North Central Region Canola Research Program
Application Cover Page
(Must fit on one page)

Project Title: Developing an NIR Calibration for Fatty Acid Profiles and Chlorophyll for the Rapid Screening of Canola Seeds.

Lead Principal Investigator and Institution: Clifford Hall III, North Dakota State University

Co-Principal Investigator(s): Mehmet Tulbek, Northern Crops Institute

Mailing Address of Lead PI: 210 Harris Hall, Fargo ND 58105

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Phone Number of Lead PI: 701-231-6359

Fax Number of Lead PI: 701-231-5171

Funds Requested for 2007: $34,512

Project Status: New __XX_____Renewal _____

Does this project involve recombinant DNA, human subjects or vertebrate animals?
____ Yes _XX__ No
If yes, please complete a CSREES Assurance Statement Form 2008 or a Research & Related Other Project Information Form that is available as part of the new application kit through Grants.gov.

Does this project involve the sale of goods or services? _____ Yes __XX___ No
If yes, please indicate the nature of the sale in this space:

By signing this proposal, the applicant certifies that the information contained herein is true and complete to the best of their knowledge and accepts as to any award the obligation to comply with the terms and conditions of the Cooperative State Research, Education and Extension Service in effect at the time of the award.

Clifford Hall III
PI Signature

James R Venette
Dept. Chair/REC Director signature
(applies only to NDSU applicants)

_____
Authorized Organizational Representative
(applies only to non-NDSU applicants)

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☐ Yes ☑ No

1.a If YES to Human Subjects

Is the IRB review Pending? ☐ Yes ☐ No

IRB Approval Date:

Exemption Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

Human Subject Assurance Number:

2. * Are Vertebrate Animals Used? ☐ Yes ☑ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☐ Yes ☑ No

4.a. * Does this project have an actual or potential impact on the environment? ☑ Yes ☐ No

4.b. If yes, please explain: Use of Lab Chemicals

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain: Categorical Exclusion (i) (A) Research w/i laboratory

5.a. * Does this project involve activities outside the U.S. or partnership with International Collaborators? ☐ Yes ☑ No

5.b. If yes, identify countries:

5.c. Optional Explanation:

6. * Project Summary/Abstract Add Attachment | Delete Attachment | View Attachment

7. * Project Narrative Add Attachment | Delete Attachment | View Attachment

8. Bibliography & References Cited Add Attachment | Delete Attachment | View Attachment

9. Facilities & Other Resources Add Attachment | Delete Attachment | View Attachment

10. Equipment Add Attachment | Delete Attachment | View Attachment

11. Other Attachments Add Attachments | Delete Attachments | View Attachments ☐

OMB Number: 4040-0001

Expiration Date: 04/30/2008

North Central Canola Research Program -FY 2007
Project Narrative

Research Objective

To develop a rapid analytical methods for determining fatty acid profiles and chlorophyll in canola using near-infrared (NIR) spectroscopy.

Procedures

1. A description of the proposed investigations

Methods: In order to develop an NIR spectroscopy method, wet chemistry methods must be used to determine the content of the fatty acids and chlorophyll. The wet chemistry data are then correlated to NIR spectra of the samples to create a calibration curve. We will scan 500 samples on the Perten and subject the spectra to chemometrics software (Grams) to develop the calibration curve. Once this preliminary calibration is developed, 200 samples to be evaluated by wet chemical methods in at least duplicates. After the wet chemical analysis, 150 of the 200 samples will be used to update the preliminary calibration curve. The remaining 50 samples will be used as the monitoring set to test the reliability of the equation. The closer the wet chemistry data is to the NIR data obtained from the updated calibration curve the more reliable is the equation and the calibration curve. In year two of the project we will continue to build the calibration robustness by evaluating additional samples. We will allow access by other research groups interested in scanning samples via the NIR. This will allow us to further develop the calibration curve.

2. Techniques to be employed, including their feasibility

Materials: Canola samples will be obtained from Art Killam from the University of Minnesota. Supplies for fatty acid and chlorophyll analysis will be purchased from Aldrich Chemical Co. (Milwaukee, WI). Some of the materials include chlorophyll standards, fatty acid methylating solvents, and solvents (methanol, acetone) for high performance liquid chromatography (HPLC) analysis of chlorophyll. Columns and vials for the HPLC and gas chromatography (GC) will be purchased from VWR Scientific.

Wet Chemistry Methods. The fatty acid profile analysis will include Soxhlet extraction of the canola to remove the oil and then GC analysis of the methyl esters. Lipid extraction will be necessary for fatty acid profile analysis. Soxhlet extraction using hexane will be completed for

16 hours to assure maximum oil extraction. After the extraction, the solvent will be removed by rotoevaporation at 35˚C. The canola oil remaining will be weighed and this oil methylated using the method of Schwarz et al. (1995). Briefly, 250 mg of oil and 5 mL of methylating reagent (2% sulfuric acid in methanol) will be heated 30 minutes in a sealed test tube at 100˚C. After cooling, 3 mL of hexane will be added to extract the methyl esters. The hexane solution containing the methyl esters will be place in vials and separated by the GC method described by Schwarz et al. (1995).

The gas chromatography (GC) system (HP 5890, Hewlett Packard, Avondale, Pa., U.S.A.) equipped with an SP-2330 fused silica capillary column (30 m × 0.25 mm i.d., and 0.20-mm film thickness)(Supelco, Bellefonte, Pa., U.S.A.), a flame ionization detector and an HP 3393A integrator will be used to determine fatty acid profiles. The operation parameters are as follows: injector and detector temperatures will be both set at 200 °C; the oven temperature will be ramped from initial 150 °C (held for 5 min) and then increase 10°C/min to a final temperature of 180 °C at a rate of 10 °C/min. The oven will be maintained at 180 °C for 12 min to complete the run. The helium flow rate will be 1.3 mL/min. The composition of fatty acids will be determined using internal standard (heptadecanoic acid, C17:0) method. The data will be converted from percent oil into mg oil/kg of canola seed value.

Chlorophyll a and b will be determined according to Forni et al.(1988), with some modifications. Ground canola (5 g) will be extracted three times with 20 mL acetone containing sodium carbonate (final pH 7). The pooled extracts (final volume 60 mL) will be dried under vacuum at 30˚C and then taken up into the mobile phase solvent acetone/ethanol/water (70/17/13 v/v/v). The samples will be injected onto a RP18 column (Phenomenex) and chlorophyll separated with a mobile phase of acetone/ethanol/water (70/17/13 v/v/v). Detection will be completed with a spectrofluorimetric detector (Waters) using an excitation λ= 413 nm, and an emission λ= 669 nm. The concentrations will be determined using a calibration of chlorophyll a and b standard solutions. Data will be reported as mg chlorophyll / kg canola.

The methods to determine fatty acid profiles and chlorophyll are well established. Dr. Hall has 18 years experience in fatty acid analysis and 9 year with the specific method proposed in this research. Dr. Hall has had 20 years of experience in using HPLC techniques to analyze food components. Although the specific HPLC method proposed has not been used in Dr. Hall's laboratory, a similar chlorophyll detection method using a different mobile phase

and detector has been used previously. The proposed method is more sensitive than the previously used method; thus, we will use the new method. The fatty acid profiles and chlorophyll contents will not be difficult to complete but will require significant time due to the length of each protocol.

We will work with Art Killam in developing the NIR calibration curve. Art is responsible for the NIR laboratory in the Department of Agronomy and Plant Genetics at the University of Minnesota. Mehmet will operate the Perten NIR in the Northern Crops Institute as he has been trained on this instrument and is the person in charge of its operation.

3. Kinds of results expected

We expect that the NIR calibration curves for fatty acid profiles and chlorophyll will be established. The expected data will be similar but not identical to that reported by Siemens and Daun (2005) who reported data for fatty acids on Canadian canola by NIR. However, they did use a different NIR instrument so differences in data may be due to the methods used to create the calibration curve.

4. Means by which data will be analyzed or interpreted

For both fatty acid profiles and chlorophyll determinations, the data will be statistically analyzed using analysis of variance (ANOVA) in which the least significant means (LSMEAN) will be used to determine the 95% confidence level ($P<0.05$). This will provide the researchers with the repeatability of the extraction and analytical protocols.

The NIR data will utilize the Partial Least Squares in the chemometric software (Grams) to develop the calibration curve. We will also use analysis of variance (ANOVA) and least significant means (LSMEAN) to determine statistical differences between the fatty acids or chlorophyll contents measure by wet chemical methods and NIR. Data that is not significantly different indicates that the calibration curve is robust.

5. Pitfalls which might be encountered

The major pitfall in this investigation would be that we might not have canola samples with a large range in fatty acids or chlorophyll contents. The robustness of the calibration curve for NIR depends on having a range in the analyte of interest. Thus, if the samples all have similar

fatty acids or chlorophyll levels then the calibration curve will not be as robust as when the samples are more diverse.

6. Limitations to proposed procedures

The limitation of any analytical protocol is the variability in the protocol itself and the individual conducting the analysis. To reduce the error associated with the protocols we employ recover studies to determine how accurate the results are expected outcome. If an individual obtains results greater than 10% from the expected, they will repeat the protocol to achieve the desired outcome (i.e. less then 10% difference). The limited samples (200) will allow us to develop a curve suitable for preliminary calibration development. However, at the end of the second year we will have sufficient number of samples analyzed to create a robust calibration curve.

Justification

McKay et al (2006) reported that 2 million acres of canola would be required to meet the current and estimated canola needs. Approximately 700,000 to 800,000 acres of production annually would be required to meet the needs of two North Dakota biodiesel plants that are now under construction. However, providing canola most suitable for the biodiesel industry will require a team effort that includes plant breeders and oil chemists. The chemical analysis of the canola is one bottleneck in the selection of canola lines suitable for biodiesel or edible oil. Chemical analyses can require substantial time to complete one assay. For example, fatty acid profiles can take up to 48 hours to complete, which includes extraction, methylation and the measurement by GC. Chlorophyll determination requires approximately 16 hours to complete, which includes extraction and HPLC analysis. In contrast, NIR can provide data within minutes once samples are placed in the instrument. In general, the total time for measuring quality traits by NIR is less than five minutes. Furthermore, multiple traits can be completed in one run; thus, substantial timesavings can be recognized and allow researchers to analyze more samples in a shorter time. Another advantage of NIR is that the raw data can be downloaded to spreadsheets for more rapid data manipulations. Other techniques require that the technician input the data into a spreadsheet prior to manipulation, which increases the chance of error during data input.

To accurately measure quality parameters by NIR, a calibration curve must be developed on test samples that represent the samples that would be characteristic of those from the breeding program or from a specific growing area. Furthermore, the robustness (i.e. accuracy and validity)

of the analysis increases as more samples are used in the calibration development. Samples evaluated over the course of several years can further enhance the robustness of the calibration because the samples best represent a seed that would be grown under different conditions. Starting this research during the fiscal year would expedite NIR calibration development for fatty acids and chlorophyll from U.S. grown canola. This curve could then be provided to others developing canola lines suitable for specific applications.

The collaboration between NDSU, the Northern Crops Institute, and the University of Minnesota is the ideal fit for this research. The Northern Crops Institute has the only Perten NIR systems at NDSU and works extensively with Perten in developing calibration curves. Art Killiam has access to close to 500 canola breeding lines, which could provide a diverse population of canola samples for the calibration development. The wet chemical methods are necessary in calibration development. Oilseed fatty acid analysis is routinely completed in Dr. Hall's laboratory at NDSU. Thus, Dr. Hall will direct the research dealing with this aspect of the study.

Literature Review

The fatty acid composition and chlorophyll contents are two components that affect the quality of canola oil and its uses. The fatty acid composition of canola makes it ideal for biodiesel. In contrast, the high chlorophyll content of the oil can create problems. While most oil contains typically less than 1 ppm chlorophyll, canola contains 13-30 ppm. The high level of chlorophyll in crude canola oil reduces the efficiency of the oil refining process. The reason being is higher amounts of bleaching clay are needed to remove the chlorophyll and in the process bind more oil; thus, less refined oils is available for the end user. Developing varieties with less chlorophyll could enhance refined oil yields. Providing the breeder with a tool that could rapidly screen large volumes of samples could reduce the time required to develop new varieties.

There have been several reports on analyzing canola components using NIR (Van Deynze and K. P. Pauls, 1994; Golebiowski et al., 2005; Siemens and Daun, 2005). As with other technologies, updates are needed to maintain the validity of the method. Thus, using calibrations on samples prior to 2000 are not useful as the technology of NIR has changed since that time. Furthermore, a calibration curve must be developed for samples that will represent a population of samples to be evaluated, as components such as fatty acid differences can influence the data

(Panford and deMan, 1990). In the 2001 Canadian Canola Quality survey, DeClercq and Daun (2001) reported chlorophyll contents of 16-18, 32-33 and 48-58 mg/kg for canola number 1, 2 and 3, respectively. If a calibration curve that was developed from samples containing between 16-33 mg chlorophyll/kg canola was used in the analysis, then the data from canola containing greater than 33 mg chlorophyll / kg canola would not be valid. Thus, measuring samples using a Canadian or European calibration curve may not be valid as the canola composition may differ from the U.S. canola.

The wet chemistry method for validating the NIR instrument is also important. Canola has a diverse fatty acid profile that includes palmitic (4%), palmitoleic (0.3), stearic (2%), oleic (61%), linoleic 20%), linolenic (10%), arachidic (0.7%), gadoleic (1.3%), eicosadienoic (0.1%), behenic (0.3%), erucic (0.1%), lignoceric (0.2%). However, development of a calibration curve that includes all fatty acids is not practical as palmitic, stearic, oleic, linoleic and linolenic are the major fatty acids. In addition, erucic acid would be the only other fatty acid that would be of interest as some countries would require this data to be able to differentiate canola from rapeseed oil. However, only the composition of the major fatty acids would be needed for the biodiesel industry, as minor fatty acids could be lost during biodiesel product.

Unlike the fatty acid profiles, calibration curves for chlorophyll are not as robust due to the wet chemistry method used to develop the curve. The traditional chlorophyll analysis uses spectrophotometer. This method allows for chlorophyll determination but not chlorophyll derivatives. Chlorophyll analysis by HPLC allows for a more accurate evaluation of cholorophyll content.

References

DeClercq, D. and Daun, J. Quality of Western Canadian canola 2001. Published by the Canadian Grain Commission. 2001.

Golebiowski, T., Leong, A. and Panozzo, J. Near infrared reflectance spectroscopy of oil in intact canola seed (Brassica napus L.). II. Association between principal components and oil content. J. Near Infrared Spectroscopy. 2005, 13, 255-264.

McKay, K. Novak, L, Johnson, B., and Henson, R. Increasing Canola Acres in North Dakota for Biodiesel Production. Abstract for the Canola: I. From Breeding to Production session at the ASA-CSSA-SSSA International Annual Meetings (November 12-16, 2006).

Panford, J.A. and deMan, J.M, 'Determination of Oil Content by NIR: Influence of Fatty Acid Composition on Wavelength Selection,' *Journal of American Oil Chemists Society*. 1990, 67, 473-482.

Schwarz, P.B.; Barr, J.M.; Gillespie, J.L. Soybean oil applied to barley for dust control: An analysis of malt, beer and by-products for residues. *Techn. Quarterly Master Brew. Association of America.* 1995, 32, 208-212.

Siemens, B. and Daun, J. Determination of the fatty acid composition of canola, flax, and solin by near-infrared spectroscopy. J. Am. Oil Chem. Soc. 2005, 82, 153-157.

Van Deynze, A. and Pauls, K. Seed colour assessment in Brassica napus using a Near Infrared Reflectance spectrometer adapted for visible light measurements. Euphytica. 1994, 76, 45-51.

Facilities and Equipment

The laboratory research will be conducted in the NDSU Pilot Plant laboratory and the grain-testing laboratory in the Northern Crops Institute. Equipment available includes high performance liquid chromatographs (including 1 LC-MS), Minolta Lab Colorimeter, ash furnaces, Soxhlet extractors, Perten NIR.

Project Timetable

The project will be 24 months in length. The length is based on the evaluating canola samples over a two-year period. The first year of the project will include the evaluation of canola from the 2007 growing season and year two will include canola samples from 2008 growing season. In each year, we will start with the characterization of the fatty acid profile evaluation. Once the wet chemistry has been completed, the samples will be evaluated using the NIR instrument. The chlorophyll will be evaluated after the fatty acid profiles have been established. Once the calibration curves have been established, 100 canola samples will be selected and the fatty acid profiles and chlorophyll contents determined by NIR as a means to re-affirm the sample traits. At the end of each year, reports will be written.

Personnel Support

Clifford Hall III (Project Director): Ph.D. in Food Science and Technology with specific interests in lipid and natural products chemistry. Projects on flaxseed in functional food systems have been the primary area of research since 1999. However, he has supervised several projects dealing with fatty acid analysis of oilseeds. Time commitment = 5%

Mehmet Tulbek: is a Crop Quality Specialist at the Northern Crops Institute (NCI). He is responsible for developing educational materials for specialty commodities at the NCI. As part of his duties, he is responsible for collecting compositional data on oilseeds. In particular, he is responsible collecting data using NIR and development of calibration curves. Time Commitment = 10%

Clifford Hall III

Education

M.S., Ph.D. Food Science		University of Nebraska-Lincoln, May 1991, 1996
B.S. Food Science;	minor in Chemistry	University of Wisconsin - River Falls, May 1988

Experience-Current Position

July 2005-present: Tenure track Assistant Professor. Responsibilities of the job include teaching, research and administrative activities.

August 2003 - present: Associate Director Great Plains Institute of Food Safety

August 2002-present: Food Science Coordinator

Experiences - Teaching

North Dakota State University

August 1998 - present: Instructor for the Food Chemistry Lecture (CFS 460/660) and Laboratory (CFS 461/661), Food Processing (470/670) and Laboratory (471/671), and Advances Cereal and Food Chemistry II (Lipids Section) (CFS 766) courses.

Experience - Industry

ConAgra Frozen Foods Omaha, NE - March 1991 - January 1992: Internship position dealt with formulation of new food products and reformulation of existing food products. The majority of the internship dealt with modifications to the Kid Cuisine frozen dinners and Banquet adult dinner products. Several projects focused on starch chemistry, color chemistry and nutritional comparisons.

Grants Funded (2005-2006)

Wolf-Hall, C.E., J.Garden-Robinson, **C. A. Hall III**, V. Hinsz, M.Khaitsa, W. Nganje, C. Wachenheim. Great Plains Portal to Food Safety: Educating Tomorrow's Leaders. USDA-CSREES-Food and Agricultural Sciences National Needs Graduate Postgraduate Fellowship Grants Program. $156,000 (2007-2011)

Hall III, C.A., Antimicrobial Activity of Flaxseed. North Dakota Agriculture Products Utilization Commission. $30,000 (2006-2007)

Hall III, C.A., C. Wolf-Hall, and G. Lardy. Soybean Substrates in Mushroom Production and Animal Feed Supplements. North Dakota Soybean Councils $24,600 (2006-2007)

Hall III, C.A., and B. Zhao. Graduate School Dissertation Fellowship. North Dakota State Graduate School. $15,330 (2006)

M. Tulbek, and **C.A. Hall III**. Development of Omega-3 Fortified Extruded Bean Snacks. Northharvest Bean Growers. $35,900 (2006-2007).

Activities in Professional Organization

Lipid Oxidation and Quality (LOQ) Division of the American Oil Chemist's Society:

- Best Paper Competition Committee Chair (2003-2006)
- Chairperson for the LOQ Division (2005-present)
- Secretary for the LOQ Division (2003-2005)
- Membership committee representative for the LOQ Division (2001-2003)

Senior Associate Editor for the American Oil Chemist's Society (2006-present).

Peer reviewer for manuscripts submitted to the American Oil Chemist's Society, Institute of food Technologists, and American Chemical Society (J. Agric. Food Chem.).

External reviewer for the National Research Initiative (NRI) Competitive Grants Program of the CSREES-USDA.

Publications (2005-2006)

Manthey, F. and **C.A. Hall III**. Effect of processing and cooking on the contents of minerals and protein in pasta containing buckwheat bran flour. Journal of the Science of Food and Agriculture. (accepted)

Hall III, C.A., Tulbek, M.C. and Xu, Y. 2006. Flaxseed. In: Advances in Food Science and Human Nutrition. Edited by S. Taylor. Elsevier Inc. Volume 5 pp 1-97.

Kangas, N, M. Niehaus and **C.A. Hall III**. 2006. Utilization of Flaxseed Gum In Dairy Foods. Proceedings of the 61st Flax Institute of the United States. Edited by J. Carter. Published by North Dakota State University, Fargo, ND. pp 205- 212.

Schorno, A., F. Manthey, **C.A. Hall III**, D. Wiesenborn and J. Hammond. 2006. Immature and Off-Colored Seeds Affects Oil Quality in Milled Flaxseed. Proceedings of the 61st Flax Institute of the United States. Edited by J. Carter. Published by North Dakota State University, Fargo, ND. pp 186- 192.

Xu, Y., **C.A. Hall III**, C. Wolf-Hall and F. Manthey. 2006. Antifungal Activity of Flaxseed Flours. Edited by J. Carter. Published by North Dakota State University, Fargo, ND. pp 177-185.

Ghosh, P., F. Manthey, M. Chakraborty and **C.A. Hall III.** 2006.Effect of Flaxseed Flour on the Quality of Fresh Wheat Tortillas. Proceedings of the 61st Flax Institute of the United States. Edited by J. Carter. Published by North Dakota State University, Fargo, ND. pp 170-176.

Wiesenborn, D., Kangas, N., Tostenson, K., **Hall III, C. A.**., and Chang, K. 2005. Sensory and Oxidative Quality of Screw-Pressed Flaxseed Oil. *J. Am. Oil Chem. Soc.* 82: 887-892.

Liu, J., C. Vijayakumar, **C.A. Hall III**, M. Hadley and C.Wolf-Hall. 2005. Sensory and Chemical Analyses of Oyster Mushrooms (*Pleurotus sajor-caju*) Harvested from Different Substrates J. Food Science 70 (9):S586-592.

Hall III, C.A., F.A. Manthey, R.E. Lee and M. Niehaus. 2005. Stability of α-Linolenic Acid and Secoisolariciresinol Diglucoside in Flaxseed-Fortified Macaroni. J. Food Science 70 (8): C483-489.

Wiesenborn, D., K. Tostenson, N. Kangas, Y. Zheng, **C. A. Hall III**, M. Niehaus, P. Jarvis, J. Schwarz and W. Twombly. 2005. Processing Flaxseed for Food and Feed Uses. Food Sci. Biotechnol. 14(3):305-310.

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E.mail: mehmet.tulbek@ndsu.edu http://www.northern-crops.com

EDUCATION

Ph. D.: May 2001- Expected December 2006 (GPA: 3.78)
North Dakota State University Department of Cereal Science Fargo, ND
Ph.D. Dissertation: Development and characterization of fermented chickpea in sour dough bread
M.Sc.: 1996-1999 (GPA: 3.23)
Istanbul Technical University Department of Food Engineering Istanbul, Turkey

M.Sc. Thesis: Relationship between some quality parameters of the Turkish wheat flours and their noodle making
B.Sc.: 1991-1996 (February)
Ankara University Faculty of Agriculture Department of Food Science and Technology Ankara, Turkey

WORK EXPERIENCE & ACCOMPLISHMENTS

Pulse and Oilseed Specialist: January 2006 - Present
Northern Crops Institute Fargo, ND
Research and Teaching Assistant: April 2001 – September 2005
North Dakota State University Department of Cereal Science Fargo, ND
Research and Teaching Assistant: May 1997- April 2001
Istanbul Technical University Department of Food Engineering Istanbul, Turkey

MEMBERSHIP TO PROFESSIONAL SOCIETIES

- American Oil Chemists Society — March 2006-Present
- NDSU Food Science Club — Sep. 2001-Present
- Institute of Food Technologists — May 2001-Present
- American Association of Cereal Chemists — May 1998-Present

AWARDS & SCHOLARSHIPS

- AACC Student Product Development Competition 2nd Place (Bison R Flax) 2004
- IFT Minnesota Section Travel Scholarships 2002, 2003 and 2004
- NDSU Department of Cereal Science Roman Meal Scholarships 2002 and 2004
- Istanbul Technical University Turkey Young Scientist Support Award 1999
- Gödöllö Agricultural University Hungary Summer Internship Scholarship 1994

SELECTED PUBLICATIONS

Book Chapters

Hall, C., **Tulbek, M.C.** and Xu, Y. 2006. Flaxseed. In: Advances in Food Science and Human Nutrition. Vol 51. pp 1-97. Elsevier Inc.

Proceedings and Presentations

Tulbek, M.C., Hall C., Wolf-Hall C.E. and Schwarz, J.G. 2005. Evaluation of sour-dough chickpea bread. American Association of Cereal Chemists (AACC) 2005 Orlando FL
Tulbek, M.C., Schorno, A., Meyers, S., Hall, C. and Manthey, F.A. 2004. Chemical and sensory analysis and shelf stability of roasted flaxseed. 60th Flax Institute Meeting. March 17-19 2004 Fargo ND
Boyacioglu, M.H., **Tulbek, M.C.**, Dexter, J.E., Preston, K.R., Hatcher, D.W. and Unal, S.S. 2002. The effects of extraction rate, dough mixing time and improvers on Turkish hearth bread quality of some Canadian durum wheat cultivars. American Association of Cereal Chemists (AACC) 2002 Montreal, CA.
Dexter, J.E., Preston, K.R., Hatcher, D.W., Boyacioglu, M.H., **Tulbek, M.C.** and Unal S.S. 2000. Flat bread, chapati and hearth bread quality of milled products varyıng in extraction rate and particle size from Canadian durum wheat cultivars, 11th Cereal and Bread Congress (CHL2000), September 8-15 2000 Australia

Budget

A fund of $46,780 is request for the completion of the project "Determination of fatty acid profiles and chlorophyll in canola using near-infrared (NIR) spectroscopy". Below is an estimated budget for the 24 month project.

	Year 1	Year 2	Total
A, and B. Salaries and Wages:	**7,140**	**7,140**	**14,280**

Graduate Students

We request a quart time stipend to fund one graduate student who will work on this project. A 2% fringe benefit rate for graduate students is charged at NDSU and is requested. The M.S. salary is $14,000/ year plus $140/year in fringe benefits. This student will be funded for 2 years, which comes to $14,280.

	Year 1	Year 2	Total
C. Equipment:	**21,872**	**0**	**21,872**

	Year 1	Year 2	Total
D. Travel:	**0**	**1000**	**1,000**

Travel for the graduate student to present the results of the experiments at a professional meetings. The PI recently attended a professional meeting of the American Oil Chemist's Society and Institute of Food Technologists. The average total expense for the was $1,000-1,200, thus the $1,000 request.

F Other Direct Costs

1. Materials and Supplies:	Year 1	Year 2	Total
	3,000	**3,000**	**6,000**
HPLC Solvents:	1,000	1,000	2,000
Chemicals/supplies for extractions:	1,000	1,000	2,000
Chromatographic supplies - GC:	1,000	1,000	2,000

	Year 1	Year 2	Total
2. Publication:	**0**	**500**	**500**

Cost for publishing in scientific journal. An average cost of $100 / page would be expected with the average article of five pages or and average cost of $500.

	Year 1	Year 2	Total
6. Fee	**2,500**	**2,500**	**5,000**

Art Killam will collaborate with us on the project. He will conduct some NIR analysis for a fee. The analysis cost $5 per sample. Each year we will have 500 samples to evaluate thus the cost per year will be $2,500.

	Year 1	Year 2	Total
Total Amount Requested:	**34,512**	**15,140**	**49,652**

RESEARCH & RELATED BUDGET - SECTION A & B

*ORGANIZATIONAL DUNS: 80-388-2299

*Budget Type: X Project ☐ Subaward/Consortium

Enter name of Organization: North Dakota Agricultural Experiment Station

Reset Entries *Start Date: 1-Oct-07 *End Date: 9/30/2008 Budget Period: 1

(If the Reset entries button is pressed, please navigate to previous year to enable the submission of the form.)

A. Senior/Key Person

	Prefix	*First Name	Middle Name	*Last Name	Suffix	*Project Role	Base Salary	Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
1						PD/PI							0
2													0
3													0
4													0
5													0
6													0
7													0
8													0
9	Total Funds requested for all Senior Key Persons in the attached file												0
												Total Senior/Key Person	0

B. Other Personnel

	*# of Personnel		Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
		Post Doctoral Associates						0
	1	Graduate Students	12			7000	140	7140
		Undergraduate Students						0
		Secretarial/Clerical						0
								0
								0
								0
								0
								0
								0
	1	Total Number Other Personnel					Total Other Personnel	7140
							Total Salary, Wages and Fringe Benefits (A+B)	7140

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment Item	*Funds Requested ($)
1	Shimadzu CG	21,872
2		
3		
4		
5		
6		
7		
8		
9		
#		
#	Total funds requested for all equipment listed in the attached file	
	Total Equipment	21872

Additional Equipment

D. Travel

		*Funds Requested ($)
1	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2	Foreign Travel Costs	
	Total Travel Cost	0

E. Participant/Trainee Support Costs

		*Funds Requested ($)
1	Tuition/Fees/Health Insurance	
2	Stipends	
3	Travel	
4	Subsistence	
5	Other	
☐	Number of Participants/Trainees	Total Participant/Trainee Support Costs 0

F. Other Direct Costs

		*Funds Requested ($)
1	Materials and Supplies	3000
2	Publication Costs	
3	Consultant Services	
4	ADP/Computer Services	
5	Subawards/Consortium/Contractual Costs	
6	Equipment or Facility Rental/User Fees	2,500
7	Alterations and Renovations	
8		
9		
#		
	Total Other Direct Costs	5500

G. Direct Costs — Total Direct Costs (A thru F): 34512

H. Indirect Costs — Total Indirect Costs:

I. Total Direct and Indirect Costs — Total Direct and Indirect Institutional Costs (G + H): 34512

J. Fee: 0

K. *Budget Justification (Only attach one file)

RESEARCH & RELATED BUDGET - SECTION A & B

*ORGANIZATIONAL DUNS: 80-388-2299

*Budget Type: X Project ☐ Subaward/Consortium

Enter name of Organization: North Dakota Agricultural Experiment Station

Reset Entries *Start Date: 10/1/2008 *End Date: 9/30/2009 Budget Period: 2

(If the Reset entries button is pressed, please navigate to previous year to enable the submission of the form.)

A. Senior/Key Person

	Prefix	*First Name	Middle Nam	*Last Name	Suffix	*Project Role	Base Salary	Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
1						PD/PI							0
2													0
3													0
4													0
5													0
6													0
7													0
8													0
9	Total Funds requested for all Senior Key Persons in the attached file												0
												Total Senior/Key Person	0

B. Other Personnel

*# of Personnel		Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
	Post Doctoral Associates						0
1	Graduate Students	12			7000	140	7140
	Undergraduate Students						0
	Secretarial/Clerical						0
							0
							0
							0
							0
							0
							0
1	Total Number Other Personnel					Total Other Personnel	7140
						Total Salary, Wages and Fringe Benefits (A+B)	7140

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment Item	*Funds Requested ($)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11	Total funds requested for all equipment listed in the attached file	
		Total Equipment 0

Additional Equipment

D. Travel

	*Funds Requested ($)
1 Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1000
2 Foreign Travel Costs	
Total Travel Cost	1000

E. Participant/Trainee Support Costs

	*Funds Requested ($)
1 Tuition/Fees/Health Insurance	
2 Stipends	
3 Travel	
4 Subsistence	
5 Other	
☐ Number of Participants/Trainees	Total Participant/Trainee Support Costs 0

F. Other Direct Costs

	*Funds Requested ($)
1 Materials and Supplies	3000
2 Publication Costs	500
3 Consultant Services	
4 ADP/Computer Services	
5 Subawards/Consortium/Contractual Costs	
6 Equipment or Facility Rental/User Fees	2,500
7 Alterations and Renovations	
8	1000
9	
10	
	Total Other Direct Costs 7000

G. Direct Costs — Total Direct Costs (A thru F) 15140

H. Indirect Costs — Total Indirect Costs

I. Total Direct and Indirect Costs — Total Direct and Indirect Institutional Costs (G + H) 15140

J. Fee 0

K. *Budget Justification (Only attach one file)

RESEARCH & RELATED BUDGET - SECTION A & B

*ORGANIZATIONAL DUNS: 80-388-2299

*Budget Type: x Project ____ Subaward/Consortium

Enter name of Organization: North Dakota Agricultural Experiment Station

Reset Entries *Start Date: 10/1/2007 *End Date: 9/30/2010 Budget Period: _____

(If the Reset entries button is pressed, please navigate to previous year to enable the submission of the form.)

A. Senior/Key Person

	Prefix	*First Name	Middle Name	*Last Name	Suffix	*Project Role	Base Salary	Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
1						PD/PI							0
2													0
3													0
4													0
5													0
6													0
7													0
8													0
9	Total Funds requested for all Senior Key Persons in the attached file												0
												Total Senior/Key Person	0

B. Other Personnel

*# of Personnel		Cal. Months	Acad. Months	Sum. Months	*Requested Salary ($)	*Fringe Benefits ($)	*Funds Requested ($)
	Post Doctoral Associates						0
2	Graduate Students	24			14000	280	14280
	Undergraduate Students						0
	Secretarial/Clerical						0
	Research Associate						0
							0
							0
							0
							0
							0
	Total Number Other Personnel					Total Other Personnel	14280
						Total Salary, Wages and Fringe Benefits (A+B)	14280

0

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment Item	*Funds Requested ($)
1	Shimadzu GC	21,872
2		
3		
4		
5		
6		
7		
8		
9		
10		
11	Total funds requested for all equipment listed in the attached file	
	Total Equipment	21872

Additional Equipment

D. Travel

	*Funds Requested ($)
1 Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	1000
2 Foreign Travel Costs	
Total Travel Cost	1000

E. Participant/Trainee Support Costs

	*Funds Requested ($)
1 Tuition/Fees/Health Insurance	
2 Stipends	
3 Travel	
4 Subsistence	
5 Other	
Number of Participants/Trainees	Total Participant/Trainee Support Costs 0

F. Other Direct Costs

	*Funds Requested ($)
1 Materials and Supplies	6000
2 Publication Costs	500
3 Consultant Services	
4 ADP/Computer Services	
5 Subawards/Consortium/Contractual Costs	0
6 Equipment or Facility Rental/User Fees	
7 Alterations and Renovations	
8	1000
9	
10	
	Total Other Direct Costs 7500

G. Direct Costs — Total Direct Costs (A thru F) 44652

H. Indirect Costs — Total Indirect Costs 0

I. Total Direct and Indirect Costs — Total Direct and Indirect Institutional Costs (G + H) 49652

J. Fee 0

K. *Budget Justification (Only attach one file)

SHIMADZU PRICE QUOTATION

Shimadzu Scientific Instruments, Inc.
CORPORATE OFFICE
7102 Riverwood Drive
Columbia, MD 21046
Phone: (410) 381-1227
Wats: (800) 477-1227
FAX: (410) 381-6781

MIDWEST REGIONAL OFFICE
8052 REEDER STREET
LENEXA, KS 66214
PHONE: (913) 888-9449
WATS: (877) 698-7923
FAX: (913) 888-8388

Terms: Net 30 days upon approval
Prices valid 30 days

Please Indicate This Number When Ordering: MWR 21555

TO: MARY NIEHAUS

NDSU
CEREAL & FOOD SCIENCE
HARRIS HALL ROOM 111
FARGO, ND 58105
Phone: 701-231-1044
Fax: 701-231-8044
Email: mary.niehaus@ndsu.edu

Date:	3/2/2006
Your Inquiry:	
Proposed Shipping Date:	30/45 Days ARO
Ship Via:	BEST WAY
Salesperson:	JOHN CAMPBELL
SalesPhone:	(612) 801-1542
F.O.B.	SHIPPING POINT
PPD & ADD:	XX
PPD:	

Thank you for your interest in Shimadzu.

PLEASE SEE ATTACHED TERMS AND CONDITIONS

DISTRIBUTION:
_____ Customer
_____ Sales Engineer
_____ Regional Office
_____ Corporate Office

Reference: NIEHAUS106

Item	Part #	NAME AND DESCRIPTION	Qty	Unit Price	Total Net
1	221-47700-92	**GC-2010AF SPL-2010 FID** The GC-2010 is a state-of-the-art Gas Chromatograph giving unsurpassed performance and reliability. This GC includes one AFC, one linear FID with electronic detector gas control and standard temperature and pressure control values.· Number of temperature programming steps: 20 The AFC (Advanced Flow Control) provides control of all parameters related to the retention time and split ratio. The GC-2010 ensures that all of the operating parameters are set reproducibly. The operation temperature profile, flow profile and heat zone temperatures are stored in 10 non-volatile memory files in the GC for easy access. Column Oven Cooling Time: 300° C to 50° C < 5 minutes, Temp. Column oven Programming Rate: to 250° C/min; Constant Linear Velocity, Pressure and flow program ramps with flow Control Range: 0 to 1200ml/min, Programming Rate: -400 to 400 ml/min., Atmospheric Pressure Compensation and Ambient Temperature Monitoring is standard, Operational oven Temperature maximum is 450° C, FID Detection Limit: 3pgC/s ., The standard linear FID has a dynamic range: 10 to the 7th, Dimensions and Weight (GC-2010): 515x437x 20mm WxHxD, Weight: 35kg. The standard AFC-2010 provides pressure up to 970kpa and flow up to 1200mL per minute. The SPL-2010 is suitable for columns from .1-.75. mm.id in split or splitless operation. No Data Station Included.	1	$14,492.00	$12,318.20
2	221-49213-91	**OCI/PTV-2010** Injection module for direct temperature programmable cool-on-column injection onto a 0.53mm I.D. capillary column. Compatible with smaller capillary columns via use optional glass insert (221-49381-01) or silanized glass insert (221-49381-02). The OCi/PTV-2010 is capable of Large Volume Injection (LVI) with LVI/PTV insert (221-49300-00). Cooled via forced air cooling fan. Includes AFC-2010 and all connecting tubing for plug and play operation A 0.47mm O.D. needle syringe is required	1	$5,923.00	$5,034.55
3	220-91390-00	**Shimadzu EZStart v7.3 Chromatography Software (Lite) License** One instrument fixed configuration only 32-bit Chromatography software package designed to run in Windows 2000 SP1, NT4 SP6 or higher and Windows XP. This package performs data acquisition, processing and reporting for a single chromatograph with up to 4 detectors simultaneously active, including PDA and RID. Includes VP Series LC control with PDA (required RS-232 I/F cable and SCSI parts for PDA) or LC-2010 /w optional PDA, or GC control with analog acquisition through SS420x (220-97231-00). Also RS-232 I/F cable required for GC control. For GC-17A, use 220-90843-00 and for GC-14A, use 220-90844-00. Includes SEC/GPC option and supports SIL-HT.	1	$2,100.00	$1,785.00

Item	Part #	NAME AND DESCRIPTION	Qty	Unit Price	Total Net
4	228-35397-92	**RS-232C Cable (2m)** 9 pin (female) - 9 pin (female) cross cable.	1	$53.00	$45.05
5	221-99999-99	**column, 15 m x .32mm id x .1um film thickness**	1	$500.00	$500.00
6	221-99999-99	**guard column, 5m x .53 mm high temp**		$200.00	$200.00
7	220-90001-06	**Gas Regulator, 19H350, H2 for GC** Gas Regulator for hydrogen; includes 1/8" outlet.	1	$402.00	$361.80
8	220-90001-07	**Gas Regulator, 19H580 He or N2 for GC** Gas Regulator for helium or nitrogen; includes 1/8" outlet.	1	$402.00	$361.80
9	220-90001-05	**Gas Regulator, 19H580 Air for GC**	1	$433.00	$389.70
10	220-90211-00	**Filter, molecular sieve** Also called an oxygen scrubber, used to remove impurities in carrier gas. Replace or recondition if saturated, indicated by unstable or noisy baseline.	1	$204.00	$183.60
11	220-90623-02	**Filter, H2** UOP high capacity molecular sieve filter specially formulated for cleaning H2gas. Has ⅛" swagelok fittings.	1	$304.00	$273.60
12	220-90623-00	**Filter, He** UOP high capacity molecular sieve filter specially formulated for cleaning He carrier gas. Highly recommended for use with QP-5000. Has ⅛" swagelok	1	$319.00	$287.10
13	220-90212-00	**Copper Tubing** Copper tubing 1/8" 50 feet.	2	$73.00	$131.40
14	INST & FAM	**Installation and Customer Familiarization**	1	$1,500.00	$0.00
15	1Y Warranty	**ONE YEAR WARRANTY** ONE YEAR WARRANTY: To cover non-consumable parts and labor to correct defects in workmanship and/or materials when defects have not been caused by misuse or abuse.	1	$0.00	$0.00
		DATA SYSTEM NEEDED--- NOT INCLUDED--- SPECS BELOW			
16	DATASYSTEM	**Standard Data Station (Win XP) /w 15"LCD** Micron - Pentium4 2.80GHZ w/HT (800FSB) Mini Tower PC with Windows XP and 15" LCD flat Panel . The system has an Intel®Pentium® 4 processor with NetBurstTM technology, 256MB Dual Channel DDR2 400 SDRAM, 512K L2 CACHE, 40 GB ATA100 hard drive, 52x Variable Speed CD-ROM, Memory is expandable to 2GB PC3200 SDRAM, 1.44 MB 3.5" floppy drive, 104-key enhanced keyboard, Microsoft Intellimouse, 64MB ATI Radeon X300 SE PCI Express Graphics Card, Intel Integrated Gigabit 10/100/1000 network connection, Four PCI slots, Two Serial Port, One Parallel Port, 8 USB 2.0 (2 front and 6 back), Integrated Flex 6 Audio (no speakers). Warranty includes 5/3 year limited parts warranty with 36 months Next Business Day On-site service• Micron (AmTran) 15" Dual-Input LCD Flat Panel w/ DVI-I, 15" (15.0" viewable), Flat Panel:TFT (Thin Film Transistor), Active Matrix Panel w/antiglare glass surface, Color Filter: Pixel RGB vertical stripe, Dot Pitch: Variable .297mm pixel pitch, Maximum Resolution:1024 x 768 @ 75Hz Number of Display Colors:16.7 million colors (True color)	1		

Item	Part #	NAME AND DESCRIPTION	Qty	Unit Price	Total Net
		Total List Price		**$26,778.00**	
		Total Discount		**$4,906.20**	
		Total Sales Price		**$21,871.80**	

AUTHORIZED SIGNATURE: _____