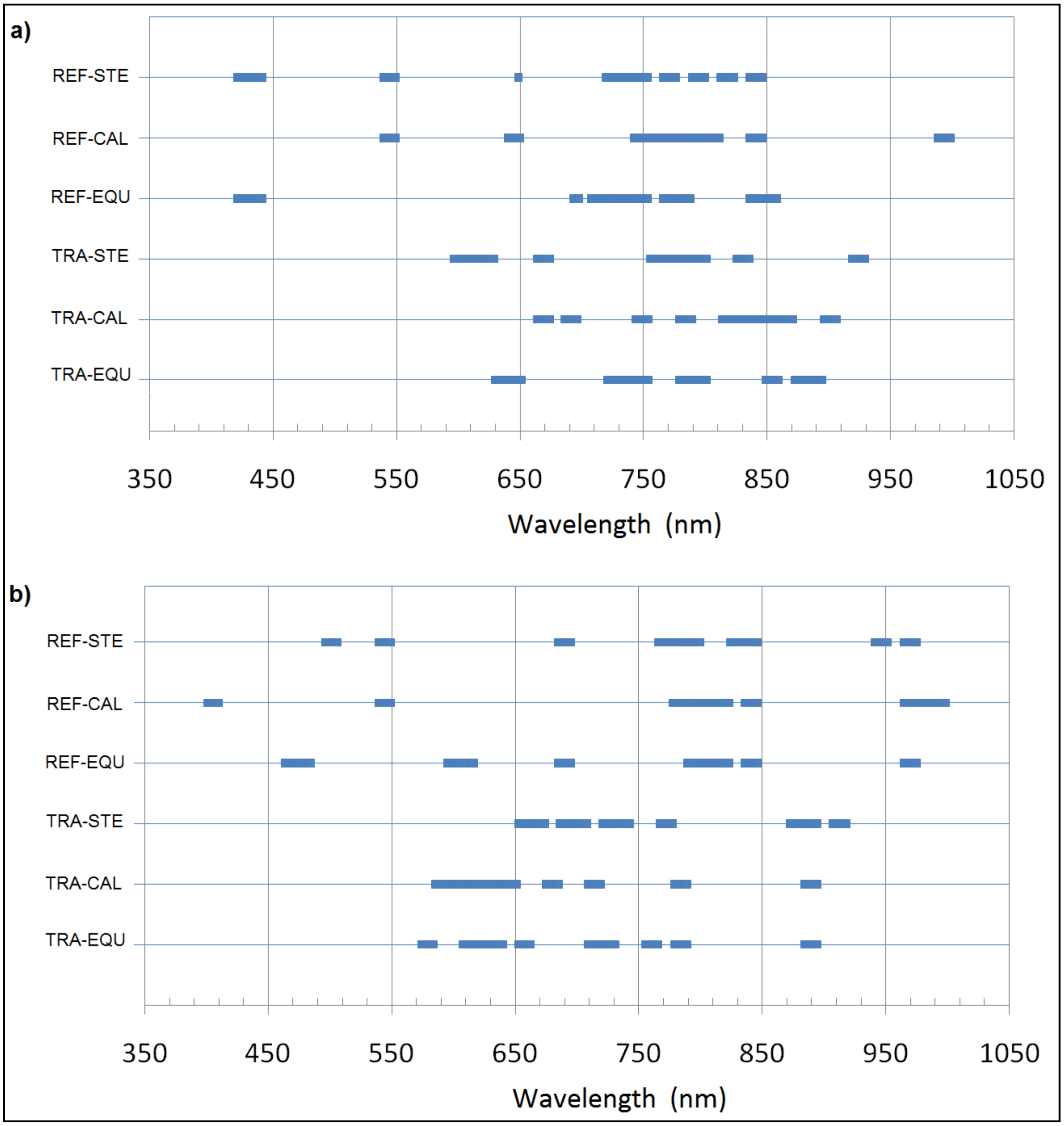

Figure 5.7. Selected wavelength intervals for a) predicting soluble solids content (SSC) and b) firmness index (FI) using reflectance (REF) and transmittance (TRA) images for the three fruit orientations (i.e., stem end or STE, calyx end or CAL and equator or EQU).

Table 5.3. Calibration and prediction results for soluble solids content (SSC) and firmness index (FI) for blueberries using partial least squares regression for 10 selected intervals each of nine wavelengths

Orientation	PC	R_c	RMSECV	R_p	RMSEP	RPD	% Dif
SSC prediction, using reflectance images							
Stem end	21 ±1	0.918 ± 0.008	0.79 ± 0.03	0.882 ± 0.029	0.95 ± 0.09 A	2.1 ± 0.2	-0.82
Calyx end	17 ± 1	0.862± 0.008	1.01 ± 0.03	0.832 ± 0.037	1.11 ± 0.10 B	1.8 ± 0.2	-9.50
Equator	19 ± 2	0.888 ± 0.008	0.91 ± 0.03	0.840 ± 0.012	1.11 ± 0.06 B	1.8 ± 0.1	-20.46 **
SSC prediction, using transmission images							
Stem end	8 ± 2	0.742 ± 0.021	1.33 ± 0.04	0.721 ± 0.028	1.38 ± 0.05 C	1.4 ± 0.1	-0.89
Calyx end	10 ± 1	0.792± 0.010	1.21 ± 0.03	0.770 ± 0.018	1.29 ± 0.02 D	1.5± 0.0	-1.57
Equator	6 ± 1	0.726 ± 0.017	1.37 ± 0.04	0.714 ± 0.049	1.39 ± 0.10 C	1.4 ± 0.1	-0.25
FI prediction, using reflectance images							
Stem end	16 ± 0	0.817 ± 0.011	0.30 ± 0.01	0.772 ± 0.041	0.33 ± 0.03 E	1.6 ± 0.1	-1.71
Calyx end	15 ± 1	0.765 ± 0.010	0.33 ± 0.01	0.689 ± 0.041	0.38 ± 0.02 EF	1.4 ± 0.1	0.78
Equator	17 ± 1	0.787 ± 0.008	0.32± 0.01	0.708 ± 0.046	0.37 ± 0.02EF	1.4 ± 0.1	3.99
FI prediction, using transmission images							
Stem end	9 ± 3	0.648 ± 0.032	0.39 ± 0.01	0.597 ± 0.070	0.41 ± 0.02 F	1.2 ± 0.1	-0.88
Calyx end	11 ± 1	0.683± 0.009	0.38 ± 0.00	0.641 ± 0.036	0.40 ± 0.01 EF	1.3 ± 0.0	-0.55
Equator	7 ± 2	0.596 ± 0.064	0.41 ± 0.03	0.559 ± 0.067	0.43± 0.02 F	1.2 ± 0.1	6.85

PC: Number of principal components; R_c: Average correlation coefficient of calibration over four calculations; R_p: Average correlation coefficient of prediction over four calculations; RMSECV: Average root mean squares error of cross validation over four calculations; RMSEP: Average root mean squares error of prediction over four calculations. RPD: Ratio of standard deviation to RMSEP; RMSEPs with the same letter(s) are not significantly different at p<0.05 using Duncan test. % Dif: Percent difference in the RMSEP between using all wavelengths and selected wavelengths. Positive values indicate better predictions using selected wavelengths, ** Denote statistic differences between whole and reduced spectra predictions.

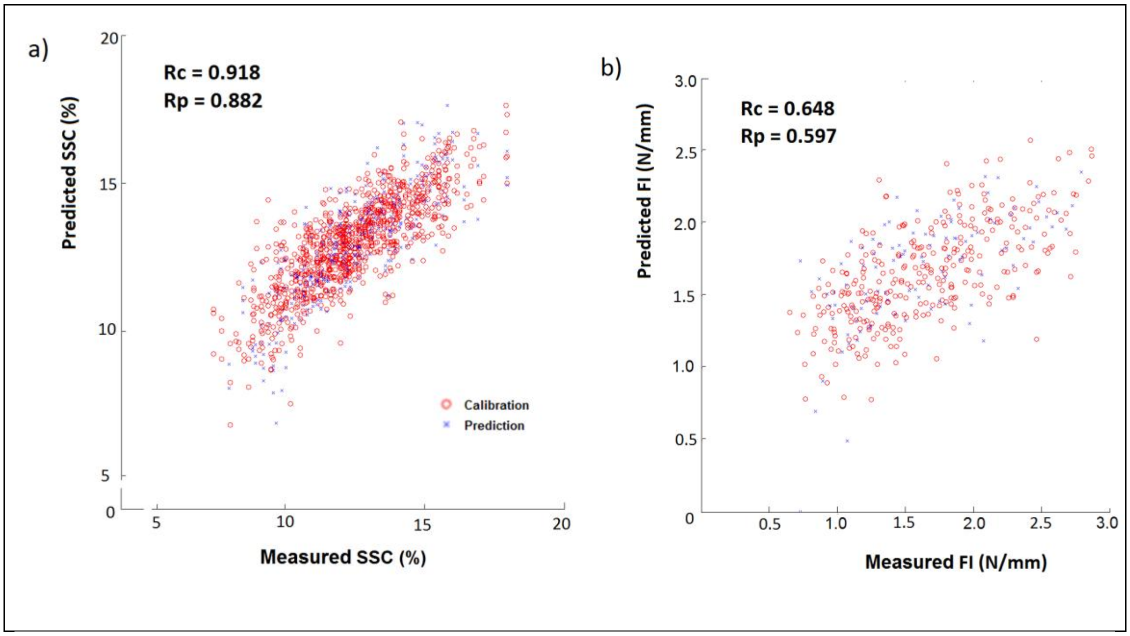

Figure 5.8. Calibration and prediction results from using selected wavelength intervals for the stem end spectra for: a) soluble solids content (SSC) in reflectance and b) firmness index (FI) in transmittance.

5.3. 4. Discussion

The findings of the research have demonstrated that it is technically feasible to sort blueberries into two SSC or sweetness classes using reflectance mode, since the RPD values for the three fruit orientations were between 2.1 and 2.6. There is no need to orient blueberries for online sorting in reflectance mode since fruit orientation had only small or insignificant effect on the reflectance prediction of SSC and FI.

While lower correlations were obtained for firmness prediction in the current study due in part to the relatively small range of sample firmness distributions, it is still possible to sort blueberries into two classes of firmness using reflectance, in view of the overall findings from this study and our previous one (Leiva-Valenzuela et al., 2013). Further improvements in image processing by integrating other image features like image textures and entropies etc. could lead to better prediction of FI and SSC.

Compared to reflectance, transmittance generally performed less satisfactorily for SSC and firmness prediction. Hence this sensing mode is not recommended for SSC or firmness sorting since it is also more difficult to implement in the commercial application.

Finally, using selected wavelengths only resulted in small or no significant decreases in SSC and FI prediction accuracy. It is, therefore, feasible to implement hyperspectral imaging technique with a few optimal wavelengths for commercial online sorting of blueberries.

5.5. Conclusion

This study compared SSC and FI prediction results obtained using hyperspectral reflectance and transmittance modes for whole or reduced spectra. The effect of three fruit orientations (i.e., stem end, calyx end and equator) on the prediction model performance was also evaluated. Overall, reflectance mode performed significantly better than transmittance mode in predicting SSC and FI. Good predictions of SSC were obtained for the whole reflectance spectra, with the best R_p of 0.93 and an RPD of 2.6. Fruit orientation only had small or insignificant effect on SSC and FI prediction in reflectance mode with the better results for SSC and FI being obtained for the equator and stem end directions, respectively, and it had insignificant influence on transmittance measurement.

Lower correlation coefficients were obtained for FI prediction using the reflectance data, with the best R_p of 0.78 and an RPD of 1.6, which were in part attributed to smaller firmness variations in the test samples. Results from the iPLS wavelengths selection showed that using the reduced data set, in most cases, increased the standard error by no more than 5% for prediction of FI and SSC. Hence it is feasible to implement hyperspectral imaging technique with a few selected wavelengths for online sorting of blueberries into two classes of SSC and possibly firmness.

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6. GENERAL CONCLUSION AND FUTURE PROSPECTS

6.1 Conclusion

Since small fruits are highly susceptible to postharvest damage during transportation or storage, their quality assessment is critical to ensure optimal commercialization. Hence, developing non-destructive technologies is not only a challenge, but also a necessity.

There are several techniques based on computer vision, NIR spectroscopy, mechanical, impact or vibration firmness detection, electric nose and nuclear resonance, which have been or could be implemented in in-line sorting systems in packing houses. Mechanical methods to assess firmness are widespread in sorting lines, however, they may induce slightly superficial bruises which might trigger fruit deterioration during the long shipping of blueberries. Still slowly and expensive to be implemented in commercial lines, aroma-based systems allow determining the overall condition of a whole batch of fruit. High cost of magnetic resonance imaging systems impedes their implementation. Differently, computer vision and NIR spectroscopy are fast, have an affordable cost in implementation and are suitable to or detect external attributes (CV) or detect both internal and external attributes correlating with chemical and textural information (NIR). However NIR measurement requires careful positioning of the fruit in order to obtain reliable signals and make it representative. Hyperspectral imaging systems, on the other hand, are slower and expensive than computer vision and NIR spectrophotometers; however, they allow both external and internal characterization of fruit. Therefore, computer vision, NIR spectrometry and hyperspectral imaging are suitable technologies for detection or classification of small fruit attributes for external and internal quality. Further studies should, thus, actively explore their implementation in sorting lines.

In the evaluation of visible attributes of blueberry, a representative small fruit, a computer vision system was used to acquire color images of different types of diseased or defective blueberries along the stem and calyx-end orientations. A large number of original features were reduced using features selection algorithms such SFS-Fisher. The best classifiers were linear discriminant analysis, support vector machine and probabilistic neural network. Good orientation detection was obtained. Also, fruit with visible damage were detected, with over the overall classification accuracy of 90% for shriveled blueberries, fungally decayed blueberries, and mechanically damaged blueberries, respectively. This research shows that the proposed statistical pattern recognition methodology is promising for inline sorting and grading of blueberries for different defects and orientations.

This research, for the first time, used hyperspectral imaging technique to assess the internal quality of blueberry. Reflectance images for blueberries in the stem and calyx end orientations were acquired. Relatively good predictions of firmness were obtained, suggesting the feasibility of sorting blueberries into two firmness classes. Fruit orientation did not have a significant effect on firmness prediction, and hence there is no need to orient fruit for hyperspectral imaging. Lower correlations were obtained for soluble solids content prediction, compared with those reported for large fruits such as apple and citrus. This study showed that hyperspectral imaging is promising for online sorting and grading of blueberries for firmness and perhaps soluble solids content as well.

The third study compared soluble solids content and firmness prediction results obtained using hyperspectral reflectance and transmittance modes for whole or reduced spectra. This is important since hyperspectral imaging system acquired a large amount of data for each fruit. In addition, the effect of three representative fruit orientations (i.e., stem end, calyx end and equator) on the prediction model performance was also evaluated. Overall, reflectance mode performed significantly better than transmittance mode in predicting soluble solids content and firmness. Good predictions of soluble solids content were obtained for the whole reflectance spectra, Fruit orientation only had small or insignificant effect on soluble solids content and firmness prediction in reflectance mode with the better results for soluble solids content and firmness being obtained for the equator and stem end directions, respectively, and it had insignificant influence on transmittance measurement.

Results from the wavelengths selection showed that using the reduced data set, in most cases, increased the standard error by no more than 5% for prediction of firmness and soluble solids content. Hence, it is feasible to implement a hyperspectral imaging technique with a few selected wavelengths for online sorting of blueberries into two classes of soluble solids content and possibly firmness.

6.2 Future prospects

The findings of the research have demonstrated that it is technically feasible to detect externally diseased fruit using a visible computer vision system; also, it is feasible to use hyperspectral imaging to sort blueberries into two sweetness and two firmness classes in reflectance mode with whole or reduced wavelengths. Results also showed that there is no need to orient blueberries for online sorting in reflectance mode since fruit orientation had only small or insignificant effect on the reflectance prediction internal quality. Further improvements in image processing by integrating other image features like image textures, entropies and tri-dimensional characterization of hypercubes could lead to better prediction of internal quality. More research is also needed to assess other blueberry quality attributes or properties (e.g., antioxidant compounds, internal bruising) and other common defects such as insect and foreign material presence. Additionally efforts should be focused on the implementation of high performance sorters using spectral images able to segregate small fruits quickly (not only blueberries) for internal quality (i.e. soluble solids content and firmness) as well as external attributes.

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