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A COMPARISON OF ANALYTICAL TECHNIQUES FOR MEASURING DAIRY SOIL ON SPRAY DRYER FILTER BAGS

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A COMPARISON OF ANALYTICAL TECHNIQUES FOR MEASURING DAIRY SOIL ON SPRAY DRYER FILTER BAGS

By

Eric E. Graf

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ABSTRACT

A COMPARISON OF ANALYTICAL TECHNIQUES FOR MEASURING DAIRY SOIL ON SPRAY DRYER FILTER BAGS

By

Eric E. Graf

Visual inspection, ultraviolet (UV) fluorescence with visual inspection, three rapid swab methods, liquid extraction followed by measurement of total protein in the extract, total nitrogen analysis with a nitrogen analyzer, light microscopy, and Fourier-transform infrared (FTIR) spectroscopy were investigated for their usefulness in measuring dairy food soil residue on spray dryer filter bags. The rapid swab methods used were the AccuPoint ATP bioluminescence-based device, the Pro-tect protein-based device, and the SpotCheck^{Plus} glucose/lactose-based device. The nitrogen analyzer was the most useful technique for measuring dairy food soil residue on filter bags, because of its sensitivity, speed and robustness. Light microscopy and FTIR spectroscopy were found to be valuable adjunct techniques for confirming nitrogen analyzer results, and for investigating the types and distributions of residual soil present on filter bags.

The distribution of residual soil on clean-in-place (CIP) cleaned spray dryer filter bags was also investigated. A significant ($p<0.0001$) trend was found whereby residual soil levels increased as one moved down a filter bag from top to bottom. Five sub-samples appeared to be adequate for determining the overall cleanliness of spray dryer filter bags.

ACKNOWLEDGEMENTS

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This project would not have been possible without the cooperation of Mr. Arne Jensen of Simatek A/S and Dr. Thomas Riepe of Intensiv-Filter GmbH, who supplied the filter bags used in this study. Mr. Robert Soule of Neogen Corporation was very helpful with the various swab tests used. Thank you to Neogen Corp. (Lansing, MI) for providing an AccuPoint luminometer for use during this project, and to Carlyn Dairy Products (Mundelein, IL) for providing sample dairy powders.

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ABBREVIATIONS USED

AOAC:	Association of Official Analytical Chemists
ATP:	Adenosine triphosphate
ATR:	Attenuated total reflectance
CIP:	Clean-in-place
CIPed:	Cleaned-in-place
ESCA:	Electron spectroscopy for chemical analysis
FTIR:	Fourier-transform infrared spectroscopy
IDF:	International Dairy Federation
IR:	Infrared
ISO:	International Organization for Standardization
NFDM:	Non-fat dry milk
PAS:	Photoacoustic spectroscopy
SPC:	Standard plate count
UV:	Ultraviolet
XPS:	X-ray photoelectron spectrometry
XRF:	X-ray fluorescence

INTRODUCTION

Spray drying is commonly used in the dairy industry to produce dry, powdered products such as non-fat dry milk (NFDM). In a spray dryer, heated air and liquid feed are introduced concurrently at one end of the dryer chamber, and the product exits the other end of the dryer as powder entrained in the process air. Cyclones are used to separate powder particles from the process air by centrifugal force. However, a small percentage of the powder particles may remain entrained in the process air even after centrifugal separation in one or several cyclones. Cloth filter bags housed in a filter bag collector are used to remove the remaining powder from the process air before the air is exhausted. During spray dryer operation, powder gradually builds up on these cloth filter bags, reducing their efficiency. Periodically, pulses of reverse air flow are used to dislodge powder buildup on the filter bags, allowing extended operation of the spray dryer.

Wet cleaning of filter bag collectors is performed infrequently, usually when changing products, after extended shutdown of the dryer, or when maintenance is required. Before wet cleaning, filter bags are removed and sent to commercial laundries for cleaning. The filter bag collector is then wet cleaned either manually or with spray devices. Clean filter bags are reinstalled following wet cleaning of the filter bag collector.

A new generation of filter bag collectors has been designed to clean filter bags while they remain installed in the filter bag collector. This type of cleaning

process is henceforth referred to as clean-in-place, or simply CIP. These CIP ready filter bag collectors help processing plants minimize down time during cleaning. The effectiveness of the CIP process has been well established in the dairy industry for cleaning stainless steel pipelines and tanks in processing plants. However, CIP technology has not previously been used to clean cloth filters in the dairy industry. Thus, there is industry and regulatory interest in monitoring the effectiveness of CIP cleaning in filter bag collectors.

Commonly, bacterial growth is used as an indicator of the effectiveness of cleaning processes in the dairy industry, since food residues support bacterial growth. In filter bag collectors, however, the temperature is approximately 185-200°F during operations (R. Semerad, personal communication and Carić, 1994), and the moisture level of the food product is very low (below 5% wet basis). These conditions are too extreme for bacteria to grow, and in fact the high temperature causes bacteria to die off. The purpose of this study was to evaluate several alternative analytical methods for their effectiveness in measuring the cleanliness of spray dryer filter bags.

Visual inspection, ultraviolet (UV) fluorescence with visual inspection, several rapid hygiene test swab devices, light microscopy, Fourier-transform infrared (FTIR) spectroscopy, liquid extraction followed by measurement of total protein in the extract, and a nitrogen analyzer measuring total nitrogen by the combustion method were all examined for their ability to measure cleanliness of spray dryer filter bags. The rapid hygiene test swab devices used were the AccuPoint swab, an ATP bioluminescence-based swab device, the SpotCheck^{Plus}

swab, a glucose and lactose sensitive swab which changes color in the presence of either glucose or lactose, and the Pro-tect swab, a protein-based swab which changes color in the presence of protein.

LITERATURE REVIEW

Dairy Powder Characteristics

Typical gross compositions of several common dairy powders are given in Table 1 (Walstra *et al.*, 1999). Milk is a biological product and thus its composition varies somewhat, but in general, the values given in the table are representative of dairy powder products.

Table 1: Typical gross compositions (% w/w) of several common dairy powders.

Constituent	Whole milk powder	Skim milk powder	Whey powder	Sweet cream buttermilk powder
Fat	26	1	1	5
Lactose	38	51	72	48
Casein	19.5	27	0.6	26
Serum Protein	4.8	6.6	8.5	6.2
Ash	6.3	8.5	8	8
Water	2.5	3	3	3

Source: Walstra *et al.* (1999).

Dairy powders are composed predominately of organic material, namely lipids, proteins, and carbohydrate (lactose). Inorganic components such as calcium and phosphorus make up a relatively small fraction of dairy powders.

Several drying methods are available for producing dairy powders, but on a commercial scale, most dairy powders are produced by either roller drying or spray drying (Carić, 1994). Spray drying involves introduction of a finely dispersed liquid into a drying chamber with hot air flowing through the chamber.

As liquid droplets travel through the drying chamber, which can be either vertical or horizontal, moisture is evaporated from the droplets and at the exit of the dryer chamber, the product has been dried to powder particles. A system of centrifugal cyclones is commonly employed to separate the resulting powder from the process air. Figure 1 shows a vertical spray dryer with several cyclones for powder recovery. If a filter bag collector was also employed to separate fine particles from the exhaust air, the bag collector would be located downstream from the cyclones, near where number (8) is in Figure 1.

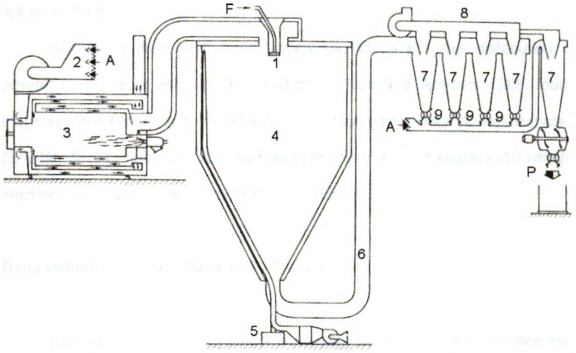

Figure 1: Vertical spray dryer with indirect air heater and powder recovery cyclones. (1) Atomizer, (2) Fan with air filter, (3) air heater, (4) spray drying chamber, (5) power source for pneumatic collector, (6) discharging pipeline for powder and air, (7) cyclone separators system, (8) air discharging pipeline, (9) valve. F, feed; A, air; P, product.
Source: Carić (1994).

Dairy powder particles vary in shape and size depending on the product but especially on the drying method (Carić, 1994). Buma (1971) described spray

dried whole milk particles as being approximately spherical and having diameters ranging from 5 to 150 μm. Buma (1971) also states that in whole milk particles, "fat globules and aircells are dispersed in a continuous phase which probably consists of amorphous lactose, milk salts and whey proteins." Walstra *et al.* (1999) agree with the view that lactose forms the continuous phase of spray dried whole milk particles by saying that "a milk powder particle generally consists of a continuous mass of amorphous lactose and other low-molar mass components in which fat globules, casein micelles, and serum protein molecules are embedded."

Several studies have been undertaken to assess the distribution of powder constituents within powder particles. Fäldt and Sjöholm (1996) found that spray dried whole milk particles had a surface composition of approximately 55% fat, 30% protein, and 15% lactose in the near surface region (~10 nm) by electron spectroscopy for chemical analysis (ESCA).

Dairy Industry Clean-in-Place (CIP) Practices

CIP cleaning technology has been used in the dairy industry for more than 50 years (Seiberling, 2002). Three major types of CIP units exit: single use, re-use, and multi-use (Sharp, 1985 and Timperley, 1989). These three unit types differ in the number of times a cleaning solution is used before being discarded.

Although CIP cycles vary depending on such factors as the type of equipment being cleaned and the product being processed, in general, a CIP

cycle in the dairy industry will consist of the following basic steps (Timperley, 1989 and Mauck *et al.*, 1993):

1. Complete draining of the equipment to remove as much product as possible.
2. Pre-rinsing with water, preferably warm (about 43°C), as soon as possible to prevent product residue from drying onto equipment and to remove as much soil as possible. Warm water helps remove fatty soils by keeping them from solidifying.
3. Recirculation of an alkaline detergent, usually chlorinated, at between 130°F (54.4°C) and 175°F (79.4°C) depending on the equipment surface to be cleaned.
4. Post-rinsing with cold water.
5. Recirculation of a sanitizer before production the following day. Common chemical sanitizers in the dairy industry are chlorine, iodine, peracetic acid, and acid anionic sanitizers (DiverseyLever, 2002).

Detergent Industry Test Methods for Fabric Cleanliness

Optical reflectance is commonly used to evaluate detergency (Cramer, 1972; Tai, 2000; and van Dalen, 2000). The method usually involves artificially soiling test fabric swatches and measuring the change in optical reflectance after washing. The method is simple, but does not distinguish among soil components (van Dalen, 2000). Visual inspection by trained panelists is also used to evaluate

the appearance of laundered test cloths (Tai, 2000), but again, this method offers little insight into the composition of any residual soil on the cloths.

Van Dalen (2000) attempted to measure protein on cloth using a nitrogen analyzer (discussed below under "Nitrogen Analyzer"), X-ray fluorescence spectrometry (XRF), X-ray photoelectron spectrometry (XPS), and Fourier-transform infrared (FTIR) spectroscopy (discussed below under "Fourier-transform Infrared Spectroscopy"). The study used blood and hemoglobin stains on cotton fabric to evaluate the techniques, and the author concluded that XRF was the most suitable technique for measuring protein build-up on cloths because of its accuracy and speed.

Various other analytical methods have been applied to soil analysis on cloths. These include gravimetric analysis, XRF, microscopy, field emission scanning electron microscopy, radiotracers, atomic absorption spectroscopy, gas chromatography, and liquid chromatography (Cramer, 1972; Kissa, 1987; and van Dalen, 2000).

Dairy Industry Hygiene Monitoring

Until recently, the usual method of assessing surface hygiene in dairy processing plants was to swab the surface with sterile cotton swabs stored in sterile buffer, and then to use microbiological tests on the buffer solution. Common microbiological procedures used with the sterile surface swab technique are the standard plate count (SPC) and coliform count (Murphy *et al.*,

1998) by the methods in *Standard Methods for the Examination of Dairy Products* (Wehr and Frank, 2004). Results from microbiological swabs are not available for 24-48 hours, however. Increasingly, rapid hygiene tests based on adenosine triphosphate (ATP) bioluminescence have been used in the dairy industry. These tests are based on reaction of ATP from microorganisms and food residue with the luciferin/luciferase enzyme complex to generate light, which is measured by a luminometer (Murphy *et al.*, 1998 and Carrick *et al.*, 2001). Results are available in minutes, allowing on-the-spot evaluation of surface cleanliness. The test typically involves swabbing a surface with a special swab containing all of the reagents necessary for the reaction, activating the swab, and placing the swab into a luminometer for measurement.

Several authors have found a correlation of about 70% between traditional SPC microbiological swab tests and ATP-based swab tests (Griffiths, 1996 and Murphy et al., 1998). That is, the two methods were in agreement whether a surface was clean or not 70% of the time. However, Carrick *et al.* (2001) tested four commercial ATP test systems and found that none produced linear results for detecting increasing known concentrations of ATP standards pipetted directly onto the swabs. However, one of the meters was tested without swabs using ATP standards in test tubes, and the resulting luminescence was linear. The authors also found that none of the test systems produced linear or consistent results when testing increasing levels of bacteria dried on a hard surface. The authors concluded that the inconsistent and nonlinear readings they observed were the result of the swab method and not of inherent faults with the

luminometers, since they observed linear results when testing ATP standard solutions. No attempt was made to compare results of the ATP swabs with microbiological swabs in this study.

Other non-instrument based hygiene monitoring tests are available to the dairy industry. These are normally swab-based tests which rely on some type of color change that is detectable visibly by the user, usually within minutes. Several tests based on detection of protein in this manner are available commercially (Moore *et al.*, 2001). Swab devices based on glucose or glucose plus lactose are manufactured by Hygiena International (Hygiena International Ltd., UK).

Nitrogen Analyzer

The nitrogen analyzer operates under a fairly simple principle. Samples are combusted at high temperature in a furnace and the combustion gases are collected. Carbon dioxide and water vapor are purged from the combustion gas, and nitrogen oxides are reduced to nitrogen gas by heating and exposure to copper. Nitrogen is then quantified by thermal conductivity detection of the resulting nitrogen gas.

The International Dairy Federation (IDF) has published a standard method for Determination of Nitrogen Content of milk and milk products by the combustion method (International Standard IDF, 2002). This standard has also been accepted by the International Organization for Standardization (ISO)

(International Standard ISO, 2002). However, the Association of Official Analytical Chemists (AOAC) has yet to recognize combustion nitrogen analysis as an official method for nitrogen/total protein analysis of dairy products, although combustion nitrogen analysis is a recognized AOAC method for crude protein in many other foodstuffs, including meats, cereal grains, oil seeds, and animal feedstuffs (AOAC, 2000). According to LECO Corporation, manufacturer of combustion nitrogen analyzers, the AOAC Dairy Collaborative is delaying adoption of a standard method for nitrogen/total protein in dairy products for "at least two years due to the inability to determine efficiency of current Kjeldahl method" (LECO Corp., 2005). A large interlaboratory study by Wiles *et al.* (1998) found the Kjeldahl and combustion methods produced similar results for 8 dairy products and 2 pure reference compounds among 11 laboratories.

Overall, the combustion nitrogen analyzer method appears to be suitable for analysis of dairy products for total nitrogen and protein by calculation.

One published study used a combustion nitrogen analyzer to measure nitrogen content of stains on cotton cloth (van Dalen, 2000). The author prepared soiled cotton cloths by pipetting known dilutions of whole blood or hemoglobin solutions onto cotton cloth, and found excellent correlation between nitrogen measured by a nitrogen analyzer and the relative concentration of blood or hemoglobin on cotton cloth. The author also found that the detection limit of the nitrogen analyzer was limited to about 0.02% nitrogen by the relatively high blank level of nitrogen on cotton cloth.

Fourier-Transform Infrared Spectroscopy

Absorptions in the infrared (IR) portion of the electromagnetic spectrum correspond to the vibrations of atoms in a molecule (Ismail *et al.*, 1997 and Stuart, 2004). There must be a change in the electric dipole moment of a bond in order for the bond to appear in an infrared spectrum (Sorrell, 1988 and Stuart, 2004). Thus, homonuclear diatomic molecules do not absorb infrared radiation because the dipole moment of such a bond is zero regardless of how long the bond is. For infrared active molecules, the wavelength of an absorption band depends on the relative masses of the atoms involved, the force constants of the bonds, and the geometry of the atoms (Silverstein and Webster, 1998).

Vibrations of atoms in a molecule due to absorption of IR radiation can be either a stretching (change in bond length) or a bending (change in bond angle) vibration (Stuart, 2004). Figure 2 illustrates stretching and bending vibrations in simple molecules. Some vibrations may be coupled over a larger portion of or even an entire molecule, giving rise to a complex absorption pattern that is characteristic of the molecule as a whole, not only a specific group within the molecule. This is known as an IR fingerprint of a molecule because most molecules possess fairly unique IR spectra (Stuart, 2004). Due to the complexity of the coupled vibrations within a molecule, the IR fingerprint of a molecule is very useful for identification of unknown molecules by comparison of spectra from unknowns with library spectra. Many commercial IR spectral libraries are

available, including libraries from Aldrich and Sadtler, for comparison of unknown spectra with reference library spectra.

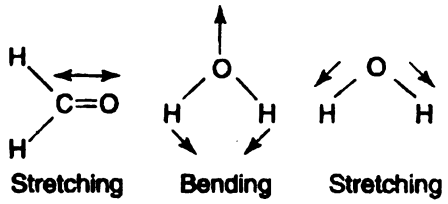

Figure 2: Stretching and bending vibrations of simple molecules.
Source: Stuart (2004).

Many IR absorption bands from certain groups of atoms occur at nearly the same wavelength regardless of the structure of the rest of the molecule they occur in (Silverstein and Webster, 1998). These absorption bands that are characteristic of certain groups of atoms within a molecule provide a very powerful tool for obtaining structural information about a molecule via its IR spectra. Several tables of characteristic group wavenumbers are available (Silverstein and Webster, 1998; Smith, 1999; and Stuart, 2004).

In infrared spectroscopy, spectra are normally given in wavenumbers rather than wavelengths. Wavenumber is normally expressed in cm^{-1}, and is simply the reciprocal of the wavelength expressed in centimeters. Wavenumber is popular because it is linear with energy. The region from 4000 to 400 cm^{-1} is called the mid-IR region (Stuart, 2004) and is considered the most useful portion of the IR region for qualitative analysis (Silverstein and Webster, 1998).

Infrared spectroscopy is currently dominated by Fourier-transform infrared (FTIR) spectrometers. Fourier-transform infrared spectroscopy relies on using

an interferometer to generate an interferogram of the interaction of a sample and IR radiation. A Michelson interferometer is most commonly used in FTIR spectroscopy (Stuart, 2004). A Michelson interferometer uses a semi-reflector called a beamsplitter to distribute IR radiation from the instrument's source to a set of two perpendicular mirrors, one of which is fixed and one of which moves during analysis. The mirrors reflect the IR radiation, which recombines at the beamsplitter. When the two beams of radiation recombine at the beamsplitter, they interfere with each other and a portion of this recombined beam is reflected by the beamsplitter towards the detector. A Michelson interferometer is shown in Figure 3 below.

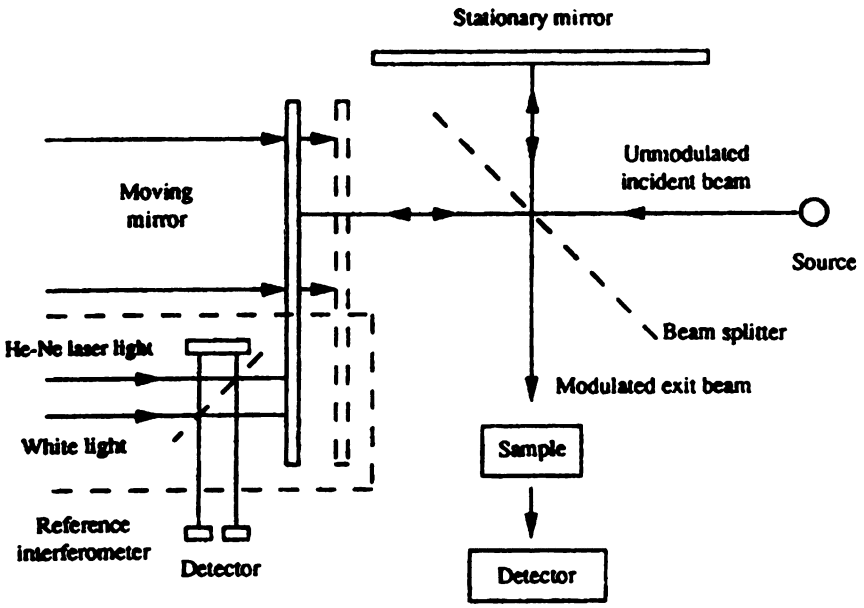

Figure 3: Schematic of a Michelson interferometer.
Source: Stuart (1997).

The interferogram is a response signal produced by the change of pathlength between the two beams of radiation emerging from the interferometer.

The interferogram is converted by a computer to a spectrum by a mathematical process known as Fourier-transformation. Fourier-transform IR instruments have numerous advantages over dispersive instruments. Dispersive IR spectrometers rely on a monochromator to allow only very narrow bandwidths of radiation to reach the detector at any one time. Fourier-transform infrared spectrometers offer more rapid scanning, higher signal to noise ratio, and better resolution than dispersive instruments (Ismail *et al.*, 1997 and Stuart, 2004).

Several methods are available for obtaining IR spectra from samples. For samples which allow sufficient transmission of IR radiation, transmission spectroscopy may be used. However, many solid samples are not amenable to transmission spectroscopy. Reflectance spectroscopy must be used for these samples. Reflectance techniques can be divided into two types, internal reflectance and external reflectance (Stuart, 2004). External reflectance techniques rely on reflecting an IR beam from the surface of a sample, and include specular reflectance and diffuse reflectance. Photoacoustic spectroscopy (PAS) is another reflectance technique. Photoacoustic spectroscopy utilizes a microphone to detect acoustic waves generated by heat released from a sample exposed to IR radiation in an acoustically isolated chamber (Stuart, 2004).

Attenuated total reflectance (ATR) spectroscopy is an internal reflectance technique that is rapidly gaining in popularity (Sedman *et al.*, 1999). Attenuated total reflectance relies on total internal reflection of an IR beam within a crystal of high refractive index, commonly zinc selenide (ZnSe), germanium (Ge), or

diamond. When an IR beam enters such a crystal at an appropriate angle, the beam will undergo internal reflection rather than pass through the crystal. Figure 4 shows an ATR system schematically.

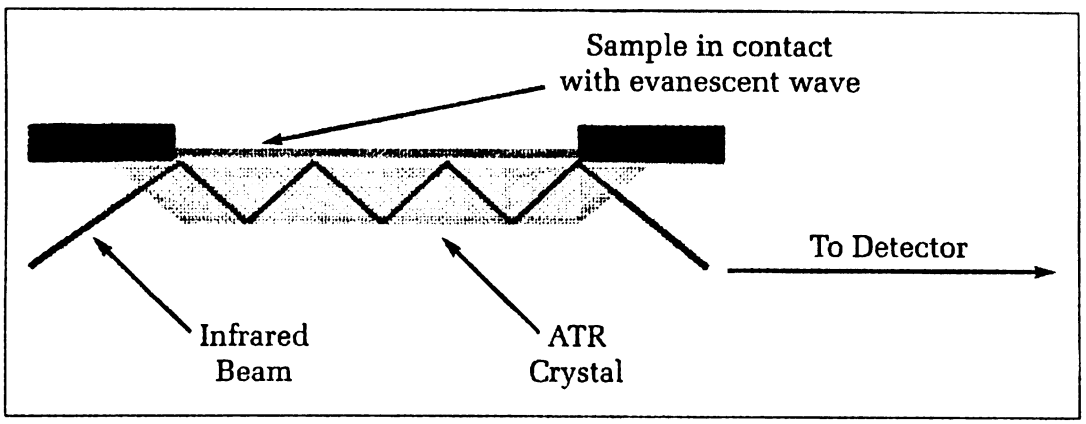

Figure 4: An attenuated total reflectance (ATR) sampling accessory.
Source: PerkinElmer (2004).

At the surface of the crystal, an evanescent wave is formed which decays exponentially as it travels away from the crystal surface (Ismail *et al.*, 1997). The evanescent wave penetrates a fraction of a wavelength past the surface of the crystal, so that if a sample is in close contact with the crystal, the evanescent wave is attenuated by absorption of radiation by the sample (Stuart, 2004). The depth of penetration, d_p, of the IR wave into a sample is a function of the refractive indexes of the sample and the crystal, the wavelength of the radiation, and the angle at which the IR beam strikes the crystal surface (Ismail *et al.*, 1997 and Stuart, 2004). The effective pathlength of an ATR cell is the product of the depth of penetration and the number of internal reflections of the IR beam in the crystal. Because d_p varies directly with wavelength, the effective pathlength in an

ATR spectrum decreases with decreasing wavelength, i.e., d_p decreases with increasing wavenumber. This causes a difference in the relative intensities of bands in an ATR spectrum compared to those in a transmission spectrum. This can usually be overcome by mathematical correction of the ATR spectrum (Sedman *et al.*, 1999).

The depth of penetration into most samples in the mid-IR with FTIR-ATR is 1-4 μm (Sedman *et al.*, 1999). This shallow depth of penetration is both an advantage and a disadvantage for FTIR-ATR. With highly absorbing samples, the ATR technique allows the collection of spectra that would not be possible to collect in transmission cells. However, the micrometer scale depth of penetration can be very limiting with inhomogeneous samples, especially when attempting quantification by ATR. The FTIR-ATR spectrum of a sample will only be truly representative of a sample when the sample is homogenous on the order of the depth of penetration (Sedman *et al.*, 1999). Also, the very small depth of penetration means that a sample must be held in very close contact with the ATR crystal, which is not always possible with rigid solid samples.

Fourier-transform infrared spectroscopy has benefited from improvements in data handling and processing, and notably from the use of chemometric techniques to analyze spectra (Brereton, 2003 and Stuart, 2004). Both quantitative and qualitative (discriminatory) data analysis techniques are available, and have recently been widely applied to FTIR spectroscopy of foods (Wilson and Tapp, 1999; Sedman *et al.*, 1999; and Stuart, 2004). For example, FTIR-ATR has been used successfully to detect adulteration of honey with invert

cane sugar (Sivakesava and Irudayaraj, 2001 and Irudayaraj *et al.*, 2003), detect adulteration of olive oil with vegetable oil (Tay *et al.*, 2002), evaluate the quality of frying oil (Innawong *et al.*, 2004), detect and classify adulterants in maple syrup (Paradkar *et al.*, 2002), and predicting functionality of whey and casein hydrolysates (van der Ven *et al.*, 2002). Fourier-transform infrared spectroscopy with ATR sampling was used with less success for the measurement of protein concentration in raw milk (Etzion *et al.*, 2004), where the prediction errors proved to be higher than acceptable for determination of payment. However, FTIR-ATR was used with good success to differentiate and quantitate bacteria (Gupta *et al.*, 2004).

Van Dalen (2000) applied FTIR spectroscopy using both ATR and PAS sampling techniques is his study on measuring protein on cotton cloths. He found both techniques to be capable of measuring the protein in blood and hemoglobin on cotton cloths, using calibration curves generated from artificially soiled cloths. The detection limit of FTIR-PAS was about 0.1%N and for FTIR-ATR it was about 0.2%N. The author also found that the repeatability of the ATR technique, especially at low protein concentrations, was somewhat limited by the inhomogeneity of the stain due to the small surface area measured by the ATR technique (about 150-200 μm in diameter).

MATERIALS AND METHODS

Filter Bags

Filter bag samples for this study were provided by Simatek A/S, Jerslev, Denmark, and Intensiv-Filter GmbH & Co. KG, Velbert, Germany. All filter bags were of polyester construction with stitched seams. For organizational purposes, the filter bags used in this study have been categorized into three groups.

Group 1. Each manufacturer originally provided one each of a brand new filter bag; a brand new, commercially laundered filter bag; a used, uncleaned filter bag; a used, cleaned-in-place (CIPed) filter bag; and a used, commercially laundered filter bag. The samples provided by Simatek A/S came from a four bag pilot scale CIP-able filter bag collector and were soiled with cream powder prior to cleaning. The samples provided by Intensiv-Filter GmbH came from a commercial plant in Germany that was producing a product containing vegetable fat in a whey base. These 10 filter bags were used to compare the various analytical methods.

Group 2. A second group of 13 filter bags was provided by Intensiv-Filter GmbH. This group consisted of 12 used, CIPed filters and one used, uncleaned filter. These filters came from a plant in eastern Germany that had been producing whey powders and non-fat dry milk. This group of filter bags was used with a nitrogen analyzer to evaluate the distribution of any residual soil present

on CIPed filter bags. Also, this group of filter bags was used to further evaluate the Pro-tect and SpotCheck^{Plus} swab methods.

Group 3. Simatek A/S provided a third group of three filter bags. These filters were all brand new and had been CIPed at Simatek's four bag pilot filter bag collector facility. This group of filter bags was used with a nitrogen analyzer to measure any cleaning chemical residues resulting from the CIP process.

Sub-Sampling of Filter Bags

Filter bags were sub-sampled by using a medical scalpel with No. 11 blades to excise samples of approximately 3x3 cm. Nitrile or latex single-use gloves were worn during handling of all filter bags and sub-samples. All sub-samples were stored in sealed plastic bags at room temperature until further analysis. For the Group 1 and Group 3 filter bags, five sub-samples were collected per filter bag, from the locations given in Figure 5 below.

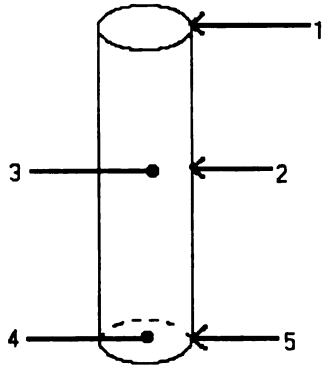

Figure 5: Sub-sampling locations for Groups 1 and 3 filter bags.

1. Sampling location 1 was located at the interface of the filter bag with the tube sheet, at the top of the filter bag.
2. Sampling location 2 was located at a point midway between the top and bottom of the filter bag, along the side seam of the bag.
3. Sampling location 3 was also located at a point midway between the top and bottom of the filter bag, except was from the sidewall of the bag, not from along the seam.
4. Sampling location 4 was located in the center of the bottom of the filter bag.
5. Sampling location 5 was located at the seam between the sides and the bottom of the filter bag.

These sub-sampling locations were selected to represent the different regions of the filter bags, without prior knowledge of the distribution of any soil residue.

Five assays – AccuPoint swab, SpotCheck^{Plus} swab, Pro-tect swab, liquid extraction followed by protein quantification, and total nitrogen with a nitrogen analyzer – were performed on the Group 1 filter bags. Each of the five assays was performed once on each of the five filter bags from each supplier. Sub-sample locations were randomized so that in the set of five filter bags from each supplier, each assay was performed once each on every sub-sample location.

Six of the 12 CIP cleaned Group 2 filter bags were sub-sampled at 12 locations per bag. These filter bags were sub-sampled more extensively than the other groups because these Group 2 filter bags were used to investigate the

distribution of residual soil after CIP cleaning of filter bags. The sub-sampling locations for Group 2 filter bags are given in Figure 6.

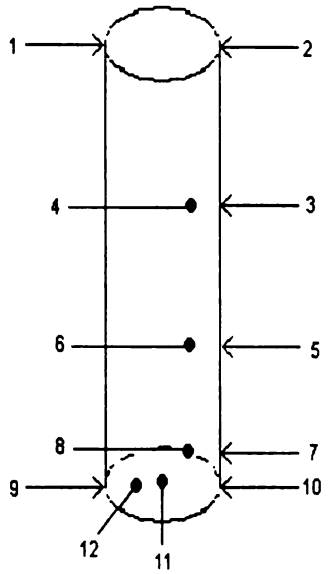

Figure 6: Sub-sampling locations for Group 2 filter bags.

1. Sampling location 1 was located at the top of the filter bag and included the fabric that encloses the metal ring sewn into the bag. This sample was taken from 180° opposite the side seam of the filter bag.
2. Sampling location 2 was identical to location 1 except that location 2 was from where the side seam of the filter bag met the top ring.
3. Sampling location 3 was located 4 feet (1.2 meters) from the top of the filter bag (one-third of the total length of the filter bag) and included the side seam of the bag.
4. Sampling location 4 was also located 4 feet (1.2 meters) from the top of the filter bag and was offset 5 inches (12.7 cm) from the side seam of the bag.

5. Sampling location 5 was located 4 feet (1.2 meters) from the bottom of the filter bag and included the side seam of the bag.
6. Sampling location 6 was also located 4 feet (1.2 meters) from the bottom of the filter bag and was offset 5 inches (12.7 cm) from the side seam of the bag.
7. Sampling location 7 was located 4 inches (10 cm) up from the bottom of the filter bag and included the side seam of the bag.
8. Sampling location 8 was also located 4 inches (10 cm) up from the bottom of the filter bag and was offset 5 inches (12.7 cm) from the side seam of the bag.
9. Sampling location 9 was located at the bottom of the filter bag along the seam where the bottom and sidewall of the filter bag are stitched together. This sample was taken from 180° opposite the side seam of the filter bag.
10. Sampling location 10 was identical to location 9 except that location 10 was from where the side and bottom seams meet.
11. Sampling location 11 was located at the center of the bottom of the filter bag.
12. Sampling location 12 was located on the bottom of the filter bag, halfway between the center and side of the filter bag.

Visual Inspection

Filter bags were laid out on tables covered with clean paper and examined visually for color, general appearance, and for any visible product residue, especially along seams and inside the filter bags.

UV Fluorescence with Visual Inspection

Both short (254 nm) and long (365 nm) wavelength ultraviolet light sources were used. The UV light sources were passed over representative sections of filter bags at a distance of 5-10 cm and any fluorescence was noted by visual inspection.

Swab Devices

SpotCheck^{Plus} swab devices (Hygiena International Ltd., UK) were purchased from Weber Scientific Inc. (Hamilton, NJ). Pro-tect swab devices (Biotrace International, UK) were purchased from Neogen Corporation (Lansing, MI) and were used with the provided swab moisturizer for use with dry surfaces. AccuPoint swab devices (Neogen Corp.) were purchased from Neogen Corp. and were used with an AccuPoint luminometer provided by Neogen Corp. All three swab devices were used according to the manufacturer's instructions, except that the swab sampling area used for all three devices was reduced to

3x3cm to keep the swabbed areas equal to the sample sizes analyzed by the other analytical methods. AccuPoint swabs were used only with Group 1 filter bags, while SpotCheck^{Plus} and Pro-tect swabs were used with Group 1 and then with Group 2 filter bags.

In addition, a subsequent experiment was done to attempt to further measure the response of the SpotCheck^{Plus} and Pro-tect swab devices to dairy powder residue on filter bags, and to attempt to establish a limit of detection for the devices when used to detect a dairy powder residue on a filter bag. Swatches of a clean, new filter bag from Intensiv-Filter were weighed to the nearest 0.1 mg and then clamped at the junction of two steel vacuum cleaner pipes which had been thoroughly washed and dried. Pre-weighed amounts of NFDM powder were then vacuumed into the system using a commercial vacuum cleaner to distribute powder onto the filter bag swatches; the vacuum was shut off and the swatches were removed and weighed to the nearest 0.1 mg. The soiled area was approximately 3 cm in diameter. One half of the soiled area was swabbed with a SpotCheck^{Plus} swab and the other half with a Pro-tect swab.

Light Microscopy

A Nikon stereoscopic zoom microscope, model SMZ 800, (Nikon Instruments Inc., Melville, NY) connected to a Schott ACE 1 150W halogen light source (Schott North America, Inc., Auburn, NY) was used to examine filter bag samples. The microscope is fitted with a C-W 10X eyepiece and a Plan Apo 1X

objective, for a total magnification range of 10-63X. The microscope is mated to a Nikon digital camera, model DXM 1200F, and several images of samples were recorded. The digital camera is interfaced to a Dell Dimension 4550 computer using Nikon ACT-1 software to control the digital camera. Filter bag sub-samples were placed on a clean sheet of white paper during examination under the microscope.

Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared spectra were collected on a PerkinElmer Spectrum ONE FTIR spectrometer (PerkinElmer Inc., Shelton, CT) equipped with a PerkinElmer Universal attenuated total reflectance (ATR) sampling accessory with a diamond crystal. The spectra were collected from 4000-650 cm^{-1} with a resolution of 4 cm^{-1} and averaging of four scans. The Spectrum ONE is interfaced to a Dell Optiplex GX260 computer with PerkinElmer Spectrum software. Filter bag sub-samples were placed on the cleaned ATR crystal and 100 N of force was applied using the attached sample foot. Acetone applied to Kimwipe delicate task wipers (Kimberly-Clark, Roswell, GA) was used to wipe the ATR crystal to clean it between samples. The spectrum of the clean ATR crystal was used as the reference background spectrum. Spectra were collected from both the product side and the clean air side of the filter bag sub-samples.

Liquid Extraction and Measurement of Total Protein

Bag sub-samples were weighed into 250ml screw-top HDPE bottles, 100ml of 6M urea was added, the bottles were capped tightly, and the samples were extracted for 20 minutes in a shaking water bath at 60°C. The sample extracts were then allowed to cool to room temperature on the lab bench for two hours before aliquots were pipetted out for assay. The solvent was assayed for total protein by the Bio-Rad microtiter plate protein assay based on the commonly used Bradford dye-binding procedure (Bradford, 1976). Dye reagent was purchased was purchased from Bio-Rad Laboratories Inc. (Hercules, CA). Reagent grade urea was purchased from Invitrogen (Carlsbad, CA). Urea solution (6M) was prepared using laboratory grade reverse osmosis water and filtered through Whatman No. 1 filter paper before use. Polystyrene 96 well microplates were purchased from Nalge Nunc International (Rochester, NY). Microplates were read at 595 nm on a μQuant microplate reader (Bio-Tek Instruments Inc., Winooski, VT) interfaced to a Dell Dimension 4600C computer running KCjunior software (Bio-Tek Instruments Inc.). A standard curve was prepared using dilutions of NFDM solubilized in 6M urea. Triplicate blanks of 6M urea plus dye reagent were performed on every microplate and the mean optical density of the blanks was subtracted from all other wells on the plate.

An initial experiment was performed to validate this extraction procedure by spiking filter bag swatches with a solution of NFDM in 6M urea, allowing the swatches to dry, and checking recovery of protein after extraction. A stock

solution containing approximately 25 mg protein/ml was prepared by weighing out commercially obtained instant NFDM and diluting with 6M urea. Because this stock solution was subsequently used to spike the filter bags and to prepare the standard curve for the assay, relative recovery of protein from the swatches could be measured without standardizing the stock solution by another assay method. Ten dilutions containing between approximately 0.01 mg protein/ml and 1 mg protein/ml were prepared volumetrically from the stock solution. These dilutions were used to prepare the standard curve during the Bio-Rad protein assay. Only the linear portion of the standard curve was used, and this was from 0.05 mg protein/ml to 0.4 mg protein/ml.

Five spiked filter bag swatches were prepared by pipetting two 500 μl aliquots onto clean, new filter bag material from Intensiv-Filter. During spiking and drying, filter bag swatches were supported on large beakers to avoid adsorption of protein onto the lab bench or other surfaces. Bags were allowed to air dry between aliquots. The swatches were marked with pencil 1 cm beyond the visible extent of the stained area and were cut out of the surrounding bag. These bag swatches were then extracted according to the procedure given above. Five control samples were also prepared by pipetting two 500 μl aliquots directly into the HDPE bottles and subsequently subjecting these bottles to the same extraction procedure. Spiked swatches and control samples were assayed at the same time.

Total nitrogen

The total nitrogen content of filter bag sub-samples was measured using an FP-2000 nitrogen analyzer from Leco Corporation (St. Joseph, MI). The furnace temperature was 1050°C. Empty combustion crucibles were used as instrument reagent blanks, and the instrument was drift calibrated daily with crystalline EDTA (9.59%N) according to the manufacturer's recommended procedure. Filter bag sub-samples of 0.3-0.4g were trimmed to fit into ceramic combustion crucibles and weighed to the nearest 0.1 mg before analysis.

The nitrogen analyzer does not discriminate among sources of nitrogen in a sample; only the total nitrogen in the sample is measured. For the filter bag samples, three potential sources of nitrogen in the cleaned samples were identified, namely, background nitrogen in the filter bag fabric itself, protein from any food product residue remaining after cleaning, and nitrogen from any cleaning chemical residue remaining after final rinsing. This is represented in the equation below:

$$\text{Total N} = \text{background N in fabric} + \text{food residue} + \text{cleaner residue} \qquad (1)$$

In order to use the nitrogen analyzer to make any meaningful conclusions about the effectiveness of a cleaning process at removing food soils from spray dryer filter bags, the contributions of the three sources of nitrogen (the terms on the right side of Equation 1) to the total nitrogen measured by the nitrogen analyzer

must be separable. Samples of a brand new, unused filter bag were used to measure the background level of nitrogen in filter bag cloth, and to determine the limit of detection of the instrument. Subsequently, Simatek provided three Group 3 filter bags which had been brand new and were installed and cleaned by a typical CIP cycle in their pilot filter bag collector facility. These Group 3 filter bags were used to assess the contribution of CIP cleaning chemical residue to total nitrogen in CIP cleaned filter bags. A mixed linear model in the Statistical Analysis Software (SAS) package (SAS Institute Inc., Cary, NC) was used to test for differences among the Group 3 filter bags and a brand new filter bag.

The distribution of residual soil on CIP cleaned filter bags was explored using 6 of the 12 Group 2 filter bags provided by Intensiv-Filter. These filter bags were sub-sampled at 12 locations each (Figure 6) and total nitrogen was measured for each sub-sample location. Overall significance of sub-sample locations and of filter bag number on total nitrogen measurements were compared using a mixed linear model in SAS. When differences were detected with the mixed linear model, individual locations or bags were tested for differences with other locations or bags by t-test.

RESULTS AND DISCUSSION

Visual Inspection

Group 1. As expected, the brand new, unused filter bags provided by both manufacturers were uniformly bright white with no visible soil or extraneous material anywhere on or in the filter bags. New, laundered filter bags possessed the closest to brand new appearance among the other four filter bag types, being also uniformly bright white but showing small amounts of extraneous material such as lint and hair. Presumably this extraneous material originated from the laundering process, since these filter bags had never been used in production at a dairy plant. The laundered filter bags of both types (new and used) showed evidence of slight pilling of the fabric when compared to brand new filter bags. Of the used filter bags, the laundered bags in general had whiter appearances than CIPed bags, although it is noted that whiteness is not necessarily indicative of overall cleanliness. CIPed bags from both manufacturers were slightly gray throughout, with the CIPed filter bag from Intensiv-Filter possessing several brown stains around the top ring of the bag. The origin of these stains could not be determined visually, although they were thought to be either from hard water during cleaning or from product residue. The used, uncleaned filter bags provided by both manufacturers had plentiful amounts of visible product powder distributed uniformly over the filters. The powder on the used, uncleaned filter bag from Intensiv-Filter was often present in large cakes, whereas the powder on

the used, uncleaned filter bag from Simatek was all individual particles. This was interpreted to be a function of the soiling procedures, because the bags provided by Intensiv-Filter came from a manufacturing plant while the Simatek bags came from a pilot filter bag collector which did not have a spray dryer upstream from the filter bag collector. Simatek personnel therefore used a fan beneath their pilot bag collector to blow cream powder onto the filter bags in the collector to simulate soiling during normal production.

Group 2. All 12 of the CIPed filter bags in this group possessed slightly off-white, uniform color. Evidence of visible powder residue could be found on the product side (outside) of all of these bags. The residue was present as small amounts of crusted, caked powder on the sidewalls and bottoms of the bags, as well as in the side seam of the filters. All 12 bags possessed abundant dark brown particles on the clean air side (inside) of the filters. These particles appeared to be scorched, agglomerated powder.

Group 3. These three filter bags were slightly gray and uniform in appearance. There was no evidence of any product residue, as expected since these filters were not used during manufacture of any product.

UV Fluorescence with Visual Inspection

UV light sources of both short (254nm) and long (365nm) wavelength ultraviolet light were passed over Group 1 filter bags. No UV fluorescence due to product residue was noted with any of the ten Group 1 filter bags, even on used,

uncleaned filter bags where product residue was plentiful. Some fluorescence of individual bag fibers was noted, however. It does not appear that UV fluorescence is useful for indicating the cleanliness of spray dryer filter bags.

Swab Devices – AccuPoint ATP Bioluminescence

The AccuPoint swab device failed to detect even gross levels of product residue on used, uncleaned bags provided by either manufacturer. Results from AccuPoint swab tests on 10 Group 1 filter bags are shown in Table 2. The AccuPoint results indicating that the used, uncleaned filter bags were not soiled was surprising, so additional measurements were made in triplicate on the sidewalls of both of the used, uncleaned Group 1 filter bags. These results are shown in Table 3. The manufacturer of this device recommends interpreting a reading of below 150 relative light units (RLU) as showing that the swabbed surface is clean, a reading between 150 and 300 RLU as showing a surface of questionable cleanliness, and a reading of greater than 300 RLU as showing an unclean surface. All of these swab samples should therefore be interpreted to mean that the filter bags were clean, when, in fact, these filter bags had product applied to them and were not subsequently cleaned and had large amounts of visible product soil remaining on them.

The AccuPoint swab device is not suitable for indicating the cleanliness of spray dryer filter bags. This finding is supported in part by the results of Carrick *et al.* (2001), who tested four ATP luminometer systems and found that all of the

systems tested gave erratic results for detecting known quantities of microorganisms dried on a solid surface and concluded that the swab method itself is unreliable.

Table 2: AccuPoint ATP swab measurements on 10 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	Swab reading (RLU)	Sub-sample location	Swab reading (RLU)
New	1	21	5	0
New, laundered	5	34	3	0
Used, laundered	4	64	1	37
Used, ClPed	3	12	4	0
Used, uncleaned	2	0	2	23

* - Sub-sample locations refer to the locations given in Figure 5.

Table 3: Additional AccuPoint ATP swab measurements on two used uncleaned Group 1 filter bags.

Swab Sample Number	Filter Bag Manufacturer	
	Intensiv	Simatek
	Swab Reading (RLU)	
1	0	112
2	10	0
3	0	96

Swab Devices - SpotCheck^{Plus} Glucose/lactose and Pro-tect Protein

Both of these swab tests are interpreted visually by the user based on color changes. For the Group 1 bags, both of these swab tests showed positive

(unclean) results for the used, uncleaned filter bags, and negative (clean) results for all of the other filter bags. These results are shown in Tables 4 and 5.

Table 4: SpotCheck^{Plus} swab device responses for 10 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	Swab response (+/-)	Sub-sample location	Swab response (+/-)
New	3	-	3	-
New, laundered	2	-	4	-
Used, laundered	1	-	5	-
Used, CIPed	5	-	2	-
Used, uncleaned	4	+	1	+

* - Sub-sample locations refer to the locations given in Figure 5.

Table 5: Pro-tect swab device responses for 10 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	Swab response (+/-)	Sub-sample location	Swab response (+/-)
New	5	-	1	-
New, laundered	4	-	5	-
Used, laundered	3	-	2	-
Used, CIPed	2	-	3	-
Used, uncleaned	1	+	4	+

* - Sub-sample locations refer to the locations given in Figure 5.

Both of these swab devices successfully differentiated between uncleaned bags and cleaned bags, with no differentiation between bags cleaned by either of the two processes, CIP cleaning or laundering. Since it was unknown whether a

difference in fact existed between the CIPed and laundered bags in Group 1, these swab tests were further examined using the Group 2 filter bags provided by Intensiv-Filter.

The Group 2 bags came from a manufacturing facility producing difficult to clean products, and showed visual evidence of product powder residue caked onto bag sidewalls and trapped in seams after CIP cleaning. This product residue appeared to be tightly held by the fabric of the filter bags as it could not be dislodged by shaking or handling of the filter bags, making these areas with visible product residue suitable to test the SpotCheck^{Plus} and Pro-tect swabs for their ability to recover residue from filter bags.

Six 3x3 cm areas with visible product residue on the sidewall of one Group 2 filter bag were selected at random. Three of these six areas were swabbed with SpotCheck^{Plus} swabs, and the remaining three were swabbed with Pro-tect swabs. Neither swab device recovered enough product residue from any of the swabbed areas to produce a positive reaction. These failures to recover product residue from visibly dirty locations indicate the unsuitability of either of these swab devices for detecting dairy product residues on spray dryer filter bags. The swab device was incapable of recovering product residue from the fabric surface after CIP cleaning of the filter bags. This is likely due to the nature of the soil that remains after CIP cleaning, since both of these swab devices showed positive reactions to visible soil from uncleaned filter bags where the soil was more loosely deposited on the filter bag. After CIP cleaning, residual soil that was visible was tightly bound to the fabric, and could not be dislodged by shaking or

handling of the bags, whereas before cleaning, large quantities of loose powder which could be shaken free of the bag very easily were present on the filter bags.

The results from the experiment using a vacuum cleaner to artificially soil filter bag swatches with NFDM are shown in Table 6. The SpotCheck^{Plus} device appears to be more sensitive for detecting NFDM than the Pro-tect device. The

Table 6: Response of SpotCheck^{Plus} and Pro-tect swab devices to artificially soiled filter bag swatches.

Trial	Mass of NFDM (g)	Increase in Mass of Filter Bag Swatch (g)	SpotCheckPlus Response *	Pro-tec Response *
1	0.2626	0.2386	+	++
2	0.0681	0.0610	+	++
3	0.0233	0.0194	+	++
4	0.0120	0.0108	+	+
5	0.0125	0.0099	+	+
6	0.0063	0.0058	+	?
7	0.0024	0.0019	+	?
8	0.0012	0.0007	+	-

*: For the SpotCheck^{Plus} device, two responses are possible, namely, no color change or clean (-), and color change to green or dirty (+). For the Pro-tect device, four responses are possible, namely, no color change or clean (-), slight color change to gray or questionable cleanliness (?), color change to purple (+), and strong color change to deep purple (++).

manufacturer of Pro-tect swabs claims that the device is sensitive to a minimum of about 50 μg of protein (Biotrace International, 2002). No detection limit information was available for the SpotCheck^{Plus}. For NFDM of typical composition, namely 34% protein content, 50 μg of protein corresponds to 147 μg of NFDM powder. The data in Table 6 imply that the Pro-tect device is therefore not recovering all of the NFDM present on the test swatches, since it's limit of detection under the conditions of the experiment is somewhat greater than 350 μg. No such comparison is possible for the SpotCheck^{Plus} device, as

the manufacturer does not provide a detection limit for the device. However, the conditions of this experiment did not necessarily truly replicate the distribution of product soil on filter bags during real world use in spray dryers. Presumably, the difference in mass between the NFDM powder used and the increase in mass of the filter bag swatch was due to either the adhesion of NFDM particles to the wall of the pipe before the filter, or to a fraction of the smallest NFDM particles passing through the filter swatch.

The failure of the Pro-tect swab device to recover NFDM from filter bag fabric in quantities down to its limit of detection, as well as the failures of both the Pro-tect and SpotCheck^{Plus} devices to recover visible residual soil from CIP-cleaned filter bags, show that the swab is not capable of full recovery of soil from filter bag fabric. The porosity of the fabric provides many pockets and crevasses that are not accessible to the swab, and as a result, not all residue is effectively recovered by the swab.

Light Microscopy

Images of a clean, new Group 1 filter bag from Simatek (Figure 7), and two used, CIP cleaned Group 2 filter bags from Intensiv-Filter (Figures 8 and 9) are shown below. Figures 8 and 9, although at low magnification, clearly show residual product residue after CIP cleaning. No such residue was evident under the microscope in the two new, unused Group 1 filter bags, or in the two used, laundered Group 1 filter bags.

Light microscopy could be a valuable technique for evaluating filter bags. As presented, light microscopy is useful for obtaining some idea of the distribution of residual soil on filter bags. There is a possibility that image analysis techniques could be applied to photographs taken with a light microscope, allowing the residual soil to be measured quantitatively. The microscope allows much detail of the filter bags to be seen, and this can potentially be used as a confirmatory test method in conjunction with other, quantitative analyses. Microscopy is also non-destructive, allowing samples to be examined under the microscope before other analyses if desired, and because of its rapidness, microscopy can easily be used on samples adjacent to samples previously analyzed with another technique to help confirm earlier results.

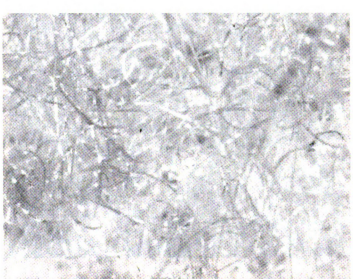

Figure 7: Simatek brand new, unused filter bag, clean air side. 30X.

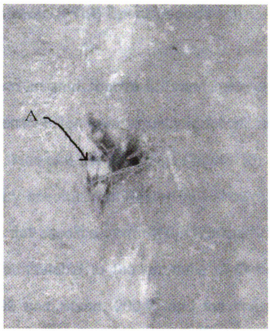

Figure 8: Intensiv-Filter Group 2 filter bag, sample location 3, product side, showing powder particles (A) trapped in a needle hole along the side seam. 20X

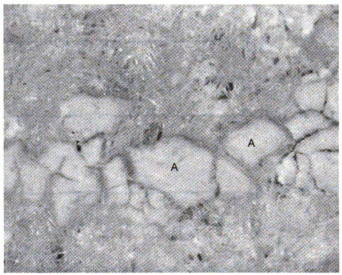

Figure 9: Intensiv-Filter Group 2 filter bag, product side along side wall of filter bag at a location showing visible powder residue (A). 20X

Fourier-Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared spectra of brand new, unused filter bags from both Simatek and Intensiv-Filter were virtually identical, as were spectra of the product and clean air sides of these filter bags (Figure 10). These spectra agree very closely with library spectra of polyester (not shown). Notable features of these spectra are the flat baseline from 4000-1750cm^{-1}, the sharp peak in the vicinity of 1712cm^{-1} attributable to the aromatic C=O bond stretching in the polyester (Smith, 1999 and Stuart, 2004), and the characteristic attributes of polyester in the fingerprint region from 1500-650cm^{-1}.

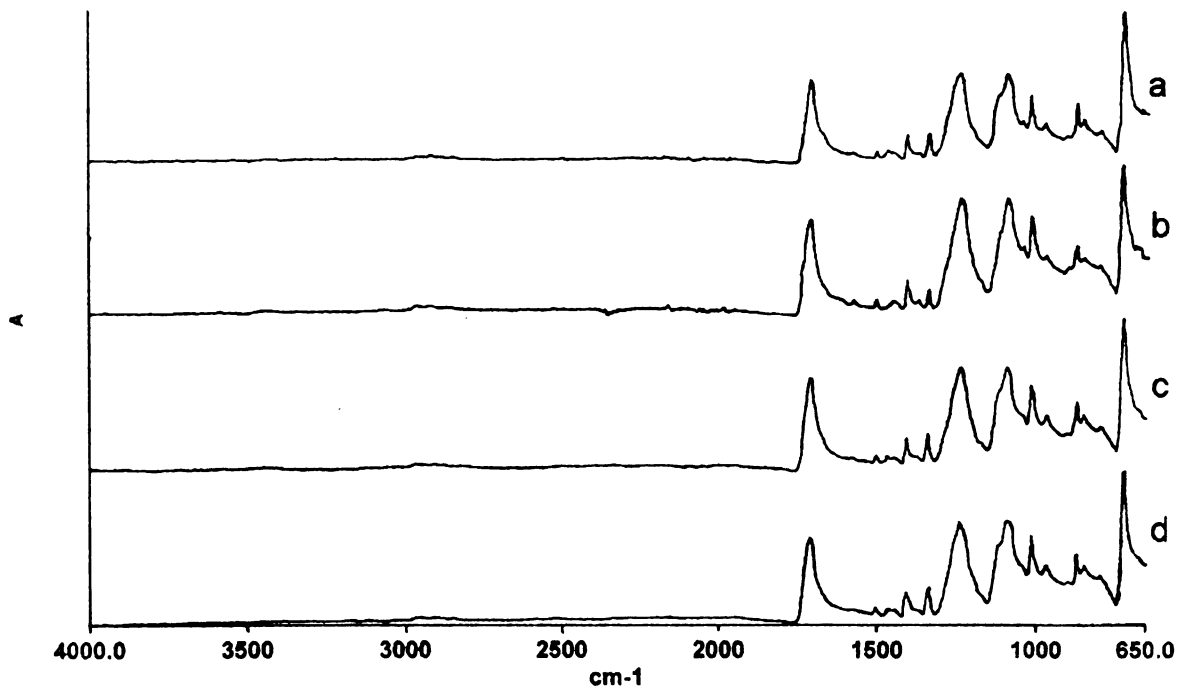

Figure 10: FTIR-ATR spectra. (a) Simatek brand new filter bag, clean air side; (b) Simatek brand new filter bag, product side; (c) Intensiv brand new filter bag, clean air side; (d) Intensiv brand new filter bag, product side.

Fourier-transform infrared spectra of several common dairy powder products are shown in Figure 11. The products shown are non-fat dry milk (NFDM), whole milk powder, buttermilk powder, and cream powder. Notable features of these spectra are summarized in Table 7. Note that not all of the peaks listed in the table are evident in spectra of all of these powders. Wavenumbers may be shifted as much as $10cm^{-1}$ in individual spectra due to matrix effects on bond vibrations. The broad peak around $1020cm^{-1}$ in NFDM, whole milk powder, and buttermilk powder agrees with the shape and location of the FTIR spectra of amorphous lactose found by Ottenhof *et al.* (2003). The broadness of this peak is due to the lack of an ordered three-dimensional network of lactose molecules in amorphous lactose (Listiohadi, 2004). This

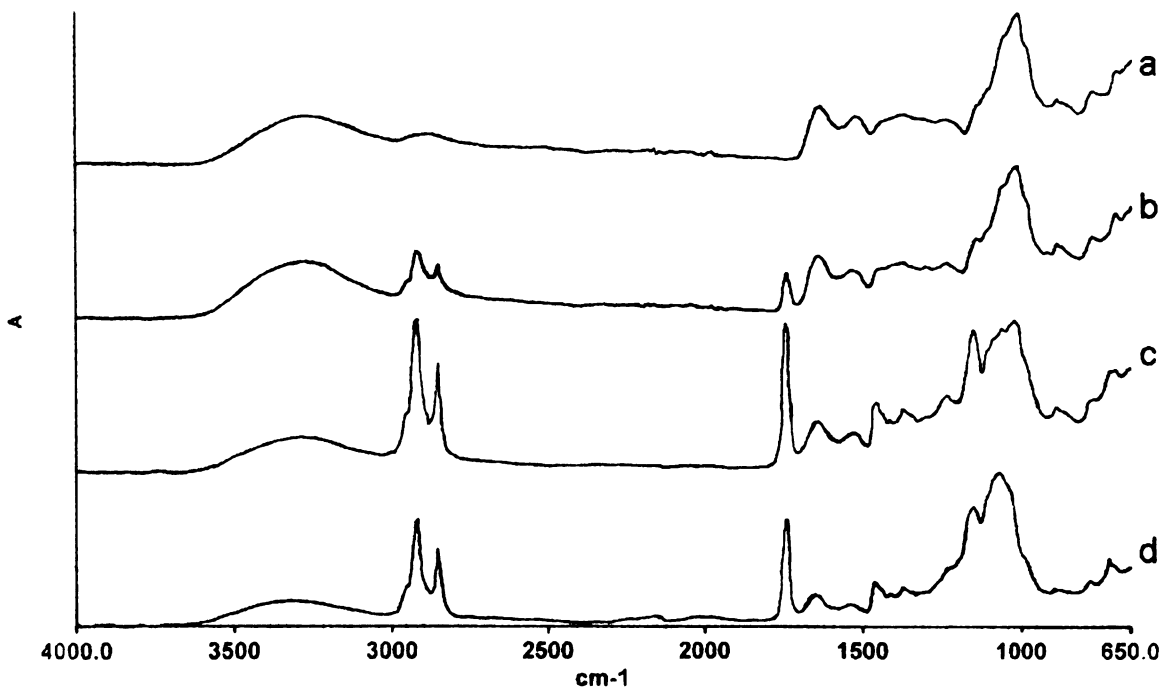

Figure 11: FTIR-ATR spectra. (a) non-fat dry milk (NFDM); (b) whole milk powder; (c) buttermilk powder; (d) cream powder.

Table 7: Prominent Features of FTIR-ATR spectra of NFDM, whole milk powder, buttermilk powder, and cream powder.

Wavenumber (cm^{-1})	Feature	Reference
3280-3310	O-H stretching	Smith (1999), Stuart (2004)
2920	CH_2 asymmetric stretching	Smith (1999), Stuart (2004)
2850	CH_2 symmetric stretching	Smith (1999), Stuart (2004)
1743	C=O stretching of triglycerides	Smith (1999), Stuart (2004)
1640	Amide I (from protein)	Smith (1999), Stuart (2004)
1535	Amide II (from protein)	Smith (1999), Stuart (2004)
1020	C-O stretching of CH_2-OH in lactose	Ottenhof (2003)

allows the bonds in lactose to have a wider distribution of vibration frequencies than they would have in the more ordered structure of a crystal. The presence of amorphous lactose in these powders is consistent with the spray drying literature (Buma, 1971 and Walstra *et al.*, 1999). Walstra (1999) states that the lactose in spray dried dairy products generally remains amorphous because there is not enough time during drying to allow crystallization to occur. All of the peaks attributable to lipid molecules (fats), i.e., the peaks at 2920, 2850, and 1743cm^{-1}, are limited in magnitude or even wholly absent from NFDM, which is consistent with the low fat content of NFDM.

The appearance in FTIR-ATR spectra of characteristic peaks that can be attributed to the various primary constituents of dairy products (fats, proteins, and lactose) shows potential for using FTIR-ATR to identify milk residues on spray dryer filter bags. However, there is overlap between the spectrum of the

polyester in the filter bag and many of the common features of milk products, especially the peaks due to protein (the amide I and II peaks) and the broad peak of amorphous lactose. This can make it difficult to resolve these spectral features of milk powders when they occur against a polyester background. However, spectral features of lipid molecules are easily distinguished from the polyester peaks of the filter bags due to the sharpness of peaks from lipid molecules and the flat baseline of the polyester spectra of brand new, clean filter bags at wavenumbers beyond the aromatic ester peak at 1712cm^{-1}. Because the penetration depth of the infrared beam into the sample during FTIR-ATR spectroscopy is very shallow, the amount of interference between the spectra of polyester and any milk powders present on the polyester filter depends on the amount of milk powder present on a filter sample. For large amounts of powder residue such as can occur in used, uncleaned filter bags, the IR beam does not penetrate past the powder residue, so there is little or no interference of polyester in the spectra obtained from such samples. For the more common cases where there may be small amounts of milk powder residue present on a filter bag after cleaning, the FTIR-ATR spectra show features of both the powder residue and the polyester. Both of these situations are illustrated in Figure 12, which presents spectra from the product and clean air sides of a used, uncleaned Group 1 filter bag and spectra from used, CIPed filter bags. The used, uncleaned filter bag from Simatek did not have heavily caked powder on it (see explanation under "Visual Inspection. Group 1" above) and thus features of both the product powder and the filter bag are present in the FTIR-ATR spectra.

These FTIR-ATR spectra as presented provide no quantitative information about the amount of product residue present on a filter bag. There are several data analysis techniques that have the potential to be successfully applied to

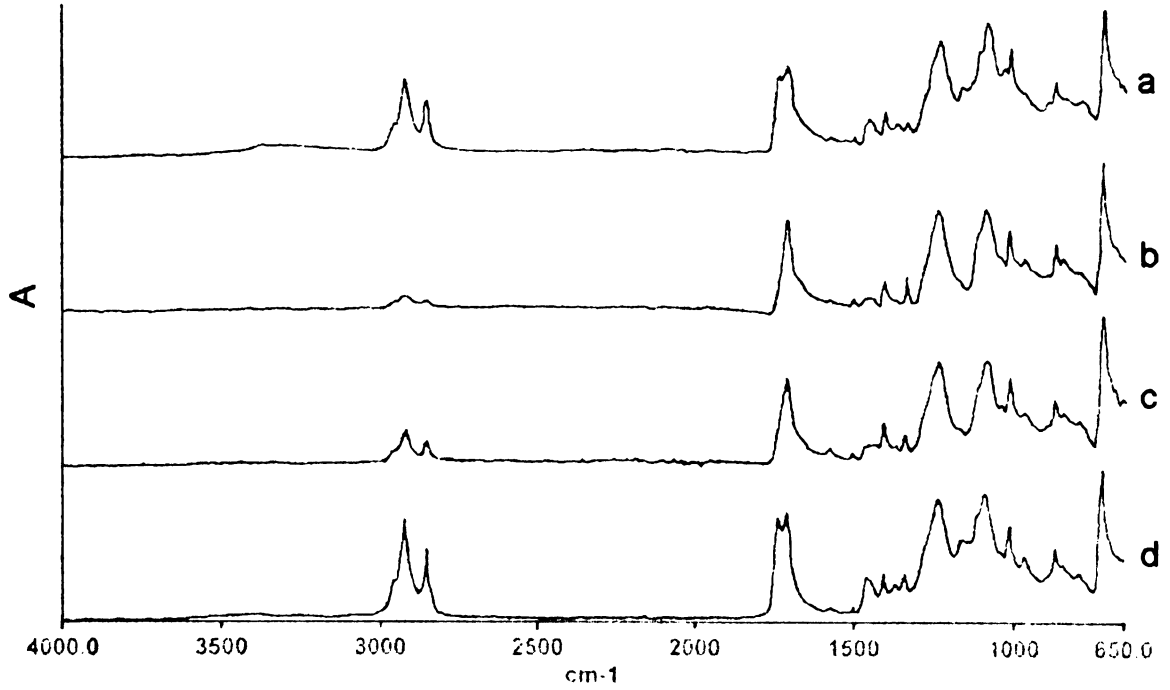

Figure 12: FTIR-ATR spectra. (a) Simatek Group 1 used uncleaned bag, product side, sub-sample 5; (b) Simatek Group 1 used uncleaned bag, clean air side, sub-sample 5; (c) Simatek Group 1 used CIPed bag, product side, sub-sample 2; (d) Simatek Group 1 used CIPed bag, product side, sub-sample 5.

measuring product residue with FTIR-ATR, but they were not explored during this preliminary phase of work. One large potential drawback to using FTIR-ATR quantitatively for this project is the small sampling diameter of the technique – about 0.2 mm (van Dalen, 2000). The small sampling diameter could make accurate quantification of inhomogeneous residues difficult. Also, because FTIR-ATR is a surface technique, the spectra only represent features of a sample that are present very near to the surface of that sample. Deeply embedded soil residues may not appear in FTIR-ATR spectra. Fourier-transform infrared

spectroscopy is a very rapid, non-destructive technique and is useful for correlating spectral information with subsequent quantitative data measured on the same sample.

Liquid Extraction and Measurement of Total Protein

Results from the initial experiment to validate the extraction procedure by measuring recovery of protein from artificially soiled filter bag swatches are given in Table 8. There was no significant difference by t-test between the means of the spiked swatches and the control samples with p=0.05. The fact that both spiked swatch and control sample means are slightly greater than 100% recovery is likely indicative of a source of systematic error such as a miscalibrated pipet used to deliver the NFDM in urea solution.

Table 8: Relative recoveries (%) of protein from spiked filter bag swatches and from control samples.

	Relative Recovery (%) by Sample Type	
Sample Number	Spiked Swatch	Control
1	107.1	108.4
2	107.5	102.8
3	96.9	108.4
4	109.6	99.0
5	109.6	101.6
Mean	106.1*	104.0*
Standard Deviation	5.3	4.2

* - the means are not significantly different by t-test with p=0.05.

Although this experiment demonstrated the efficacy of this extraction technique for recovering milk protein from filter bag cloth under the conditions of the experiment, the results are not necessarily applicable to the conditions of any residual food soil remaining after laundering or CIP cleaning of filter bags. There are many differences between the conditions of this experiment and possible real world conditions found on used filter bags. The protein used in this experiment was solubilized in urea and pipetted onto the filter bag swatches, unlike the protein in solid particles that is likely to be present as residual soil on used filter bags. Presumably, any soil remaining on filter bags after laundering or CIP cleaning is fairly tightly bound to the cloth of the filter. The effect of the spatial distribution of soil on the cloth, which results from the method of the application of that soil, on the efficiency of this extraction method is unknown. However, real world bag collector conditions could not be simulated in the laboratory in order to explore this effect further.

When applied to the analysis of the 10 Group 1 filter bags, the liquid extraction/total protein method was only capable of detecting protein residue on the used, uncleaned bag from Intensiv-Filter. There was no protein detected in any of the other 9 Group 1 filter bags, although there very likely was in fact protein on the sub-sample of the used, uncleaned filter bag from Simatek. The results from the analysis of Group 1 filter bags by liquid extraction and total protein quantification are shown in Table 9. These results were discouraging, and because of this and other reasons explained below, work with this analytical method was not continued.

Table 9: Liquid extraction and measurement of total protein in 10 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	% Protein (w/w)	Sub-sample location	% Protein (w/w)
New	2	0	4	0
New, laundered	1	0	2	0
Used, laundered	5	0	3	0
Used, ClPed	4	0	1	0
Used, uncleaned	3	0	5	0.222

* - Sub-sample locations refer to locations given in Figure 5.

The calculated limit of detection for this liquid extraction and measurement of total protein method using 1 gram filter bag samples is 0.50% protein. This is nearly 8 times higher than the limit of detection of the nitrogen analyzer, calculated as three times the standard deviation of ten samples from a brand new filter bag (explained in the "Nitrogen analyzer" section below). There was the potential to improve the sensitivity of the extraction technique by changing the solvent:sample ratio or by changing the protein assay method. However, the liquid extraction/total protein technique suffered from other limitations when compared with the nitrogen analyzer. The liquid extraction technique relies on extraction of protein from samples, whereas the nitrogen analyzer measures the total nitrogen in a sample *in situ* by combusting the whole sample. Thus, the extraction technique may not necessarily recover all residue from a sample, while the nitrogen analyzer should. The ability to extract protein from residual soil on a filter bag may vary with product type, whereas the nitrogen analyzer should be

able to accurately determine nitrogen in a variety of matrices. Also, the Bio-Rad total protein assay is sensitive to proteins with a molecular mass of approximately 3,000 to 5,000 daltons and higher (Bio-rad Protein Assay kit instructions). Proteins and peptides smaller than this do not interact with the dye used in the assay. The nitrogen analyzer measures nitrogen from all proteins and peptides without regard to their molecular mass. During cleaning of spray dryer filter bags, hydrolysis of dairy proteins by cleaning chemicals is likely. Incompletely removed, partially hydrolyzed proteins may not be detected with the Bio-Rad total protein assay when their molecular mass is below 3,000 to 5,000 daltons. Because of these technical limitations of the liquid extraction and measurement of total protein method when compared with the nitrogen analyzer, the liquid extraction method was not explored further.

Nitrogen Analyzer

The first term on the right side of Equation 1 (given in the discussion of the nitrogen analyzer in the "Materials and Methods" chapter) was straightforward to account for, as background levels of nitrogen in the polyester filter bags provided by both manufacturers were uniformly low and below the reliable limit of detection of the nitrogen analyzer. The limit of detection of the nitrogen analyzer was calculated to be very near 0.01% (w/w) nitrogen, equal to three times the standard deviation of 10 samples from a brand new filter bag supplied by Intensiv-Filter. The background level of nitrogen in the brand new

filter bag provided by Simatek was similar. According to Leco Corp., the manufacturer of the nitrogen analyzer used in this work, the FP-2000 instrument is capable of detecting a range of 0.03-300 mg of nitrogen in samples. For a 0.3 g sample, 0.03 mg corresponds to 0.01% (w/w) nitrogen. Thus, the calculated detection limit is in good accordance with the instruments specifications. The filter bag cloth appears to contain no significant amounts of nitrogen.

Results from the analysis of Group 3 filter bags are shown in Figure 13. The data have been corrected so that the mean of the measurements made on the brand new filter bag is 0% nitrogen. There is no statistical difference among the three Group 3 filter bags tested by a mixed linear model in SAS with p=0.05, and no difference between any of the Group 3 filter bags and the new filter bag by t-test with p=0.05. In other words, CIP cleaning chemicals appear to be rinsed away thoroughly and do not contribute significant amounts of nitrogen to measurements made with the nitrogen analyzer.

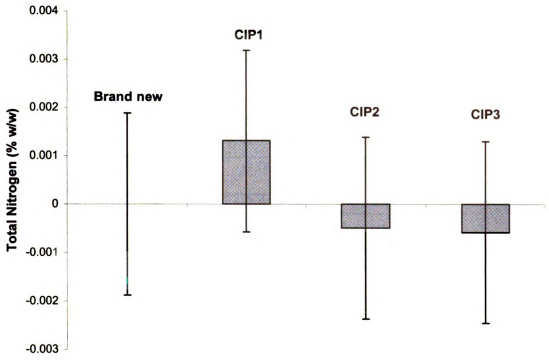

Figure 13: Total nitrogen contents (% w/w) of a brand new filter bag and three CIP cleaned Group 3 filter bags. CIP1, CIP2, and CIP3 are Group 3 filter bags.

Measurements made with the nitrogen analyzer on 10 Group 1 filter bags are shown in Table 10. Although there are not enough data points to test for significant differences among bags, only the used uncleaned and the used CIP cleaned filter bags had higher nitrogen levels than new filter bags. This observation was explored further by measuring the nitrogen content of used uncleaned, used laundered, and used CIP cleaned Group 1 filter bags from both Simatek and Intensiv-Filter at all five of the sampling locations shown in Figure 5. This data is presented in Table 11.

Table 10: Nitrogen analyzer measurement of total nitrogen in 10 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	% Nitrogen (w/w)	Sub-sample location	% Nitrogen (w/w)
New	4	0.01236	2	0.00769
New, laundered	3	0.01489	1	0.00650
Used, laundered	2	0.00900	4	0.01101
Used, CIPed	1	0.03938	5	0.02283
Used, uncleaned	5	0.02783	3	0.09829

* - Sub-sample locations refer to locations given in Figure 5.

The results in Table 11 show a fairly clear trend of higher nitrogen levels in used CIP cleaned filter bags than in new filter bags, while used laundered filter bags appeared to have nitrogen levels similar to new filter bags. However, the data set was very small, containing only one used CIP cleaned bag and one used laundered bag from each of two facilities, so final conclusions about the two cleaning processes were not made based on this data set.

Results from the analysis of 6 Group 2 filter bags are shown in Figures 14 and 15. Figure 14 shows the mean total nitrogen by sub-sample location, while Figure 15 shows the mean total nitrogen means for each entire filter bag. The raw data for this experiment is presented in Table A.1 in Appendix 1.

Table 11: Nitrogen analyzer measurement of total nitrogen in 6 Group 1 filter bags.

Filter Bag Type	Simatek		Intensiv-Filter	
	Sub-sample location*	% Nitrogen (w/w)	Sub-sample location	% Nitrogen (w/w)
Used, uncleaned	1	0.01520	1	0.05939
	2	0.01787	2	0.07603
	3	0.03455	3	0.1055
	4	0.04336	4	0.08094
	5	0.03734	5	0.06878
	Mean	0.02966	Mean	0.07813
	St. dev.	0.01244	St. dev.	0.01732
Used, laundered	1	0.00809	1	0.00934
	2	0.00694	2	0.00770
	3	0.00657	3	0.00676
	4	0.01034	4	0.00907
	5	0.00788	5	0.00821
	Mean	0.00796	Mean	0.00822
	St. dev.	0.00147	St. dev.	0.00105
Used, ClPed	1	0.04464	1	0.02379
	2	0.02689	2	0.01703
	3	0.02945	3	0.01539
	4	0.08816	4	0.04156
	5	0.05923	5	0.02496
	Mean	0.04967	Mean	0.02455
	St. dev.	0.02513	St. dev.	0.01037

* - Sub-sample locations refer to locations given in Figure 5.

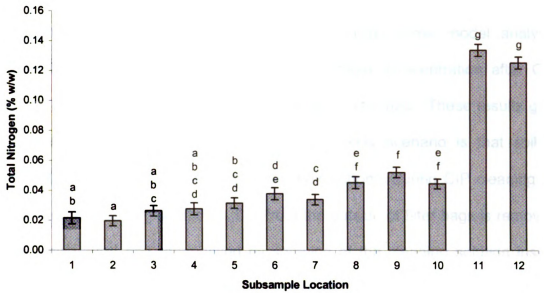

Figure 14: Total nitrogen in 6 Group 2 filter bags by sub-sample location. Letters indicate significant differences between locations ($p<0.05$) by t-test.

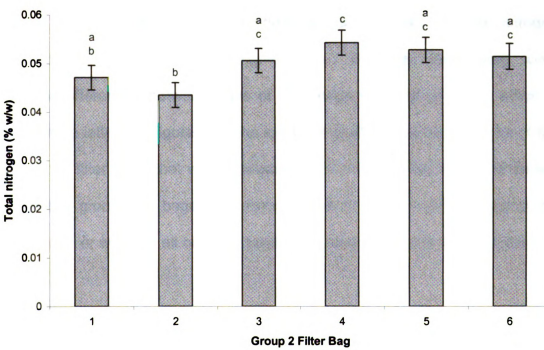

Figure 15: Total nitrogen in 6 Group 2 filter bags by filter bag. Letters indicate significant differences between filter bags ($p<0.05$) by t-test.

The sampling region of a CIP cleaned filter bag had a significant effect on total nitrogen measurements according to the mixed linear model analysis ($p<0.0001$). There is a trend of increasing nitrogen concentration after CIP cleaning as one moves from top to bottom down a filter bag. These results give no indication of the mechanism for this. One likely scenario is that soil is deposited on the inside of filter bags near their bottoms during CIP cleaning by recirculation of cleaning solutions. Soil from the outside of filter bags is removed and is recirculated and redeposited by spraying of the cleaning solution inside the filter bag, where it is more difficult to remove because it must pass through the filter bag fabric to be removed.

On the other hand, there do not appear to be large differences in total nitrogen levels among entire filter bags. According to the mixed linear model analysis by filter bag number, the effect of the filter bag on total nitrogen measurements is not pronounced ($p=0.0502$). However, there were some significant differences between pairs of filter bags by t-test ($p<0.05$), which is shown by the letters in Figure 15. The locations inside of the bag collector of the six Group 2 filter bags that were analyzed were not reported, so it is unknown whether this group of 6 bags represents a random sample of filter bags in the bag collector or whether all of these bags were taken from one region of the bag collector.

CONCLUSIONS

Of the analytical methods examined in this work, the measurement of total nitrogen with a nitrogen analyzer appears to be the most suitable for measuring residual dairy food soil on spray dryer filter bags. The nitrogen analyzer method had adequate sensitivity (to 0.01% w/w nitrogen) and was shown to be unaffected by background nitrogen in filter bag cloth or by cleaning chemical residue.

Light microscopy and FTIR-ATR spectroscopy also may be useful in future studies of spray dryer filter bags. Both are rapid, non-destructive methods that can potentially provide additional information about types and distribution of soils on spray dryer filter bags. Neither method is quantitative, however, so these methods are most useful as adjunct methods to help support measurements made with another technique such as the nitrogen analyzer.

The AccuPoint, Pro-tect, and SpotCheck^{Plus} swab devices do not appear to be suitable for measuring residual food soil on spray dryer filter bags. The failure of all three devices to respond to soil which was visible on the filter bags seems to indicate that the swab itself is not adequate for recovering soil from filter bag fabric. This is probably a combination of the soil adhering to the fabric, and that only the very surface of the fabric is accessible to the swab.

Liquid extraction followed by total protein measurement in the extract is not as useful as a nitrogen analyzer for measuring dairy food soil on spray dryer filter bags. The nitrogen analyzer is more rapid and offers greater sensitivity, and

in theory is less sensitive to matrix effects of changes in bag construction or product type.

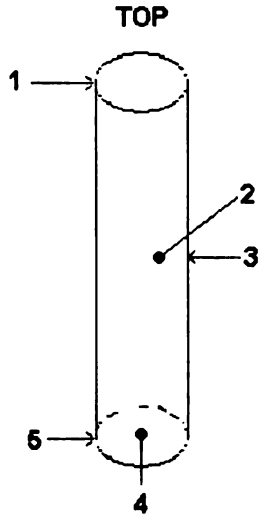

Figure 16: Recommended sub-sample locations for spray dryer filter bags. (1) top of the filter bag 180° opposite of side seam, including the fabric that encloses the metal ring sewn into the bag; (2) halfway between top and bottom of filter bag, 10 cm from side seam; (3) halfway between top and bottom of filter bag, on side seam; (4) center of bottom of filter bag; (5) at the bottom of the filter bag 180° opposite the side seam at the seam where the bottom and sidewall of the filter bag are stitched together.

Based on the distribution of soil observed in Group 2 filter bags from Intensiv-Filter and on the results of the various analytical methods examined in this work, recommendations for measuring residual soil after cleaning of spray dryer filter bags are as follows:

1. Sub-sampling spray dryer filter bags at 5 locations is adequate. To provide a representative sample, these 5 locations should represent all areas of the filter bag, although the majority of residual soil will be found at the bottom of the filter bags. Figure 16 shows the recommended sub-sampling

locations. These locations are very similar to what was used for sub-sampling Groups 1 and 3 filter bags.

2. Sub-samples should be 4x4 cm in area (about 0.5 g, except at seams, where sub-samples will weigh more due to overlapped fabric), and should be stored individually in sealed plastic bags to prevent contamination before analysis. The filter bag sub-samples were quite stable in our experience and may be stored for several weeks before analysis.
3. Between 0.3 g and 0.4 g of each sub-sample should be analyzed with a suitable nitrogen analyzer. The remaining portion of each sub-sample should be saved for future FTIR-ATR or microscopic examination, if necessary. Fourier-transform infrared spectroscopy and/or microscopy may be used to supplement nitrogen analyzer results to identify soil types and distribution within the filter bag fabric, when desired.

RECOMMENDATIONS FOR FUTURE RESEARCH

While this work has helped to answer several questions about measuring residual dairy food soil on spray dryer filter bags, several areas may warrant future research. Recommended future studies are:

1. Examine the distribution of residual food soil on laundered spray dryer filter bags. While the mechanical agitation of a washing machine will help to provide equal cleaning of all parts of a filter bag, the possibility that areas such as inside the bottom of the filter bag and along seams will clean at different levels than other parts of the filter bag should be investigated.
2. Explore the use of image analysis techniques to quantify powder residues in pictures taken with a light microscope.
3. Investigate the use of chemometric calibration techniques such as partial least square (PLS) analysis with Fourier-transform infrared spectroscopy to measure food residues on spray dryer filter bags.. This is worth investigating, as the speed and low cost of FTIR data collection, as well as its non-destructiveness, would make this a very desirable analytical technique, especially for large-scale, routine analysis of many samples.
4. The ability to quantify food residues on spray dryer filter bags opens the door to many further studies that could explore the effects of numerous cleaning variables, such as cleaning methods (e.g., CIP cleaning versus laundering) and cleaning parameters (solution strength, temperature, time applied, etc.), on the overall cleanliness of spray dryer filter bags.

APPENDIX

Table A.1: Raw data for total nitrogen analysis of 6 Group 2 filter bags.

Sub-Sample Number	Bag 1	Bag 2	Bag 3	Bag 4	Bag 5	Bag 6
1	0.03054	0.00945	0.01087	0.04834	0.01586	*
	0.01910		0.00620	0.03985		
2	0.02618	*	0.02921	0.03783	0.03062	0.02941
	0.01476	0.01653	0.00849	0.02335	0.01660	0.00585
	0.02599	0.00899	0.02353	0.01927	0.01170	0.01131
3	0.01875	0.02252	0.03014	0.03501	0.02804	0.02659
	0.01919	0.02258	0.02592	0.03061	0.02918	0.02705
4	0.02204	0.02513	0.03133	0.02783	0.02955	0.03068
5	0.03375	0.02465	0.02364	*	0.02478	0.03367
	0.03357	0.02458	0.02249	0.05020	0.01890	0.04650
			0.02481			
6	0.03296	0.03330	0.02790	0.03938	0.05797	0.03617
7	0.03944	0.02620	0.03576	0.04476	0.02719	0.03495
	0.03838	0.02581	0.03032	0.03871	0.02723	0.03887
8	0.03543	0.04028	0.05169	0.04590	0.06147	0.03658
9	0.04755	0.05053	0.05146	0.04847	0.05508	0.06519
	0.05009	0.04318	0.04211	0.05100	0.05071	0.07042
10	0.04365	0.04940	0.04639	0.05544	0.05382	0.05317
	0.03339	0.02750	0.03805	0.03672	0.03774	0.04188
	0.04237	0.04088	0.05417	0.05525	0.01309	0.04727
11	0.1287	0.1240	0.1474	0.1297	0.1503	0.1260
12	0.1117	0.1164	0.1603	0.1063	0.1386	0.1207

Note: All values are in % nitrogen (w/w). Blank cells indicate locations were there was not enough material to perform another analysis on that sub-sample.

* - Data not valid due to error during analysis (e.g., incomplete combustion or other instrument error).

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