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DISCOVERY

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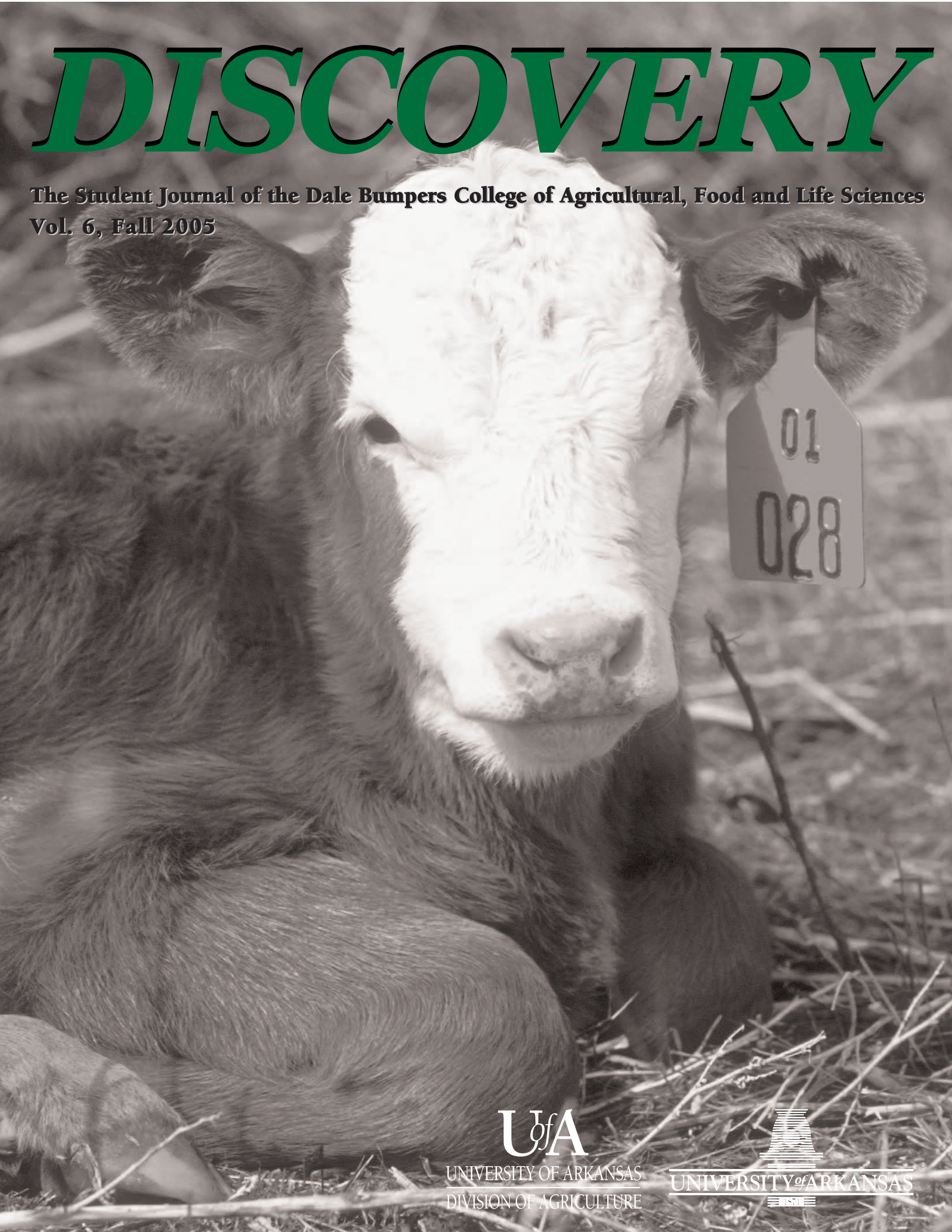
Vol. 6, Fall 2005



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DISCOVERY

The Student Journal of the Dale Bumpers College of Agricultural, Food and Life Sciences
Vol. 6, Fall 2005

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Contents

Undergraduate Research Articles

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1871

Letter from the Dean

Gregory J. Weidemann

The University of Arkansas continues to make remarkable progress towards our goal of being recognized as a as a nationally competitive, student-centered research university serving Arkansas and the world. The Dale Bumpers College of Agricultural, Food and Life Sciences and the UofA System's statewide Division of Agriculture provide a rich research and scholarly environment for all who enroll in our college.

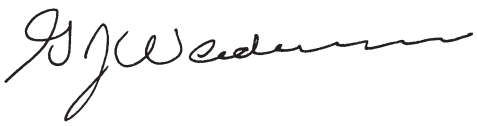
The opportunity students have to work directly with renowned faculty in a research environment helps them prepare to enter the work force after graduation or to continue their education in graduate school. Some 60 faculty members presently serve as mentors to students engaged in research projects that address real-world problems. Most, but not all, student research projects are designed to meet the requirements of an honors thesis in the Bumpers College Honors Program. Whether in the Honors Program or not, students who take advantage of research opportunities gain scientific and professional skills that can strengthen their contributions to society. Conducting a research project and authoring the results add value to their university education and enrich their educational experience.

Bumpers College encourages student research by awarding undergraduate research grants, including the Carroll Walls Undergraduate Research Fellowship, which provides a grant of $1,000. We awarded 14 Undergraduate Research Grants in Fall 2004.

DISCOVERY is a dedicated journal for our student scientists that documents their research efforts and outcomes. Being published in *DISCOVERY* does not supersede publication elsewhere; this journal provides a forum for students and faculty to share their methods and findings in a citable publication.

The six articles in this sixth annual volume of *DISCOVERY* explore research and production issues in food science, animal science, tomato cultivation, rice milling, and the soybean industry. We are proud to present these articles as examples of the research accomplishments of our undergraduate students.

I heartily congratulate the student authors on their accomplishments and extend thanks to their faculty mentors and to the editors who reviewed their manuscripts. Thanks also to the Honors Committee for providing a structured program that encourages our students to pursue the scientific method in service to society.



Gregory J. Weidemann, Dean
and Associate Vice President for Agriculture

Inhibitory activity against *Listeria monocytogenes* by soy-protein edible film containing grape seed extract, nisin, and malic acid

Brittany Adams[*], *T. Sivarooban*[†], *N.S. Hettiarachchy*[§], *and M.G. Johnson*[‡]

ABSTRACT

The frequent outbreaks of food-borne illness necessitate development of intervention strategies, including the use of natural antimicrobials. *Listeria monocytogenes* is one of the most important bacterial pathogens that recently has caused a significant number of outbreaks. With the aim of finding potent natural agents that can minimize pathogen contamination concerns, this study evaluated the inhibitory activities against *L. monocytogenes* of grape seed extract (GSE), malic acid (M), nisin (N), and combinations thereof incorporated into soy-protein edible films. Soy-protein films with/without addition of antimicrobial agents (GSE: 1%, Nisin: 10,000 IU/g, Malic acid: 1%, and their combinations) were prepared and evaluated for anti-listerial activities. The highest inhibitory activity after 1 h incubation at 25°C was found in the treatment containing GSE, nisin, and malic acid, which produced reductions of log 3.7 colony-forming units (CFU)/ml as compared to control film without the addition of antimicrobial agents. These data demonstrated that the GSE, nisin, and malic acid combination incorporated into soy-protein edible films is very effective in inhibiting *L. monocytogenes* growth at 25°C and has potential for applications on a variety of food products to help prevent *L. monocytogenes* contamination and growth.

[*] Brittany Adams is a sophomore majoring in food science.

[†] T. Sivarooban is a Ph.D student in the Department of Food Science.

[§] Navam S. Hettiarachchy, teacher and faculty mentor, is a professor in the Department of Food Science.

[‡] M.G. Johnson, teacher and faculty mentor for guiding microbiological procedures, is a professor in the Department of Food Science.

MEET THE STUDENT-AUTHOR

Brittany Adams

I graduated from Jonesboro High School in 2003 and enrolled at the University of Arkansas in the fall as a food science major. I was awarded the Chancellor's scholarship as well as the Governor's Distinguished Scholarship and since then, I have also been awarded an Institute of Food Technologists scholarship. I am a member of the Food Science Club and the Institute of Food Technologists. I am also an active member of the IFT college bowl team for the University of Arkansas.

Spring semester of my freshman year I began working for Dr. Hettiarachchy conducting research in the area of utilizing proteins and anti-microbial plant extracts to inhibit pathogens, which led me to this research project. I have competed twice in the Gamma Sigma Delta undergraduate research competition and received 1st place twice in the poster category and 2nd place in the oral category. I also competed in the Ozark Food Processors Association undergraduate poster competition and received 1st place for my research in anti-microbials for food safety. I plan to continue with graduate studies after completing my B.S. in food science and become a director of research in R&D.

INTRODUCTION

Food-borne pathogens, which cause illness, death, and great economic losses, are of great concern to consumers and the food industry. The annual economic loss associated with food-borne disease is approximately $5-6 billion (Murphy et al, 2003). *Listeria monocytogenes* is one of the most significant pathogens that has caused numerous outbreaks (Janes et al., 2002). *L. monocytogenes* is a cold-loving (psychrophilic) pathogen that causes an average of 2,500 illnesses and 500 deaths each year (Mead et al., 1999; CDC, 2003). In 2003 and 2004, respectively, 45,251 and 502,845 pounds of refrigerated ready-to-eat (RTE) meat products including chicken, turkey, and beef meats were recalled due to *L. monocytogenes* contamination (FSIS/USDA, 2005).

L. monocytogenes is of particular concern for the elderly, unborn fetuses, and those who are immuno-compromised (Lorber, 1990). Food products can be cross contaminated with *L. monocytogenes* during processing. This causes the most problems in RTE products such as luncheon meats, which require no further treatment post-packaging. These RTE meat products become prime candidates for food-borne disease transmission if they are contaminated during processing, and thus can cause illness in consumers.

The food industry of late has been showing an increased interest in edible films to control various spoilage challenges of foods and increase shelf life. Research regarding these films has centered on casein, collagen, corn zein, gelatin, soy protein, wheat gluten, calcium alginate, and methylcellulose (Eswaranandam et al., 2004; Gennadios and Weller, 1991; Guilbert, 1997; Hoffman et al., 1998; Janes et al., 2002; Lungu and Johnson, 2005; Were et al., 1998&1999). These edible films can be used as carriers for various beneficial compounds including antimicrobials. A slow release of these antimicrobials from the film to the food product would control bacterial growth, and thus increase the shelf life of the products.

There is an increasing interest in natural antimicrobial compounds, especially those of plant origin, since they are considered as safe and economical. Phenolic compounds in plant extracts are responsible for antimicrobial and antioxidant activities. Grape seeds have been recognized to have health benefits for human beings. Hettiarahchy et al., (2003a and 2003b) and Adams et al., (2004) have demonstrated the antimicrobial and antioxidant qualities of grape seed extract. Grape seed extract is commercially available and is currently used in a variety of food products.

Organic acids and their salts are also promising antimicrobial agents because of their established acceptance in food products and low cost (Miller et al., 1995). Organic acids are either naturally present in fruits and vegetables or synthesized by microorganisms. Eswaranandam et al., (2004) reported that malic acid incorporated into a soy protein film was very effective in controlling *L. monocytogenes*.

Nisin is a hydrophobic protein containing 34 amino acids. It is a bacteriocin produced by Lactococcus lactic sub sp. Lactis (Jung et al., 1991; Montville et al., 2001). It is recognized as a safe biological food preservative by the Food and Drug Administration and is effective in controlling a wide range of gram-positive bacterial growth, including *L. monocytogenes*. Currently, nisin is approved for use in the U.S. for soft cheeses to control *Clostridium botulinum* growth.

No literature is available on the antimicrobial activity of grape-seed and tea extracts, nisin, and their combinations with malic acid-incorporated edible films. Therefore, the objective of this study was to evaluate the inhibitory effects at 25°C against *L. monocytogenes* of grape seed extract (GSE) and the combined effects of grape seed extract with nisin and malic acid incorporated into soy-protein edible films.

MATERIALS AND METHODS

Pathogenic *L. monocytogenes* (strain V7 serotype 1/2a) was obtained from the Food Microbiology Research Laboratory in the Department of Food Science at the University of Arkansas, Fayetteville. *Listeria* Selective Agar (Oxford formulation) and malic acid were obtained from EM science, (EM Industries, Gibbstown, N.J.) Glycerol was purchased from Sigma Chemical Co. (St. Louis, Mo.) Soy protein isolate (AR-DEX®) was obtained from Archer Daniel Midland, Decatur, Ill. Commercial grape seed extract (GSE) powder was obtained from MegaNatural Inc. (Madera, Calif.) Nisin (Nisaplin®) was obtained from Aplin and Barrett Ltd (Dorset, U.K.)

Antimicrobial testing of grape seed extract / nisin / malic acid combinations in BHI broth model system

The anti-microbial effects of grape seed extract, nisin, and malic acid were evaluated for their effectiveness against *L. monocytogenes* in a model system. One loop of the organism was transferred from the frozen stock at -70°C to 10 mL of Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 h. Thereafter, 10 µL of this culture was transferred into 10 mL of fresh BHI and incubated for activation at 37°C for 18 h after which 20 _L of the culture was added to 10 mL BHI for the tests (approximately 106 log C F U / ml). Precisely 0.5 mL of this culture was combined with 0.5 mL of a test solution (GSE, nisin, malic acid, or a combination) and incubated at 37°C for 24 h. The samples were diluted in phosphate buffer solution (PBS) and spread plated onto Listeria Selective Agar and incubated for 48 h at 37°C and the colonies were counted.

Preparation of soy protein films

To prepare the films, 10 g of soy protein were added to and mixed well with 90 g of de-ionized water in each sample (GSE, nisin, malic acid, combinations, and control). A concentration of 3.5% glycerol (plasticizer) was added and stirred with a magnetic stirrer for 30 min. The solutions were then heated and stirred in an 85°C water bath for 30 min and were then allowed to cool to room temperature. After cooling, GSE (1%w/w), nisin (10,000 IU/g), malic acid (1%w/w), or one of their combinations were added to the film solutions. A control film was prepared which contained soy protein isolate and glycerol with no additional substances.

Casting films

The resulting film solutions prepared were cast onto 19 x 28 cm^2 mylar plastic sheets coated with silicone (Richard Mistler, Inc., Morrisville, Penn.) by way of draw-down equipment (Paul N. Gardner Co., Inc.,Pompano Beach, Fla.) The solutions formed a film with uniform thickness and were then dried for 4 h in a controlled humidity chamber (Hot Pack, Philadelphia, Penn.) at 50°C and 40% RH. The films were then removed from the chamber and placed between sheets of wax paper and into a 5°C, 50% RH desiccator over a NaBr-saturated solution (Sanplatec Corp., Osaka, Japan) for storage until testing of antimicrobial activity.

Thickness of films

At three different locations on each of the films, a small section was measured for thickness. The three values were averaged to determine the thickness of each film. These measurements were performed with a micrometer (Model 2804 – 10, Mitutoyo, Japan) and determined to the nearest 2.5 mm.

Puncture strength of films

Puncture strength measures the ability of the film to hold up under various stresses after being applied to the food product. This measurement was taken using a texture analyzer (TA-XT2I, Texture Technologies Corp., Scarsdale N.Y.) The film samples were allowed to reach room temperature and a RH of 50% for 48 h before the test was conducted. A 30-mm piece of film was placed on a 10-mm film-testing rig (TA- 108S Mini) and punctured with a 2-mm probe (TA-52). The puncture strength of the film (given as a force in Newtons) was measured at the point when the probe pierced the film.

Antimicrobial testing of soy protein films

To determine the killing effect of each film, bacterial cultures were inoculated onto a film disc and total plate counts were enumerated. The bacterial suspensions were prepared once more and diluted in PBS to yield approximately 1.5 x 10^6 bacteria. Thereafter, 15 µL of this suspension was inoculated onto each film disc (1 cm diameter). The inoculated discs were then transferred to stomacher bags and 985 µL of PBS were added to each bag. These bags were stomached for 2 min to completely dissolve the film. The resulting mixture was diluted up to a 10^6 dilution with PBS and spread plated onto *Listeria* Selective Agar plates. These plates were incubated at 37°C for 48 h and the colonies were counted.

RESULTS AND DISCUSSION

Anti-Listerial activities of grape seed extract, nisin and malic acid, and their combinations in BHI broth medium at 37°C

It was found that an initial *L. monocytogenes* inoculation level of about 6.7 log CFU/ml grew to 8.9 log CFU/ml after 24 h at 37°C in BHI (control) medium (Fig. 1.) In the presence of GSE or nisin alone at the same initial inoculation level of 6.7 log CFU/ml, *L. monocytogenes* changed to 4.4 and 8.6 log CFU/ml, respectively, (Fig. 1.) The growth was reduced to non-detectable levels after 6 h incubation in the presence of malic acid alone. The highest inhibitory activity was found in the treatment with GSE combined with nisin and malic acid or GSE combined with malic acid or malic acid combined with nisin, (Fig. 1.) The logs CFU/ml at 0 h and 3 h for treatments with GSE with malic acid / malic acid with nisin / GSE, nisin, and malic acid were 6.7 and non-detectable levels, respectively. No growth was observed in these extracts after incubation of 3 to 24 h.

Thickness and puncture strength of films

Incorporation of GSE increased the thickness and puncture strength of soy protein film, (Fig. 2 and 3.) Higher molecular-weight individual phenolic constituents including catechin, genestic acid, epicatechin, and syringic acid; and gallic acid, protochatechuic, and caffeic acid (Rababah et al., 2004) present in the GSE might have contributed to the thickness and puncture strength of soy protein films. The higher molecular-weight, hydrophilic nature of these phenolics and their hydroxyl groups may participate to increase the protein-protein inter-chain interactions and reduce the plasticizing effect in the film-forming solution.

When nisin was incorporated with soy protein film, thickness of the film significantly increased ($p<0.05$), (Fig. 2.) The nisin molecules could have contributed to hydrophobic and electrostatic interactions with protein molecules. No significant differences were observed in the addition of nisin (10,000 IU/g) on puncture strength of soy protein film, (Fig. 3.)

Incorporation of malic acid reduced the thickness and puncture strength of soy protein film, (Fig. 2 and 3.) The lower molecular-weight and plasticizing properties of malic acid may have disturbed the higher molecular-weight protein-polymer chain interactions. The addition of malic acid to the film solutions also decreased the pH of the film.

Anti-Listerial activities of soy-protein edible film containing grape seed extract, nisin, malic acid, and their combinations

The counts of *L. monocytogenes* were approximately 6.6 log CFU/ml after 1 h incubation at 25°C in the soy-protein edible film which had no addition of antimicrobial agents (control), (Table 1). The count of *L. monocytogenes* was reduced to log 5.7 CFU/ml in the presence of GSE alone (Table 1). Of all possible combinations of the compounds, the highest inhibitory activity was found in the combination of GSE, nisin, and malic acid, with reductions of 3.7 log CFU/ml compared to the soy-protein film control, (Table 1) The hydroxyl groups present in the phenolic compounds of GSE were likely responsible for its antimicrobial activity. Nisin has the ability to form pores in the cell membrane of the bacteria, thereby allowing a free flow of ions into and out of the cell, which causes an imbalance and eventually cell death. Malic acid has a very small molecular weight and can easily enter the bacterial cell. This helps disrupt the internal pH of the cell and reduce the protein motive force, thereby causing death. Nisin and malic acid enhanced the effectiveness of anti-listerial activities of GSE. The killing mechanism of these three natural antimicrobial compounds incorporated into soy protein film was very effective against *L. monocytogenes*.

These findings will have applications for controlling *L. monocytogenes* contamination in various food products including raw and ready-to-eat poultry and beef products as well as fresh, whole, and minimally processed vegetables and fruits.

ACKNOWLEDGMENTS

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Table 1. Effect of grape seed extract, nisin, malic acid and their combinations incorporated in soy-protein edible film against *Listeria monocytogenes* at 25°C

Edible film	*L. monocytogenes* (Log CFU/ml)
Control (SPI)	6.6 ± 0.1^{a}
GSE (1% w/w)	5.7 ± 0.1^{b}
N	4.8 ± 0.2^{de}
M	5.1 ± 0.1^{cd}
GSE + N	4.6 ± 0.1^{e}
GSE + M	5.3 ± 0.1^{c}
N + M	4.0 ± 0.2^{f}
GSE + N + M	2.9 ± 0.1^{g}

Film disc (1cm diameter) was inoculated (appoximately10^{6} CFU/ml) and incubated at 25°C for 1 h. Log numbers of *L. monocytogenes* in control film was 6.6 ± 0.1 CFU/ml.

All means were measurements of three separate experiments in duplicates. Means within a column followed by same superscript are not significantly different ($p<0.05$).

Abbreviation of treatments are as follows: SPI=soy protein film, GSE=grape seed extract (1% w/w), N=nisin (10,000IU/g), M=malic acid (1% w/w).

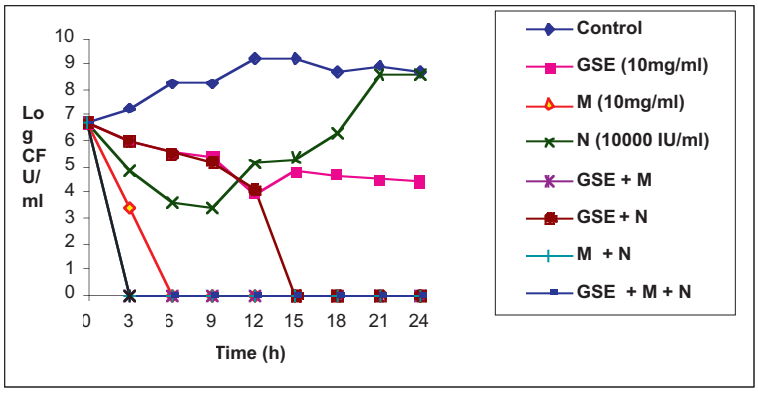

Fig. 1. Anti-listerial activities of grape seed extract (GSE), nisin (N), malic acid (M), and their combinations in BHI broth medium at 37°C. (Values represent means of three separate experiments.)

Abbreviation of treatments are as follows: SPI=soy protein film, GSE=grape seed extract (1% w/w), N=nisin (10,000IU/g), M=malic acid (1% w/w).

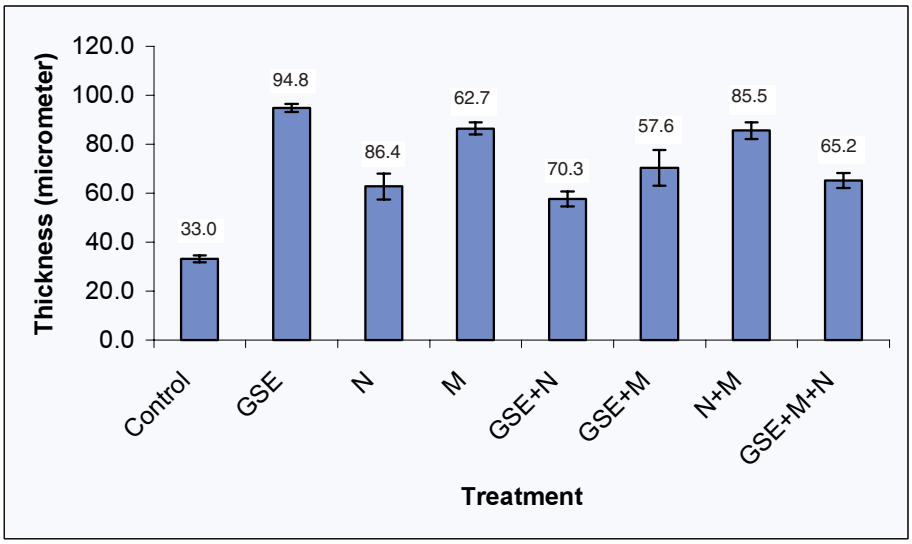

Fig 2. Thickness of soy protein films containing grape seed extract (GSE), nisin (N), and malic acid (M) and their combinations

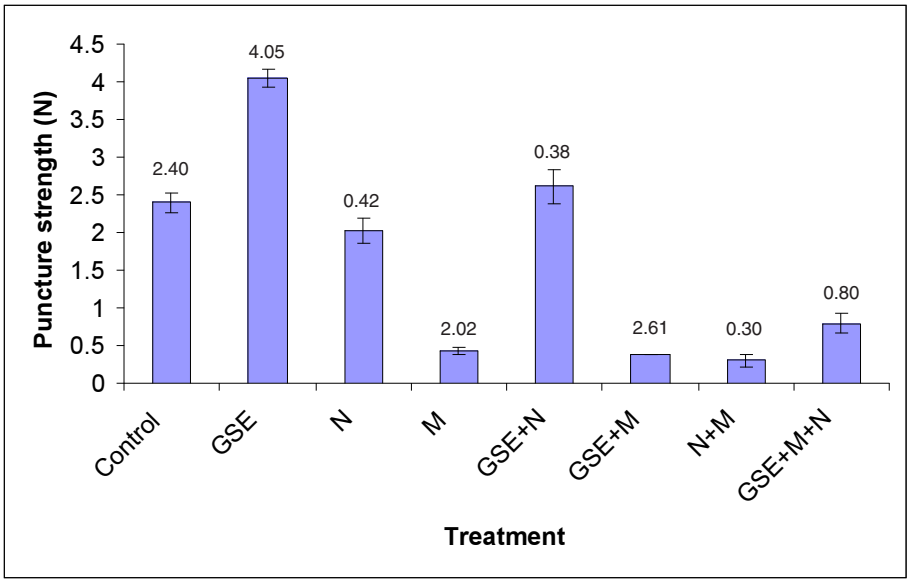

Fig 3. Puncture strength of soy protein films containing grape seed extract (GSE), nisin (N), and malic acid (M) and their combinations

Abbreviation of treatments are as follows: SPI=soy protein film, GSE=grape seed extract (1% w/w), N=nisin (10,000IU/g), M=malic acid (1% w/w).

Effect of social status of dairy heifers on expression of estrus and subsequent fertility

Dawn A. Elkins[*] *and Rick W. Rorie*[†]

ABSTRACT

A study was conducted to determine if social status of heifers within a herd influences estrus activity and subsequent fertility. Thirty cyclic dairy heifers were observed over a 14 d period and ranked by social status, based on a displacement index. The estrous cycles of the heifers were synchronized by treatment with two injections of prostaglandin F2a (PGF2a; Lutalyse, 25 mg) given 14 d apart. At the second PGF2a injection, HeatWatch transmitters were placed on the heifers for continuous monitoring of mounting (estrus) activity over the next 45 d. All heifers were artificially inseminated at estrus, using semen from a single sire. Pregnancy status was determined by ultrasonography post-insemination. For analysis, heifers were placed into three groups based on social status. Subordinate heifers, ranked in the bottom one-third of the herd, exhibited a shorter estrus ($P = 0.001$) than more dominant heifers. The number of mounts recorded during estrus declined with decreasing social status ($P = 0.009$). There was a trend for the number of mounts per h to be greatest for heifers in the top one-third of the social hierarchy ($P = 0.074$). Of those heifers detected in estrus and inseminated, pregnancy rate was similar among the social groups for the first and subsequent inseminations ($P = 0.315$ and 0.608, respectively). Preliminary results indicate that social hierarchy of dairy heifers influences length and expression of estrus. However, social standing within the herd does not influence fertility of heifers detected in estrus.

[*] Dawn A. Elkins is a senior majoring in animal science.

† Rick W. Rorie, faulty sponsor, is a professor in the Department of Animal Science.

MEET THE STUDENT-AUTHOR

As a 2002 graduate from Valley View High School in Jonesboro, Ark., I left in search of great knowledge and found the University of Arkansas was going to be the place where I would begin my college career. I never grew up around livestock and only started studying animal science in hopes of becoming a veterinarian. I soon found out that was not the path I wanted to pursue, so I became more involved in different aspects of the animal science field. I took a job under Dr. Elizabeth Kegley, who allowed me to work in the Animal Science Nutrition Lab. I really enjoyed this aspect of animal science and soon asked Dr. Kegley who in the department might be interested in taking on an undergraduate for a research project that I could use as my honors research project. She introduced me to Dr. Rick Rorie, who became my mentor. Upon graduating in 2006, I plan to enroll in graduate school and study more about reproductive physiology.

Dawn A. Elkins

INTRODUCTION

A successful artificial insemination program in cattle is dependent on accurate and efficient estrus detection. Visual observation for estrus, which is based on mounting activity, is accurate but not very efficient without continuous observation of the animals. During any given estrous cycle, 30-50% of cows or heifers within a herd are not detected as in estrus by visual observation (Rorie et al., 2002). A reproductive examination of animals failing to exhibit estrus usually does not reveal any physiological abnormalities. Failure to detect estrus has been attributed to infrequent mounting activity and/or short estrus periods (Dransfield et al., 1998). It is possible that failure to express estrus activity could be correlated to an individual animal's social status within the herd.

When a group of cows or heifers are placed together, they will establish a "pecking order" or social hierarchy. The social hierarchy can be dependent upon age, breed, temperament, weight, and presence or absence of horns (Ewing et al., 1999). The number of animals in the group can affect the amount of stress in a particular herd. Individuals at the lower end of the social hierarchy can be subjected to considerable levels of stress which in turn can result in adverse effects on all production traits, including reproduction (Dobson et al., 2001). Social stresses might explain why some animals within a group fail to express sexual receptivity (estrus) and thus have poor fertility. While studies have been conducted to determine how social status in bulls affects reproductive efficiency within a herd (Garcia et al., 1986), very limited research has been conducted to determine the effects of social status in cows on expression of estrus and fertility. Therefore, the objective of this study was to determine if the social status of dairy heifers within a herd influences their expression of estrus and subsequent fertility.

MATERIALS AND METHODS

A group of 30 crossbred dairy heifers, predominantly of Holstein or Jersey breeding, ranging from 14 to 16 months of age and weighing between 287 and 376 kg, were used for this study. The heifers were maintained on pasture and fed supplemental grain and hay to achieve a gain of ~ 0.77 kg/d. Prior to the start of the study, the heifers were examined via rectal palpation and ultrasonography to confirm that they were cyclic (based on the presence of a corpus luteum on one ovary) and free of any obvious reproductive tract abnormalities.

The heifers were observed over a 14 d period for expression of dominant or submissive behaviors in individual confrontations. Dominant behavior included

butting, charges, and pushing, whereas submissive behavior included avoidance either of an individual or of a situation, and submission to or displacement by the aggressor (Galindo and Broom, 2000; Phillips and Rind, 2002). Data collected were used to determine the social status of individual heifers based on a displacement index (Galindo and Broom, 2000).

The displacement index was calculated based on the number of times a heifer displaced another individual divided by the number of times a heifer displaced another individual plus the number of times the heifer herself was displaced. This formula gave a continuous range of numbers between 0 and 1, with the higher the number the more dominant the individual animal (Galindo and Broom, 2000).

To aid in breeding, the estrous cycles of the heifers were synchronized by treatment of two injections of prostaglandin F2a (PGF2a; Lutalyse, 25 mg, Pfizer Animal Health, New York, N.Y.) given 14 d apart. At the time of the second PGF2a injection, HeatWatch (DDx Corp., Denver, Colo.) transmitters were placed on the heifers for continuous monitoring of mounting (estrus) activity over the next 45 d. The HeatWatch system electronically recorded the time of onset of estrus, the length of estrus, and the number of mounts during the estrus period. The HeatWatch parameters for estrus were three or more mounts of at least 2 sec duration each within a 4-h period. The time of the first mount within the 4-h period was considered the onset of estrus.

All heifers were artificially inseminated approximately 12 h after onset of estrus, using frozen-thawed semen from a single Jersey sire. An experienced technician performed all inseminations. Pregnancy status was determined for heifers failing to return to estrus by ultrasonography, using an Aloka 500V (Aloka Corp., Tokyo, Japan) ultrasound with a 5 MHz trans-rectal transducer at approximately 35 d post-insemination. Heifers that returned to estrus after the first insemination were inseminated again and pregnancy status was determined by ultrasonography approximately 30 d later.

Data were analyzed using JMP statistical software (SAS Institute, Cary, N.C.). For analysis, heifers were placed into three groups (top, middle, and bottom one-third of the herd) based on displacement index scores. Analysis of variance was used to determine any differences among the three groups of heifers for length of estrus, number of mounts during estrus, and number of mounts/h. Treatment means were compared, using Student's t test. Pregnancy rate was compared among groups using chi-square analysis.

RESULTS AND DISCUSSION

Overall, 27 of 30 (90%) heifers were detected in estrus by the HeatWatch system (Table 1). Visual observation of heifers for estrus (i.e., mounting activity) can only detect about 50-70% of animals in estrus during any given estrous cycle (Rorie et al., 2002). The use of the HeatWatch system to continuously monitor animals for estrus illustrates the greater efficiency of an electronic estrus-detection system over that reported for visual observation alone.

With a range approaching 100 kg difference in body weight among individual heifers, it might be assumed that larger heifers might be the more dominant animals in the herd. However, the mean weight of heifers in the three social groupings only ranged from 320 to 333 kg and were similar ($P = 0.369$), regardless of the group's social rank. These results are an agreement with others who report that several factors in addition to weight contribute to dominance, including age, breed, temperament, and presence or absence of horns (Ewing et al., 1999).

Subordinate heifers, ranked in the bottom one-third of the herd, exhibited a shorter estrus ($P = 0.001$) than more dominant heifers (Table 1). The number of mounts recorded during estrus declined with decreasing social status ($P = 0.009$). The number of mounts/h tended to be greater for heifers in the top one-third of the social hierarchy ($P = 0.074$). Previous research (Dransfield et al., 1998) reports that ~ 25 % of cows have infrequent mounts during estrus and/or estrus periods of short duration. With visual observation, cows are typically observed for about an hour twice per day for signs of estrus, and thus this method may fail to detect cows undergoing infrequent mounting activity. Study results suggest that the animals not detected as in estrus by visual observation could be the more subordinate animals in the herd.

Of those heifers detected in estrus and inseminated, pregnancy rate (Table 2) was similar among the social groups after the first insemination ($P = 0.315$), as well as for the cumulative pregnancy rate after the second insemination ($P = 0.608$). Therefore, duration and intensity of estrus had no effect on subsequent fertility. These results are in agreement with previous findings of Rorie et al. (2002), who evaluated estrus parameters of over 500 beef cows and found no relationship between the length of estrus or mounting activity and pregnancy status after artificial insemination.

In summary, this study indicates that the social status of dairy heifers within a herd influences the length and expression of estrus. Subordinate heifers have less

mounting activity during estrus and a shorter duration of estrus. Social status does not influence subsequent pregnancy rates of heifers detected in estrus. However, social status could reduce the overall pregnancy rate due to failure to detect subordinate heifers in estrus by visual observation. It would likely be advantageous to manage cows or heifers in small rather than large herds for estrus detection and artificial insemination. Smaller groupings should reduce social stress and increase the chances of detecting subordinate animals in estrus.

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Table 1. Effect of social status of heifers on length and mounting activity during estrus.

Social group	Detected in estrus	Length of estrus (h)	Number of mounts	Mounts per hour
Top one-third	10/10	15.0 ± 1.4^{a}	36.0 ± 5.2^{c}	2.4 ± 0.3^{f}
Middle one-third	9/10	13.7 ± 1.0^{a}	22.4 ± 5.3^{d}	1.6 ± 0.2^{g}
Bottom one-third	8/10	8.1 ± 1.1^{b}	13.8 ± 2.4^{e}	1.9 ± 0.3^{g}

Numbers within columns with different superscripts differ ($^{ab}P = 0.001$; $^{cde}P = 0.009$; $^{fg}P = 0.074$).

Table 2. Effect of social status of heifers on pregnancy rate after artificial insemination*.

Social group	Pregnancy rate (first insemination)	Pregnancy rate (second insemination)
Top one-third	4/10 (40.0%)	7/10 (70.0%)
Middle one-third	4/9 (44.4%)	6/9 (66.7%)
Bottom one-third	6/8 (75.0%)	7/8 (87.5%)

*There were no significant differences among means ($P \geq 0.315$)

Level and source of supplemental selenium for beef steers

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ABSTRACT

Selenium (Se) is deficient in many Arkansas soils; therefore, an experiment was conducted on steers to evaluate the effects of two supplemental Se sources on performance, blood metabolites, and immune function. Thirty Angus-crossbred steers were blocked by weight and assigned within block to one of 15 pens (two steers/pen). Pens were assigned randomly within blocks to one of three dietary treatments consisting of a corn-soybean meal supplement devoid of supplemental Se (negative control, NC) or corn-soybean meal supplements providing 1.7 mg supplemental Se/d as sodium selenite (inorganic Se, ISe) or as Se yeast (organic Se, OSe). Steers were offered fescue hay to allow for approximately 10% refusals, and 1.1 kg/d (as fed basis) of the appropriate grain supplement. Level and source of supplemental Se did not affect average daily gain for the 105-d trial. By d 42, steers fed both sources of supplemental Se had greater blood Se concentrations than those fed the NC. On d 63 and 84, blood Se concentrations differed among all dietary treatments (NC < ISe < OSe), and on d 105 steers fed both sources of supplemental Se had greater blood Se concentrations than NC. Antibody response to vaccination for bovine respiratory viruses, or in vitro lymphocyte blastogenesis did not differ among steers fed the different diets. Both sources of supplemental Se increased blood Se concentrations, the organic source more rapidly than the inorganic source; however, Se level and source had minimal effects on immune function of weaned beef steers.

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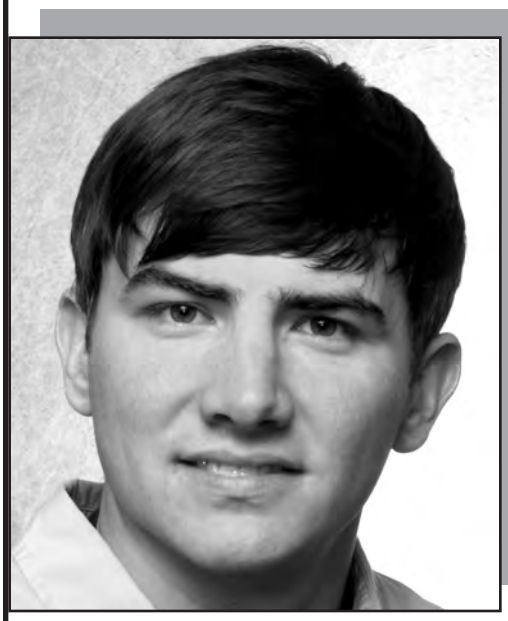
MEET THE STUDENT-AUTHOR



R. Scott Fry

I graduated from Quitman High School in May 2001, and I began my college career at the University of Arkansas in August 2001. I come from a strong agricultural background in rural Arkansas. I have always had a great deal of knowledge about the production aspects of the cattle industry, and I saw an honors research project as a way to explore the world of research in agriculture.

This research project helped me obtain an internship position at USDA-ARS in Beltsville, Md., where I was involved in research with reproductive physiology in turkeys. Experiences like these have really brought out in me a great appreciation for research. I feel that the educational experiences and my involvement on campus at the U of A have really opened many windows of opportunity for me. The U of A and the Bumpers College have proven to be outstanding scholarly environments in which to become more knowledgeable and prepared for the real world.

I am a senior and will graduate in May 2005, and I plan to attend graduate school in the field of animal science. I plan to focus my research on ruminant nutrition and the immune function of beef cattle.

I would like to thank Dr. Beth Kegley and Dr. Ellen Davis for their guidance in my research project. Also I would like to acknowledge Michael Ratcliff, Doug Galloway, and Pete Hornsby for their assistance in the completion of the selenium trial. I would also like to thank Alltech Inc. for the donation of their product. All involved are greatly appreciated.

INTRODUCTION

Selenium deficiency is a serious problem in livestock production worldwide. Severe cases can be found in Finland, New Zealand, China, Japan, and the United States. The most Se-deficient areas in the United States are found in the Northwest, Southeast, and the Northeast, including many of the states adjoining the Great Lakes (McDowell, 2003). Large areas of Arkansas' soil are deficient in trace minerals, including Se. With these problems in mind it is important to pay attention to the effects Se deficiency has on the agricultural/beef industry in Arkansas. Arkansas ranks fifteenth nationally in beef cattle production with a cattle population exceeding 1.8 million on 30,000 farms (Arkansas Farm Bureau, 2003). Arkansas' cattle production is mainly a cow/calf system with some grazing of feeder and stocker animals that are ultimately shipped to the high plains and Midwest for finishing and processing. It is very important that producers in Arkansas, as well as throughout the U.S., manage mineral deficiency problems.

Cattle consuming forages from Se-deficient soils are at risk of developing Se deficiency-associated disorders, including white muscle disease; ill thrift syndrome; reproduction dysfunction (neonatal weakness, retained placenta, calf scours, calf pneumonia, abortion, stillbirths, decreased fertility); and diarrhea (McDowell, 2003). Selenium functions to prevent oxidative damage to body tissues (Hoekstra, 1974), and Se deficiency can inhibit antibody production to foreign challenges (Mulhern et al., 1985) and the detoxification of certain toxins (Burk, 1983). Studies have indicated that calves can be severely depleted of Se and Se-dependent glutathione peroxidase (Arthur, 1981) yet exhibit no clinical signs until they are subjected to a stress.

Selenium deficiencies are frequently subclinical, and for this reason, the lack of this mineral may limit calf

performance without the producer being aware of the deficiency. Research shows that Se deficient cattle grow slower and are less efficient than non-deficient cattle (McDowell, 2003). Selenium deficient cattle can also be unresponsive to medication (McDowell, 2003). Borderline deficiencies are often very costly to the producer. Estimates indicate that the use of Se in all animal diets would save about one billion dollars annually in the United States (Phillips et al., 1989).

Different mineral sources differ in bioavailability, which is the proportion of the mineral in the feed that is absorbed and utilized by the animal. Organic Se is potentially a more bioavailable source of Se; however, currently research is limited in this area. The objectives of this study were to determine the effects of supplemental Se from two sources on growth and immune function of weaned beef steers.

MATERIALS AND METHODS

Thirty weaned steers weighing between 215 and 279 kg were obtained from the University of Arkansas Beef Cattle Facility, Savoy. Steers were grazed for 80 d without any supplemental Se, with the intention of them being marginally deficient in Se at the beginning of the trial on 22 Jan., 2004.

Steers were weighed on two consecutive days at the beginning of the trial. Steers were blocked by weight (five blocks), and within the block they were assigned randomly to a pen (three pens/block, two steers/pen). Steers were housed in 3.7 m by 30 m pens with a 3 m concrete feedbunk in the front of each pen. Water was available ad libitum.

Three dietary treatments were used, 1) a control diet (no supplemental Se), 2) the control diet plus 1.7 mg/d supplemental Se from an inorganic source as sodium selenite, or 3) the control diet plus 1.7 mg/d supplemental Se from an organic source as a high Se yeast (Sel-Plex, Alltech, Nicholasville, Ky.). A grain supplement was fed at 1.1 kg/d on an as-fed basis (Table 1). Grain supplements were mixed three times, on d 0, 32, and 66. The grain supplement met the NRC (1996) requirements for protein, vitamins, and other minerals. Grain supplements were sampled at all mixing times. Fescue hay was available ad libitum to allow approximately 10% refusals and feed intake was recorded daily. Each bale of hay was sampled and five composite samples made over the 105 d trial. Hay was fed once daily at 0800 h and the supplement was fed twice daily at 0800 and 1600 h.

Steers were weighed prior to feeding every 21 d and weights were taken on two consecutive days at the end of the trial. Blood was drawn from each steer every 21 d to determine the concentration of Se and the activity of glutathione peroxidase (d 1, 21, 42, 63, 84, and 105). Blood samples were taken via jugular veinpuncture into 10-mL heparinized tubes (BD Vacutainer‘, Franklin Lakes, N.J.). The steers were observed daily for any signs of Se deficiency symptoms.

Blood samples were used to monitor immune function by analysis of in vitro lymphocyte proliferation, macrophage phagocytosis, and in vivo antibody production. On d 98, blood was collected for lymphocyte proliferation assays. Isolation, incubation, labeling with tritiated thymidine, and cell harvesting for lymphocyte proliferation followed the procedures by van Heugten et al. (1994). Cells were incubated for 48 h at 39°C with 5% CO_2, tritiated thymidine was added to each well, and cultures were incubated for an additional 18 h. Cells were harvested on glass fiber mats and the radioactivity was measured as counts/minute on a liquid scintillation analyzer (TRI-CARB 2200CA, Packard Instrument Co., Downers Grove, Ill.).

On d 38 and 98, monocytes were isolated and phagocytic ability was determined by methods adapted from Monteleone et al. (1999) and Nibbering et al. (1987). In short, peripheral blood mononuclear cells were isolated. Two milliliters of cells suspended in LM Hahn medium (2×10^6 cells/mL) were incubated for 16 h at 39°C in duplicate in six-well plates containing a glass coverslip. Isolation of monocytes occurred because of the propensity of the cells to adhere to the glass surface of the cover slip. Following the 16 h incubation, excess medium was removed, and 2 mL of a 5% porcine-red blood cell (PRBC) suspension were added to the monocytes/macrophages remaining on the glass coverslips. Cells were incubated with PRBC at 39°C and 5% CO_2 for 2 h, after which the coverslips were removed and rinsed with warmed LM Hahn medium to remove non-adherent cells and non-phagocytized PRBC. Cells were then fixed on coverslips and stained (Heme-3, Fisher Scientific, Pittsburgh, Penn.) for visualization under a microscope at 100x. The percentage of phagocytic monocytes and number of PRBC consumed by each phagocytic monocyte were determined by visual assessment of 200 monocytes on each duplicate coverslip.

Glutathione peroxidase (GTH-Px) was measured in whole-blood samples collected through jugular veinpuncture into 10-mL heparinized tubes. The red blood cells (RBC) were washed three times in an isotonic saline solution. The samples were then frozen in 5-mL tubes containing four parts RBC and one part deionized water. Glutathione peroxidase activity was expressed in enzyme units, and results were expressed as units per gram of hemoglobin (Hb). Determination of GTH-Px was performed using a commercial assay kit (OxisResearch™

BIOXYTECH®GPx-340™, Portland, Ore.). Hemoglobin was analyzed via commercial assay (Hemoglobin B, Wako Chemicals USA, Inc., Richmond, Va.).

To measure antibody production, all steers were vaccinated on d 42 with a modified live viral vaccine for infectious bovine rhinotracheitis virus (IBR), parainfluenza 3 (PI3), bovine viral diarrhea (BVD), and bovine respiratory syncytial virus (BRSV) (Titanium, AgriLabs LTD, St. Joseph, Mo.). Blood samples were taken via jugular venipuncture into plain glass tubes prior to vaccination on d 42 and on d 63 (d 21 after vaccination). Steers were revaccinated on d 63 to evaluate the secondary immune response and samples taken on d 70, 77, and 84. Blood samples were stored on ice, taken to the lab, and centrifuged. Serum was stored frozen until analysis for IBR, PI3, BVD, and BRSV. Samples were sent to the Oklahoma Animal Disease Diagnostic Laboratory for analysis.

Steer weights, blood and serum Se, GTH-Px activity, lymphocyte proliferation, macrophage function and antibody production were statistically analyzed using the mixed procedures of SAS (SAS Inst., Cary, N.C.). The experimental unit was pen. The random statement included block, and repeated statement was used for blood data. Least squares means were separated using pair-wise t-test when the F-test was significant ($P < 0.05$).

RESULTS AND DISCUSSION

Negative control supplements mixed on d 0 and d 32 had Se concentrations (dry-matter basis) of 0.22 mg/kg; however, the control supplement mixed on d 66 had Se concentration of 1.14 mg/kg. The hay had Se concentrations ranging from 0.13 to 0.2 with a mean of 0.16 mg/kg. These levels were higher than the analysis received of 0.036 mg/kg on core samples taken prior to the study. When supplement and hay intakes were calculated, the negative control steers were receiving an average of 0.17 mg Se/kg of diet until d 67 when the control diet contained approximately 0.36 mg Se/kg of diet, thus the Se concentrations in all diets were greater than the NRC (1996) requirement for Se of 0.1 mg/kg. There were no symptoms of Se deficiency or morbidity recorded.

There were no differences ($P > 0.10$) between steers fed the different dietary treatments for average daily gain, dry-matter intake, and gain-to-feed ratio (Table 2). Phillips et al. (1989) gave Se boluses to calves grazing Se-deficient forage and reported no positive effect on average daily gain until after d 60; however, blood Se concentrations were increased by d 30 with the Se boluses.

The analysis of blood Se (Fig. 1; day x treatment interaction, $P < 0.001$) revealed that on d 0 the steers to be fed organic Se had the lowest initial blood Se ($P < 0.05$). On d 21 there were no differences in blood Se concentrations between steers fed the different diets. By d 42, steers fed both sources of supplemental Se had greater ($P < 0.05$) blood Se concentrations than those fed the negative control diet. On d 63 and 84, blood Se concentrations differed ($P < 0.05$) among all dietary treatments (with negative control < inorganic Se < organic Se). Also, the increase in Se in the grain supplement on d 66 related to an increase in all blood Se concentrations on d 84 and 105. On d 105, steers fed both sources of supplemental Se had greater ($P < 0.01$) blood Se concentrations than the negative control. Beck et al. (2005) reported that supplementation of calves with Se yeast increased Se concentration in whole blood by 3.1 times compared with the negative controls and 1.6 times compared with calves fed inorganic Se.

The serum Se (Fig. 2; day x treatment interaction, $P < 0.001$) concentrations on d 0 were similar to that of the blood Se concentration with the steers to be fed organic Se being lower than that of the steers to be fed the control diet ($P < 0.05$). On d 21, 42, and 63, steers fed both sources of supplemental Se had greater ($P < 0.05$) serum Se concentrations than those fed the negative control diet. On d 84, serum Se concentrations differed among all the dietary treatments ($P < 0.05$), with the steers fed the organic source having the highest concentration and the steers fed the control diet having the lowest concentrations. On d 84 and 105, an increase occurred that was closely related to that of the increase in Se concentration seen in the grain supplement after mixing on d 66. On d 105, the steers fed the inorganic source had higher concentrations of serum Se than those of steers fed the negative control diet ($P < 0.05$).

Glutathione peroxidase (GTH-Px) activity (Fig. 3; day x treatment, $P < 0.05$) differed on d 0 compared to that of the blood- and serum-Se concentrations, when the steers to be fed the organic Se had higher GTH-Px activity than steers to be fed the negative control diet ($P < 0.05$). On d 21, 63, and 84 there were no differences in GTH-Px activity between the dietary treatments. However, on d 42 there was an increase in the GTH-Px activity in steers fed the negative control diet ($P < 0.05$), and on d 105 steers fed the organic source had higher GTH-Px activity than those steers fed the negative control ($P < 0.05$). Beck et al. (2005) found that supplemental Se did not enhance glutathione peroxidase activity. This variation may reflect the slower turnover rate of GTH-Px in the red blood cell compared to serum Se concentrations, which respond to Se supplementation more rapidly (Underwood and Suttle, 1999).

Lymphocyte proliferation on d 98 (Table 3) was not affected by the dietary treatments ($P < 0.48$). Beck et al.

(2005) found that there were no differences (P > 0.47) in lymphocyte proliferation with either unstimulated cultures or cultures stimulated with ConA, PWM, or PHA. Supplemental Se has resulted in varying effects on lymphocyte proliferation of ruminants as reported in the literature (Beck et al., 2005). Cao et al. (1992) reported that Se deficiency in dairy cows resulted in a decreased response of isolated peripheral blood lymphocytes to mitogen stimulation using ConA. Low dietary Se does not consistently affect cell-mediated immune response in ruminants (Stabel and Spears, 1993).

The functions of macrophages are very critical to the immune function of the animal. The function of macrophages (called monocytes when found in blood) is to engulf foreign materials that are invading the system. There was a tendency (P = 0.15) for diet to affect the number of PRBC ingested in vitro (Table 3). Macrophages from steers supplemented with inorganic Se phagocytized more (P = 0.06) porcine red blood cells than did macrophages from negative control steers, with macrophages from organic Se-supplemented steers being intermediate. However, the percentage of phagocytic macrophages was not affected by diet. In contrast, Beck et al. (2005) found supplemental Se did not affect the number of PRBC ingested by macrophages; but the percentage of phagocytic macrophages was increased by supplemental Se yeast as compared with the control or with supplemental sodium selenite.

Antibody response to vaccination for bovine respiratory viruses did not (P > 0.35) differ among steers fed the different diets nor were there any day x treatment interactions (P≥0.58) (data not shown). The steers at the beginning were all naïve to IBR and BVD, but not BRSV and PI_3. Selenium level and source had minimal effects on the antibody response of these weaned beef steers. There were day effects revealed in all titers over the 42 d period, with titers peaking at d 35. Humoral response has been improved by increasing dietary Se and/or vitamin E in several studies (Finch and Turner, 1996; Stabel and Spears, 1993; Swecker et al., 1989). Antibody responses have been most consistent when Se is supplemented with vitamin E in the diet (Spears, 2000).

In conclusion, the level and source of supplemental Se did not affect average daily gain of steers over the 105 d trial. Both sources of supplemental Se increased blood Se, the organic source increasing more rapidly than the inorganic source of Se. Selenium level and source had minimal effects on the immune function of the beef steers, perhaps due to the basal level of Se in the control diet.

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Table 1. Ingredient and nutrient composition of grain supplement (dry-matter basis).

Item	%
Ingredient	
Corn	70.86
Soybean meal	12.60
Dicalcium phosphate	0.94
Limestone	1.74
Salt, white	1.53
Rumensin premix[a]	+
Vitamin premix[b]	+
Trace mineral premix[c]	+
Molasses	1.94
Fat	10.4
Calculated nutrient composition[d]	
Crude protein	14.86
NE_m, Mcal/kg	2.16
NE_g, Mcal/kg	1.42

[a]Provided 150 mg of monensin/d.

[b]Provided 11,000 IU vitamin A, 2,200 IU vitamin D, and 26 IU vitamin E/d.

[c]Provided 200 mg zinc as zinc sulfate, 60 mg copper as copper sulfate, 0.6 mg cobalt as cobalt carbonate, and 2.9 mg iodine as calcium iodate/d.

[d]Values calculated with the Oklahoma State Univ. Ration Calculator 1999 (as-fed version) software (www.ansi.okstate.edu/software/OSUNRCAF.xls).

Table 2. Growth performance, feed intake, and gain:feed ratio for steers fed different levels and sources of supplemental selenium for 105 d.

Item	Control	Inorganic Se	Organic Se	Standard error
Average daily gain, kg	0.44	0.45	0.40	0.034
Dry-matter intake, kg	5.19	5.13	5.08	0.073
Gain:feed	0.085	0.089	0.078	0.0073

Table 3. In vitro lymphocyte proliferation and macrophage activity for cells isolated from steers fed different levels and sources of supplemental selenium.

Item	Control	Inorganic Se	Organic Se	Standard error
Lymphocyte proliferation, counts/min				
PHA[a]	62,771	65,549	56,610	10,376
PWM[b]	25,650	25,402	21,772	4,678
ConA[c]	25,219	19,070	16,924	4,858
Macrophage phagocytosis				
Phagocytic cells, %	9.33	11.73	10.79	2.449
Number PRBC ingested/cell	0.96	1.31	1.11	0.117

[a]Lymphocytes cultured in the presence of phytohemagglutinin (PHA; 20μg/mL) to stimulate proliferation of peripheral blood T lymphocytes associated with cell-mediated immunity.
[b]Lymphocytes cultured in the presence of pokeweed mitogen (PWM; 15 μg/mL) to stimulate proliferation of B lymphocytes associated with humoral immunity.
[c]Lymphocytes cultured in the presence of concanavalian A (ConA; 40 μg/mL) to stimulate proliferation of peripheral blood T lymphocytes associated with cell-mediated immunity.

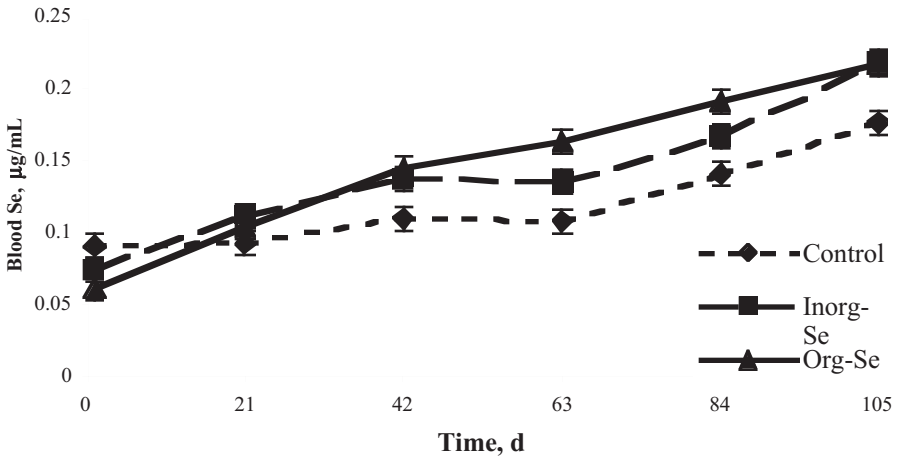

Fig. 1. Effect of dietary selenium concentration and source on steer blood selenium concentrations throughout the 105 d trial.

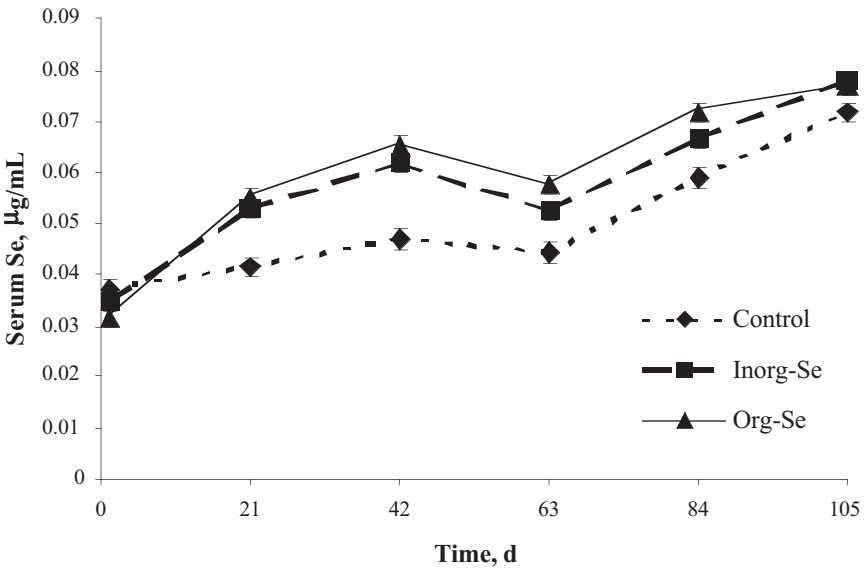

Fig. 2. Effect of dietary selenium concentration and source on steer serum selenium concentrations throughout the 105 d trial.

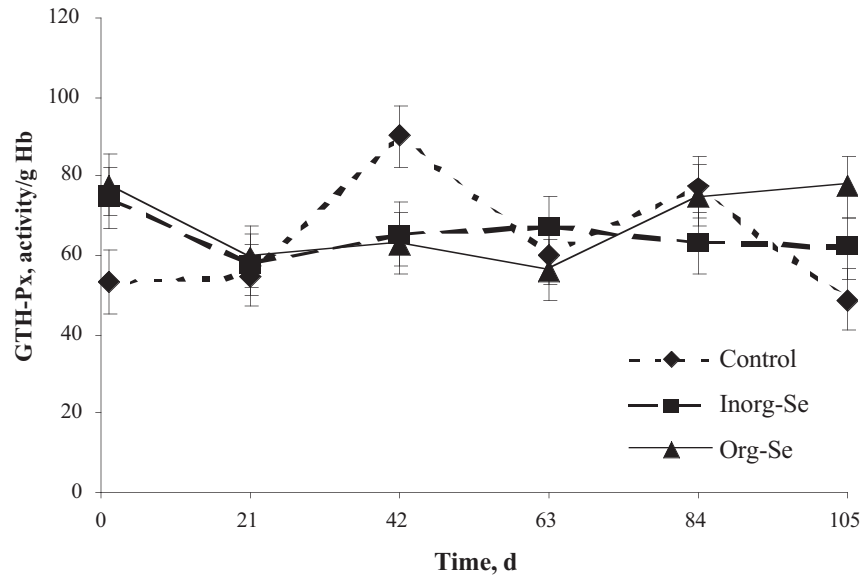

Fig. 3. Effect of dietary selenium concentration and source on steer glutathione peroxidase activity per gram of hemoglobin throughout the 105 d trial.

Growth and development of tomato seedlings in sphagnum peat, vermiculite, and processed rice hull substrates

Matthew K. Nutt[*] *and Michael R. Evans*[†]

ABSTRACT

Tomato (*Lycopersicum esculentum* 'Early Girl') seedling growth was evaluated in substrates containing varying proportions of ground rice hulls. Substrates were formulated containing 0, 30, 60, and 90% ground rice hulls with one-half of the treatments also treated with a surfactant. Seedling growth in two of the ground rice hull-containing substrates was generally similar to the two controls of 90% peat or 100% vermiculite. The germination percentages for all ground rice hull-containing substrates were similar to the two controls. Ground rice hulls are a viable alternative to peat and vermiculite seedling substrates.

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[†] Michael R. Evans, faculty mentor, is an associate professor in the Department of Horticulture.

INTRODUCTION

Artificial substrates are most commonly used in greenhouse crop production (Nelson, 1998). These substrates are made of various components blended in varying proportions to produce a substrate with physical and chemical properties suitable for its intended use (Blunt, 1988). These components may be naturally occurring, man-made, or a municipal or agricultural by-product. One of the commonly used natural components is Sphagnum peat (peat). Sphagnum peat is generally used in artificial substrates for its water- and nutrient-holding capacity. However, significant interest has been expressed in finding alternatives to peat due to environmental concerns (Barkham, 1993; Buckland, 1993; Robertson, 1993) and costs associated with this component.

Some research has been completed in the use of municipal waste products such as waste paper products (Chong and Cline, 1993; Norrie and Gosselin, 1996), composted yard waste (Beeson, 1996), and municipal sewage sludge (Mori et al., 1981) as alternatives to peat. Additional research has been conducted on industrial and agricultural waste products. Some of these include coconut coir (Evans and Stamps, 1996), composted rice hulls (Laiche and Nash, 1990), processed poultry feathers (Evans, 2004), kenaf (Wang, 1994), and composted animal manures (Tyler et al., 1993).

Many of these alternative substrates were discarded due to their chemical or physical properties not meeting the needed properties for the substrate mix as used by the industry. Additionally, expense eliminated or greatly slowed others, such as ground bovine bone as a replacement for perlite (Evans, 2004).

Rice hulls are a by-product of the rice milling industry in Arkansas and across the United States. It has been estimated that 31 million metric tons of fresh rice hulls are produced annually in the United States (Kamath and Proctor, 1998).

Fresh rice hulls have not been used in potting substrates in the past because it was believed they caused nitrogen depletion. However, it was recently found that nitrogen depletion did not occur to any significant extent (Evans and Gachukia, 2004) when fresh rice hulls are used as a component in substrates. Furthermore, rice seeds have been a common contaminant of rice hulls and, therefore, created a weed problem (Evans and Gachukia, 2004). However, parboiled rice hulls were found to be free of viable weed seeds (Evans and Gachukia, 2004).

MEET THE STUDENT-AUTHOR

Matthew K. Nutt

I am from Monett, Mo., where I graduated from Monett High School in 2000. I plan to graduate with honors distinction in the spring of 2005 with my B.Sc. in horticultural science. The Robert L. & Marilyn Bogle Scholarship and the Missouri Federated Garden Clubs Scholarship allowed me to pursue my degree, which I may not have been able to otherwise. Some of my collegiate activities included National Society of Collegiate Scholars, Gamma Beta Phi, and the Fraternity of Alpha Zeta.

I contacted Dr. Evans about the possibility of completing a research project with him, and he suggested that I assist him on some of his alternative horticultural media research. This project has allowed me to gain valuable skills and knowledge that I will be able to apply in future situations in my career. I plan to work in the industry after pursuing my master's degree and eventually teach in an academic institution.

All previous research conducted with rice hulls has replaced perlite and used whole parboiled fresh rice hulls to provide for drainage and air-filled pore space. Substrate particle size directly affects pore size. Large particles create large pores that drain and become air-filled after irrigation. Small particles create small pores that retain water for use by the plant. By grinding rice hulls, the particle size is reduced. The smaller-sized rice hull particles should create small pores that hold water. Thus, ground rice hulls (GRH) might be used as an alternative to peat. Further, grinding destroys any viable rice seed eliminating the weed problem and allowing for the use of non-parboiled hulls.

Surfactants are used in the horticultural industry to increase the water-holding capacity of substrates. These surfactants are used on many alternative substrates to increase water-holding capacity. Their use might allow for the use of other alternative substrates which might not otherwise be useful due to low water-holding capacity. Ground rice hulls may not provide sufficient water-holding capacity and therefore may require that a surfactant be added to increase water-holding capacity.

The objective of this research was to determine if ground rice hull products could be used as an alternative to peat in the production of seedlings.

MATERIALS AND METHODS

Rice hulls were acquired from Riceland Foods (Stuttgart, Ark.) and ground in a Wiley Hammer Mill (Arthur H. Thomas Co., Philadelphia, Penn.). This process created a product in which 98% of the particles were less than or equal to 2.0 mm in size (Fig. 1). The ground rice hulls (GRH) either remained untreated or were treated with the surfactant Soax (Scotts, Marysville, Ohio) at the recommended label rate.

Substrates were formulated by blending the GRH, peat, and perlite (4 to 6 mm). All substrates contained 10% perlite and 30, 60, or 90% GRH with the remainder being peat. Calcitic limestone was added to the peat to adjust its pH to approximately 5.5. Two control treatments were evaluated. One control consisted of 90% peat and 10% perlite with no surfactant. An additional control substrate of 100% vermiculite was also included.

Substrates were placed into five-cell-by-five-cell mini-plug trays, made from round #273 (5 ml volume per plug cell) plug trays. One tomato seed ('Early Girl') was planted per cell. Plug trays were then transferred to a bi-wall polycarbonate-glazed greenhouse. The low-temperature set point was 18°C. Light levels averaged 250 $\mu mol \cdot sec^{-1} \cdot m^{-2}$ at 12 h. The trays of substrates were misted once or twice daily to ensure a constantly moist substrate required for germination. All trays were misted at the same time, thus applying the same amount of water to all substrates. The mini-plug trays were fertilized with a 25 mg $\cdot L^{-1}$ nitrogen solution using N-P-K 15-5-15 Excel (Scotts, Marysville, Ohio) with every misting from the start of the third week until the experiment was terminated at the end of the fifth week. There were eight treatments with three replications, with a tray being a replication. The replications were placed on the greenhouse bench in a random pattern.

An analysis of variance was run to establish if there were significant differences in seedling germination and growth among the different substrates. A least significant difference mean separation test ($\alpha = 0.05$) was used to ascertain which means were significantly different.

RESULTS AND DISCUSSION

Seedlings grown in vermiculite had higher per-tray fresh shoot weights than seedlings grown in all other substrates (Table 1). Seedlings grown in the 90% GRH without surfactant and 60% GRH with surfactant had similar fresh shoot weights as the 90% peat substrate. All other GRH-containing substrates had lower fresh shoot weights than the 90% peat control.

Vermiculite, 90% GRH without surfactant, and 90% peat all had similar per-tray fresh root weights (Table 1). The 30% GRH with surfactant, 60% GRH without surfactant, and 90% GRH with surfactant had lower fresh root weights per tray than the 90% peat control.

Seedlings grown in 90% GRH without surfactant, 60% GRH with surfactant, 90% peat, and 100% vermiculite all had similar dry shoot weights. The 90% GRH with surfactant, 60% GRH without surfactant, and 30% GRH with surfactant had similar dry shoot weights per tray but were significantly lower than the controls. All seedlings had similar per-tray dry root weights regardless of the substrate. The germination percentage was similar for all substrates except the 30% GRH with surfactant, which was significantly lower than the 90% peat or 100% vermiculite controls.

Fresh shoot weights per plant for vermiculite and 90% GRH without surfactant were similar (Table 2). The fresh shoot weights per plant were similar for seedlings grown in 90% peat, 90% GRH without surfactant, and 60% GRH with surfactant. However, the 90% peat and 60% GRH with surfactant were significantly lower than vermiculite. Seedlings grown in 30% GRH-without-surfactant, 60% GRH-without-surfactant, 30% GRH-with-surfactant, and 90% GRH-with-surfactant substrates had lower per-plant fresh shoot weights than those grown in the 90% peat and vermiculite controls (Table 2).

The average fresh root-weights per plant for seedlings grown in the two controls of 90% peat or 100% vermicu-

lite were similar to the 90% GRH without surfactant. All other seedlings grown in GRH-containing substrates had similar average fresh root-weights per plant and were significantly lower than the 90% peat control and 100% vermiculite control.

Seedlings grown in 30% GRH with surfactant and 90% GRH with surfactant had significantly lower average dry shoot-weights per plant compared to the two control substrates of 90% peat or 100% vermiculite. Seedlings grown in 90% GRH without surfactant and 60% GRH with surfactant had similar average dry shoot-weights per plant compared to the two control substrates of 90% peat and vermiculite. All seedlings had similar per-plant average dry root-weights regardless of the substrates.

This study shows the use of GRH in seedling production substrates is a viable replacement for peat. Seedlings grown in substrates containing 90% GRH without surfactant and 60% GRH with surfactant showed on a per-tray and per-plant basis results similar to the two controls of 100% vermiculite and 90% peat. The germination percentages in all GRH-containing substrates were similar to the seedlings grown in 90% peat except for those in 30% GRH with surfactant, which were significantly lower.

Surfactants possibly increased the water-holding capacity of the substrate, therefore exceeding the seedlings' moisture requirement. Also the surfactant used could have caused a slight phytotoxic reaction in the seedlings. This requires further investigation.

ACKNOWLEDGMENTS

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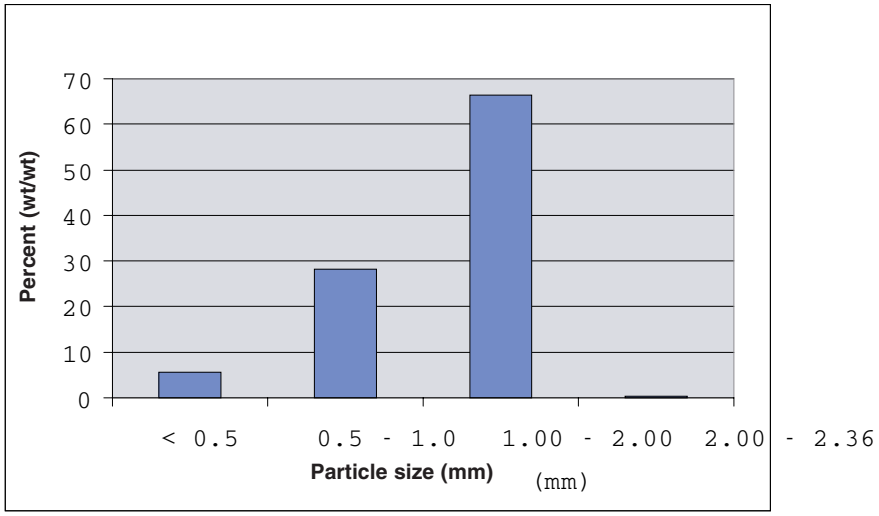


Fig. 1. Particle size distribution of ground rice hulls.

Table 1. Growth per tray of tomato seedlings in Sphagnum-peat-based substrates amended with ground rice hulls (GRH).

Substrate component (% vol/vol)									
Sphagnum peat	GRH [z]	GRH w/ wetting agent [z]	Perlite	Vermiculite	Fresh shoot weight (g) [y]	Fresh root weight (g) [y]	Dry shoot weight (g) [y]	Dry root weight (g) [y]	Germination %
90	0	0	10	0	1.30	1.05	0.21	0.10	89
60	30	0	10	0	0.95	0.73	0.16	0.07	92
30	60	0	10	0	0.88	0.62	0.15	0.07	92
0	90	0	10	0	1.41	1.16	0.21	0.11	79
60	0	30	10	0	0.60	0.51	0.11	0.07	69
30	0	60	10	0	1.26	0.81	0.19	0.09	87
0	0	90	10	0	0.77	0.57	0.09	0.10	81
0	0	0	0	100	1.75	1.29	0.22	0.11	92
Significance					***	**	***	NS	***
Substrate					NS	NS	NS	NS	NS
Block					0.29	0.39	0.05	0.04	10
LSD (α=0.05)									

NS, **, *** Nonsignificant or significant at the 0.01 or 0.001 level, respectively

[z] Ground rice hulls

[y] Weights for combined 5 x 5 plug tray

Table 2. Average per-plant growth weights of tomato in Sphagnum-peat-based substrates amended with ground rice hulls (GRH).

Substrate component (% vol/vol)								
Sphagnum peat	GRH [z]	GRH [z] w/ wetting agent	Perlite	Vermiculite	Avg[y] fresh shoot weight (g)	Avg[y] fresh root weight (g)	Avg[y] dry shoot weight (g)	Avg[y] dry root weight (g)
90	0	0	10	0	0.06	0.05	0.009	0.004
60	30	0	10	0	0.04	0.03	0.007	0.003
30	60	0	10	0	0.04	0.03	0.007	0.003
0	90	0	10	0	0.07	0.06	0.011	0.005
60	0	30	10	0	0.03	0.03	0.006	0.004
30	0	60	10	0	0.06	0.04	0.009	0.004
0	0	90	10	0	0.04	0.03	0.005	0.005
0	0	0	0	100	0.08	0.06	0.009	0.005
Significance					***	**	***	NS
Substrate					NS	NS	NS	NS
Block					0.01	0.02	0.002	0.002
LSD (α=0.05)								

NS, **, *** Nonsignificant or significant at the 0.01 or 0.001 level, respectively

[z] Ground rice hulls

[y] Weights are per-plant averages.

Incorporating glass transition concepts to explain rice milling-quality reductions during the drying process

Derek A. Schluterman[*] *and Terry J. Siebenmorgen*[†]

ABSTRACT

Previous research has indicated that while drying rough rice using air temperatures above the glass transition temperature (Tg), head rice yield (HRY) reductions are incurred if a state transition occurs when severe intra-kernel moisture content (MC) gradients are present. State transitions can occur by extended drying using high-temperature air or by cooling kernels below Tg before sufficient tempering has occurred. The objectives of this experiment were to determine the maximum MC removal per initial drying pass and the associated tempering durations required to prevent HRY reduction. Two long-grain cultivars, 'Francis' and 'Wells', at two harvest moisture contents (HMC) were used. Samples were dried with air conditions of either 60°C/17% RH or 50°C/28% RH for various durations to create a range of intra-kernel MC gradients and were subsequently tempered in sealed bags for durations ranging from 0 to 160 min. After tempering, samples were cooled to cause a state transition, and then slowly dried to 12.2% MC. Samples were then milled to determine HRY. Control samples were dried at 21°C/60% RH. Results showed that the amount of moisture that could be removed in the initial drying pass was directly related to the HMC and the drying air condition. The tempering duration required to prevent HRY reductions increased with the amount of MC removed from the kernel in a drying pass. The HRY reduction patterns concur with a hypothesis that explains fissure formation during the drying process based on the Tg of rice kernels.

[*] Derek A. Schluterman, who graduated in May 2005 with a B.S. in biological engineering, is program assistant and lab manager for the University of Arkansas Rice Processing Program.

[†] Terry J. Siebenmorgen, faculty sponsor, is a professor in the Department of Food Science and coordinator of the University of Arkansas Rice Processing Program.

INTRODUCTION

In the United States, rough rice is typically harvested at moisture contents (MCs) ranging from 14% to 24%, and subsequently dried to approximately 12% for safe long-term storage. High-temperature drying creates temperature and MC gradients within kernels, which induces tensile stresses at the kernel surface and compressive stresses at the kernel interior (Sharma and Kunze, 1982). These stresses can lead to fissure formation within the kernel and subsequently reduce quality due to reduction in head rice yield (HRY). In order to reduce these stresses, tempering is typically practiced, during which kernels are held in a non-drying condition in order to allow MC gradients within kernels to subside. Intermittent drying/tempering cycles are often used to avoid fissure formation and HRY reductions.

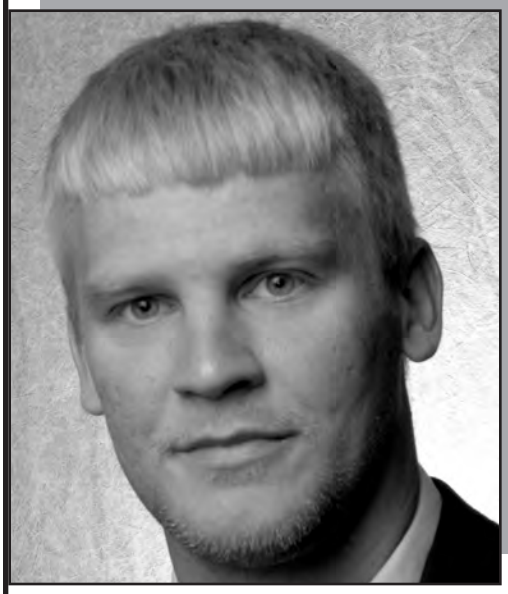
Rice drying and tempering have been studied extensively (Chen, 1997; Chen et al., 1997; Cnossen and Siebenmorgen, 2000; Cnossen et al., 1999; Kunze, 1979: Mossman, 1986) toward the goal of drying rice more quickly while maintaining high HRY. When drying rough rice, the glass transition temperature (Tg), the temperature at which a state transition occurs causing the rice to change from a 'glassy' to a 'rubbery' state, plays a significant role in the rate at which moisture can be removed from the kernel (Cnossen and Siebenmorgen, 2002) and in the occurrence of fissure formation (Cnossen and Siebenmorgen, 2000). Cnossen and Siebenmorgen (2002) found that the drying rate was greater if the rice kernel temperature was above Tg.

Figure 1 shows the inverse relationship between Tg and the MC of rice. If the rice kernel temperature is below Tg, the starch exists in a 'glassy' state with a high viscosity and modulus of elasticity, but low specific heat, specific volume, and expansion coefficient. If the kernel temperature is above Tg, the starch exists in a 'rubbery' state with a much higher specific heat, specific volume, and expansion coefficient (Perdon et al., 2000). Cnossen and Siebenmorgen (2000) presented a hypothesis incor-

MEET THE STUDENT-AUTHOR

I graduated from Subiaco Academy in Subiaco, Ark., in 2000. I then enrolled at the University of Arkansas where I majored in Biological Engineering and worked part time for Dr. Terry Siebenmorgen with the University of Arkansas Rice Processing Program within the Food Science Department. I will graduate in May 2005 with a B.S. in Biological Engineering and have recently accepted a position as program assistant and lab manager for the Rice Processing Program.

I am a member of the American Society of Agricultural Engineers (ASAE). I was part of a four-person team who submitted a design project in the AGCO National Student Design Competition in the summer of 2004 as part of the ASAE annual meeting. We received an award for second place for our project entitled: The Design of a System for the Rapid Pasteurization of Carcasses Contaminated with High-Risk Pathogens. This project was sponsored by the Arkansas Livestock and Poultry Commission and involved designing and testing a pilot-scale pasteurization system and the design of a commercial-scale, portable system that could be used throughout the United States.



Derek A. Schluterman

My primary research area in the Rice Processing Program focuses on drying rough rice to achieve the highest quality rice possible. This includes evaluating both infrared and fluidized bed driers as possible new and more efficient ways to dry rough rice. I am also evaluating the use of infrared energy as a means of controlling insects in stored rice as an alternative to fumigation. Another project that I am working on, with a group of researchers and farmers, is the validation of a computer model used for predicting performance in on-farm bin drying of grains.

porating the Tg concept to explain rice kernel fissuring during drying and tempering. To explain this hypothesis, Fig. 2 shows hypothetical temperature and MC gradients created within a rice kernel during drying. When drying using air temperatures above Tg, rice transitions from the 'glassy' to the 'rubbery' state. This transition dramatically changes kernel material properties. During this high-temperature drying, the outer layer of the kernel will dry much more quickly than the center of the kernel, causing an MC gradient within the kernel. This can cause the surface and the center to be at different material states (Fig. 3). Extended drying causes a sufficient volume of the kernel surface to transition to the 'glassy' state, thereby creating an imbalance in the expansion rate that initiates fissure formation.

During tempering and/or cooling, depending on the temperature to which the kernel is exposed, the outer kernel layer may transition to the 'glassy' state, while the center remains in the 'rubbery' state, causing portions of the kernel to experience different magnitudes of material properties (Cnossen and Siebenmorgen, 2000). Figure 4 shows this process, which can also lead to fissure formation. During tempering, if the kernels are cooled below the Tg temperature before the MC gradient is allowed to subside, fissures will occur due to the surface and center conforming to different properties; this is shown with situation 'B' in Fig. 4. Once the MC gradient subsides, the rice kernels can be exposed to temperatures below the Tg temperature without incurring fissures.

Most commercial rice dryers try to safely remove the maximum amount of MC in as short a period as possible without incurring HRY reductions. Given the Tg hypothesis, the objectives of this experiment were to determine the maximum MC removal per initial drying pass and the associated tempering durations required to prevent HRY reduction using air temperatures that produce kernel states both above and below the Tg. This information is intended to help optimize performance of commercial rice driers.

MATERIALS AND METHODS

In the fall of 2003, two long-grain rice cultivars, Francis (with HMC of 19.5 and 17.4%) and Wells (with HMC of 21.6 and 16.1%), were obtained from the University of Arkansas Rice Research and Extension Center near Stuttgart, Ark. Immediately after harvest, the rice was transported to the University of Arkansas Rice Processing Laboratories and was cleaned with a dockage tester (Model XT4, Carter Day Co., Minneapolis, Minn.) and stored at 4°C for six weeks until drying tests were conducted.

Rice samples were dried using a temperature and relative humidity (RH) control unit (Climate Lab AA: 300 CFM, Parameter Generation & Control, Inc., Black Mountain, N.C.). The air conditions were monitored using a hygrometer (Hygro-M2, General Eastern, Woburn, Mass.). Air from the temperature and RH control unit was supplied to a laboratory drying chamber, which included 16 trays (25 cm x 14 cm x 6.5 cm) with perforated bottoms. The 16 trays were arranged as two eight-tray sets, which served as two repetitions. Approximately 110 g of rough rice was added to each tray to form a layer of two to three kernels deep. Three different drying air conditions were tested:

Condition H (high temperature)	60°C, 17.0% RH	Equilibrium MC 5.5%
Condition L (low temperature)	50°C, 28% RH	EMC7.2%
Condition C (control)	21°C, 60% RH	EMC 12.2%

Extended drying using the control conditions has been shown to produce no reductions in HRY (Fan et al., 2000). For each drying air condition, samples were dried for various durations to produce a range of MC gradients within the kernels. The different magnitudes of the MC gradients formed during drying would indicate the maximal amounts of MC that could be removed before fissures were formed due to differential stresses formed within the kernels when crossing the Tg line, as depicted in Fig. 3. After each drying duration, samples from the two repetitions were tempered, which consisted of placing samples in an oven set at either 50°C or 60°C in sealed bags for various durations ranging from 0 to 160 min in increments of 30 to 40 min depending on the drying duration; the longer increments, 40 min, were used for the extended drying durations. After tempering, the samples were placed into a conditioning chamber maintained at 21°C and 60% RH to cool and continue to dry to 12.2% MC. The purpose of tempering the samples for different increments was to determine the shortest duration needed to allow the MC gradient that was created during drying to subside. If the tempering duration was too short resulting in the kernel cooling below Tg before the gradient subsided, fissures would result. This transition is illustrated with Situation 'B' in Fig. 4. After each drying duration, the MC was determined in triplicate using an oven method, which comprised drying 15 g of rough rice for 24 h in a convection oven set at 130°C (Jindal and Siebenmorgen, 1987).

To determine the effect of the drying and tempering treatments on milling quality, 150 g samples of rough rice were dehulled using a laboratory huller (THU, Satake, Tokyo, Japan), and the resultant brown rice was milled in a laboratory mill (McGill #2, RAPSCO, Brookshire, Texas). During milling, a 1.5 kg weight was placed on the lever arm of the mill, 15 cm from the cen-

terline of the mill chamber. The samples were milled for 30 s. The amount of head rice, milled kernels that are at least three-fourths of the original kernel length (USDA 1997), in each milled rice sample was determined with an image analysis system (Graincheck 2312 Analyzer, Foss Tecator, Höganäs, Sweden). Head rice yield was then calculated as the mass percentage of rough rice that remained as head rice.

For the control, five 200 g samples of rice from each of the four cultivars/HMC lots were gently dried in the conditioning chamber described above from the HMC to 12.2% MC, resulting in minimal breakage and therefore the highest possible HRY. The five HRYs from each variety/HMC lot were averaged to represent the control HRY of each lot. The HRYs of the different drying/tempering treatments were compared to the respective control HRYs to determine the amount of HRY reduction caused by drying and/or tempering.

RESULTS AND DISCUSSION

Figure 5 shows the HRY data of 'Wells' (HMC of 21.6%) versus tempering duration for various drying durations ranging from 10 to 55 min using drying air at 60°C/17% RH. When drying for 10, 20, and 31 min and tempering for at least 90 min, no HRY reductions were measured compared to the control HRY. Thus, as much as 6.4 percentage points of MC (PPMC) were removed without appreciable damage, given sufficient tempering before cooling. However, when drying for 43 min and removing 7.7 PPMC, a reduction of 5 percentage points of head rice yield (PPHRY) resulted compared to the control HRY, even after extended tempering durations. A reduction of 18 PPHRY resulted after drying for 55 min, removing 8.8 PPMC, and tempering for over 2 h. Therefore, the maximum amount of MC that could be safely removed in a single pass with air at 60°C/17% RH from 'Wells' at 21.6% HMC was 6.4 PP. It is speculated that beyond this amount of MC removal, MC gradients and resultant transitioning of sufficient portions of the kernel surface to the 'glassy' state created stresses within the kernel during extended drying that were too great to overcome during tempering, resulting in permanent HRY reductions.

A Tg diagram is shown in Fig. 6 for 'Wells' (HMC 21.6%) dried using air at 60°C/17% RH for the various durations indicated in Fig. 5. The points in fig. 6 indicate the rice temperature (60°C), the corresponding PPMC removed for each drying duration, and the associated HRY reductions (after tempering for 90 min), in relation to the Tg line. As indicated above, drying for 10, 20, and 31 min, removing 3.1, 4.7, and 6.4 PPMC, respectively, and tempering for at least 90 min resulted in no HRY reductions compared to the control HRY. For these drying durations, the average MC after drying caused most of the kernel to be in the rubbery state, which would also indicate that a significant portion of the kernel surface had not transitioned from the 'rubbery' to the 'glassy' state (Fig.6). However, drying for 43 min and removing 7.7 PPMC resulted in HRY reductions compared to the control HRY, even after extended tempering durations. For this situation, the average kernel MC and temperature after drying positioned the kernel material state very near the Tg line, which would indicate that a large portion of the kernel periphery had transitioned into the 'glassy' region while the kernel center remained in the 'rubbery' region (Fig. 6). As reported by Cnossen and Siebenmorgen (2000), this condition results in kernel fissuring and reduced HRYs. Proportionately greater HRY reductions occurred (17.1 PP) as greater MC gradients were produced, caused by removing 8.8 PPMC (Fig. 6).

The HRY data for 'Francis' (HMC of 17.4%) at various tempering durations and drying durations ranging from 23 to 88 min using drying air at 50°C/28% RH are shown in Fig. 7. Even drying for 88 min, removing 5.6 PPMC at this condition, and tempering for at least 120 min resulted in little to no HRY reduction compared to the control HRY. Therefore the amount of MC removal required to reach a safe storage level of less than 12% MC was removed in a single pass given the required tempering duration of 120 min. This can be explained because the low HMC of 17.4% and mild drying condition placed the kernel state near the Tg line at the start of drying. This resulted in insufficient MC gradients when the kernel transitioned from the 'rubbery' to the 'glassy' state, which occurred during drying, resulting in high HRYs compared to the control HRY with sufficient tempering.

A Tg diagram is shown in Fig. 8 along with the drying and tempering data of Fig. 7 for Francis. The points in Fig. 8 indicate the rice temperature (50°C), the corresponding PPMC removed for each drying duration, and the associated HRY reductions (after tempering for 120 min), in relation to the Tg line. Because of the low HMC and the mild drying condition placing the kernel state near the Tg line at the start of drying, the amount of MC removed had little effect on HRYs, given sufficient tempering before cooling. Drying up to 68 min and removing 4.5 PPMC resulted in no HRY reductions; less than 1 PPHRY reduction was measured for 88 min of drying compared to the control HRY. Thus, drying low-HMC rice with air conditions starting at the transition line resulted in little to no HRY reduction. This is due to the fact that while MC gradients were created inside kernels, the kernel had not initially transitioned into the 'rub-

bery' state so as to create the fissure formation scenario described above.

Figures 6 and 8 illustrate that the HMC has a large role in affecting fissure formation according to the Tg hypothesis. To summarize this role, the HRY reductions for 'Wells' with HMCs of 21.6 and 16.1% and for 'Francis' with HMCs of 19.5 and 17.4% versus MC removed using air at 60°C/17% RH are illustrated (Fig. 9); the data represent samples that were tempered for 90 min at 60°C immediately after drying and before cooling and subsequent drying. As a clarification of how Fig. 9 was developed, the HRY reductions and the PPMC removed for 'Wells' (HMC 21.6%) in Fig. 9 were obtained from Fig. 6. HRY reduction began after 2.3, 4.2, 4.8, and 6.4 PPMC were removed for 'Wells' (16.1% HMC), 'Francis' (17.4% HMC), 'Francis' (19.5% HMC), and 'Wells' (21.6% HMC), respectively. During drying with high HMC rice, even though a severe MC gradient formed within the kernel, fissuring did not occur until a sufficient amount of the kernel surface transitioned into the 'glassy' state. Thus, as the MC at which drying began increased, more moisture could be removed per drying pass without incurring HRY reductions given sufficient tempering immediately after drying. Thus for this air condition, or any given drying air condition, the amount of MC that could be removed without HRY reduction was directly related to the HMC of the rice.

The following conclusions were drawn from this study:

• Drying with air temperatures below the Tg of rice, with sufficient tempering, produced little to no HRY reduction, due to the lack of an MC gradient within the kernel during the state transition. This is because the center and surface of the kernel remains in the 'glassy' region as opposed to drying above Tg, where the center and surface of the kernel could be in different regions resulting in an MC gradient and a difference in material properties, which could lead to fissure formations and HRY reductions without sufficient tempering.

• Tempering rice for at least 90 min at the drying air temperature immediately after high-temperature drying was sufficient to cause intra-kernel MC gradients to subside and thus prevent HRY reduction upon cooling and further drying.

• The amount of MC that could be removed in the initial pass with sufficient tempering was directly related to the HMC. This is illustrated by Figs. 5 through 9 and concurs with the Tg hypothesis developed by Cnossen and Siebenmorgen (2000).

• These results confirm the importance of monitoring both the rice and drying conditions in order to obtain the greatest HRY possible.

ACKNOWLEDGMENTS

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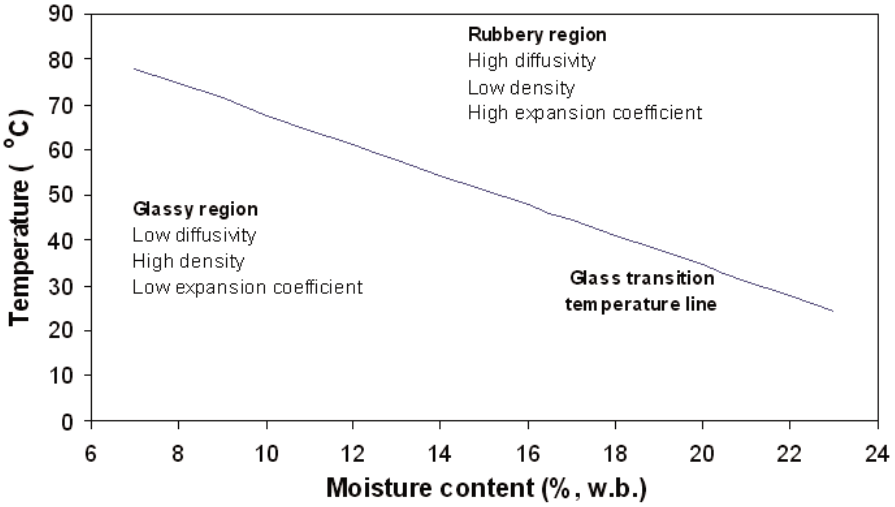

Fig. 1. Glass transition temperature relationship for brown rice, indicating the glassy and rubbery regions, as well as the general property trends associated with each region (Siebenmorgen et al., 2004).

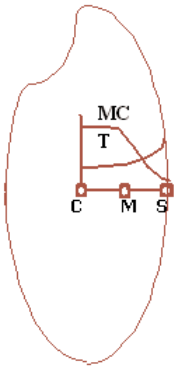

Fig. 2. Hypothetical temperature (T) and moisture content (MC) distribution within a rice kernel during the drying process. Points (C), (M), and (S) correspond to the center, mid-point, and surface locations of the rice kernel, respectively.

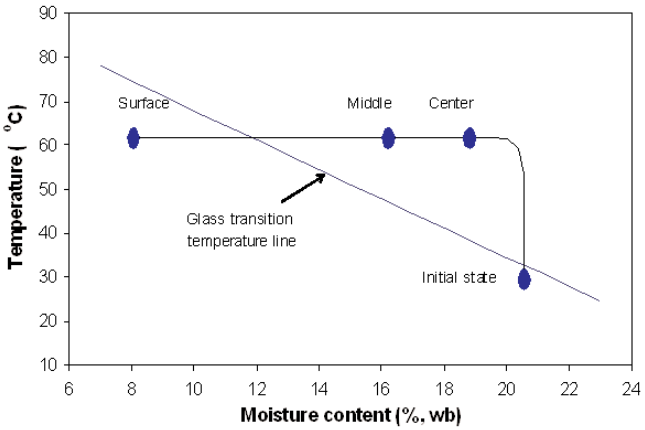

Fig. 3. Hypothetical temperature and moisture content gradients within a rice kernel at the locations depicted in Fig. 2, after extended high-temperature drying.

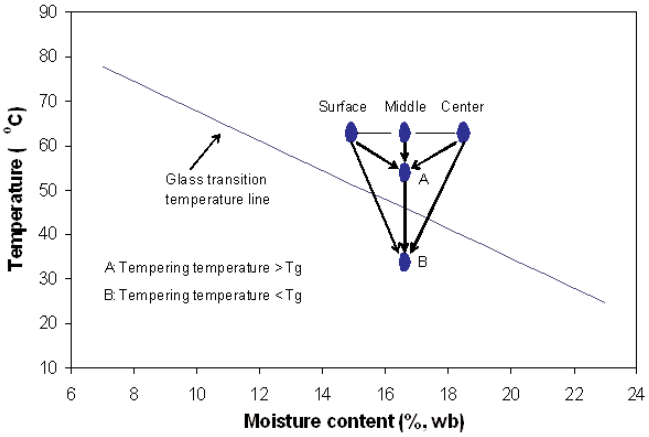

Fig. 4. Hypothetical tempering situations above and below the glass transition temperature (Tg) for a rice kernel that had been dried using air temperatures above Tg. Surface, middle, and center correspond to the kernel locations depicted in Fig. 2.

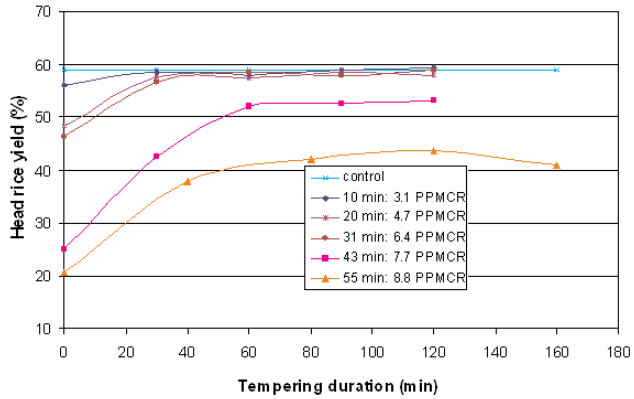

Fig. 5. Head rice yield versus tempering duration for cultivar Wells, with a harvest moisture content of 21.6%. Samples were dried using air at 60°C/17% RH for 10, 20, 31, 43, and 55 min, removing 3.1, 4.7, 6.4, 7.7, and 8.8 percentage points of moisture content (PPMCR), respectively, tempered at 60°C, and then cooled to 21°C. Each data point represents the average of two replicate sample HRYs.

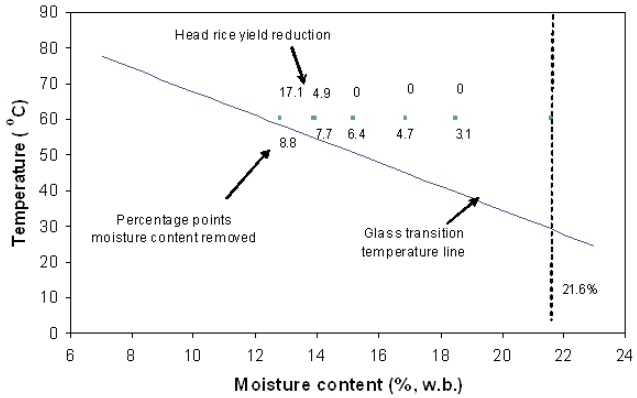

Fig. 6. Head rice yield reductions corresponding to the indicated percentage points moisture content removed plotted onto a Tg diagram of temperature versus moisture content. Samples of cultivar Wells with a harvest moisture content of 21.6% were dried using air at 60°C/17% RH. All samples were tempered for 90 min at 60°C immediately after drying and before cooling and subsequent drying. Each data point represents the average of two replicate sample HRYs.

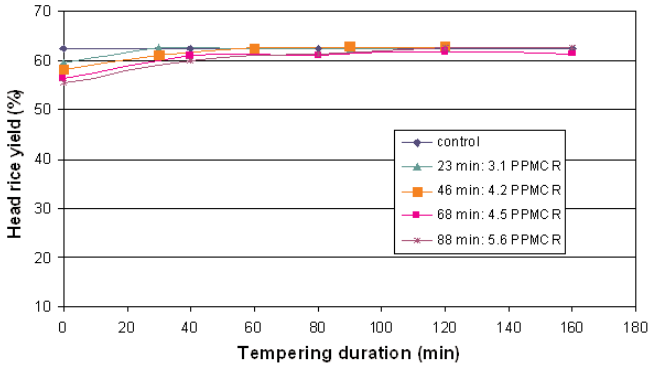

Fig. 7. Head rice yield versus tempering duration for cultivar Francis, with a harvest moisture content of 17.4%. Samples were dried using air at 50°C/28% RH for 23, 46, 68, and 88 min, removing 3.1, 4.2, 4.5, and 5.6 percentage points of moisture content (PPMCR), respectively, tempered at 50°C, and then cooled to 21°C. Each data point represents the average of two replicate sample HRYs.

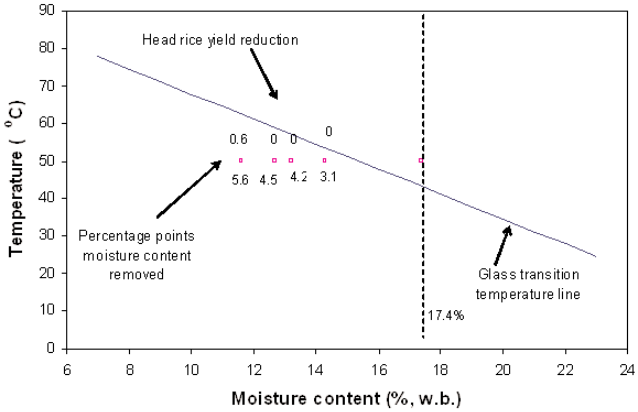

Fig. 8. Head rice yield reductions corresponding to the indicated percentage points moisture content removed plotted onto a Tg diagram of temperature versus moisture content. Samples of cultivar Francis with a harvest moisture content of 17.4% were dried using drying air at 50°C/28% RH. All samples were tempered for 90 min at 50°C immediately after drying and before cooling and subsequent drying. Each data point represents the average of two replicate sample HRYs.

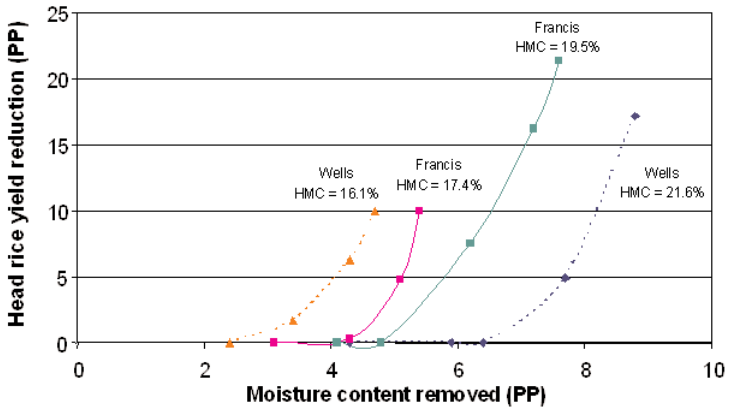

Fig. 9. Head rice yield reduction versus percentage points moisture content removed for cultivars Wells and Francis at the indicated harvest moisture contents (HMCs) using drying air at 60°C/17% RH. Each data point represents the average of two replicate sample HRYs. All samples were tempered for 90 min at 60°C immediately after drying and before cooling and subsequent drying.

Effects of heating on hydrophobicity, viscosity, and gelling properties of soy products

Robert S Walnofer[*], *Navam S. Hettiarachchy*[†], *Ronny Horax*[§]

ABSTRACT

The co-product of soybean after oil extraction is the meal, which is rich in protein. From this meal, protein concentrate and protein isolate are prepared and are commercially available as functional ingredients. Thermal treatment is the most common step applied to foods during processing. Changes in structural and functional properties can be affected by thermal or chemical treatments. The objective of this study was to evaluate the effect of heat on surface hydrophobicity, gelling properties, and viscosity of soy meal (SM), soy protein concentrate (SPC), and soy protein isolate (SPI). The soy products were subjected to heat at varying temperatures and heating times. Viscosity of soy protein products treated with heat increased for SM when temperature and heating times increased, but decreased for SPC and SPI. This may be due to the polysaccharides present in SM that could form starch gelation and increase meal viscosity. The surface hydrophobicity of the soy products increased when the proteins were treated with heat, possibly due to heat exposing the hydrophobic amino acids buried within the protein molecule making them become more hydrophobic on the surface of the molecule. When 8% suspensions (protein basis) were heated at 100°C, all soy products formed firm gels, indicating that protein plays an important role in gel network formation. Precaution must be taken to maintain functionality when heat processing is applied to food systems that contain soy protein products as functional ingredients.

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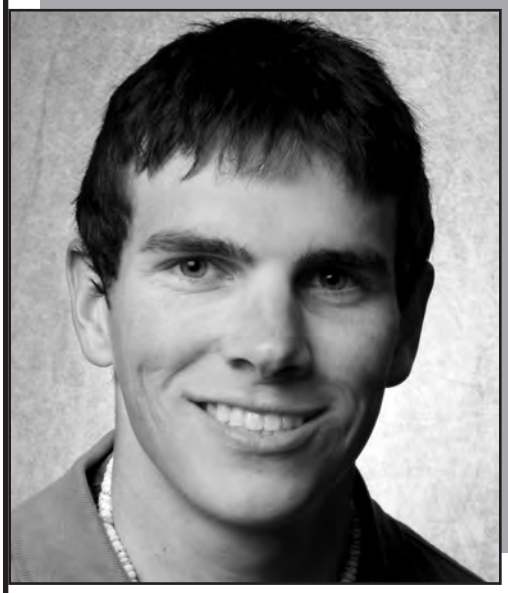
MEET THE STUDENT-AUTHOR

I am from Charleston, Ark., and a graduate of Charleston High School. At present, I am a senior honors student at the University of Arkansas majoring in food science. Through the Food Science Department, I have received the Carolyn S.Q. Sharp Scholarship from the Ozark Food Processors Association and a Mid-South Institute of Food Technologist Scholarship. My future plans include attending pharmacy school. I got the opportunity to work with my mentor, Dr. Navam Hettiarachchy, after participating in her Honors Proposal Development class. Under her guidance and the help of several of the protein chemistry lab M.S. and Ph.D. candidates, I learned a great deal about research and had the opportunity to work with many of the leading experts in the world of food science. I was able to present my research and compete in the Ozark Food Processors Association poster competition and came away with a third-place prize. My experiences at the University of Arkansas have not been limited to research. Other activities that I have participated in include serving as a Bumpers College Ambassador and a member of Alpha Gamma Rho Fraternity; holding a position on the executive board for Up 'til Dawn; competing on the U of A Cycling Team; serving as a Greek Life Facilitator; and being inducted into the Alpha Zeta and Gamma Beta Phi honor societies. Financial support of my project has been provided by the Dale Bumpers Agricultural, Food and Life Sciences Undergraduate Research award and a Silo Undergraduate Research Fellowship (SURF).



Robert S. Walnofer

INTRODUCTION

Soybeans (*Glycene max*) are an excellent source of protein. The use of soybean in the United States is expected to grow more than 10 % annually. Soy protein use is expected to reach nearly 50 million bushels by 2010 (USDA, 2004). Additionally, soybean is an important export product for U.S. processors. Soybean production has traditionally been one of the largest agricultural enterprises in Arkansas. Arkansas ranks eighth nationally in soybean production (ASPB, 2003). Due to its abundance and use as an inexpensive ingredient, soybean is also an important product in the food industry (Añón et al., 2001).

Soy products are commercially available to the food industry in the form of flours, concentrates, and isolates. Soy protein has received substantial publicity because of the U. S. Food and Drug Administration's claim that 25 g per day of soy protein can reduce the incidence of heart disease. This is important because heart disease is the number one cause of premature death in the U.S. Numerous products in the grocery store contain soy protein as a functional ingredient. A functional ingredient is that property of a substance that exhibits any property other than nutrition. The use of these products in a wide variety of foods has been increasing due to their desirable nutritional, nutraceutical, and functional properties, such as high essential amino-acid contents, and good emulsifying, foaming, fat absorption, and gelling properties. Soy protein as a functional ingredient has been studied extensively (Kalapathy et al., 1996; Kalapathy et al., 1997; Qi et al., 1997; Wu et al., 1998; Wu et al., 1999; Xie et al., 1998a; Xie et al., 1998b). Soy protein products can be used in food as water-binding agents, to increase viscosity, and to form protein gel (Kinsella et al., 1985). Soy flours or soy meals are prepared from defatted ground seed and usually contain about 40-50% protein. These are mostly used in food products such as bakery products and cereals. Protein content of soy protein concentrates usually varies from 60% to near 90%. These protein concentrates are prepared from defatted soy flour by removing the oligosac-

charides, fiber, and part of the minerals. Protein isolates contain more than 90% protein. Protein isolates are prepared from defatted flour by separating protein from polysaccharides, fiber components, and other low molecular-weight compounds.

In order to increase its use in the food industry, modification of soy protein is widely used to improve functional properties. Structural and functional property changes in soy protein can be achieved by thermal, enzymatic, or chemical treatments (Kalapathy et al., 1996; Kalapathy et al., 1997; Qi et al., 1997; Sorgentini et al., 1995; Wu et al., 1998; Wu et al., 1999; Xie et al., 1998a; Xie et al., 1998b). Thermal modification is much preferred due to the use of fewer chemicals in the process. During food processing, thermal treatment is the most common step that may affect the properties of soy products in the food system. However, information on changes in physicochemical properties of soy products after thermal modifications and treatments is limited.

MATERIALS AND METHODS

Protein determination

Protein contents of soy meal (SM), soy protein concentrate (SPC), and soy protein isolate (SPI) obtained from Archer Daniels Midland Company (Decatur, Ill.) were determined by an Automatic Kjeldahl method (AACC, 1990). The Kjeldahl 2006 Digestor (Foss Tecator, Hoganas, Sweden) was used for digesting the soy products in concentrated sulfuric acid with Kjeldahl tablet® as catalyst at 420°C for 1 h, and the Kjeltec® 2300 Analyzer Unit (Foss Tecator, Hoganas, Sweden) was used to determine the protein contents of the digested soy products. The protein contents were automatically calculated using 6.25 as the protein conversion factor commonly used in soybean industries.

Molecular size determination

Molecular sizes of the soy products were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970). The SDS-PAGE was carried out on a slab gel in an SDS-Tris-Glycine discontinuous buffer system. Protein solutions were prepared in non-reducing buffer solutions. Twelve microliters of the solution containing approximately 2 mg/mL of protein were loaded onto the gel performing at a constant current of 60 mA per gel for approximately 45 min. The gel was stained using a 0.1% Coomassie brilliant blue solution in acetic acid/ethanol/water (10/40/50,v/v/v) and de-stained in the same solvent in the absence of Coomassie brilliant blue. The approximate molecular sizes were determined by comparing the sample bands with Bio-Rad molecular size standard bands ranging from 6.5 to 200 kDa (Mysosin 200 kDa, β-galactosidase 116.25 kDa, Phosphorylase B 97.4 kDa, Serum albumin 66.2 kDa, Ovalbumin 45 kDa, Carbonic anhydrase 31 kDa, Trypsin inhibitor 21.5 kDa, Lysozyme 14.4 kDa, and Aprotinin 6.5 kDa) (Bio-Rad Laboratories, Hercules, Calif.).

Preparation of soy solutions

Soy meal, SPC, and SPI were suspended in deionized water (6%, based on protein content). Each suspension was heated in a water bath at 50/70/90°C for 30, 60, 90, and 120 min and then cooled to room temperature before viscosity and hydrophobicity determinations.

Viscosity determination

Viscosities of the thermally treated soy products were determined by a rotational rheometer (Haake VT 550, Germany) equipped with a MVDIN measuring spindle (radius = 19.36 mm, height = 58.08 mm) at room temperature (26°C). Samples (30 mL, 6% protein basis) were loaded into the cylindrical cup (radius = 21.0 mm). The samples were subjected to a constant shear rate (400 s^{-1}) and the viscosity was determined automatically using Rheowin Pro Data manager version 2.84 (Haake Mess Tech, Germany). All experiments were carried out in triplicates at room temperature.

Hydrophobicity determination

Surface hydrophobicity of the thermally treated soy products was determined by using an 8-anilino-1-naphthalene sulfonate (ANS) method adopted from Hayakawa and Nakai (1985). Concentrations ranging from 0.0005 to 0.003% (protein basis) were prepared by serially diluting the solution in 0.01 M phosphate buffer (pH 7). Ten microliters of 8 mM ANS (in 0.01 M phosphate buffer pH 7) were added to 2.0 mL of soy product solution. Fluorescence intensity of the ANS-protein conjugates was measured with a Shimadzu Model RF-1501 Spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) at excitation and emission wavelengths of 390 nm and 470 nm, respectively. The slope of the fluorescence intensity versus the soy product concentration was calculated by linear regression and was used as an index of the soy product hydrophobicity.

Gelling property determination

Gelling properties of the soy product solutions in water were determined by a slightly modified method of Coffmann and Garcia (1977) as described by Sathe et al. (1982). A series of concentrations of soy product suspensions from 2 to 20% w/v with 2% increments were prepared in 5 mL deionized water to determine the least or lowest gelation concentration of soy protein products in water. The test tubes containing these suspensions were then heated in a boiling water bath for 1 h followed by rapid cooling under running cold tap water. The test

tubes were further cooled for 30 min at 4°C, and the cooled suspension in the tubes was considered to form a firm gel if the suspension of inverted test tube did not slip or spill.

Statistical analysis

Data were analyzed for variance with multiple mean comparisons using JMP 5 software package (SAS Inst., 2002). The significance of means was determined by the Tukey Honestly Significant Difference (HSD) procedure at $P \leq 0.05$.

RESULTS AND DISCUSSION

Protein Content

Before functional protein analysis, protein contents of soy products had to be determined because protein of these products plays an important role in food systems (Table 1). From the Kjeldahl analysis, the protein contents in SM, SPC, and SPI were 51.2%, 65.2%, and 84.7%, respectively. The SM of 51% was above the expected range of 40-50% and the SPI of 85% was under the expected range of slightly >90% due to the samples being commercially produced compared with lab-scale soy protein isolate. Because protein isolates are prepared from defatted flour by removing the polysaccharides and other low molecular-weight compounds, the residual polysaccharides and fiber could have influenced protein levels.

Molecular Size

An electrophoretogram obtained using SDS-PAGE electrophoresis showed the molecular sizes of the proteins in SM, SPC, and SPI (Fig. 1). Soy products consisted of more than one type of protein with varying molecular size. SDS-PAGE was used because it promotes a separation based on the size of protein molecules. Based on the molecular size, the protein molecules move in an electric field and different size of proteins are separated. The major bands of all soy products ranged from 14.4-35 kDa as compared by Bio-Rad molecular size standard (Fig. 1). The SPI, SPC, and SM showed similar bands located at 35, 22, and 14.4 kDa, even though lighter bands at 14.4 kDa were observed for SPC and SPI in comparison to SM. The SM had larger amounts of proteins at 14.4 kDa and < 6.5 kDa than those of SPI and SPC, while more proteins with the molecular size of > 200.0 kDa were observed in SPC and SPI. This is important because the molecular size of a protein plays a role in gel formation in that disulfide linkages form cross-links, and the cross-linking of protein molecules forms gel. The larger the protein, the firmer the gel.

Viscosity

The viscosity of a product is simply its resistance to flow, which is an important factor in food processing. The determination of viscosity is important for the type of product application and to design needed equipment. Highly viscous products have a thicker solution and can cause clogging of narrow tubes and piping in a production facility. The control was determined by recording the viscosity of non-treated samples of each protein type. The viscosities of the SM heated at 50°C for up to 90 min and at 70°C for up to 60 min did not significantly differ in comparison to untreated SM ($P > 0.05$) (Table 2). This result indicated that these treatments were not enough to cause changes in the viscosity of SM. Yet when SM was heated longer and at higher temperature (up to 120 min at 50°C, up to 90 min at 70°C, or only 30 min at 90°C), its viscosity was significantly higher ($P < 0.0001$) than unheated SM. The viscosities of heat treatments at 90°C were much higher than those of heat treatments at 50 and 70°C. This result clearly showed that heating time and temperature affect the viscosity of SM in water. On the other hand, the viscosity of SPC and SPI treated with heat showed the opposite results. The results for SPC showed that SPC treated at 50°C and 90°C across all the heating times had significantly lower viscosity in comparison to untreated SPC ($P < 0.0001$). However, even though the viscosities of SPC treated at 70°C for up to 60 min were significantly lower than control ($P < 0.0001$), there were no significant differences between the viscosities of SPC treated up to 90 and 120 min and that of untreated SPC ($P > 0.05$). The results for SPI were quite similar to those for SPC. However, the viscosities of SPI treated with heat across all the temperatures and for all the heating times were significantly lower than that of untreated SPI ($P < 0.0001$). Heating the SPI at 90°C for any time greatly decreased its viscosity in water suspension. The results indicated the viscosity of soy products was affected by the protein-polysaccharide ratio. When the polysaccharide content was high, as occurred in SM, the polysaccharide affected the viscosity more than the protein by forming starch gelation that decreased the ability of the suspension to flow and increased viscosity. When there was no polysaccharide in the soy product, which happened in SPI, the protein characteristics considerably affected the viscosity of its suspension in water. This may be due to protein denaturation. At high temperature, the protein is denatured and its structure is opened up to expose the hydrophobic residues, along with some hydrophilic residues of protein. This unfolded protein with more hydrophilic amino acids on the surface of the molecules probably could interact more with water mol-

ecules by forming hydrogen bonds that in turn could cause the increase of its viscosity due to the hydration of the protein molecules.

Hydrophobicity

The surface hydrophobicity was determined from a linear relationship between the protein concentrations and fluorescence intensity. By plotting the line of regression, the surface hydrophobicity was expressed as the slope of fluorescence intensity versus protein concentration. The surface hydrophobicities of untreated soy protein products (control) showed that the surface hydrophobicity of SM was significantly lower than those of SPC and SPI ($P < 0.0001$) (Table 3). This could be due to proteins in SPC and SPI undergoing partial denaturation during preparation that could open up some buried hydrophobic residues to the surface of the protein molecules. Overall, surface hydrophobicities of the SM and SPC treated with heat across all temperatures were significantly higher than those of untreated samples with the exception of SPC at 70°C with up to 120 min heating ($P < 0.0001$). For SM, the higher the temperatures applied, the higher the surface hydrophobicities of the protein. This was due to the increase in degree of denaturation. This result exhibited that when more heat was applied to the protein, this could cause more protein to be unfolded and expose more hydrophobic amino acids of the protein to the surface of the protein molecules. At lower temperature (50°C and 70°C), longer heating time was needed by SM to unfold more of its protein molecules, which in turn increased the surface hydrophobicity value of this soy protein product. Similar results were obtained for SPC, which had a significantly higher surface hydrophobicity value for a heat-treated sample than that for untreated sample (control) ($P < 0.0001$). However, unlike SM, different temperature and heating time treatments conducted on SPC did not show considerable effects on the surface hydrophobicity, probably due to maximal hydrophobic residues that had been exposed to the surface of the proteins even at low temperature (50°C). Unlike SM and SPC, surface hydrophobicities of SPI treated at 50°C, particularly for longer heating time (up to 60 min and longer), were significantly lower than that of untreated sample ($P < 0.0001$). This was probably due to hydrophobic interaction between proteins, which may be thermodynamically favorable at this temperature, reducing the amount of hydrophobic residues on the surface of the protein structure. However, when the sample was heated at higher temperature (90°C), the surface hydrophobicities increased significantly, with the exception of heating time up to 60 min ($P < 0.0001$). When hydrophobicity values of the soy protein products treated at 90°C were observed, all types of the soy products showed the same pattern over the heating times. Even though this result is not clearly understood, these fluctuation values could be caused by protein-protein interactions either between hydrophobic residues or hydrophilic residues, depending on the time duration of temperature applied to the protein.

Gelation

The lowest solution concentrations required to form gels for SM, SPC, and SPI were 14%, 10%, and 10% (weight basis), respectively. To determine the heating time needed by the soy protein products to form a firm gel at the lowest solution concentration, the soy product suspensions were heated at 70, 80, 90, and 100°C for 10, 20, 30, 40, 50, and 60 min. SM formed a firm gel by heating 14% (weight basis) of SM at 80°C for 30 min, and at 90°C and 100°C for 10 min, but it did not form gel at 70°C even for 60 min heating. On the other hand, 10% (weight basis) of SPC and SPI formed gel after heating at 70°C for 20 min and 10 min, respectively, and needed only 10 min for both to form gel when heated at 80°C and above. When suspensions were made on the basis of protein content, 8% (protein basis) for all soy products could form gel when the suspensions were heated at 100°C for 1 h. This result indicated that even though polysaccharide is present in a significant amount in SM for gel formation, the proteins of the soy products play a more important role in the formation of a gel network when their suspensions in water are heated.

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Table 1. Protein contents of soy meal, soy protein concentrate, and soy protein isolate.[z]

Protein type	Protein content (%)
Soy meal	51.2 ± 0.6c
Soy protein concentrate	65.2 ± 0.7b
Soy protein isolate	84.7 ± 0.9a

[z]Values are means ± standard deviations of three replications; mean values with different lower cases in the same column are significantly different ($P < 0.05$).

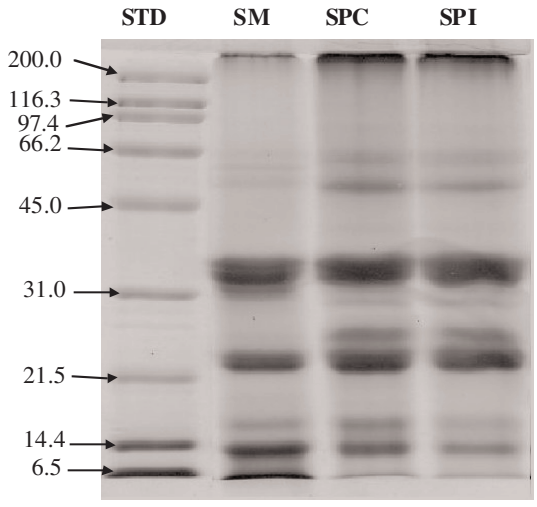

Fig 1. Electrophoretogram of soy meal (SM), soy protein concentrate (SPC), soy protein isolate (SPI), and Bio-Rad standard (Std).

Table 2. Viscosities (cps) of soy meal, soy protein concentrate, and soy protein isolate treated with heat at varying temperatures and heating times.[z]

		Temperature (°C)												
		50				70				90				
Protein type[y]	Control	30[x]	60	90	120	30	60	90	120	30	60	90	120	P-Value
SM	0.0095 cFGH	0.0093 cGH	0.0075 cH	0.0080 cH	0.0113 cEFG	0.0113 cEFG	0.0122 cEF	0.0124 cE	0.0129 cE	0.0384 bD	0.0506 bB	0.0472 aC	0.0804 aA	<.0001
SPC	0.0613 aA	0.0496 aFG	0.0507 aEFG	0.0512 aEFG	0.0535 aDE	0.0535 aDE	0.0574 aBC	0.0635 aA	0.0607 aAB	0.0485 aG	0.0551 aCD	0.0359 bH	0.0527 bDEF	<.0001
SPI	0.0458 bA	0.0385 bC	0.0400 bBC	0.0406 bBC	0.0265 bF	0.0265 bF	0.0406 bB	0.0357 bD	0.0332 bE	0.0143 cG	0.0092 cH	0.0091 cH	0.0075 cH	<.0001
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

[z]Values are means of three replications; mean values with different upper cases in the same row and different lower cases in the same column are significantly different (P < 0.05).

[y]SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

[x]Heating times (min).

Table 3. Surface hydrophobicities of soy meal, soy protein concentrate, and soy protein isolate treated with heat at varying temperatures and heating times.[z]

Protein type[y]	Control	Temperature (°C)												P-Value
		50				70				90				
		30[x]	60	90	120	30	60	90	120	30	60	90	120	
SM	35194 cH	45909 cG	46244 cG	54439 cF	42688 cG	71226 cE	73729 cE	73269 cE	78158 bD	100421 bB	92815 bC	131772 bA	91181 bC	<.0001
SPC	89463 aI	119603 aD	127381 aC	131282 aB	118383 aDE	116105 aE	125996 aC	112437 aFG	76611 bJ	115271 aEF	98775 aH	153488 aA	111106 aG	<.0001
SPI	81578 bD	79666 bDE	61401 bF	74020 bE	66133 bF	92781 bC	89310 bC	78051 bDE	81851 aD	87835 cC	76880 cDE	120588 cA	113325 aB	<.0001
P-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0027	<.0001	<.0001	<.0001	<.0001	

[z]Values are means of three replications; mean values with different upper cases in the same row and different lower cases in the same column are significantly different (P < 0.05).

[y]SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

[x]Heating times (min).

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