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, expensive 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

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 µmol . sec⁻¹ . 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 . 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 (α = 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**

The authors wish to acknowledge the financial support provided for this study by a Dale Bumpers College of Agricultural, Food and Life Sciences Undergraduate Research Award.

**LITERATURE CITED**


Particle size distribution of ground rice hulls.

![Particle size distribution of ground rice hulls](image)

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

<table>
<thead>
<tr>
<th>Substrate component (% vol/vol)</th>
<th>Sphagnum peat</th>
<th>GRH w/ wetting agent</th>
<th>Perlite</th>
<th>Vermiculite</th>
<th>Fresh shoot weight (g)</th>
<th>Fresh root weight (g)</th>
<th>Dry shoot weight (g)</th>
<th>Dry root weight (g)</th>
<th>Germination %</th>
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<td>1.29</td>
<td>0.22</td>
<td>0.11</td>
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</table>

| Significance                   | ***           | **                     | ***     | NS           | ***                    | NS                   | NS                   | NS                | ***          |
| Substrate                      | NS            | NS                    | NS      | NS           | NS                     | NS                   | NS                   | NS                | NS           |
| Block                           | 0.29          | 0.39                  | 0.05    | 0.04         | 10                     |                      |                      |                   |              |

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

GRH = Ground rice hulls

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).

<table>
<thead>
<tr>
<th>Sphagnum peat</th>
<th>GRH z w/ wetting agent</th>
<th>Perlite</th>
<th>Vermiculite</th>
<th>Avg' fresh shoot weight (g)</th>
<th>Avg' fresh root weight (g)</th>
<th>Avg' dry shoot weight (g)</th>
<th>Avg' dry root weight (g)</th>
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<td>90</td>
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<td>0</td>
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<td>0.06</td>
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Significance:
- **: Nonsignificant or significant at the 0.01 level
- ***: Nonsignificant or significant at the 0.001 level

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.

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.

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:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Temperature</th>
<th>RH</th>
<th>Equilibrium MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>H (high)</td>
<td>60°C, 17.0% RH</td>
<td></td>
<td>5.5%</td>
</tr>
<tr>
<td>L (low)</td>
<td>50°C, 28% RH</td>
<td></td>
<td>7.2%</td>
</tr>
<tr>
<td>C (control)</td>
<td>21°C, 60% RH</td>
<td></td>
<td>12.2%</td>
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</table>

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-

**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.
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 (PPPMC) 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

The authors wish to thank the Arkansas Rice Research and Promotion Board and the corporate sponsors of the University of Arkansas Rice Processing Program for the financial support of this research. The authors also wish to thank Ms. DeCee Bowman for conducting milling analyses and for helping conduct the drying experiment.

LITERATURE CITED


Perdon, A., T.J. Siebenmorgen, and A. Mauromoustakos. 2000. Glassy state transition and rice drying: develop-
ment of a brown rice state diagram. Cereal Chem. 77: 708-713.

**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 (PMPMC), 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 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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INTRODUCTION

Soybeans (Glycine 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 (Anón 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 a 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<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 51.2% 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 crosslinks, 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-
molecules 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, the higher the surface hydrophobicities of the 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 ($P < 0.0001$). 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.

**ACKNOWLEDGMENTS**

The authors would like to thank Archer Daniels Midland Company for supplying soy protein products. Financial support of this project was provided by the Dale Bumper’s College of Agricultural, Food and Life Sciences Undergraduate Research Grant and the Silo Undergraduate Research Fellowship.

**LITERATURE CITED**


Coffmann, C.W. and V.V. García. 1977. Functional properties and amino acid content of a protein isolate...


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

<table>
<thead>
<tr>
<th>Protein type</th>
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<td>Soy meal</td>
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<td>Soy protein concentrate</td>
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<td>Soy protein isolate</td>
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*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.†

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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).

†SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

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

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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).

‡SM = soy meal; SPC = soy protein concentrate; SPI = soy protein isolate.

§Heating times (min).
Instructions for Authors

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For research in disciplines where professional journals use style guides that differ significantly from the CBE, please consult the DISCOVERY managing editor for guidance. Please follow the most recent issue of DISCOVERY Instructions for Authors, also available at http://www.uark.edu/depts/agripub/Publications/Discovery/index.html.

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• authors’ first names, middle initials (if any), and last names (faculty sponsor should be listed as a coauthor)
• an abstract
• a footnote identifying each author by classification and major for students; rank and department for faculty and staff
• a footnote identifying faculty sponsor or mentor
• a footnote acknowledging financial support and other assistance. Note support by any companies or parties with a vested interest in the research results.

The Abstract summarizes the purpose, procedures, and main findings in 250 words or less.
The Introduction states the purpose of the study, the hypothesis, and pertinent background information.
The Materials and Methods section describes the experimental design, materials used, statistical analysis (required), and any other details needed for another researcher to reproduce the study and to confirm the validity of findings and conclusions.
The Results and Discussion section presents appropriate data, but not all data, in text, tables, and figures and places the findings in context with other research in the field. The discussion emphasizes new and important aspects of the research and conclusions that follow from them. Include implications and impact of the findings. Relate your findings to observations of other studies. State new hypotheses when warranted, but avoid unqualified statements not completely supported by your data.
The Literature Cited section lists the complete references corresponding to those cited in the text. Within the text, references are indicated by (Last Name, Year), e.g., (Jones, 2000) (Smith and Jones, 2000) (Brown et al., 2000) The complete citation is listed at the end of the manuscript in alphabetical order by the first author’s last name. Multiple citations of the same author are listed chronologically or by order of reference in the text if dated the same year. Journal references are written as follows:
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Length should be limited to about 2000 words, but no minimum or maximum length is required.

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