Authentication of Andean Flours using a Benchtop FT-IR System and a Portable FT-IR Spectrometer.

THESIS

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By

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Abstract

Andean indigenous grains such as quinoa (*Chenopodium quinoa*), cañihua (*Chenopodium pallidicaule*), and kiwicha (*Amaranthus caudatus* L.) have high nutritional value for the Andean region serving as principal protein sources, substituting for scarce animal proteins. The importance of these grains is based on their relatively high protein content with excellent composition of essential amino acids, gluten-free type, good source of dietary fiber, and bioactive compounds and minerals (calcium, zinc and iron). Because of their nutritional and economical importance, there is a risk for adulteration with less-expensive grains. Our objective was to develop a rapid analytical tool to characterize and detect adulteration of native Andean indigenous grains by combining infrared spectroscopy and pattern recognition analysis. Pure flours produced from the Andean region (quinoa, cañihua, kiwicha, maca, sacha inchi) and other (maize, mashua, soybean, wheat, linseed, algarroba, canary grass, sesame, barley, fava bean) ingredients were provided by Universidad Nacional Agraria la Molina (UNALM) (Lima, Peru). In addition, commercial samples were obtained from various local markets (Lima, Peru) and used for predictions. Unique spectral data was collected with a portable attenuated total reflectance (ATR) mid-infrared spectrometer equipped with a diamond crystal; data was also collected on a benchtop ATR mid-infrared spectrometer equipped with a ZnSe crystal. All analysis was done by soft independent modeling of class analogy (SIMCA). Pure flours formed distinct clusters allowing for the evaluation of commercial samples from Peruvian markets, which showed some prevalence of adulteration. Samples were well separated indicated by interclass distances greater than 3. Spectral differences responsible for the separation of
classes were attributed to C-O, C-C stretching and C-O-H and C-O-C deformation of carbohydrates (950-1000 cm\(^{-1}\)) and the amide (1560-1665 cm\(^{-1}\)) region of proteins. ATR-IR spectroscopy in combination with chemometrics was a viable tool in characterization of Andean flour samples allowing for the rapid, “in-field”, and reliable detection of adulteration of food ingredients, making it a great alternative to traditional testing methods; results were also comparable to the traditional benchtop method.
Dedicated to my Mom and Dad.

Mom, Thank you for always supporting me and being such an inspiration.

Dad, thank you for supporting me and I hope you are proud of everything I’ve done.
Acknowledgments

I would like to acknowledge my advisor Dr. Rodriguez, and my other committee members for their guidance and support. Also I would like to acknowledge my friends and family for their encouragement and support during this process.
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Chapter 1: Literature Review

1.1 Infrared Spectroscopy

The interaction of matter with electromagnetic radiation is known as spectroscopy, which forms the basis of spectra production, measurement, and analysis. Many different spectroscopic methods exist in order to solve a variety of problems; the species analyzed, the region of the electromagnetic spectrum used, and type of radiation are factors that need to be considered when choosing a method. The most commonly used spectroscopic approaches for the food industry are those that are based on the absorption or emission of radiation in the radio (nuclear magnetic resonance, NMR), infrared (IR), visible (Vis), and ultraviolet (UV) frequency ranges. Electromagnetic radiation in the UV-Vis range has wavelengths (λ) from 200 to 700 nm, while the visible range, which can be seen by the human eye, ranges from 350-700 nm (Penner, 1998). The infrared region of the electromagnetic spectrum contains longer wavelengths than visible or UV light, but shorter wavelengths than microwaves and radio waves (Wehling, 1998).

Infrared spectroscopy (IR) is often used in the food industry for analysis of food ingredients (Wehling, 1998). Infrared spectroscopy can further be broken down into 3 regions: near-IR (14,000-4000 cm\(^{-1}\)), mid-IR (4000-400 cm\(^{-1}\)), and far-IR (400-40 cm\(^{-1}\)) (Ellis et al., 2012). The near and mid infrared regions are most commonly used for both qualitative and quantitative analysis of foods. A molecule can absorb infrared radiation if it vibrates in a way that its electric dipole moment changes during the vibration. Many different vibrations exist, but only stretching and bending motions cause a change in the dipole moment. The frequency of the vibration is
directly proportional to the strength of the bond; therefore different functional groups will vibrate at varying energies. A vibrating functional group can absorb radiant energy to move from the lowest (ν=0) vibrational state to the first excited state (ν=1). The frequency of radiation that causes this occurrence is the same as the beginning frequency of vibration of the bond which is referred to as a fundamental absorption. Molecules that can absorb radiation to move to a higher state (ν=2 or 3) are overtones. The absorption intensities involved in overtones are greatly lower than fundamental absorptions because they are not favored (Wehling, 1998). Different compounds have different vibrations, resulting in unique spectra (Smith, 2011). The IR spectrum of a compound is characteristic of that compound, therefore it can be known as its chemical fingerprint. Infrared spectroscopy is especially beneficial because it requires little sample preparation, rapid, simple to use, and accurate (Ismail, van de Voort, & Sedman, 1997).

1.1.1 Mid-Infrared Spectroscopy

Mid-infrared spectroscopy is an analysis tool most often used for quality control and screening. The chemical bonds in a molecule vibrate at specific frequencies which relate to certain energy levels. A specific vibration has to be related to a change in the dipole in order for there to be an absorption in the infrared region of the spectrum. Molecules containing multiple bonds can lead to different modes of vibrations, such as stretching and bending. Those vibrations lead to absorptions in the infrared region at characteristic frequencies that could be affiliated with specific chemical groups. An infrared beam of light is used to measure a sample, and the light travels through the sample and the energy that was absorbed at every wavelength is documented. This can be achieved by scanning the entire spectrum with a monochromatic
beam, or by the use of a Fourier transform infrared spectroscopy system (FT-IR) which can measure all of the wavelengths at one time. The results take the different functional groups into effect and an absorbance or transmittance spectrum is collected; the spectrum shows the wavelengths at which the different functional groups were absorbed, and provides information about the chemical bonds present. A unique fingerprint is obtained which can be used to identify the sample (Karoui, Pierna, & Dufour, 2008).

Dispersive instruments and Fourier transform (FT) instruments are the two types of spectrometers used in mid-infrared spectroscopy. Dispersive instruments use a monochromator to disperse the individual frequencies of radiation and successively pass them through a sample so that the absorption can be measured and recorded at each frequency (Wehling, 1998). The dispersive systems which relied on monochromators have been replaced by interferometry technology, leading to the development of the FT-MIR spectrometer (Karoui et al., 2008). In Fourier transform instruments, all wavelengths arrive at the detector concurrently, and an FT transforms the results into a typical IR spectrum. An interferometer is used instead of a monochromator, the most common being a Michelson interferometer. In a Michelson interferometer, an infrared beam is split and then combined again by using a mirror to reflect back the split beams. The pathlength of one beam can be changed by moving its mirror, and the two beams will interfere either positively or negatively as they are joined, depending on their phase difference. So the intensity of the infrared radiation that reaches the detector changes as a function of the optical path difference and the diagram of the intensity of the energy obtained from the optical path difference is called an interferogram. When a sample
is placed in the recombined beam in front of the detector, the occupancy of the sample changes the radiation that reaches the detector. The generated interferogram demonstrating intensity vs. pathlength is then transformed by Fourier transformation into an infrared spectrum showing absorbance vs. frequency which can be done quickly by the computer (Wehling, 1998). Figure 1 shows a Fourier transform spectrometer using a Michelson interferometer.

![Diagram of Fourier transform spectrometer using Michelson interferometer](image)

**Figure 1. General setup of a Fourier transform spectrometer using a Michelson Interferometer (Saptari, 2003).**

Analysis can be done on liquid or solids samples with mid-infrared spectroscopy. Transmission IR spectroscopy is used to measure liquid samples and also thinner solid samples, while attenuated total reflectance (ATR) cells are used for solid samples, thicker pastes such as peanut butter, or very viscous liquids (Wehling, 1998). Figure 2 shows an attenuated total
reflectance device. ATR is used to measure the amount of energy reflected from the surface of a sample with an IR transmitting crystal (Wehling, 1998). The sample is in contact with a high refractive index crystal. The crystal is usually made up of zinc selenide (ZnSe), germanium (Ge), zinc sulfide (ZnS), silicon (Si), or diamond (Karoui et al., 2008). The infrared radiation passes through the crystal to the sample, and the radiation slightly enters the sample and is then reflected back into the crystal. At the wavelengths where the sample absorbs more radiation, the magnitude of the reflected radiation is decreased, which allows a spectrum to be collected which is akin to a transmission spectrum (Wehling, 1998).

![Diagram of ATR device](image)

Figure 2. Horizontal attenuated total reflection sampling device (ATR) (Karoui et al., 2008).

ATR is a very functional and useful tool for infrared sampling. It is useful for samples that are too thick or not transparent enough for transmission infrared measurements. Substances that are ideal to be analyzed using the ATR include cheese, meat, soft powders, as well as oils and dyes. ATR is beneficial because it doesn’t damage the sample, little sample preparation is needed, and it allows for quick and easy sampling. Despite the benefits that the ATR crystal offers, there are some cons as well; it absorbs energy at low energy levels, most crystals have
pH limitations, and good contact needs to be achieved between the sample and the crystal in order to receive accurate data. Many organic functional groups absorb radiation in the mid infrared region (Karoui et al., 2008). Table 1 shows the functional groups, their associated absorbing feature, and the frequency at which they absorb.

Table 1. Mid-IR absorption frequencies of various organic functional groups (Wehling, 1998).

<table>
<thead>
<tr>
<th>Group</th>
<th>Absorbing Feature</th>
<th>Frequency (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkanes</td>
<td>-CH stretch and bend</td>
<td>3000-2800</td>
</tr>
<tr>
<td></td>
<td>-CH(_2) and -CH(_3) bend</td>
<td>1470-1420 and 1380-1340</td>
</tr>
<tr>
<td>Alkenes</td>
<td>Olefinic-CH stretch</td>
<td>3100-3000</td>
</tr>
<tr>
<td>Alkynes</td>
<td>Acetylenic-CH stretch</td>
<td>3300</td>
</tr>
<tr>
<td>Aromatics</td>
<td>Aromatic-CH stretch</td>
<td>3100-3000</td>
</tr>
<tr>
<td></td>
<td>-C=C- stretch</td>
<td>1600</td>
</tr>
<tr>
<td>Alcohols</td>
<td>-OH stretch</td>
<td>3600-3200</td>
</tr>
<tr>
<td></td>
<td>-OH bend</td>
<td>1500-1300</td>
</tr>
<tr>
<td></td>
<td>C-O stretch</td>
<td>1220-1000</td>
</tr>
<tr>
<td>Ethers</td>
<td>C-O asymmetric stretch</td>
<td>1220-1000</td>
</tr>
<tr>
<td>Amines</td>
<td>Primary and secondary -NH stretch</td>
<td>3500-3300</td>
</tr>
<tr>
<td>Aldehydes and Ketones</td>
<td>-C=O stretch</td>
<td>1735-1700</td>
</tr>
<tr>
<td></td>
<td>-CH (doublet)</td>
<td>2850-2700</td>
</tr>
<tr>
<td>Carboxylic Acids</td>
<td>-C=O stretch</td>
<td>1740-1720</td>
</tr>
<tr>
<td>Amides</td>
<td>-C=O stretch</td>
<td>1670-1640</td>
</tr>
<tr>
<td></td>
<td>-NH stretch</td>
<td>3500-3100</td>
</tr>
<tr>
<td></td>
<td>-NH bend</td>
<td>1640-1550</td>
</tr>
</tbody>
</table>

A typical infrared spectrum has absorbance or percent transmittance values on the y-axis and usually wavenumbers (cm\(^{-1}\)) or wavelengths expressed on the x-axis (Wehling, 1998). Figure 3 shows a mid-infrared spectrum of virgin olive oils. On the x axis is wavenumbers and the y-axis
is absorbance. Peaks seen in the figure relate to the absorption of these specific functional groups that are present in the sample (Dupuy, Galtier, Ollivier, Vanloot, & Artaud, 2010). The functional groups labeled on the figure correlate to those that are listed in Table 1. MIR has been used to monitor the quality of cheese during ripening, to differentiate between cuts of meat, to discriminate flour samples of different cereals which were treated to different technological treatments (such as toasting or puffing), adulteration of extra virgin olive oil, and others. It has started to become widely used in the food industry and is a growing analytical tool useful in a variety of applications (Karoui et al., 2008).

![Mid-Infrared spectra of virgin olive oils (Dupuy et al., 2010).](image)

Overall the benefits of FT instruments are far greater than dispersive instruments and are the primary type of spectrometers used today. They provide a much better signal to noise ratio as compared to dispersive systems, as well as the ability to scan all frequencies at the same time,
which helps to reduce scan time without compromising resolution; other benefits include better wavelength resolution and accuracy. The advantages previously mentioned in combination with the ATR make FT-IR instruments a viable quantitative analysis tool for food systems. Proximate analysis of food is one sector in which FT-IR can really be utilized because food matrices are mainly composed of protein, fat, carbohydrates, and moisture, all of which show distinct absorption peaks in a spectrum. The use of the ATR enables analysis of food because it allows thick liquids, pastes, and solid food items to be measured (Van de Voort, 1992). The development of FT instruments and sample presentation options (such as the ATR) has dramatically increased the use of mid-infrared spectroscopy as a tool in the food industry (Downey, 1998).

Benefits of mid infrared analysis is the ability to identify unknown species, the presence of characteristic bands for organic functional groups, and is in a part of the spectrum that relies on fundamental vibrations. Some negatives include the expense of transmitting materials, the energy that is available lowers with wavelength, and cells need to have a short path length due to the fact that many compounds absorb in this mid-infrared region. If compounds cannot be measured using MIR, they may be able to be analyzed using near infrared spectroscopy (NIR) (Karoui et al., 2008).

1.1.2 Near Infrared Spectroscopy

NIR is used more for quantitative analysis of food as compared to MIR. A major benefit to using NIR is its use of diffuse reflection techniques to directly measure the composition of solid food products. Specular reflection often occurs in NIR, which is when radiation hits a solid material, a
part of the radiation is reflected from the surface of the sample; it often does not provide much useful information. Most of that radiation goes back to the energy source; however some of it will penetrate through the sample and then be reflected off several sample particles before leaving the sample. This is known as diffuse reflection and arrives at the surface of the sample at random angles through 180°. Every time the radiation connects with sample particles, the chemical components present in the sample can absorb part of the radiation. So, the diffusely reflected radiation possesses information about the chemical makeup of the sample, which is determined by the quantity of energy absorbed at specific wavelengths. Overtones and combination bands are the prominent absorption bands present in the NIR area, which are weaker in intensity. The weak intensity is advantageous because absorption bands that have enough intensity to be observed in this region come from functional groups that have a hydrogen atom attached to a carbon, oxygen, or nitrogen atom; these are functional groups present in protein, water, lipids, and carbohydrates, which are the main components of food. NIR spectra are more complex because the bands often overlap and are rather broad. Reflectance or transmittance measurements can be made using NIR instruments, which tend to use monochromators as opposed to interferometers to disperse the frequencies of radiation. Reflectance measurements are used for solid samples while transmittance measurements are used for liquid samples. Although NIR can be used both for qualitative and quantitative analysis, it is most commonly used for quantitative applications because of its ability to directly measure the amounts of different food components in a sample based on the absorption at different wavelengths (Wehling, 1998).
NIR is widely used in the food and beverage industry for analysis. It can be used for quantification of food ingredients, food adulteration, detection of defects in foods, and sensory determination. It is widely used for cereal products, coffee, fruit products, honey, meat products, dairy products, tea, and so on (Karoui et al., 2008).

1.1.3 Portable Infrared Systems

There has been a rapid development in smaller FT-IR instruments, which are compact, reliable, and versatile, such as the Cary 630 FT-IR. It operates the same way as a traditional benchtop FT-IR system, but is much smaller and versatile than these traditional systems. It can be used to measure solids, liquids, or gasses, with different sampling accessories such as the ATR which can easily be snapped into place or switched out for a different accessory (Agilent Technologies, 2013). The Cary 630 FT-IR is pictured below in Figure 4.

Figure 4. Cary 630 FT-IR Spectrometer with sample accessories (Agilent Technologies, 2013).

These types of systems were originally intended for use in identification of illegal drugs,
weapons of mass destruction, and other hazardous material (Rein, 2008). The employment of these systems in recent times has branched out into the food industry and has a variety of applications. Agilent, the manufacturer of the Cary 630 FT-IR, has reported uses of this system on quality control of coffee, tea, sugars, flours, and dairy powders; it can also be used in the pharmaceutical industry, chemical industry, and in academia (Agilent Technologies, 2013). Portable systems will continue to grow in different industries because of the desire for more in-field and rapid testing techniques (Rein, 2008).

### 1.2 Crops Indigenous to the Andean region and South America

The Incans cultivated many different plants before the Spanish conquest, almost as many as the growers of Europe and Asia. Once the Spaniards conquered Peru, they suppressed the culture of the Incans including the crops that were of such high value. A lot of these important crops were forced into obscurity, while some of the native crops remained in the hills for over 500 years since the conquest. These crops have not received much attention from the scientific community and have not been investigated for their potential health benefits, adaptability, overall nutrition, and taste. These crops did not reach extinction due to the fact that the natives still cultivated these crops high up in the Andean region. Some of these crops that escaped from this region have grown to become very popular worldwide, i.e. tomatoes and potatoes. Even though some crops have reached notoriety, most still remain unknown to the world. If more research is done on these native crops, then they could become used worldwide for their different functionalities, tastes, and health benefits (National Research Council, 1989).
1.2.1 Roots and Tubers

Maca (*Lepidium meyenii*) is relatively unknown and is grown in regions of high altitudes and extreme conditions (intense sunlight, violent winds, and very cold temperatures). It is a tuberous root with a tangy taste and has an aroma that is very similar to butterscotch. Once it is cultivated it can grow in regions where even bitter potatoes cannot grow, making it an essential and durable crop for the region. It closely resembles a radish, which is in the same family, and can be yellow, purple, or yellow with purple bands. Maca contains higher amounts of sugars, starches, protein, iron, and iodine. It is sweeter than cocoa when cooked in water, and when dried it is sweet and spicy and considered a delicacy. Maca has been linked to enhanced fertility in both humans and livestock (National Research Council, 1989). Maca has been shown to reduce psychological symptoms such as depression and anxiety, and lowers measures of sexual dysfunction in postmenopausal women independent of hormones (Brooks et al., 2008). Peru’s maca harvest has been growing, and much more exportation is being done to regions such as the United States and Japan (Brinckmann & Smith, 2004). The market for maca is ever-growing and with more research it can become a highly touted commodity.

Mashua (*Tropaeolum tuberosum*) is one of the most popular root crops in the Andean region and is very resistant to pests, insects, and cold temperatures and grows throughout the area. The tubers of the mashua plant are similar in size to small potatoes, and can be eaten raw, in which it has a taste similar to that of a radish, or can be cooked where the flavor becomes mild, and can even be used in desserts. It is rich in vitamin C and also contains a variable percentage of protein depending on the type. The tubers vary in color from yellow and white to sometimes
purple or red (National Research Council, 1989). Mashua has been used as folk remedies for various conditions including kidney and liver pain, skin eczemas, and prostate disorders. A lot of the health benefits associated with mashua is due to its presence of antioxidant phenolic compounds. Mashua tubers that were purple were shown to have the highest antioxidant activity, which could be related to the high amount of anthocyanins present as well. Although further studies have shown that the phenolic compounds present play a bigger role in the antioxidant capacity than previously expected (Campos et al., 2006). Phenolic compounds can have antibacterial, antimutagenic, and anticarcinogenic effects on the body (Chirinos et al., 2006). Not much information exists on the health benefits of mashua, but from the research that has been done, it appears to be a good option especially for people who live in the area in which it grows.

Algarrobo (Prosopis pallida) pods were a vital part of the diet for the indigenous people of Peru, Argentina, and the deserts of North America. The mesocarp portion of the pod is often used to produce flour, usually by milling or sieving. In terms of energy and protein content, it is similar to wheat flour, but it varies from wheat flour because of its distinct flavor and its lack of gluten does not make it ideal to provide texture to products; it has a taste and aroma similar to cinnamon and mocha and is used more for flavoring in desserts. It can also be used as a coffee substitute and be processed into syrups for flavoring (Felker, 2003). The pods are rich in sugars, proteins, and carbohydrates and are desirable for consumption, although it is mostly given to livestock (Choge et al., 2007). It is an underutilized food that has the potential for marked success mainly in the dessert and beverage industry (Felker, 2003).
1.2.2 Exotic Grains

Many of the crops that the Incans relied on for sustenance included many grains. Many of these grains were on the brink of extinction, only being cultivated by the natives of the region. However, due to the increasing concern for more natural products that are nutrient dense, the use of some of these ancient grains are seeing a resurgence. The most well-known Incan grain that has seen worldwide success is quinoa (National Research Council, 1989).

Quinoa (Chenopodium quinoa) was one of the most vital grains in the Incan culture and was even considered sacred (National Research Council, 1989). It is a pseudo cereal that has been cultivated in the area for thousands of years. It is referred to as a pseudo-cereal because it produces a cereal like seed but comes from a broad leaved plant. After the Spanish conquest, quinoa was pushed to the side in favor of potatoes and barley. Due to the destruction of these other crops by drought and the failure of green revolution, these grains such as quinoa were renewed as viable crops due to the crop’s ability to withstand severe conditions. It is grown in a wide range of regions in South America, mostly around the Andean region, at varying latitudes and sea levels. It has been introduced to most European countries as well as to Africa, Asia, and North America. The quinoa plant has large leaves that vary in color due to betacyanins present in the leaves. The highly developed root system helps to protect the plant against drought conditions (Bhargava, Shukla, & Ohri, 2006). Several varieties of quinoa contain saponins, which are bitter antinutritional compounds; if these saponins reach the bloodstream they could cause cells to lyse (Mastebroek, Limburg, Gilles, & Marvin, 2000). Because of the presence of these saponins, quinoa is thoroughly washed or milled to remove the outer layers of the seed coat.
(National Research Council, 1989). Quinoa is a very versatile grain that can be prepared and consumed in a variety of ways. It has been made into flour and is being used in soups, alcohol, or can be fermented to make beer. It is used to make quinoa tortillas, pancakes, and puffed grains, as well as food for livestock (Bhargava et al., 2006). Quinoa is highly sought after because of its exceptional nutritional content. It is considered a complete protein, containing all of the essential amino acids, comparable to that of casein the milk protein. Quinoa is a great thickener for frozen foods due to its good freeze-thaw stability. It is also rich in calcium, magnesium, iron, copper, and zinc, which are in higher amounts than most other grains (Vega-Gálvez et al., 2010). Quinoa is becoming increasingly popular for those who have to follow a gluten free diet because of Celiac’s disease; it is a gluten free grain that is more nutrient dense, containing a variety of minerals, vitamins, and a balanced amino acid profile (Alvarez-Jubete, Arendt, & Gallagher, 2009). It has also been shown to have intermediate antioxidant activity, which helps the body decrease forms of damage caused by free radicals (Peñarrietaabc, Alvaradoa, Akesson, & Bergenstahl, 2005). Overall with the exceptional nutritional profile of quinoa, the market for it is ever growing and there is an increase in demand for a product that is gluten-free as well as providing a balanced amino acid profile for those who do not consume meat (Alvarez-Jubete et al., 2009).

Kiwicha or commonly known as amaranth (Amaranthus caudatus L.) has been a staple crop in the Incan culture, with a popularity similar to corn in the Americas. The plant is well known for its beauty, with vibrantly colored stems, leaves, and flowers; it can also withstand drought, heat, and pests, but can also easily adapt to new environments. Amaranth can be prepared and
consumed in a variety of ways; it can be popped like popcorn, consumed like a cereal, can be used as a breading for fish or meat, and it can also be ground into flour to make other products. Because of its lack of gluten, it is a great gluten-free option for a grain like product in the diet (National Research Council, 1989). It is also considered a pseudo-cereal, related to both quinoa and cañihua (Bhargava et al., 2006). Amaranth has a higher protein content than most other cereal grains, making it a more desirable grain to incorporate into the diet. It also has a higher lysine content which makes it more useful to increase the nutritional value of some processed foods. The starch present in the amaranth grains are quite small as compared to other grains, and because of this it may have different freeze/thaw characteristics as well as gelatinization characteristics which could be useful in the food industry. The lipid portion of amaranth has a high proportion of squalene and tocotrienols as compared to other grains (Stallknecht & Schulz-Schaeffer, 1993). Squalene is an intermediate in cholesterol biosynthesis and has been found to lower cholesterol levels and to act as an anticancer agent (Tikekar, Ludescher, & Karwe, 2008). Tocotrienols have also been shown to be effective in lowering cholesterol levels (Stallknecht & Schulz-Schaeffer, 1993). Amaranth has been growing in popularity and will continue to grow in the market due to its exemplary health benefits.

Cañihua (*Chenopodium pallidicaule*) is a native food plant to the Andean region and has been used as a food source for centuries in the Incan culture (Repo-Carrasco, Espinoza, & Jacobsen, 2003). It often grows in areas where other crops would not survive; it is more resistant than most other grain crops to frost, drought, salt, and pests. It often grows in the extreme highland areas where most other plants cannot grow because of the extreme cold. Cañihua produces a
cereal like seed, although it is actually a pseudo cereal because it botanically is related to quinoa and is a broad leaved plant. Unlike quinoa, its seeds contain little saponins and require less processing, although the husking process is quite troublesome (National Research Council, 1989). Both cañihua and quinoa are often substituted for sparse animal protein in the Andes region. Cañihua has a high chemical score, because of its balanced essential amino acid profile similar to that of casein. It also has a high amount of oil as well, mostly being composed of linoleic acid (an omega-6 fatty acid). Cañihua also has a higher proportion of γ-tocopherol, which acts as an antioxidant and will help extend the shelf life of the product. It also has higher amounts of the minerals calcium and phosphorous. Overall, cañihua has a similar nutritional profile to quinoa and is a great substitute for those that follow a gluten free diet, or those who need protein from non-animal sources, or just overall as a healthy item to include in the diet (Repo-Carrasco et al., 2003).

Sacha Inchi (*Plukentia volubilis* L.) is commonly known as the Incan peanut, and is a crop native to the Andean region; it has been a part of the diet for thousands of years (Fanali et al., 2011). The seeds of the plant are generating interest due to the high oil content present in these seeds (35-60%), which have higher levels of both linoleic and linolenic acids. The ratios of these fatty acids are of particular interest because they are important in the prevention of coronary heart disease and hypertension, having a cholesterol lowering effect when taken as a supplement. The protein content in sacha inchi is also similar to other oil seeds like soybean, cottonseed, and sunflower. The sacha inchi seeds also contained higher amounts of magnesium, calcium, zinc, and potassium, making it a viable source for a variety of minerals. Although not much information is available on sacha inchi, it is starting to reach more recognition due to its fatty
acid profile as well as the ability to be processed into flour for cooking purposes or its use as an oil (Gutiérrez, Rosada, & Jiménez, 2011).

1.3 Domesticated Plants

Barley (*Hordeum vulgare*) belongs to one of the most important plant families which include wheat, rye, and crop rye wheat; it is produced throughout the world, from the Andes to China and the Middle East. It can be used for livestock feeding, food for humans, or in the production of alcoholic beverages (Horsley, Franckowiak, & Schwarz, 2009). Barley can be further processed in order to make flour, flakes, or grits, which can be used in a variety of products from cereals to soups and flatbreads. Barley is mostly used in the production of alcoholic beverages, and its use in the food industry has declined over the years in favor of other grains such as rice and wheat. Barley grains are mostly composed of starch (around 65-68%), are low fat, and provide a significant amount of fiber to the diet; they also provide a relatively well balanced amino acid profile, vitamin E, and antioxidants such as polyphenolics. The endosperm cell walls are rich in B-glucans which can help lower cholesterol levels as well as have a positive effect on glucose levels. Due to its availability and great nutritional profile, the market for barley in the food industry is growing (Baik & Ullrich, 2008).

Soybean (*Glycine max* L.) is one of the most popular crops worldwide. Cultivation of soybeans originally started in China, and then in the 1950’s the United States soybean production rapidly increased, and the U.S. is now the largest producer of soybeans in the world (Qiu & Chang, 2010). Currently, soybean is mostly used for its oil as well as the protein rich de-oiled cake, which is an important byproduct of the oil extraction process (Singh & Shivakumar, 2010).
The soybean is touted for its high protein content (~40%) and its oil content (~20%), making it a viable crop for consumption (Mishra & Verma, 2010; Panthee, 2010; Rao & Reddy, 2010; Ghosh & Jayas, 2010). In North America and Europe, it is mostly used for oil production and for protein-rich feed for animals; however in other parts of the world it is used for human consumption, from tofu and sprouts, to soymilk and soy nuts (Panthee, 2010). Soybeans contain many essential amino acids including lysine, tryptophan, methionine, and cysteine. The oil also contains lecithin, which can be used in a variety of ways from emulsification to lubrication and even for pharmaceuticals. Soybean is also one of the most inexpensive sources of protein (Rao & Reddy, 2010). Soybeans can also be processed into flour, grits, protein isolates, and used in a variety of products such as soups, bakery products, snack foods, and salad dressings (Ghosh & Jayas, 2010). Research has shown that the phytochemicals present in soybeans are health promoting; although isoflavones have garnered the most attention. Isoflavones appear to work together with soy protein to have anti-carcinogenic, anti-atherogenic, and anti-osteoporotic effects, among various other health benefits (Omoni & Aluko, 2005). Overall, the health benefits of soybean are vast, and the market for soybeans is growing, and does not appear to be slowing down (Ali, 2010).

Sesame seeds (*Sesamum indicum* L.) are one of the oldest cultivated plants in the world, but production has been low due to its low yield. It is an important oil seed crop due to its high oil content, a balanced fatty acid profile with generally equal amounts of linoleic and oleic acids; the oil is also resistant to oxidation because of the presence of antioxidants, both sesamol and sesaminol as well as tocopherols (Baydar, 2005). They also contain lignans, sesamin and
sesamolin, which have been shown to have positive health effects as well (Chen et al., 2005). Sesame seeds are also rich in protein (~19-25%), and have a desirable amino acid profile, similar to that of soybean protein. It can be used as cooking oil, in bakery and confectionary products, can be processed into flour, and also the oil could be used for margarine, paints, and varnishes (Tunde-Akintunde & Akintunde, 2004). The sesame seeds and their hull fractions have been shown to possess some antioxidant activity (Shahidi, Liyana-Pathirana, & Wall, 2006). Sesame has also been shown to lower cholesterol levels and enhance antioxidant activity in people with high cholesterol (Chen et al., 2005).

Flaxseed (*Linum usitatissimum*) has been part of the diet in Asia, Europe, and Africa for centuries (Williams et al., 2007). The flax crop produces seeds that vary in color from reddish brown to golden yellow. Flaxseed is found as whole seeds, ground seeds (meal), or flaxseed oil. The whole flaxseed contains around 40% fat, 28% fiber, 20% protein and multiple vitamins and minerals. Flaxseed oil is garnering attention due to its high percentage of polyunsaturated fatty acids (~73%), with 18% monounsaturated fatty acids, and about 9% saturated fatty acids; it is one of the highest sources of ALA, an omega 3 fatty acid, which makes up about half of the fatty acids present. Flaxseed also contains both soluble and insoluble fiber; the soluble fiber mucilage contributes to some of the cardioprotective effects from flax. It also contains a large amount of phytoestrogens called lignans, which can help lower cholesterol. Flax can protect against cardiovascular disease by reducing cholesterol levels, platelet aggregation, and inflammatory markers as well as acting as an antioxidant and improving glucose tolerance (Bloedon & Szapary, 2004). Numerous studies have been done on the health benefits of both.
flaxseed and flaxseed oil, most of which have shown promising effects (Jenkins et al. 1999; Spence, Thornton, Muir, & Westcott, 2003).

Fava bean (*Vicia faba* L.) is a very popular legume in Africa, Europe, and Asia. It contains higher quality proteins and is relatively inexpensive, contains a fair amount of carbohydrates, vitamins, and minerals. Fava beans contain many anti-nutritional compounds such as trypsin inhibitors, tannins, and phytic acid, although these levels can be decreased upon germination, which causes an increase in calcium, zinc, and iron; germinated legumes also contain more vitamin C, fiber, minerals, as well as functional non-protein amino acids L-DOPA and GABA. GABA is an inhibitory neurotransmitter that alleviates pain and anxiety, as well as regulating blood pressure and heart rate (Li, Bai, Jin, Wen, & Gu, 2010). There is interest in the phenolic acid L-DOPA, due to its preventative effect against Parkinson’s disease. During Parkinson’s disease there is a lack of dopamine present, and L-DOPA can be transformed into dopamine upon arrival into the neurons in the brain (Shetty, Atallah, & Shetty K., 2003). Previous studies have confirmed the higher content of L-DOPA present in fava beans (Shetty, Atallah, & Shetty, K., 2001). Fava beans can be dangerous because of the presence of divicine, isouramil, and convincine, toxic compounds in fava beans which can increase the activity of the hexose monophosphate shunt, promoting hemolysis in glucose 6 phosphate dehydrogenase (G6PD) deficient individuals; this condition is called favism, which is subsequently named after the fava bean. It is only harmful for those individuals who suffer from G6PD deficiency (Irccs & Via, 2008). Overall, consumption of dry beans, peas, and lentils, could improve the quality of the diet in the United States (Mitchell, Lawrence, Hartman, & Curran, 2009).
Reed canary grass (*Phalaris arundinacea* L) is native to North America, Europe, and Asia but is now more widely dispersed throughout the world (Lavergne & Molofsky, 2004). It can be cultivated in low value areas, like bogs and fields not needed for food production (Kallioinen et al., 2012). It is a very invasive plant that can take over wet prairies, stream banks, and wetlands; it can also outcompete with native plants (Lavergne & Molofsky, 2004). Canary grass is also gaining popularity due to its potential use for electricity production, heat by combustion, and also for ethanol production. This is especially useful because of the invasiveness and prevalence of canary grass (Kallioinen et al., 2012). Its value for food production is limited to the young succulent shoot stage, and older stems are not desirable to either humans or livestock (Christian, Yates, & Riche, 2006). Although information is limited on this grass, its invasiveness and prevalence throughout the world make it a viable crop for usage in electricity and ethanol production as well as its use in paper making (Kallioinen et al., 2012; Christian et al., 2006).

Wheat and corn are considered 2 of the 3 major cereal crops, with vast amounts being harvested yearly (Shewry, 2009). Maize or corn (*Zea mays* L.) was first cultivated in Mexico, thousands of years ago, and became the staple crop in that area (Hallauer & Carena, 2009); breeding of maize started to occur when people realized the value of maize for food, feed, fiber, and fuel (Carena, Hallauer, & Filho, 2010). It has become one of the most popular crops worldwide as well as in the United States; each American consumes about 50 pounds of corn products per year, from popcorn, to corn chips, and even corn syrup (Grivetti, Corlett, & Lockett, 2001). Corn is mostly composed of starch, with smaller amounts of protein and fiber, and can be processed to produce oil (Davis, 2001). The phenolic phytochemicals present in corn
can have potential antioxidant effects and could play a role in management of hyperglycemia and hypertension associated with diabetes (Kwon, Apostolidis, Kim, & Shetty, 2007).

Wheat (*Triticum spp.*) is cultivated in a wide range of areas, from Russia, to Asia, and even North America. It is used in a wide variety of products, from breads and baked goods, to pastas and other products. Wheat contains a high amount of starch, with protein amounts similar to that of soybeans (~8-15%), and is also a good source of both iron and zinc. Whole grain wheat has significantly more nutrients as compared to white flour products, such as phenolic acids, lignans, fiber, and others. Wheat has garnered much attention because of a surge in Celiac’s disease, an allergy to gluten protein found in wheat, rye, and barley. Despite of this information, wheat will continue to remain a dominant force in the cereal crop industry due to its wide range of uses and its nutrition (Shewry, 2009).
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Chapter 2:

Authentication of Andean Flours using Benchtop FT-IR System and a Portable FT-IR Spectrometer

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2.1 Abstract

Andean indigenous grains such as quinoa (*Chenopodium quinoa*), cañihua (*Chenopodium pallidicaule*), and kiwicha (*Amaranthus caudatus* L.) have high nutritional value for the Andean region serving as principal protein sources, substituting for scarce animal proteins. The importance of these grains is based on their relatively high protein content with excellent composition of essential amino acids, gluten-free type, good source of dietary fiber, and bioactive compounds and minerals (calcium, zinc and iron). Because of their nutritional and economical importance, there is a risk for adulteration with less-expensive grains. Our objective was to develop a rapid analytical tool to characterize and detect adulteration of native Andean indigenous grains by combining infrared spectroscopy and pattern recognition analysis. Pure flours produced from the Andean region (quinoa, cañihua, kiwicha, maca, sacha inchi) and other (maize, mashua, soybean, wheat, linseed, algarrobo, canary grass, sesame, barley, fava bean) ingredients were provided by Universidad Nacional Agraria la Molina (UNALM) (Lima, Peru). In addition, commercial samples were obtained from various local markets (Lima, Peru) and used for predictions. Unique spectral data was collected with a portable attenuated total reflectance (ATR) mid-infrared spectrometer equipped with a diamond crystal, data was also collected on a benchtop ATR mid-infrared spectrometer equipped with a ZnSe crystal; all analysis was done by soft independent modeling of class analogy (SIMCA). Pure flours formed distinct clusters allowing for the evaluation of commercial samples from Peruvian markets, which showed some prevalence of adulteration. Samples were well separated indicated by interclass distances greater than 3. Spectral differences responsible for the separation of classes were attributed to C-O, C-C stretching and C-O-H and C-O-C deformation of
carbohydrates (950-1000 cm\(^{-1}\)) and the amide (1560-1665 cm\(^{-1}\)) region of proteins. ATR-IR spectroscopy in combination with chemometrics was a viable tool in characterization of Andean flour samples allowing for the rapid, “in-field”, and reliable detection of adulteration of food ingredients, making it a great alternative to traditional testing methods; results were also comparable to the traditional benchtop method.
2.2 Introduction

Before the Spanish conquest, the Incans had cultivated a wide variety of plants with exceptional nutritional benefits. During the Spanish conquest however, the Spanish suppressed the Incan culture as well as their native crops in favor of their own crops such as barley, wheat, and carrots. These plants were isolated to the highland region, where they were still cultivated by the natives who held onto the traditions of their culture; they even sold these products in the local marketplace. Some of these native crops have escaped the confines of the area and have reached massive success like potatoes, tomatoes, peppers, and lima beans. Many more of these crops remain unnoticed by the world, with the inability to take full advantage of their unique flavor profiles, functionalities, as well as their health benefits (National Research Council, 1989).

One of the most recognizable grains coming from the region is quinoa (Chenopodium quinoa), which has seen unprecedented success throughout the world. Quinoa is closely related to amaranth and beets and is a stress tolerant plant that has been cultivated in the Andean region for centuries. It is not considered a true cereal grain because it is actually a fruit, and also because of its balance of oil, protein, and fat, as well as unusual composition; it is instead referred to as a pseudo-cereal grain. It is highly touted because of its exceptional nutrition profile, containing all of the essential amino acids needed in the diet, making it comparable to casein, a protein found in milk. The starch granules make quinoa an ideal candidate for thickeners, especially because of its excellent freeze-thaw stability; it also has a high content of calcium, magnesium, iron, copper, and zinc, most of which are found in higher amounts than other grains. It also has a desirable fatty acid profile containing larger amounts of linoleic acid, a
polyunsaturated fatty acid; polyunsaturated fatty acids are beneficial against cardiovascular disease (Vega-Galvez et al., 2010). The seeds and the sprouts of quinoa have been shown to have high antioxidant activity, in addition to significant sources of total phenolics and anthocyanins, both beneficial for health (Pasko et al., 2009; Gorinstein et al., 2008).

Although quinoa is the most studied crop from this Andean region, many others exist that have exceptional nutritional benefits as well. Amaranth, which belongs in the same family as quinoa has been garnering much attention for its nutrition profile. It has a higher protein content compared to other grains, and a higher lysine content. The starch granules are similar in size to quinoa, providing the same freeze/thaw stability, making it a viable option as a thickener. The predominant fatty acid is linoleic acid, which has been shown to have positive health effects (Stallknecht & Schulz-Schaeffer, 1993). Amaranth has also been shown to possess some antioxidant activity (Gorinstein et al., 2008; Tikekar et al., 2008). Other native crops such as maca, mashua, cañihua, and sacha inchi also have unique properties making them desirable additions to the diet. Maca can withstand extreme weather conditions, making it a very durable and reliable crop for harvest. It has been linked to enhanced fertility in humans and livestock (National Research Council, 1989). It has also been shown to reduce psychological symptoms such as depression and anxiety, and lowers measures of sexual dysfunction in postmenopausal women independent of hormones (Brooks et al., 2008). Mashua is a tuberous root that varies in color from yellow and white to sometimes purple or red (National Research Council, 1989). It also possesses antioxidant activity and has been used as a folk remedy throughout the years (Campos et al., 2006; Chirinos et al., 2006; National Research Council, 1989). Some of these
crops are especially valuable to the region due to the higher protein content, which natives rely on for their main sources of protein (National Research Council, 1989).

There is a lack of information available on most of these crops, making it difficult to determine the functionality and uses of these products (National Research Council, 1989). It has been shown that mid-infrared spectroscopy in combination with chemometrics has been a viable tool in classifying different flours. Information can be obtained about the functional groups present and can be used to characterize raw materials and the composition of food matrices (Cocchi et al., 2004).

Adulteration in the food industry is a growing concern, although it has occurred throughout time (Schieber, 2008). Economic adulteration is one of the most common types of adulteration, defined as the substitution, partly or fully, of cheaper or inferior products for high cost foods in order to defraud the customer. Although rarely causing health hazards, it is driven by the demand for higher cost products and the ability for companies to make more money by adulterating with less expensive ingredients (Arvanitoyannis, 2008). Due to the exceptional health benefits of these Andean flours, as well as their limited availability, adulteration with inexpensive ingredients can likely occur. Infrared spectroscopy is a fast and non-destructive method that has been shown to be a valuable technique in the detection of adulteration in a variety of different foods (Schieber, 2008).
The objective of this study was to develop a rapid test combining ATR-MIR spectroscopy with chemometrics to characterize and detect adulteration in native Andean flours and flours obtained from markets in the Andean region in Peru.

2.3 Materials and Methods

Flour samples for analysis were supplied by UNALM, Peru. There were 45 samples total including standards and samples from different markets in Peru. Standards were obtained from various regions in Peru to account for varietal differences in the samples. Flour samples included: maize (corn), maca, algarrobo, trigo (wheat), sacha inchi, ajonjoli (sesame), alpiste (canary grass), linaza (flaxseed), quinoa, haba (fava bean), soya (soy), kiwicha (amaranth), cañihua, mashua, and cebada (barley).

2.3.1 FT-IR Spectroscopy

Infrared spectral data was collected on an ATR-MIR benchtop and portable spectrometers. A total of 4 replications each were done using the benchtop system, while 3 replications each were done with the portable system. The portable Cary 630 FT-IR spectrometer (Agilent Technologies Inc, Santa Clara, CA) had a single bounce diamond ATR crystal. The portable spectrometer collected data over the spectral range of 4000-700 cm\(^{-1}\) and the data was collected by co-adding 64 scans at a resolution of 4 cm\(^{-1}\). A background was taken after each sample run. Spectral data was collected in absorbance measurements and was viewed using MicroLab FTIR software. Other infrared spectra were collected on a benchtop Digilab Excalibur FTS 3500GX spectrometer (Varian, Randolph, MA) equipped with a KBr beamsplitter and
deuterated triglycine sulfate (DTGS) detector. A triple bounce ATR ZnSe crystal was used for benchtop data collection. Spectra were collected over a range of 4,000-700 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution and an interferogram of 64 scans were co-added. A background was taken after each set of samples. Spectral data was collected in absorbance measurements and was viewed using Resolutions Pro Software. The instrument was purged continuously with CO\(_2\) from a CO\(_2\)RP140 dryer (Dominic Hunter, Charlotte, NC, USA) to prevent interference in the spectra.

2.3.2 Data Analysis

The data was analyzed using multivariate statistical analysis software (Pirouette version 4.0, Infometrix Inc., Woodville, WA, USA). The spectra were imported into the software from the instruments as GRAMS (.spc) files and analyzed by normalizing and taking the 2\(^{nd}\) derivative for each spectra. Matching flour samples were identified by the same class number. Soft independent modeling of class analogy algorithm (SIMCA) was used to characterize the flour samples. A SIMCA plot was made of the standard flours which were used to predict the flour samples from the marketplace. If the flours from the marketplace deviated from the standards, then adulteration was suspected. SIMCA’s discriminating power plot was used to determine which bands in the infrared region were responsible for classification. Interclass distances greater than 3 (>3) indicated a significant difference between classes.
2.4 Results and Discussion

2.4.1 Characterization and Adulteration of Exotic Flours

In spectroscopy, specifically FT-IR spectroscopy, chemometrics is a vital tool for analyzing spectra (Shiroma & Rodriguez-Saona, 2009). Figure 5 shows the spectra of 3 different Andean flours, Quinoa, Cañihua, and Kiwicha. The use of chemometrics is important in analysis because these spectra appear very similar, but chemometrics can analyze the minor differences present between samples.

![Spectra of Quinoa, Kiwicha, and Canihua](image)

Figure 5. Mid- infrared spectra of Quinoa, Kiwicha, and Canihua using a benchtop FT-IR system.

The use of FT-IR with chemometrics has been widely used in food industry for a variety of purposes including characterization, authentication, and detection of adulterants in food samples (Rodriguez-Saona & Allendorf, 2011). SIMCA is a classification method that is based on Principle Component Analysis (PCA) that classifies samples based on differences in their
composition as observed by absorbancies in the infrared region (Allendorf, Subramanian, & Rodriguez-Saona, 2012).

The SIMCA plots of the standard flours using the ATR-MIR portable spectrometer and the benchtop ATR-MIR can be seen in Figure 6. The different colored classes indicate different types of flours, with several data points in each group indicating replications. Flours indicated as standards were used in the model to predict flours obtained from the marketplace for authenticity. The SIMCA plot shows the location of the classes in relation to each other. Samples that have more similarities in composition would lie closer together on the SIMCA plots; those that are vastly different would lie farther apart. However a better way to analyze the distances between the different classes are to look at interclass distances (ICD). The discriminating power plot provides information about what functional groups or compounds are the most vital for classification (Shiroma & Rodriguez-Saona, 2009). Figure 6 shows the discriminating power plot for the portable FT-IR spectrometer and the benchtop FT-IR spectrometer.
Figure 6. SIMCA and discriminating power plot of standard flours using portable (A,B) and a benchtop FT-IR spectrometers (C,D).
The fingerprint region for mid-infrared analysis is 900-1500 cm\(^{-1}\), where many compounds absorb. The 1500-1700 cm\(^{-1}\) region is associated with proteins, specifically the amide portion of proteins (Karoui, Downey, & Blecker, 2010). Major peaks in that region were observed in the portable system, specifically peaks around 1562 and 1666 cm\(^{-1}\); more minor peaks in that region were observed in the benchtop system. This is expected because the flours have different protein compositions, containing different ratios of varying amino acids. The other major peaks observed in both systems are around the 950-1000 cm\(^{-1}\) region which are associated with C-O, C-C stretching and C-O-H and C-O-C deformation of carbohydrates (Shiroma & Rodriguez-Saona, 2009); the results are expected because carbohydrates are a main constituent of flour.

The interclass distances were used to analyze the separation of the flours, with distances > 3 indicating a significant difference between samples (He, Rodriguez-Saona, & Giusti, 2007). Table 2 shows the interclass distances between all standard flour samples for the portable and benchtop systems. Interclass distance values for the portable system are reported in bold. The most notable value is 2.6, the interclass distance between cañihua and kiwicha. There are many similarities between these 2 Andean crops. Cañihua contains ~12-16% protein, while kiwicha contains ~15-19% protein, both consisting of sulfur containing amino acids (Mujica, 1994); Also from the interclass distances we can start to understand the similarities and differences between samples. Another notable interclass distance is 3.0, which is the distance between
ajonjoli (sesame seed) and soya (soy). They have similarities in amino acid profiles, containing comparable amounts of the branched chain amino acids, valine, leucine, and isoleucine (Oomah, 2001). Table 2 also shows the interclass distances of all the flour samples using the benchtop system which are reported in a different color. All of the interclass distances are above 3, indicating that all of the samples are significantly different from each other. The most notable value is the distance between cañihua and kiwicha; as noted previously they have similar amino acid profiles.
Table 2. Interclass distances between all standard flours using portable and benchtop FT-IR spectrometers.

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<td>21.9</td>
<td>17.1</td>
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<td>8.9</td>
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<td>27.8</td>
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<td>9.1</td>
<td>21.8</td>
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<td>22.7</td>
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<td>29.4</td>
<td>28.2</td>
<td>7.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

C1 is Canihua, C2 is Cebada, C3 is Haba, C4 is Kiwicha, C5 is Maca, C6 is Maize, C7 is Mashua, C8 is Quinoa, C9 is Soya, C10 is Trigo, C11 is Ajonjoli, C12 is Algarrobo, C13 is Alpiste, C14 is Linaza, and C15 is Sacha Inchi.
Overall, both systems have shown an ability to separate the different flour classes, indicated by interclass distance values greater than 3. Interclass distances as well as the SIMCA plots can help provide information about characterizing the different flours. By assessing the interclass distances and locations on the SIMCA plot, one can gain some information on these flours. As previously mentioned, cañihua and kiwicha had smaller interclass distance values, and as seen on the SIMCA plot they are relatively close together. This means that they have some similarities in chemical composition, such as their amino acid composition, which was a defining peak classified in the discriminating power plot (Berganza et al., 2003; Repo-Carrasco et al., 2003). Some of the flours with the highest interclass distances were between Maca and Sacha Inchi (32.8) for the portable system and between Algarrobo and Alpiste (90.9) for the benchtop system. These values indicate that the samples are vastly different from each other in composition. The benchtop achieved greater separation between samples indicated by higher interclass distance values overall, with no values less than 3. The portable system has smaller interclass distance values, with 1 value less than 3. Overall the benchtop system was more successful in separating the flour samples, this is because it is a triple bounce system, so light interacts with the sample more than the single bounce portable system, and therefore more reliable results were obtained (Wilkerson, Sayajon, Santos, Rodriguez-Saona, Anthon, & Barrett, 2013).
FT-IR is a widely used technique to test for food authenticity. It is a rapid and non-destructive method that does not require the use of hazardous solvents. The standard flours seen in Figure 6 were used to predict the authenticity of flours from the different markets using FT-IR. Flours tested for authenticity were: haba, maca, cañihua, quinoa, kiwicha, soya, and cebada. Predictions were done using SIMCA. A training set is made that is used to develop the classification rules, and an evaluation set, which is the set that is being tested for reliability (Forina, Oliveri, Lanteri, & Casale, 2008). The training set is the standard flours, while the evaluation set are the flours from the market. In theory if the flours from the markets were authentic, then they would fall into the same location on the SIMCA plot as the standard flours, as well as being predicted into the same class as the standard flours. Figure 7 shows the SIMCA prediction plots for both the portable system (A) and the benchtop system (B).
Figure 7. SIMCA prediction plots of market flours using a portable FT-IR spectrometer (A) and a benchtop FT-IR spectrometer (B).
The circles on the predicted plots indicate where the standards of the flours should be located. If the market flours were in the same location as the standards, that means that they were predicted correctly as the same flour and are authentic. If they deviate from the position of the standards, then there is a chance for adulteration with cheaper flours. As seen in Figure 7 most of the market flours fell into the same location as the standards, however two of the flour samples did not. Kiwicha and quinoa fell into different locations with the portable and benchtop systems. Tables 4 and 5 show the prediction tables obtained from both the portable system and benchtop system. The information provided describes which class of standard flours the market flours were classified under. The market flours are listed horizontally while the standard flours are listed vertically in the table. For example, of the samples labeled cañihua from the market, all of them were predicted into the cañihua group with the standards; this indicates that there is no adulteration in those samples from the market. The data from the table correlates well with the SIMCA plot, showing that the troublesome samples are both kiwicha and quinoa with both systems. For both systems, some kiwicha was predicted as haba, meaning that the kiwicha could have been adulterated with haba flour which is more inexpensive; this can also be seen in the SIMCA plots as the color associated with kiwicha is overlapping with haba. Quinoa and other kiwicha samples could not be predicted into any class of standard flour, which could be due to the fact that they could have been adulterated with a mixture of different flours that formed their own separate group.
Table 3. Prediction table of market flours using a portable FT-IR spectrometer.

<table>
<thead>
<tr>
<th>Flours</th>
<th>Cañihua</th>
<th>Cebada</th>
<th>Haba</th>
<th>Kiwicha</th>
<th>Maca</th>
<th>Quinoa</th>
<th>Soya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cañihua std.</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cebada std.</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haba std.</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiwicha std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maca std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashua std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoa std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Trigo std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ajonjoli std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Algarrobo std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpiste std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linaza std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacha Inchi std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Misclassified</td>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Prediction table of market flours using a benchtop FT-IR spectrometer.

<table>
<thead>
<tr>
<th>Flours</th>
<th>Cañihua</th>
<th>Cebada</th>
<th>Haba</th>
<th>Kiwicha</th>
<th>Maca</th>
<th>Quinoa</th>
<th>Soya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cañihua std.</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cebada std.</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haba std.</td>
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<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiwicha std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maca std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mashua std.</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoa std.</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soya std.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Trigo std.</td>
<td></td>
<td></td>
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<tr>
<td>Ajonjoli std.</td>
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</tr>
<tr>
<td>Algarrobo std.</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Alpiste std.</td>
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<tr>
<td>Linaza std.</td>
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<td>Sacha Inchi std.</td>
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<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These flours are susceptible to adulteration because of their high value on the marketplace. Quinoa is highly sought after because of its exceptional nutritional content. It is considered a complete protein, containing all of the essential amino acids, comparable to that of casein the milk protein. It is also rich in calcium, magnesium, iron, copper, and zinc, which are in higher amounts than most other grains (Vega-Gálvez et al., 2010). Quinoa is becoming increasingly popular for those who follow a gluten free diet as well (Alvarez-Jubete et al., 2009). It has also been shown to have intermediate
antioxidant activity, which helps the body decrease forms of damage caused by free radicals (Peñarrietaabc et al., 2005). Kiwicha is also a highly sought after grain due to its nutritional nature. Kiwicha has a higher protein content (12-18%) than most other cereal grains (Stallknecht & Schulz-Schaeffer, 1993); Corn and wheat have around 10% protein, while barley has around 12.5% protein (Young and Pellett, 1994). It also has a higher lysine content which makes it more useful to increase the nutritional value of some processed foods. The lipid portion of amaranth has a high proportion of squalene and tocotrienols as compared to other grains (Stallknecht & Schulz-Schaeffer, 1993), with amounts averaging around 3.6% of the oil portion being composed of squalene (Berganza et al., 2003). Barley contains about .2 mg squalene per 100 grams, while corn contains 1.6 mg squalene per 100 grams (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Squalene is an intermediate in cholesterol biosynthesis and has been found to lower cholesterol levels and to act as an anticancer agent (Tikekar et al., 2008). Tocotrienols have also been shown to be effective in lowering cholesterol levels (Stallknecht & Schulz-Schaeffer, 1993).

Overall, both FT-IR systems were successful in detecting adulteration in Andean flour samples from Peru. This method has been shown to be successful in detecting adulteration in meat products, edible oils, jams, and others (Karoui, Pierna, & DuFour, 2008).
2.5 Conclusion

Portable and benchtop mid-infrared FT-IR systems were effective in determining authenticity of flour samples from the Andean region; similar results were obtained with both systems. The importance of detecting authenticity in food samples is important for economic and legislation reasons, especially if consumers are being deceived by manufacturers. A portable system can now be used for rapid, in field testing to detect adulteration in food samples, and could be used for a variety of other applications with relative ease.

2.6 Acknowledgments

The authors would like to acknowledge UNALM for providing samples for this research and also Agilent Technologies for providing the portable FT-IR spectrometer used for this research.
2.7 References


crops: native potato (Solanum sp.), mashua (Tropaeolum tuberosum Ruiz & Pavon), Oca (Oxalis tuberosa Molina) and ulluco (Ullucus tuberosus Caldas).

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