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Osmoconditioned seeds as a foodstuff

Julie Marie Goldman
Iowa State University

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Julie Marie Goldman

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Signature was redacted for privacy.

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Signature was redacted for privacy.

For the Graduate College

Members of the Committee:

Signature was redacted for privacy.

Signature was redacted for privacy.

Signature was redacted for privacy.

Signature was redacted for privacy.

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ABSTRACT

The technique of seed osmoconditioning, which consists of a controlled hydration and dehydration, was evaluated for use in foodstuff production.

In the first experiment, soybean, wheat, mungbean, and sesame seeds were hydrated in polyethylene glycol (PEG), chitosan plus water (CH), citric acid (CA), and water (W). Hydrated seeds were either dehydrated to their original moisture content or left fully-hydrated and stored at 5°C or 15°C for 2 or 4 weeks. Fully-hydrated and dehydrated seeds sprouted faster than untreated seeds, but the performance of fully-hydrated seeds declined during storage. Phytate content in soybean and mungbean seeds increased with the initial hydration. Phytate decreased when seeds were dehydrated, but phytate content in fully-hydrated seeds remained high. To assess microbiological safety, aerobic plate counts, total coliform counts, and yeast and mold counts were performed. Dehydrated seeds had lower microbial counts than fully-hydrated seeds. Polyethylene glycol and citric acid had an antimicrobial effect.

In the second experiment, soybean and wheat seeds were surface-sterilized with NaOCl, EtOH, or a control. Seeds were hydrated in PEG, CA, or W. Following hydration, seeds were treated with calcium propionate or a control, and then dehydrated. The crops were analyzed as dry, unsprouted seeds and as 48-hour sprouts. Citric acid and NaOCl had antimicrobial impacts on unsprouted seeds, but sprouted seeds had microbial counts up to 10^5 higher than unsprouted seeds

regardless of treatment. The calcium propionate treatment reduced total coliform counts in wheat by a factor of 10. Some counts declined after 16 weeks of 4°C storage. Sprouting resulted in increased phytate in soybeans (40%) and wheat (10%). Sprouts from osmoconditioned and untreated seeds were rated similarly in sensory analyses.

In the final experiment, phytate content and phytase activity of soybean and wheat seeds were determined during the osmoconditioning process and during a subsequent 144-192 hour sprouting period. While phytate content in wheat seeds and sprouts did not change during osmoconditioning or sprouting, phytase activity increased throughout the sprouting period. Phytate in soybean seeds increased at 8 hours of sprouting, and decreased at 192 hours. The reduced content at 192 hours coincided with an increase in phytase activity. Osmoconditioned and untreated sprouts did not differ with regard to phytate content or phytase activity.

GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with the General Introduction, which is comprised of this statement of organization as well as the Rationale for Research and the Literature Review. Following the introduction is the main component of the dissertation, which consists of three manuscripts that will be submitted to peer-reviewed journals for publication. The dissertation concludes with General Conclusions and Acknowledgments. All of the experiments and experimental analyses in this dissertation were conducted by the candidate, Julie M. Goldman, with the exception of the microbiological laboratory procedures.

Rationale for Research

The technique of seed osmoconditioning, traditionally used to enhance seed germination and stand establishment of agronomic and horticultural crops, has the potential to enhance food production in a more immediate manner. When sprouted seeds are used directly as a foodstuff, osmoconditioning may result in improved foodstuff quality and increased efficiency of production.

Because osmoconditioning reduces the time repuired for sprouting, the technique should decrease the time required for production of sprouts as a foodstuff. Furthermore, microbiological safety, sensory quality, and nutritional status of sprouts are likely to be impacted by the hydration and dehydration procedures of osmoconditioning and the accelerated timecourse of development. Of special interest is the

status of phytate, an antinutritional factor whose synthesis and catabolism are greatly affected by conditions of the sprouting process.

The goal of this research was to evaluate osmoconditioning for use in foodstuff production. The specific objectives included: 1) the assessment of osmoconditioning in terms of production time, nutritional status, microbiological safety, and sensory impact; and 2) an assessment of phytate metabolism during osmoconditioning and sprouting.

Literature Review

Sprouted Seeds as a Foodstuff

The ancient practice of sprouting seeds for human consumption continues as a modern-day means of obtaining a flavorful, nutritious product. Sprouted seeds, which are often referred to as "sprouts," are typically consumed as a fresh vegetable in salads, sandwiches, and casseroles. Sprouted seeds also are processed *via* retort and dehydration, thereby yielding foodstuffs with a longer storage life than fresh sprouts and with additional uses, such as incorporation into cereals and soup mixes. Following dehydration, sprouts also can be milled to flour to be incorporated into bakery products, beverages, and weaning foods.

Crop seeds used for sprouting include most of the cereal and legume crops, including soybean, wheat, mungbean, miscellaneous beans, pea, millet, corn, alfalfa, and barley. While less common, sprouts are also produced from seeds of sesame, radish, buckwheat, sunflower, almond, clover, flax, lentil, and pumpkin (Larimore, 1975).

The production of sprouts is a process with minimal inputs of time, energy, and labor. Relatively small quantities of water, oxygen, heat, and light are required for the several days of sprouting, and there are virtually no waste products from production, processing, and consumption.

Several desirable nutritional changes occur during the process of sprouting. First of all, when compared on a dry weight basis with unsprouted seeds, sprouted seeds from a wide range of crops exhibited an increased content of ascorbic acid, B-group vitamins, and some amino acids (Kylen and McReady, 1975; Pennington and Church, 1989; Fordham et al., 1975; Chen et al., 1975; Lemar and Swanson, 1976). Secondly, protein and starch digestibility improved with sprouting (Chavan and Kadam, 1989), and the PER (protein efficiency ratio) of soybeans increased (Bau and Debry, 1979). Thirdly, antinutritional factors such as trypsin inhibitor, tannins, and phytate were reduced in many crops when sprouting occurred (Bartnik and Szafranska, 1987; Kumar and Chauhan, 1993; Vidal-Valverde et al., 1994; Larsson and Sandberg, 1992; Dagnia et al., 1992; Beal et al., 1984; Mihailovic et al., 1965).

Sprouted seeds, with a high moisture content and active metabolism, supported a much higher microbial population than dry seeds (Prokopowich and Blank, 1991; Splittstoesser et al., 1983; Andrews et al., 1982). Although the high counts do not typically indicate a health concern, since pathogenic bacteria such as *Salmonellae spp.* are not commonly found in plant foodstuffs (Splittstoesser et al., 1983), appropriate sprouting methodology and antimicrobial treatments can aid in limiting

microbial growth (Sawyer et al., 1985; and Splittstoesser et al., 1983).

Sprouting Methodology

The same principles apply to sprout production even though materials and methods may differ. Seeds are soaked in water for a period of several hours to hydrate the seeds and initiate the sprouting process. Thereafter, seeds are kept moist by regular rinsing or sprinkling treatments. Seeds typically are considered to have sprouted when the radicle (first root) emerges. The length of the first root or the first shoot is often used as an indicator of maturity, which is based on consumer preference in terms of flavor, texture, and appearance. Sprouts typically reach the desired stage of development in 1 to 6 days, depending on the species, the vigor of the seeds, and the sprouting environment. Once harvested, sprouts may be stored for several days under refrigeration (Larimore, 1975; Blanchard, 1975).

When sprouts are produced in a large-scale operation, the process may utilize automated enquipment, with greater environmental control than might be found with small-scale production. The materials and methods for small-scale production are typically inexpensive but labor-intensive. Methods such as the "glass jar method," the "clay pot method," and the "plastic tub method" (Larimore, 1975) are recommended for home use and are often used, with minor modifications, for food-service production (Sawyer et al., 1985) and for experimental work (Marero et al., 1988; and Larsson and Sandberg, 1992).

Osmoconditioning

Seed osmoconditioning, or "priming," is a means of enhancing the sprouting process. The technique has traditionally been used to enhance stand establishment in field production of agronomic and horticultural crops. Osmoconditioning allows seeds to commence but not complete germination by providing for a controlled hydration, i.e., the seed moisture content is not allowed to reach the level necessary for radicle emergence. Following the hydration, seeds are dehydrated to their original moisture content to facilitate storage (Bradford, 1986). When the osmoconditioned seeds are exposed to an appropriate germination environment, the seeds typically germinate more quickly and more uniformly than untreated seeds (Heydecker and Coolbear, 1977; and Smith and Cobb, 1991), especially under adverse conditions (Bodsworth and Bewley, 1981; and Dell'Aquila and Tritto, 1990). Lalonde and Bewley (1986) attributed he faster germination to the pattern of protein synthesis, which entailed both qualitative and quantitative changes. When seeds were hydrated, but not sufficiently for germination, and then dehydrated, protein-synthesizing mechanisms reverted to production of proteins typical of earlier stages of hydration. However, the reversion was only partial, and the seeds never reverted to the metabolic status of the initial dry seed.

Controlled hydration usually is achieved by using osmotica such as polyethylene glycol (PEG) or salt solutions such as NaCl. Water alone may be used, but the treatment time and temperature must be more carefully controlled. Alternatively, water can be used in conjunction

with water-absorbing materials such as vermiculite, shale, or sphagnum moss in a process called solid matrix priming or matriconditioning (Taylor et al., 1988).

Phytate in Seeds and Sprouted Seeds

Overview Phytate is one of the antinutritional factors commonly studied in nutritional assessments of cereal grains, legumes, and other seeds. As the primary storage form of phosphorus in plant seeds, phytate comprises 50-90% of the total phosphorus in plant seeds.

The terms phytate (inositol hexaphosphate, or the mono to dodeca anion of phytic acid) and phytic acid (inositol hexaphosphoric acid) are used somewhat interchangeably, and the term phytin (a calcium-magnesium salt of phytic acid) is also used by some researchers and reviewers (Maga, 1982).

Originally, phytate was considered important because it provided phosphorus for germinating seeds and young seedlings. It is now recognized that phytate also supplies myo-inositol and cations, and plays a role in sequestering and chelating metallic cations. Additional roles will no doubt be ascribed to phytate as research continues. One such role is the function of phytate in the inositol phosphte signalling system. Also referred to as "signal transduction," the system is a mechanism for the transmission of signals across cell membranes via second messengers such as IP_3 (inositol-1,4,5-triphosphate). The messengers are regulated by receptor-controlled hydrolysis of inositol phospholipids (Billington, 1993).

Phytate acts as an antinutrient by binding divalent cations, thus reducing the bioavailability of iron, zinc, and calcium (Torre et al., 1991; Beal et al., 1984; Heaney et al., 1991; Hurrell et al., 1992). A high phytate content in foodstuffs also reduces protein and starch digestibility (Knuckles et al., 1985; and Thompson and Yoon, 1984). Phytate content may be reduced by several processes, including the leaching of water-soluble forms of phytate (Chang et al., 1977), high temperature destruction (Lehrfield, 1994; and deBoland et al., 1975), acid or alkaline extraction (Hartman, 1979), dialysis and ultrafiltration (Erdman, 1979), fermentation (Ranhotra et al., 1974), soaking and hydration (Chang et al., 1977; Beleia et al., 1993; and Jones and Boulter, 1983), and sprouting of seeds (Salunkhe and Kadam, 1989; Vidal-Valverde, 1994; Kumar and Chauhan, 1993; Sattar et al., 1990; and Dagnia et al., 1992).

Phytate Synthesis During the growth and development of field crops, phytate typically begins to accumulate after pollination and continues to do so until seeds are fully developed. When crops were grown with varying levels of nutrient phosphorus, the nonphytic phosphorus remained relatively constant in wheat (Michael et al., 1980) and soybean (Raboy and Dickinson, 1987), while the phytic phosphorus increased. In soybean, germination and growth of seeds with reduced levels of phytate was similar to that of control seeds, and this suggests that cultivated soybeans contain "luxury levels" of phytate (Raboy et al., 1985).

As phytate is synthesized, the deposition in cereal crops is

generally tissue-specific. For example, in wheat (O'Dell et al., 1972) and rice (Kennedy and Schelstraete, 1975), most of the phytate is found in the aleurone layer. In corn, approximately 90% of the phytate is in the germ (O'Dell, 1972). In dicot species, deposition is generally in the endosperm, embryonic axis, and cotyledons (Raboy, 1990), and the phytate is usually concentrated within globoids inside the protein bodies (Lott, 1984). Soybean phytate also is associated with protein bodies (Tombs, 1967), and the deposits are evenly distributed throughout the tissues of the cotyledon (Lott, 1984).

Many studies have documented phytate synthesis and accumulation during seed development, and there is also documentation of synthesis during the early stages of sprouting. Mandal and Biswas (1970) conducted a study that examined phytate synthesis in mungbean cotyledons. During a 72-hour soaking period, incorporation of ^{32}P into inositol phosphates was maximal at 36 hours of soaking. The researchers concluded that synthesis was initiated after 12 hours of soaking, after which the rate of synthesis increased up to 36 hours and then decreased. It was further concluded that the incorporation of labelled phosphorus was not due to an exchange reaction with phosphorus hydrolyzed by phytase.

Sutardi and Buckle (1985) reported that phytate content of soybeans increased by 57% following 24 hours of soaking. Moeljopawiro et al. (1988) also reported an increase in phytate content when whole raw soybeans were boiled and soaked, and they suggested that the increase was related either to a loss of solids during soaking and boiling or to

the soaking process itself.

Dmitrieva and Sobolev (1985) studied the sequence of phytate mobilization in different tissues of the castor bean and the distribution between storage tissues and growing parts of the seed. They found that there was a net accumulation of phytate within the cotyledon during seed development and subsequent germination, with accumulation due to *de novo* synthesis. The authors suggested that delivery of inorganic phosphorus to growing parts of seedlings can be regulated by the "secondary deposition" of phytate in cotyledons, which would provide a phosphorus reserve for subsequent formation of shoot tissues.

Organ et al. (1988), again working with germinating castor beans, found that the mobilization of phytate reserves in the embryonic axis, endosperm, and cotyledons was followed by an extensive synthesis of phytate, especially in the cotyledons. With adequate supplies of exogenous inorganic phosphorus, cotyledons isolated from seeds 2 days after imbibition synthesized phytate for up to 6 more days. This ability to synthesize phytate was retained by the cotyledons even when phosphate was not present.

At present, the mechanisms involved with phytate synthesis are unclear. Two hypothetical pathways of phytate synthesis in plants were summarized by Raboy (1990). In the first pathway, based on Biswas et al. (1978), myo-inositol kinase catalyzes the phosphorylation of myo-inositol to myoinositol monokisphosphate (IP_1). The pathway proceeds via a stepwise phosphorylation of IP_1 to the pentaphosphate

(IP_5), catalyzed by phosphoinositol kinase. The final step to the hexaphosphate IP_6 is catalyzed by either phosphoinositol kinase or an ADP phosphotransferase. In the second pathway, based on Irvine et al. (1986) and Majerus et al. (1988), phosphatidylinositol is phosphorylated to the triphosphate, IP_3, which is then phosphorylated in a stepwise manner with catalysis by one or more kinases or a phosphotransferase enzyme.

In studies characterizing the phosphoinositol kinase in germinating mung bean seeds (Majumder et al., 1972), the enzyme was found to be different from inositol kinase, which mediates the phosphorylation of myoinositol to inositol monophosphate. In addition, phosphoinositol kinase had a pH optimum of 7.4 and required divalent cations and ATP to carry out the phosphorylation of lower inositol phosphates to their corresponding higher homologues. In further studies (Majumder and Biswas, 1973 a), the immediate product of each step of the reaction, from IP_2 to IP_6, exhibited a competitive inhibition. Formation of the final product, IP_6, thus was encouraged. It was proposed that IP_6 is continuously removed from the reaction region or is complexed to favor its continuing synthesis.

Majumder and Biswas (1973 b) also characterized a phosphoinositol kinase inhibitor that is hypothesized to accumulate during the latter stages of seed ripening. The authors suggested that the failure to detect phosphoinositol kinase activity in mature ungerminated seeds was not due to absence of the enzyme, but to the presence of a complex of phosphoinositol kinase and the inhibitor. The labile complex was

thought to dissociate during early stages of sprouting.

Phytate Hydrolysis The enzymes and mechanisms involved with phytate synthesis are still unclear. In contrast, the enzymatic hydrolysis of phytate has been extensively studied. The enzyme of interest is phytase (myo-inositol-hexaphosphate phosphohydrolase, E.C.3.1.3.8), which catalyzes the hydrolysis of phyate to inositol and inorganic phosphate (Maga, 1982).

Studies with germinated wheat seeds (Mihailovic et al., 1965) indicated that the hydrolysis occurred in a stepwise manner -- there was an intermediate formation of penta, tetra, tri, di, and monophosphates of inositol as phosphoric acid was also being liberated. Blatny et al. (1994) noted a similar process when hydrolyzing phytate with wheat phytase. In experiments with mungbeans, the rate of dephosphorylation increased at each step, indicating that once hydrolysis of IP_6 was initiated, the complete hydrolysis of IP_6 to inorganic phosphorus would occur very quickly (Mandal et al., 1972).

The activity of phytase was typically very low or even undetectable in dry or dormant seed (Lolas and Markakis, 1977; Mihailovic et al., 1965; and Gibson and Ullah, 1988). The activity increased gradually during sprouting until peaking at about 2 to 3 days for mungbean cotyledons (Mandal and Biswas, 1970 b), 4 days for wheat (Bartnik and Szafranska, 1987), 4 to 6 days for pea (Young and Varner, 1959), 6 days for fababean (Eskin and Wiebe, 1983), 9 days for cotyledons of *Phaseolus vulgaris* var Improved Canadian Wonder (Gibbins and Norris, 1963), and 8 to 12 days for soybean cotyledons (Gibson and Ullah, 1988).

Optimum conditions for hydrolytic activity of phytase vary somewhat depending on the species. For wheat, optimum pH was cited at 5.1 (Bartnik and Szafranska, 1987) or 5.5 (Peers, 1953; and Sartirana and Biachetti, 1967), with 55°C cited as the optimum temperature. Peers (1953) noted that in the absence of substrate and hydrolysis products, some inactivation of the enzyme occurred at 55°C. He suggested that the substrate and hydrolysis products may protect the enzyme from heat inactivation. Navy beans and California small white beans had very similar pH (5.2) and temperature (60°C) optima (Chang and Schwimmer, 1977; and Lolas and Markakis, 1977), and as temperatures increased above 65°C the activity decreased markedly. Soybean phytase had optimal conditions of pH 4.8-4.9 and 60°C (Sutardi and Buckle, 1986) and pH 4.5-4.8 and 55°C (Gibson and Ullah, 1988), and activity decreased at temperatures above 60°C. Several other crops had similar optimal conditions of pH and temperature, although Mandal et al. (1972) cited a pH of 7.5 for mung bean phytase.

Several metals affect phytase activity in vivo. Enhanced activity was reported with magnesium and calcium in wheat (Peers, 1953), and magnesium, manganese, and calcium at 0.2 to 5 mM concentrations in soybean (Gibson and Ullah, 1988). Gibson and Ullah (1988) also reported that zinc and iron at 0.5 to 2 mM concentrations inhibited phytase in soybean, possibly due to metal complexation with phytic acid, leading to formation of partially insoluble metal complexes. Sutardi and Buckle (1985) found that ferrous iron enhanced phytase activity, whereas zinc, copper, mercury, manganese, magnesium, calcium, cobalt, and aluminum

were inhibitory at a concentration of 1 mM.

The reaction product of phytate hydrolysis, inorganic phosphate, acted as a competitive inhibitor in mung beans (Mandal et al., 1972), California small white beans (Chang and Schwimmer, 1977), and soybeans (Gibson and Ullah, 1988). In wheat embryos, inorganic phosphate caused a decrease in phytase activity and repressed phytase synthesis (Sartirana and Biachetti, 1967). Furthermore, high substrate concentration resulted in partial inhibition of phytase (Sutardi and Buckle, 1986; Chang and Schwimmer, 1977; and Lolas and Markakis, 1977).

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OSMOCONDITIONED SEEDS AS A FOODSTUFF: DIMENSIONS OF SPROUTING PERFORMANCE, ANTINUTRIENT STATUS, AND MICROBIOLOGICAL SAFETY

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Julie M. Goldman[1], Amadu D. Ayebo[2], Mark H. Love[3], James S. Dickson[3]

Abstract

The technique of seed osmoconditioning, which consists of a controlled hydration and dehydration, was evaluated for use in foodstuff production. Soybean, wheat, mungbean, and sesame seeds were hydrated in polyethylene glycol, chitosan plus water, citric acid, and water. Hydrated seeds were either dehydrated to their original moisture content or left fully-hydrated, and stored at 5°C or 15°C for 2 or 4 weeks. Fully-hydrated and dehydrated seeds sprouted 20 to 50% faster than control seeds, but the performance of fully-hydrated seeds declined during storage. Phytate content in soybean and mungbean seeds increased with the initial hydration. Phytate decreased when seeds were dehydrated, but phytate content in fully-hydrated seeds remained high throughout storage. Dehydrated seeds had lower lower microbial counts than fully-hydrated seeds. Polyethylene glycol and citric acid had antimicrobial activity.

Introduction

The germination or sprouting of seeds yields a food product quite

[1]Graduate Research Assistant, Iowa State University, Ames, IA

[2]Research Scientist, CIREH, University of Iowa, Iowa City, IA

[3]Associate Professors, Iowa State University, Ames, IA

different from the original dry seeds. Nutritional changes occur, with sprouted seeds from a wide range of crops exhibiting an increased content of ascorbic acid, B-group vitamins, and some amino acids (Kylen and McReady, 1975; Pennington and Church, 1989; Fordham et al., 1975; Lemar and Swanson, 1976; Chen et al., 1975). Protein and starch digestibility usually improve, and reduction of antinutritional factors occurs in many cases (Chavan and Kadam, 1989; Bartnik and Szafranska, 1987; Vidal-Valverde et al., 1994; Larsson and Sandberg, 1992; Dagnia et al., 1992; Beal et al., 1984; Mihailovic et al., 1965; Kumar and Chauhan, 1993). Since microbiological changes also occur, with sprouted seeds supporting a much higher microbial population than dry seeds (Sawyer et al., 1985; Marero et al., 1988), sprouting methodology is critical in obtaining a nutritious, microbiologically safe foodstuff.

In this study, a technique traditionally used to enhance stand establishment and field production of agronomic and horticultural crops -- seed osmoconditioning -- was evaluated for use in foodstuff production. Osmoconditioning allows seeds to begin the metabolic processes of germination as the seeds are hydrated. Germination is not completed because the hydration process is stopped before the seed moisture content is sufficiently high for radicle emergence. Following hydration, seeds usually are dehydrated to the original moisture content as a means to facilitate storage. When the osmoconditioned seeds are subsequently exposed to the sprouting environment, the seeds complete germination more quickly and uniformly than untreated seeds, especially under suboptimal environmental

conditions (Heydecker and Coolbear, 1977; Bradford, 1986).

The impact of osmoconditioning on foodstuff production was assessed by determining sprouting performance, microbiological activity, and phytate concentrations in soybean, wheat, mungbean, and sesame. Sprouting performance is of interest because of time and space constraints on food production systems. Microbial activity is a major factor in food safety, and it also has an impact the overall quality of the foodstuff. The antinutrient phytate is of special interest because treatments similar to aspects of osmoconditioning -- soaking, storage, and germination -- affect phytate concentrations in foodstuffs (Salunkhe and Kadam, 1989; Sutardi and Buckle, 1985; Beleia et al., 1993; Bartnik and Szafranska, 1987; Jones and Boulter, 1983; Vidal-Valverde et al., 1994). Phytate is the inorganic ester of the hexa-alcohol inositol, and as the primary storage form of phosphorus in plant seeds, it is found in a wide variety of foodstuffs. Phytate has been studied extensively due to its antinutrient activity. It binds divalent cations, most notably the minerals calcium, iron, and zinc, and therefore it has a substantial impact on human nutrition (Torre et al., 1991; Beal et al., 1984; Heaney et al., 1991, Hurrell et al., 1992).

Materials and Methods

Seed Treatment

Wheat (*Triticum aestivum*), mungbean (*Vigna radiata*), and sesame (*Sesamum orientale*) seeds were obtained from Arrowhead Mills (Hereford, TX) in June 1993 and were stored at room temperature until treatments were applied in July. The wheat, hard red winter high-protein, was grown

and harvested in Texas, and the seeds were nitrogen-packaged. Mungbean seeds were grown in Oklahoma. Following harvest, the seeds were nitrogen-packaged. The sesame seeds were grown in Southwest Mexico and packaged in sealed plastic bags following harvest. Hulls were not removed from the seeds. *Glycine max* var. Vinton 81 soybean seeds, grown in Iowa and harvested in the fall of 1992, were stored in sealed plastic containers at 4°C.

Imbibition media, hereafter referred to as osmotica, included two materials used for osmoconditioning agronomic and horticultural crop seed, polyethylene glycol (PEG) and a solid matrix priming material called chitosan (CH). While both materials have proven to be effective for the osmoconditioning process, neither is listed as a GRAS (generally recognized as safe) food additive. The other osmotica, citric acid (CA) and water (W), are safe for food use. Citric acid has antimicrobial properties due to its low pH and the metabolic inhibition caused by undissociated acid molecules (Jay, 1991).

The osmotica were prepared as follows: 1) polyethylene glycol 8000 (Sigma Chemical Co., St. Louis, MO), 250 g/kg distilled water; 2) Chitosan Pro Floc 123 (Protan, Inc., Raymond, WA), a deacetylated product of poly N-acetylglucosamine, at 1g/6ml distilled water; 3) citrate buffer of 0.05 M and pH 4.4, made from citric acid monohydrate and sodium citrate dihydrate; and 4) distilled water.

Quantities of 300g seed were placed in Ziploc (Dow Corning, Midland, MI) 4000 ml storage bags of 1.75 mil thickness. For each crop, the 15 combinations of replicates (3) and osmotica (4 plus 1 "untreated"

control) were then randomly assigned, one to each of 15 bags. Sampling #1 took place at this time. Six hundred ml of the assigned osmoticum was added to each bag, or in the case of CH, 600 ml water plus 100 g chitosan. Bags were randomly arranged in a dark growth chamber at 25°C. CH, CA, and W treatments were applied for 4 hr (10 hr for mungbean) and the PEG treatments were 16 hr in length. Previously, tests had been performed to determine the optimal hydration time for seeds to achieve maximum moisture content without risk of germination.

Following hydration, all the bags were drained and the seeds were rinsed once in 600 ml distilled water, except for the CH-treated seeds which required washing with approximately 1800-2400 ml water. Rinsed seeds were placed on absorbent toweling for up to 30 min to surface dry, after which sampling #2 was performed. Seeds from each bag were divided into two groups, one of which was stored in a clean Ziploc bag at 15°C for approximately 24 hr while the other group was dehydrated in a food dehydrator (Harvest Maid Model 2400, Alternative Pioneering Systems, Chaska, MN) at 40°C. The dehydration continued until the seed moisture content was approximately equal to original moisture content, a process requiring 4 to 12 hr.

After the dehydrated seeds had cooled to room temperature, they were placed in Ziploc bags and stored at 15°C for the remainder of the 24 hr period, at which time sampling #3 was performed for both the hydrated and dehydrated groups. Following sampling #3, seeds were again split into two groups, each of which was placed in a sterile 750 ml Whirlpak bag (Nasco, Fort Atkinson, WI). One group of bags was stored

at 5°C and the other group at 15°C. Samplings #4 and #5 were performed after 2 and 4 weeks of storage, respectively. Sufficient seeds were withdrawn at each sampling time to make determinations on moisture content, germination, phytate, and microbiological counts. Due to visually-apparent microbiological contamination and seed deterioration, hydrated seeds stored at 15°C for 4 weeks were not sampled.

Moisture Content

To verify the degree of hydration and dehydration of seeds, moisture content was determined. Samples of 10 seeds (approximately 100 for sesame) were placed on Kimwipes (Kimberly-Clark, Roswell, GA) until surface-dry, then weighed to determine fresh weight. After being dried to constant weight at 103°C, samples were weighed again to determine dry weight. Moisture content was computed as a percentage, on a fresh weight basis.

Sprouting/Germination

All sprouting performance tests were conducted by placing samples of 10 seeds on 2 layers of Whatman #1 filter paper (Whatman Int'l. Ltd., England) in 100 x 15 mm plastic petri plates (Fisher Scientific Co., Canada). The filter paper was moistened with the necessary amount of distilled water. Covered plates were incubated at 25°C in complete darkness. Data were taken on the time required for 50% germination, with seeds checked every 6 hr for evidence of radicle emergence. Data were taken on germination percentage after 48 hr.

Phytate

Samples of sufficient size to supply 10 g dry matter were placed in 60 ml sterile Whirlpak bags, frozen in liquid N, and stored at -18°C. Following freeze-drying (FreezeDry-5, Labconco, Kansas City, MO), samples were ground in a coffee grinder (Braun, Inc., Lynnfield, MA) for 20 s and sieved through a 20 mesh screen. Phytate analyses were performed according to the colorimetric method of Vaintraub and Lapteva (1988), which is a modification of the method of Latta and Eskin (1980).

Microbiological

Ten gram samples were placed in sterile 60 ml Whirlpak bags for microbiological quality determinations. Samples were placed in peptone buffer and processed in a stomacher (model 400 Mark II, Tekmar, Cincinnati, OH) at normal speed for 120 s. Appropriate serial dilutions in buffered peptone water (Accumedia) were plated *via* a spiral plater (Model D, Spiral Systems, Cincinnati, OH) onto duplicate plates of tryptic soy agar (TSA) for aerobic plate counts (APC), violet red bile agar (VRBA) for total coliform counts (TC), and potato dextrose agar (PDA) for determining yeast and mold counts (YM). The plates were incubated as follows: TSA at 32°C for 48 hr, VRBA at 37°C for 24 hr, and PDA at 25°C for 120 hr. Plates were visually counted using methods appropriate for spiral plates (Peeler and Maturin, 1992).

Some counts were recorded as "too many to count" in plates where the colonies exceeded the manual counting capability; numeric values for these samples were obtained by the following formula: 10,000 x 1/greatest dilution. For data analysis, the arithmetic mean of the

duplicate plates was computed and then converted to a $\log_{10}$ mean by the following formula: $\log_{10}$ mean = $\log_{10}$ of (arithmetic mean + 1.00). 1.00 was added to the arithmetic mean to allow the use of zero counts. Counts were reported as $\log_{10}$ means of colony forming units (CFU)/g of seed.

Statistical Analyses

Statistical analyses for phytate and microbiological data were performed by using ANOVA (analysis of variance) produced by the GLM (general linear models) procedure in SAS (Statistical Analysis System, version 6.07). Each crop was analyzed separately. Due to storage problems, sesame samples from sampling times 4 and 5 (2 and 4 weeks of storage) were not available for analyses.

Data from sampling time 1 were analyzed to determine if phytate content and microbial counts differed (prior to treatment) among the 15 bags designated to receive the different hydration treatments. The impact of the initial hydration was then assessed by analyzing data from sampling time #2 (following hydration). Data were analyzed as a completely randomized design, with hydration treatment (untreated, PEG, CH, CA, and W) as the factor and replications within hydration treatment as the error term. The effect of dehydration was examined by using data from sampling time 3 (following dehydration), with osmoticum as the whole-plot factor and hydration status and osmoticum by hydration status as split-plot factors in a split-plot design.

A comparison was made among seeds stored for 2 weeks (sampling time 4), with osmoticum as the whole-plot factor and hydration status,

storage temperature, and all interactions as split-plot factors in a split-plot design. Seeds stored for 2 and 4 weeks were also compared (sampling times 4 and 5) by analyzing: a) dehydrated seed data only, with osmoticum as the whole-plot factor and storage time, storage temperature, and all interactions as split-plot factors in a split-plot design; and b) fully-hydrated seed stored at 5°C only, with osmoticum as the whole-plot factor and storage time and all interactions as the split-plot factors in a split-plot design. For all split-plot designs, the error term for the whole-plot factor was replications within osmoticum. The GLM procedure generated the appropriate error term for the split-plot factors for each model.

Factors and interactions with a p value of ≤ 0.05 were considered significant. Means separations were conducted via the Student-Newman-Keuls (SNK) test.

Germination data were not distributed normally, so nonparametric analyses were performed on these data. Using the SAS frequency procedure, the effects of treatment variables on the dependent variables were assessed using row mean scores of the Cochran-Mantel-Haenszel statistic (SAS, 1990). The row mean scores, generated via contingency tables, provided probability values for accepting or rejecting the null hypothesis that a treatment variable had no effect on the dependent variable in question. If the p value was ≤ 0.05, the null hypothesis was rejected.

Results and Discussion

Sprouting/Germination

The initial hydration treatment decreased the time required for germination, but did not impact germination percentage (Figure 1). As figure 2 illustrates, the soybean and mungbean seeds that remained hydrated sprouted more quickly than the dehydrated seeds. The performance of fully-hydrated sesame seeds was poorer than that of dehydrated seeds; the high oil content may have been a factor. Figure 2 also illustrates that dehydrated seeds sprouted more quickly than untreated seeds; the difference was determined to be significant for all crops in a separate analysis which compared dehydrated and untreated seeds. Following both the initial hydration and the dehydration, osmoticum was a significant factor; PEG-treated soybeans required more time than the other osmotica to reach 50% germination, as did PEG and CA-treated mungbeans.

After 2 weeks of storage, the conditions which resulted in significantly greater time to germinate and/or decreased germination percentage were: being fully-hydrated rather than dehydrated; being stored at 5°C rather than 15°C; and having been hydrated with PEG rather than the other osmotica. Fully-hydrated seeds probably performed poorly due to an increased rate of physiological deterioration and greater microbial infestation. The poor performance at 5°C was only among fully-hydrated seeds; a sensitivity to lower temperatures may have been induced by greater physiological deterioration among fully-hydrated seeds. And, the poorer performance of PEG-treated seeds may have been

due to reduced absorption of water and oxygen as a consequence of a residue left on the seed coats after hydration with PEG.

During the full 4 weeks of storage, dehydrated seeds generally were not affected by storage time and temperature. However, PEG-treated soybeans required more time than the other osmotica to germinate and CA-treated soybeans and mungbeans had reduced germination percentages when compared with the other osmotica. When fully-hydrated seeds stored at 5°C were compared at 2 and 4 weeks of storage, the performance of mungbean seeds worsened with increasing time in storage.

Phytate

Before hydration, phytate content did not differ among the bags of seeds designated to receive different treatments. Following hydration, phytate content in soybean and mung bean seeds was higher than in untreated seeds (Table 1).

Table 2 illustrates a comparison of seeds subjected to either dehydration or a 24-hr holding period following the initial hydration. Dehydrated seeds (except sesame) had lower phytate concentrations than fully-hydrated seeds, with differences of 41% for soy, 15% for wheat, and 25% for mungbean. Fully-hydrated seeds presumably continued to synthesize phytate, while the dehydration process may have enhanced enzymatic hydrolysis or a temperature-related breakdown of phytate. Data from our preliminary studies, where heat-killed soybean and wheat seeds were hydrated and dehydrated, indicate that the changes in phytate observed with viable seeds were not caused by a matrix effect.

As further indicated in Table 2, the main effect of osmoticum was

significant for soybean, wheat, and mungbean. PEG-treated soybean and mungbean seeds had lower values than the other osmotica. Since the moisture content of the seeds hydrated with different osmotica varied somewhat, with the PEG-treated seeds tending to hydrate less fully, the impact of moisture content on phytate content was examined.

Regressions of moisture content on phytate for soybean, mungbean, and sesame were significant, and these coefficients of variations were just slightly less than the values of the ANOVAs for effect of treatment on phytate. Hence, the impact of different treatments (osmotica) on phytate content is almost entirely a function of the moisture content achieved during hydration with the particular osmoticum. Higher moisture content may play a role in enhancing phytate synthesis.

At 2 weeks of storage, phytate concentrations of PEG-treated soybean and mungbean seeds were again lower than concentrations for the other osmotica (Table 3). And, values for dehydrated soybean and mungbean remained lower than values for fully-hydrated seeds, by 36% and 30%, respectively. Soybean seeds stored at 15°C had higher concentrations than those stored at 5°C, suggesting a relationship between temperature and the metabolism of phytate. The only significant interactions were for soybean, for the factors of osmoticum and hydration status, and osmoticum and temperature. The interaction means do not indicate any trends that change the conclusions noted for the main effects.

In comparing seeds at 2 and 4 weeks of storage, fully-hydrated and dehydrated seeds were examined separately. The phytate content of

dehydrated soybean and mungbean seeds increased 15% and 12% respectively between 2 and 4 weeks. However, only PEG and CA-treated soybeans changed over time. There were some differences among osmotica, with patterns similar to those noted previously.

Among fully-hydrated seeds, the phytate content of mungbean seeds stored at 5°C decreased from 2 to 4 weeks and soybeans did not differ between 2 and 4 weeks. It is possible that for these samples phytate synthesis eventually slowed or stopped. The rate of phytate hydrolysis also may have begun to increase.

In summary, the initial hydration of soybean and mungbean resulted in an increase in phytate content. The phytate in hydrated soybean and mungbean seeds remained high for at least several weeks, even under reduced temperature storage. When seeds were dehydrated following the initial hydration treatment, phytate concentrations decreased to levels comparable with untreated seeds.

The finding of increased phytate content following hydration is not surprising, considering that phytate content of soybeans increased by 57% following 24 hours of soaking in preparation for tempeh production (Sutardi and Buckle, 1985). Furthermore, phytate synthesis occurred during the early phases of the sprouting process in studies with mungbean (Mandal and Biswas, 1970 a) and castorbean (Dmitrieva and Sobolev, 1985; and Organ et al., 1988). In addition, Mandal and Biswas (1970 b) determined that phytate synthesis peaked 12 to 24 hours earlier than did phytate hydrolysis during the sprouting of mungbean seeds.

As for the impact of dehydration on phytate content, the decrease

may be due to a temperature-related enhancement of enzymatic hydrolysis, or a re-formation of a complex that inhibits synthesis.

Microbiological Activity

Before hydration, microbial counts did not differ among the bags of seeds designated to receive different treatments. Following hydration (Table 4), CH-treated soybean and CA-treated sesame seeds had lower aerobic plate counts than the untreated seeds, and W-treated mungbean seeds had higher aerobic plate counts than untreated seeds or seeds treated with the other osmotica. The total coliform counts in the CA-treated wheat and sesame seeds were lower than counts in the untreated seeds or the seeds treated with the other osmotica, but total coliform counts were higher in CH and W-treated mungbean seeds. Yeast and mold counts in the W-treated sesame seeds were lower than the counts in untreated seeds.

The instances of increased microbial populations in mungbean seeds suggest a rapid proliferation of microbes due to the availability of water. However, despite the availability of water, CA-treated wheat and sesame seeds had reduced microbial counts.

Tables 5, 6, and 7 illustrate the effect of dehydration on microbial counts. Hydration status was a significant factor in several instances. The aerobic plate counts of dehydrated wheat and sesame seeds were lower than counts in fully-hydrated seeds, but higher than counts in untreated seeds. Total coliforms counts of dehydrated soybean, wheat, and sesame seeds were less than counts of fully-hydrated seeds, and, in the cases of soybean and wheat, less than or comparable

to untreated seed counts. For yeast and mold counts, dehydrated mungbean seeds had lower counts than fully-hydrated seeds. Some desiccation of microbes may have occurred during dehydration, and there was probably a continued proliferation of microbes on the fully-hydrated seeds during the 24-hr holding period, even though the seeds were held at 5°C.

Osmoticum had an impact in wheat, where aerobic plate counts were lower in CA-treated seeds when compared to the other osmotica, and in soybean, where total coliforms in PEG-treated seeds were lower than in untreated seeds. For yeast and mold counts, CA and W-treated mungbean seeds had higher counts than untreated seeds, as did PEG and W-treated sesame seeds. The osmoticum by hydration status interactions indicate that in all cases except one (total coliform counts in CH-treated soybean), dehydrated seeds had lower counts than fully-hydrated seeds regardless of osmoticum.

After 2 weeks of storage (Tables 8, 9, and 10), dehydrated soybean, wheat, and sesame seeds had lower microbial counts than fully-hydrated seeds except in the case of soybean yeast and mold counts. Counts of dehydrated and fully-hydrated mungbean seeds did not differ. The CA-treated wheat and sesame seeds again had lower counts than the other osmotica, and PEG-treated soybeans had lower total coliform counts. The fully-hydrated 15°C seeds had higher counts than the 5°C seeds in several instances. Among the significant hydration status by temperature interactions, the fully-hydrated-15°C soybean seeds (aerobic plate counts and yeast and mold counts), wheat seeds (aerobic plate

counts and total coliforms), and sesame seeds (aerobic plate counts and yeast and mold counts) had the highest counts. The other interactions were not significant. While yeast and mold counts for soybean and wheat were zero or near zero following hydration and dehydration, at 2 weeks of storage the counts were approximately 10^8 for soybean and 10^3 to 10^4 for wheat.

In comparing dehydrated seeds at 2 vs 4 weeks of storage (Tables 11, 12, and 13), storage time was a significant factor for soybeans, for which 4-week aerobic plate counts and yeast and mold counts were less than at 2 weeks. For mungbeans, aerobic plate counts and total coliform counts increased with time as yeast and mold counts decreased, and for wheat, aerobic plate counts increased from 2 to 4 weeks. Osmoticum was also a source of variation. PEG-treated soybeans had lower aerobic plate counts, total coliform counts, and yeast and mold counts than the other osmotica, as did PEG-treated mungbeans. For CA-treated sesame and wheat seeds, all counts were lower than counts for the other osmotica.

When fully-hydrated seeds stored at 5°C were compared at 2 and 4 weeks of storage, osmoticum was a significant factor for wheat, with counts for CA-treated seeds lower than for the other osmotica for all microbial categories. PEG-treated soybeans had lower counts for total coliforms. Storage time was significant in several cases, with an increase in one microbial category typically corresponding with a decrease in one or two of the other categories, an indication of microbial succession. For soybean, aerobic plate counts and yeast and mold counts decreased from 2 to 4 weeks, while total coliform counts

increased. With wheat, aerobic plate counts increased and total coliform counts increased. For mungbean, aerobic plate counts and total coliform counts increased while the yeast and mold counts decreased. In the case of sesame, all counts increased by a factor of 10^2.

The mean values of microbial counts reported here are comparable to the counts reported in other studies of dry and hydrated seeds (Richter et al., 1993; Prokopowich and Blank, 1991; Splittstoesser et al., 1983; and Andrews et al., 1982). However, the values reported here for fully-hydrated stored seeds are higher, indicating that fully-hydrated seeds are not well-suited for storage. The declines in sprouting performance noted earlier are further evidence that fully-hydrated seeds should not be stored if the ultimate intent is to sprout the seeds.

Conclusions

In situations that demand minimal sprouting time and improved microbial control, the technique of osmoconditioning may be of sufficient benefit to warrant treatment of the seeds. While PEG has an antimicrobial impact on soybean and mungbean seeds, the lack of GRAS status for seed osmoconditioning prevents its use as a food-grade osmoticum. Citric acid also has an antimicrobial impact, most notably on wheat and sesame seeds, and is a suitable food-grade osmoticum. Changes in phytate content indicate the need for an enzymatic assessment of seeds during the osmoconditioning treatment.

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Table 1. Effect of initial hydration on phytate concentrations of soybean, wheat, mungbean, and sesame seeds.

Source of Variation[a]	Phytate Concentration (g/100g dry seed) Soybean	Wheat	Mungbean	Sesame
Hydration Treatment		ns[b]		ns
Untreated	0.92[c]y	0.72	0.66 y	1.70
Polyethylene glycol	1.38 z	0.73	1.08 z	2.67
Chitosan plus water	1.60 z	0.74	1.16 z	nd[d]
Citric acid	1.52 z	0.74	1.13 z	2.36
Water	1.60 z	0.80	1.16 z	2.50

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values are means of three replications; different letters (y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.

Table 2. Phytate concentrations of hydrated seeds undergoing dehydration to original moisture content versus seeds being held, fully hydrated, for 24 hours.

Source of Variation[a]	Phytate Concentration (g/100g dry seed) Soybean	Wheat	Mungbean	Sesame
Hydration Status				ns[b]
Fully Hydrated	1.53[c]z	0.66 z	1.21 z	2.41
Dehydrated	0.91 y	0.56 y	0.91 y	2.37
Osmoticum				ns
Polyethylene glycol	1.07[d]xy	0.72 z	0.84 y	2.24
Chitosan plus water	1.16 xy	0.51 x	1.20 z	nd[e]
Citric acid	1.24 y	0.57 xy	1.12 z	2.59
Water	1.40 z	0.66 yz	1.08 z	2.32
Osmoticum x Hydration Status	ns	ns	ns	ns

[a]General linear models procedure, SAS.
[b]ns = not significant at the 5% level.
[c]Values are means over all replications and all osmotica; different letters (y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls (SNK) test.
[d]Values are means of three replications of hydrated seeds and three replications of dehydrated seed; different letters (x,y,z) indicate statistically significant differences at the 5% level, SNK test.
[e]nd = not determined.

Table 3. Effect of treatment and storage variables on phytate concentration of seeds after 2 weeks storage.

Source of Variation[a]	Phytate Concentration (g/100g dry seed) Soybean	Wheat	Mungbean	Sesame
Osmoticum				nd[b]
Polyethylene glycol(PEG)	1.10[c]y	0.81 y	1.05 y	
Chitosan plus water(CH)	1.31 z	0.80 y	1.18 z	
Citric acid (CA)	1.37 z	0.86 yz	1.28 z	
Water (W)	1.44 z	0.91 z	1.27 z	
Hydration Status		ns[d]		
Fully Hydrated (FH)	1.59 z		1.41 z	
Dehydrated (DH)	1.02 y		0.98 y	
Temperature		ns	ns	
15°C	1.36 z			
5°C	1.25 y			
Osmoticum x Hydration Status		ns	ns	
PEG-FH	1.26[e]x			
PEG-DH	0.94 v			
CH-FH	1.60 y			
CH-DH	1.01 w			
CA-FH	1.69 yz			
CA-DH	1.05 w			
W-FH	1.80 z			
W-DH	1.08 w			
Osmoticum x Temperature		ns	ns	
PEG-15°C	1.14 x			
PEG-5°C	1.06 x			
CH-15°C	1.28 y			
CH-5°C	1.33 yz			
CA-15°C	1.44 z			
CA-5°C	1.29 y			
W-15°C	1.56 z			
W-5°C	1.32 yz			

[a]General linear models procedure analysis of variance, SAS.
[b]nd = not determined due to insufficient numbers of samples.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]ns = not significant at the 5% level.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 4. Effect of initial hydration on aerobic plate counts, total coliforms, and yeasts and molds in soybean, wheat, mungbean, and sesame seeds.

Microbial Counts	Source of Variation[a]	Log_{10} Means of CFU/g seed Soybean	Wheat	Mungbean	Sesame
Aerobic Plate Counts	Hydration Treatment		ns[b]		
	Untreated	7.41[c]z	4.81	6.99 y	4.66 z
	Polyethylene glycol	4.95 yz	6.45	7.36 y	5.77 z
	Chitosan plus water	0.00 y	6.09	7.42 y	nd[d]
	Citric acid	2.36 yz	3.87	7.00 y	1.33 y
	Water	7.58 z	5.30	8.02 z	4.26 z
Total Coliforms	Hydration Treatment	ns			
	Untreated	2.00	4.15 z	6.58 y	4.43 yz
	Polyethylene glycol	0.00	5.71 z	7.13 yz	5.81 z
	Chitosan plus water	2.23	4.83 z	7.68 z	nd
	Citric acid	4.10	1.43 y	7.01 yz	0.00 x
	Water	2.00	5.15 z	7.69 z	2.67 y
Yeasts and Molds	Hydration Treatment	ns	ns	ns	
	Untreated	0.00	0.00	7.11	7.00 z
	Polyethylene glycol	0.00	0.00	7.26	6.53 yz
	Chitosan plus water	0.00	0.00	7.94	nd
	Citric acid	0.00	0.00	8.31	5.26 yz
	Water	0.00	0.00	8.46	4.58 y

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values are means of three replications; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.

Table 5. Aerobic plate counts of hydrated seeds undergoing dehydration to original moisture content versus seeds being held, fully hydrated, for 24 hours.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Hydration Status	ns		ns	
Fully Hydrated (FH)	6.96	6.52 z	8.45	6.45 z
Dehydrated (DH)	6.02	3.37 y	8.72	5.00 y
Untreated	4.38	0.00 x	8.10	3.17 x
Osmoticum	ns[b]		ns	ns
Polyethylene glycol (PEG)	7.25[c]	6.62 z	8.53	6.31
Chitosan plus water (CH)	5.62	5.82 z	8.39	nd[d]
Citric acid (CA)	6.57	2.72 y	8.59	4.66
Water (W)	6.53	4.61 z	8.83	6.20
Untreated	4.38	0.00 x	8.10	3.17
Osmoticum x Hydration Status	ns	ns	ns	
PEG-FH	7.33	8.56	8.33	7.39[e]z
PEG-DH	7.17	4.68	8.78	5.23 x
CH-FH	6.54	7.23	8.34	nd
CH-DH	4.69	4.42	8.44	nd
CA-FH	6.81	5.44	8.53	5.30 x
CA-DH	6.33	0.00	8.65	4.02 w
W-FH	7.17	4.86	8.60	6.65 y
W-DH	5.90	4.37	9.06	5.75 x

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 6. Total coliforms in hydrated seeds undergoing dehydration to original moisture content versus seeds being held, fully hydrated, for 24 hours.

Source of Variation[a]	Log_{10} Means of CFU/g seed Soybean	Wheat	Mungbean	Sesame
Hydration Status				ns
Fully Hydrated (FH)	7.20 z	6.34 z	8.36 z	6.50
Dehydrated (DH)	3.53 y	3.28 y	7.61 y	6.01
Untreated	7.34 z	2.07 y	2.10 x	3.08
Osmoticum		ns[b]	ns	ns
Polyethylene glycol (PEG)	4.00[c]y	5.54	7.27	6.50
Chitosan plus water (CH)	6.67 yz	5.68	8.14	nd[d]
Citric acid (CA)	6.11 yz	3.47	8.30	5.67
Water (W)	4.68 yz	4.56	8.22	6.60
Untreated	7.34 z	2.07	2.10	3.08
Osmoticum x Hydration Status		ns		ns
PEG-FH	8.01[e]z	8.31	8.30 z	7.18
PEG-DH	0.00 x	2.77	6.24 x	5.82
CH-FH	6.57 yz	7.10	8.64 z	nd
CH-DH	6.77 yz	4.26	7.65 y	nd
CA-FH	6.85 yz	5.45	8.04 yz	5.61
CA-DH	5.36 y	1.49	8.56 z	5.72
W-FH	7.36 yz	4.50	8.45 z	6.72
W-DH	2.00 x	4.62	8.00 yz	6.48

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 7. Yeasts and molds in hydrated seeds undergoing dehydration to original moisture content versus seeds being held, fully hydrated, for 24 hours.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Hydration Status	ns	ns		ns
Fully Hydrated (FH)	0.00	0.00	8.65 z	6.50
Dehydrated (DH)	1.25	0.00	8.12 y	6.35
Untreated	0.00	0.00	7.93 y	5.11
Osmoticum		ns[b]		
Polyethylene glycol (PEG)	2.50[c]z	0.00	8.08 yz	7.00 z
Chitosan plus water (CH)	0.00 z	0.00	8.26 yz	nd[d]
Citric acid (CA)	0.00 z	0.00	8.57 z	5.37 y
Water (W)	0.00 z	0.00	8.62 z	6.90 z
Untreated	0.00 z	0.00	7.93 y	5.11 y
Osmoticum x Hydration Status	ns	ns	ns	ns
PEG-FH	0.00[e]	0.00	8.00	7.01
PEG-DH	5.00	0.00	8.17	7.99
CH-FH	0.00	0.00	8.60	nd
CH-DH	0.00	0.00	7.94	nd
CA-FH	0.00	0.00	9.00	5.70
CA-DH	0.00	0.00	8.14	5.04
W-FH	0.00	0.00	9.00	6.80
W-DH	0.00	0.00	8.23	7.00

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 8. Effect of treatment and storage variables on aerobic plate counts of soybean, wheat, mungbean, and sesame seeds after two weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed Soybean	Wheat	Mungbean	Sesame
Hydration Status			ns	
Fully Hydrated (FH)	9.78 z	7.57 z	8.32	5.68 z
Dehydrated (DH)	9.23 y	2.88 y	7.85	4.51 y
Untreated (UT)	8.94 y	2.40 y	7.71	4.68 y
Osmoticum			ns[b]	
Polyethylene glycol (PEG)	9.21[c]xy	6.32 z	8.25	6.15 z
Chitosan plus water (CH)	9.46 yz	5.42 z	7.48	nd[d]
Citric acid (CA)	9.76 z	3.32 y	8.25	4.25 x
Water (W)	9.59 yz	5.84 z	8.35	4.90 y
Untreated (UT)	8.94 x	2.40 y	7.71	4.68 y
Temperature		ns	ns	
15°C	9.74 z	4.90	8.12	5.25 z
5°C	9.14 y	4.92	7.96	4.82 y
Hydration Status x Temperature			ns	
FH-15°C	10.62[e]z	8.00 z	8.07	6.37 z
FH-5°C	8.94 y	7.14 z	8.56	5.00 y
DH-15°C	9.07 y	2.23 y	7.95	4.45 x
DH-5°C	9.39 y	3.53 y	7.76	4.58 x
UT-15°C	8.90 y	3.16 y	7.62	4.32 x
UT-5°C	8.97 y	1.63 y	7.80	5.03 y

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 9. Effect of treatment and storage variables on total coliforms in soybean, wheat, mungbean, and sesame seeds after two weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Hydration Status			ns[d]	
Fully Hydrated (FH)	7.09 z	7.92 z	7.39	5.45 z
Dehydrated (DH)	3.90 y	2.64 y	7.78	4.37 y
Untreated (UT)	0.00 x	2.87 y	7.04	4.34 y
Osmoticum				
Polyethylene glycol (PEG)	2.28[b]y	5.69 yz	7.22 z	5.77 z
Chitosan plus water (CH)	6.54 z	5.52 yz	6.61 z	nd[c]
Citric acid (CA)	7.27 z	3.41 xy	8.31 z	4.10 y
Water (W)	5.89 z	6.49 z	8.21 z	4.86 y
Untreated (UT)	0.00 x	2.87 x	7.04 z	4.34 y
Temperature	ns	ns		ns
15°C	4.64	4.99	7.03 y	4.92
5°C	5.13	5.04	8.02 z	4.73
Hydration Status x Temperature	ns		ns	ns
FH-15°C	7.23[e]	7.94 z	6.28	5.90
FH-5°C	6.95	7.90 z	8.50	5.00
DH-15°C	3.20	2.58 y	7.93	4.36
DH-5°C	4.60	2.69 y	7.64	4.37
UT-15°C	0.00	2.77 y	6.49	3.65
UT-5°C	0.00	2.97 y	7.59	5.04

[a]General linear models procedure analysis of variance, SAS.
[b]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[c]nd = not determined.
[d]ns = not significant at the 5% level.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 10. Effect of treatment and storage variables on yeasts and molds in soybean, wheat, mungbean, and sesame seeds after two weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Hydration Status			ns	
Fully Hydrated (FH)	9.46 z	7.41 z	9.07	5.64 z
Dehydrated (DH)	8.44 z	3.62 x	9.69	4.33 y
Untreated (UT)	9.10 z	4.10 y	6.38	4.84 y
Osmoticum	ns[b]			
Polyethylene glycol (PEG)	8.39[c]	6.52 z	8.30 y	6.08 z
Chitosan plus water (CH)	8.81	6.19 z	8.90 yz	nd[d]
Citric acid (CA)	9.39	3.24 y	10.63 z	4.28 x
Water (W)	9.22	6.11 z	9.69 yz	4.60 xy
Untreated (UT)	9.10	4.10 y	6.38 x	4.84 y
Temperature	ns	ns		
15°C	9.32	5.55	8.38 y	5.23 z
5°C	8.62	5.17	9.72 z	4.70 y
Hydration Status x Temperature		ns	ns	
FH-15°C	10.56[e]z	7.62	8.14	6.28 z
FH-5°C	8.37 y	7.20	10.00	5.00 y
DH-15°C	8.08 y	4.05	9.60	4.42 x
DH-5°C	8.80 y	3.19	9.79	4.24 x
UT-15°C	9.31 y	3.25	4.46	4.48 x
UT-5°C	8.89 y	4.94	8.30	5.19 y

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.
[e]Values for interactions are least squares means; different letters indicate significant differences at the 5% level.

Table 11. Effect of treatment and storage variables on aerobic plate counts in dehydrated soybean, wheat, mungbean, and sesame seeds at two and four weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Storage Time				ns[b]
2 weeks	9.17[c]z	2.78 y	7.82 y	4.55
4 weeks	3.77 y	4.44 z	8.79 z	4.86
Osmoticum				
Polyethylene glycol (PEG)	5.12 y	4.60 z	7.53 w	5.53 z
Chitosan plus water (CH)	8.16 z	3.81 z	8.14 x	nd[c]
Citric acid (CA)	6.30 yz	1.42 y	8.58 y	3.17 y
Water (W)	7.78 y	4.31 z	8.87 z	5.25 z
Untreated (UT)	4.99 y	3.91 z	8.43 y	4.88 z
Temperature	ns	ns	ns	ns
15°C	6.30	3.42	8.34	4.87
5°C	6.64	3.80	8.28	4.54

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.

Table 12. Effect of treatment and storage variables on total coliforms in dehydrated soybean, wheat, mungbean, and sesame seeds at two and four weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Storage Time	ns[b]	ns		ns
2 weeks	3.12[c]	2.68	7.64 y	4.36
4 weeks	3.19	2.81	8.76 z	3.74
Osmoticum				
Polyethylene glycol (PEG)	0.56 y	3.32 yz	7.03 x	5.37 z
Chitosan plus water (CH)	6.28 z	1.99 y	8.03 y	nd[d]
Citric acid (CA)	4.55 z	0.00 x	8.80 z	2.12 x
Water (W)	3.87 z	4.59 z	8.81 z	5.03 yz
Untreated (UT)	0.50 y	3.83 yz	8.32 yz	3.69 y
Temperature	ns	ns	ns	ns
15°C	2.64	2.56	8.19	4.09
5°C	3.67	2.93	8.21	4.01

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.

Table 13. Effect of treatment and storage variables on yeasts and molds in dehydrated soybean, wheat, mungbean, and sesame seeds at two and four weeks of storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed			
	Soybean	Wheat	Mungbean	Sesame
Storage Time		ns[b]	ns	ns
2 weeks	8.57[c]z	3.72	9.03 z	4.46
4 weeks	3.23 y	4.22	8.75 y	4.72
Osmoticum				
Polyethylene glycol (PEG)	4.75 y	5.03 z	7.76 x	5.37 z
Chitosan plus water (CH)	7.96 z	4.35 z	9.00 y	nd[d]
Citric acid (CA)	6.21 yz	1.04 y	10.00 z	2.91 y
Water (W)	6.03 yz	4.77 z	10.00 z	5.07 z
Untreated (UT)	4.55 y	4.65 z	7.69 x	5.02 z
Temperature		ns	ns	ns
15°C	5.33 y	4.08	8.63	4.70
5°C	6.48 z	3.86	9.15	4.48

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]nd = not determined.

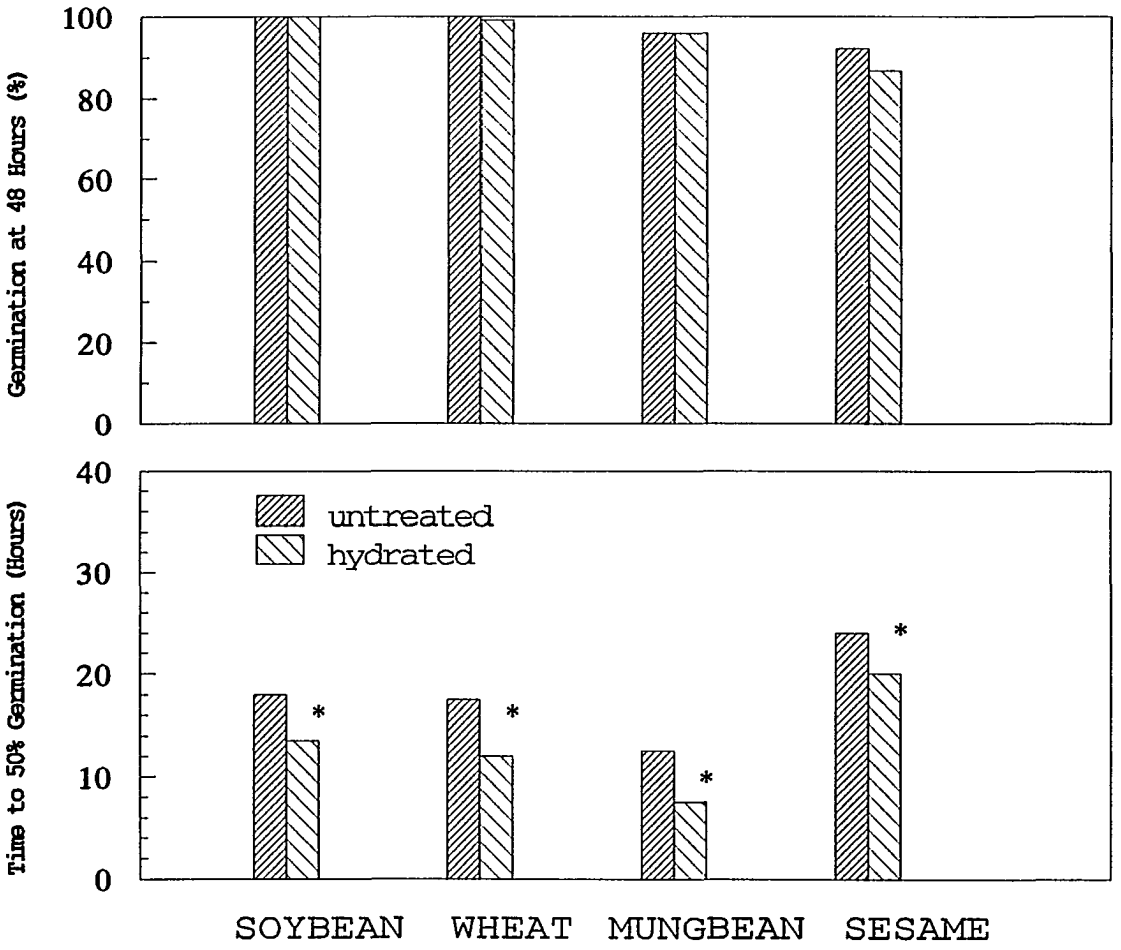

Figure 1. Effect of hydration on seed germination. Values are means of four osmotica. Crops were analyzed separately; an * indicates a statistical difference at the 5% level.

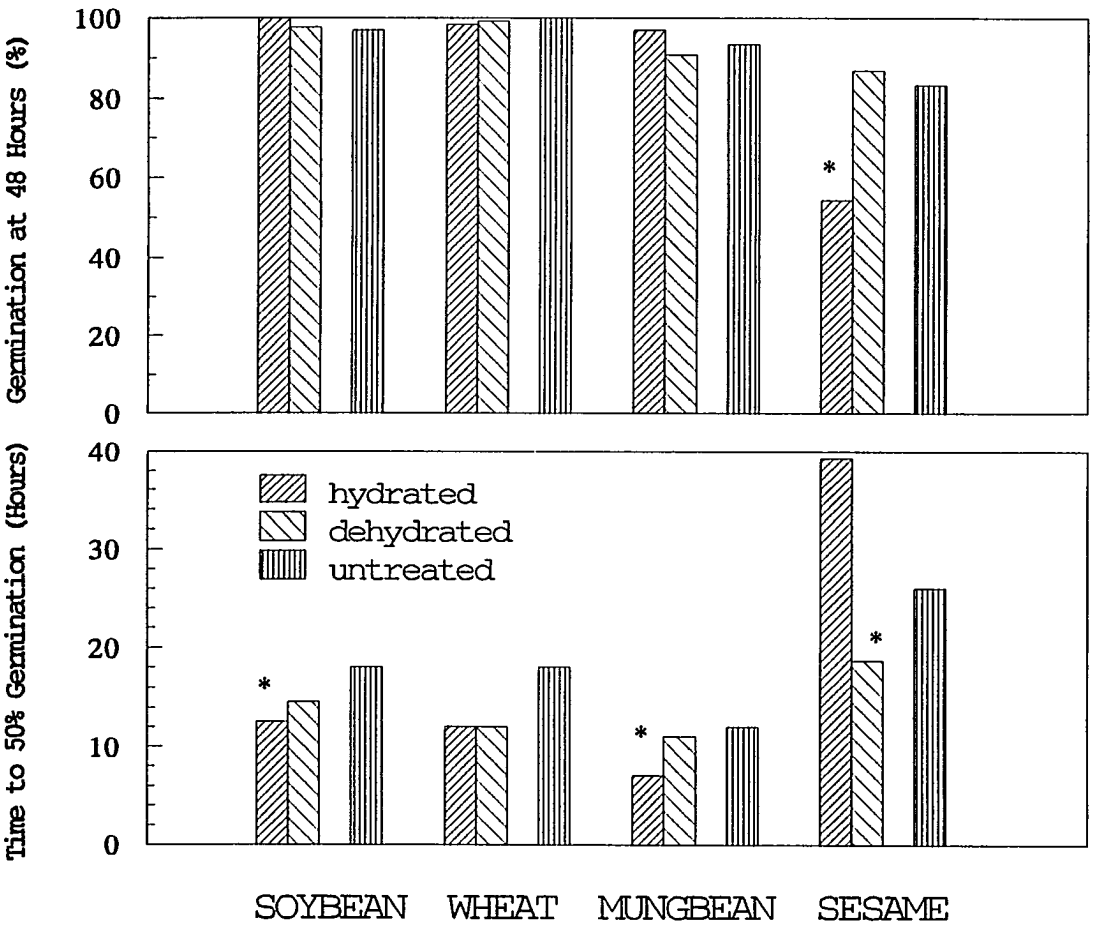

Figure 2. Effect of dehydration on seed germination. Values are means of four osmotica. Crops were analyzed separately; an * indicates a statistical difference at the 5% level. Untreated values were not included in the statistical analysis.

OSMOCONDITIONED SEEDS AS A FOODSTUFF: MICROBIOLOGICAL SAFETY, NUTRITIONAL STATUS, AND SENSORY PROPERTIES OF SPROUTED SEEDS

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Julie M. Goldman[1], Amadu D. Ayebo[2], Mark H. Love[3], and James S. Dickson[3]

Abstract

Soybean and wheat seeds were surface-sterilized with NaOCl or EtOH or left untreated. Seeds then were hydrated in polyethylene glycol, citric acid, or water. Following hydration, seeds were treated with calcium propionate or left untreated. Seeds were then dehydrated to complete the osmoconditioning process. The crops were analyzed as dry, unsprouted seeds and as 48-hour sprouts. Citric acid and NaOCl had an antimicrobial impact on unsprouted seeds, but sprouted seeds had counts up to 10^5 higher than unsprouted seeds regardless of treatment. The calcium propionate treatment reduced total coliform counts in wheat by a factor of 10, but did not affect aerobic plate counts or yeast and mold counts. Some of the microbial counts declined after 16 weeks of 4°C storage. Sprouting resulted in increased phytate in both soybean (40%) and wheat (10%). Sprouts from osmoconditioned and untreated seeds were rated similarly in sensory analyses.

[1]Graduate Research Assistant, Iowa State University, Ames, IA

[2]Research Scientist, CIREH, University of Iowa, Iowa City, IA

[3]Associate Professors, Iowa State University, Ames, IA

Introduction

Seed osmoconditioning is a technique traditionally used to enhance field production of crops. The controlled hydration and dehydration process yields a product that germinates more uniformly and more quickly when subsequently exposed to the sprouting environment (Bradford, 1986). In a study of osmoconditioning for foodstuffs (Goldman et al., unpublished), sprouting time decreased when seeds were osmoconditioned. Furthermore, osmoticum and seed moisture status affected microbial levels. The osmotica polyethylene glycol (for soybean and mungbean) and citric acid (for wheat and sesame) had some antimicrobial activity when compared with water or chitosan plus water as osmotica. If seeds were dehydrated following hydration, microbial counts were lower than in seeds remaining fully-hydrated. Phytate content of seeds increased with the initial hydration but decreased to pre-hydration levels after dehydration.

In this study, osmoconditioned seeds were sprouted for foodstuff use and assessed in terms of microbiological safety, nutritional status, and sensory properties.

Microbiological safety is of interest because sprouting methodology has an impact on microbial activity (Sawyer et al., 1985; and Marero et al., 1988). Phytate content was determined because of its antinutritional activity and the consequent impact on human nutrition (Torre et al., 1991; Bea et al., 1984; Heaney et al., 1991; and Hurrell et al., 1992). And, osmoconditioned and untreated sprouts were assessed to measure their sensory attributes.

Materials and Methods

Seed Treatment

Hard red winter high protein wheat seeds were obtained from Arrowhead Mills (Hereford, TX) in May 1994 and were stored at room temperature until treatments were applied in June 1994. For the sensory analysis only, hard red spring high protein wheat seeds were obtained from Bob's Red Mill (Milwaukie, OR) in August 1994 and stored at room temperature until treated in September, 1994. 'Vinton 81' soybean seeds, grown in Iowa and harvested in the fall of 1992, were stored in sealed plastic containers at 4°C until used.

Seeds were surface-sterilized by immersion in NaOCl or EtOH. Seeds were immersed in the 2000 ppm NaOCl (Clorox, Oakland, CA) solution for 10 min, followed by three 30 s rinses in distilled water. For the 95% EtOH treatment, seeds were immersed for 30 s, followed by three 30 s rinses in distilled water. Prior testing indicated that these treatments did not result in reduced sprouting performance.

Imbibition media, hereafter referred to as osmotica, included: polyethylene glycol 8000 (Sigma Chemical Co.) at 250 g/kg distilled water, hereafter referred to as PEG; a 0.05 M citrate buffer at pH 4.4 prepared from citric acid monohydrate and sodium citrate dihydrate, referred to as CA; and distilled water, referred to as W.

Treatment combinations were assigned randomly to quantities of 150 g seed. Following surface-sterilization, seeds were placed in Ziploc (Dow Corning, Midland, MI) 2000 ml storage bags of 1.75 mil thickness. Three hundred ml of the assigned osmoticum was added to each

bag, and the bags were randomly arranged in a dark growth chamber at 25°C. The CA and W treatments were applied for 4 hr (soybean) or 6 hr (wheat) while the PEG treatments were 16 hr in length. Following hydration, all bags were drained and the seeds were rinsed once with 300 ml distilled water. One additional treatment was then applied, consisting of seed immersion in 1000 ppm calcium propionate (Niacet, NY) for 10 min without subsequent rinsing.

Hydrated seeds were placed on absorbent toweling for 30 min to surface-dry. Seeds were dehydrated in a food dehydrator (Harvest Maid Model 2400, Alternative Pioneering Systems, Chaska, MN) at 40°C until the seed moisture content was approximately equal to original moisture content, a process requiring 9 hr for soybean and 4-5 hr for wheat. After the seeds had cooled to room temperature, a sample from each treatment group was withdrawn for analysis. The remaining seeds of each group were placed in sterile 120 ml Whirlpak bags (Nasco, Janesville, WI) and stored in sealed rigid plastic containers at 4°C for 16 weeks, at which time sampling was performed. Samples for phytate analysis were withdrawn at 4 weeks in addition to the 16 week sampling. The experiment was replicated at one week intervals for a total of 3 replications.

Moisture Content

To verify the degree of hydration and dehydration of seeds, moisture content was determined. Samples of 10 seeds were placed on Kimwipes (Kimberly-Clark, Roswell, GA) until surface-dry, then weighed to determine fresh weight. After being dried to constant weight at

103°C, samples were weighed again to determine dry weight. Moisture content was computed as a percentage, on a fresh weight basis.

Sprouting

Quantities of 20 to 30 g seed were placed in clear 450 ml plastic salad bar containers (Ecoware, U.S.). Seeds were soaked in distilled water for 8 hr at 25°C in a dark growth chamber. Covers of the containers were left slightly ajar. Following the soaking period, the water was drained and the seeds were rinsed once with 150 ml distilled water. Thereafter, rinsing and draining was done at 8 a.m., 12 p.m., and 4 p.m. After 36 hours had elapsed, fluorescent lighting was applied at 40 microeinsteins m^{-2} sec^{-1}. Forty-eight hours from the start of the soaking period, sprouts were given a final rinse and samples were taken for microbiological and phytate assessment.

Microbiological

Ten gram samples were placed in sterile 60 ml Whirlpak bags for microbiological quality determinations. Samples were placed in peptone buffer and processed in a stomacher (model 400 Mark II, Tekmar, Cincinnati, OH) at normal speed for 120 s. Appropriate serial dilutions in buffered peptone water (Accumedia) were plated via a spiral plater (Model D, Spiral Systems, Cincinnati, OH) onto duplicate plates of tryptic soy agar (TSA) for aerobic plate counts (APC), violet red bile agar (VRBA) for total coliform counts (TC), and potato dextrose agar (PDA) for yeasts and molds (YM). The plates were incubated as follows: TSA at 32°C for 48 hr, VRBA at 37°C for 24 hr, and PDA at 25°C for 120

hr. Plates were counted visually by using methods appropriate for spiral plates (Peeler and Maturin, 1992).

Some counts were recorded as "too many to count" in plates where the colonies exceeded the manual counting capability; numeric values for these samples were obtained by the following formula: 10,000 x 1/greatest dilution. For data analysis, the arithmetic mean of the duplicate plates was computed and then converted to a $\log_{10}$ mean by the following formula: $\log_{10}$ mean = $\log_{10}$ of (arithmetic mean + 1.00). 1.00 was added to the arithmetic mean to allow the use of zero counts. Counts were reported as $\log_{10}$ means of colony forming units (CFU)/g of seed.

Phytate

Samples sufficient to supply 10 g dry matter were placed in 60 ml sterile Whirlpak bags, frozen, and stored at -18°C. Following freeze-drying (FreezeDry-5, Labconco, Kansas City, MO), samples were ground in a coffee grinder (Braun, Inc., Lynnfield, MA) for 20 s and sieved through a 20 mesh screen. Phytate analyses were performed according to the colorimetric method of Vaintraub and Lapteva (1988), which is a modification of the method of Latta and Eskin (1980).

Sensory Analysis

A plan for the sensory analysis study was submitted to and approved by the Iowa State University Human Subjects Research Committee before implementation of the study. Panelists signed an informed consent statement prior to participation.

Soybean and wheat seeds used for sensory analysis received one of

the following treatments, using food-grade constituents and equipment: 1) NaOCl surface-sterilization with CA-treatment and dehydration; 2) CA-treatment and dehydration; 3) NaOCl surface-sterilization with W-treatment and dehydration; 4) W-treatment and dehydration; and 5) control -- no sterilization and no osmoconditioning. Polyethylene glycol was not used as an osmoticum in the sensory portion of this study because it does not have GRAS status for an osmoconditioning-like process.

Quantities of approximately 100 g (dry weight basis) of soybean seeds and 50 g of wheat seeds (dry weight basis) were then placed in sterile 250 ml glass jars (Ball Co., Muncie, IN). The open jar tops were covered with cheesecloth secured by the screw bands and 200 ml water was added to each jar. Seeds were soaked for 8 hr at 25°C in a fermentation cabinet (Model 505-SS, National Manufacturing Company, Lincoln, NB) from which light was excluded. Following the 8 hr soaking period, the water was drained from the jars and the seeds were rinsed once with 200 ml water. Jars were placed on their sides in the chamber. Sprouts were thereafter rinsed and drained at 8 a.m., 12 p.m., 4 p.m, and 8 p.m. After 36 hours had elapsed, wheat sprouts were exposed to fluorescent light, and after a total of 48 hr had elapsed, the wheat sprouts were given a final rinse.

Soybean sprouts, following the final rinse at 48 hr, were steamed for 3 min and allowed to cool to room temperature prior to being tasted.

Prior to the sensory analyses by the trained panel, a group of experienced sensory panelists tasted soybean and wheat sprouts. The

group recommended that the crops be rated on the characteristics of "color", "beany flavor", and "nutty flavor" for soybean sprouts, and "sweetness", "firmness", and "chewiness" for wheat sprouts.

Samples were presented to panelists in a controlled environment sensory facility with individual booths. At each session, 5 coded samples of soybean sprouts were presented, followed by a 10 minute break. Five coded samples of wheat sprouts were then presented. The 14 trained panelists used a descriptive analysis technique for flavor and texture characteristics, scaling attributes on an unstructured 15 cm line scale.

Statistical Analysis

Statistical analyses for phytate and microbiological data were performed by using ANOVA (analysis of variance) produced by the GLM (general linear models) procedure in SAS, version 6.07. Each crop was analyzed separately.

The microbiological data were analyzed to determine activity among treated seeds prior to storage, with osmoticum as the whole-plot factor and surface-sterilant, calcium propionate treatment, sprouting stage, and all interactions as the split-plot factors in a split-plot design. The impact of storage on microbiological activity was also analyzed as a split-plot design, with osmoticum (CA and W only) as the whole-plot factor and storage time, calcium propionate treatment, sprouting stage, and all interactions as the split-plot factors. For phytate, osmoticum (CA and W only) was the whole-plot factor. Storage time, sprouting stage, and the interactions were split-plot factors in a split-plot

design. Replications within osmoticum was designated as the whole-plot error term, and the GLM procedure calculated the appropriate split-plot error terms.

Factors and interactions with a p value of ≤ 0.05 were considered significant. Means separations for main effects were conducted with the Student-Newman-Keuls (SNK) test. Interaction means were compared using p values generated by the PDIFF statement in SAS, using least squares means.

Sensory data were also analyzed using ANOVA generated by GLM. Each sensory attribute for each crop was analyzed separately. The experiment was a 2 (surface sterilants) x 3 (osmotica) x 13 (panelists) factorial in a completely randomized design, with 3 replications for each treatment combination. The error term in each instance was automatically calculated with the GLM procedure.

Results and Discussion

Microbiological Activity

Table 1 illustrates the microbial activity in treated seeds before storage. Osmoticum was not a significant factor in this analysis. With regard to surface-sterilants, NaOCl-treated seeds had lower counts than other treatments for aerobic plate counts and total coliform counts of soybean and total coliform counts and yeast and mold counts of wheat. However, interactions of surface sterilant by sprouting stage for soybean indicate that the effect was on unsprouted seeds only. Since microbial cells and spores are present within seeds as well as on the surface (Caetano-Anolles et al., 1990; and Mundt and Hinkle, 1976), the

endogenous population within seeds typically cannot be removed by chemical surface-sterilization (Caetano-Anolles et al., 1990). Sprouting resulted in a large increase in counts in all microbial categories for both crops.

Calcium propionate had an impact in only one instance -- reduced total coliform counts in wheat. The interactions of all three factors (osmoticum, surface sterilant, and sprouting stage) were not significant, indicating that incorporating a surface sterilant with an antimicrobial osmoticum did not further enhance antimicrobial activity, even among unsprouted seeds.

For the analysis that included storage as a factor (Table 2), surface-sterilized seeds were not included, since the impact of the sterilants would be expected to be immediate rather than following storage. After 16 weeks of storage, calcium propionate-treated seeds that had been osmoconditioned with CA and W were sampled, as were untreated seeds that had not been osmoconditioned.

As Table 2 illustrates, CA-treated soybeans had lower total coliform counts than W-treated seeds, and the osmoticum by sprouting stage interaction indicates that the impact was on unsprouted seeds. Unsprouted CA-treated seeds had counts comparable to untreated unsprouted seeds. Sprouting resulted in large increases in microbial counts. Storage had a beneficial effect in two cases; aerobic plate counts in soybean and total coliform counts in wheat were lower after 16 weeks in storage. This was true for both unsprouted and sprouted seeds, since the interactions of storage time and sprouting stage were not

significant. The osmoticum by storage time interaction for total coliform counts in wheat illustrates a substantial decline for the CA-treated seeds at 16 weeks, but no decrease for the W-treated seeds and an increase in the untreated seeds. The CA-treated wheat at 16 weeks had total coliform counts comparable with the lowest value for untreated seeds, at 0 weeks. Calcium propionate did not exhibit an antimicrobial effect (not illustrated in this table).

The counts for both unsprouted and sprouted seeds are comparable to counts reported in the literature (Richter et al., 1993; Patterson and Woodburn, 1980). The high counts associated with sprouts do not typically indicate a health concern, since pathogenic bacteria such as *salmonellae* are not commonly found in plant foods (Splittstoesser et al., 1983).

In summary, some degree of microbial control in unsprouted seeds is achieved with the application of NaOCl prior to osmoconditioning and the use of CA as an osmoticum. Microbial control in sprouted seeds is more elusive because control of the endogenous internal microbial population is more difficult. However, the instances of lower counts following storage suggest that combining osmoconditioning treatments with a storage treatment could result in seeds and sprouts with lower microbial counts. Because seeds were stored in sealed containers with minimal gas exchange, the reduced counts may have been the consequence of a modified atmosphere. Seeds respire, and they consume O_2 and produce CO_2. Atmospheres with increased amounts of CO_2 (up to about 10%) extend the storage life of fruits, meats, and other products by, among other

actions, inhibiting microbial growth and development (Jay, 1991), which may have been partly responsible for the reduced counts noted here.

Phytate

After 0, 4, and 16 weeks of storage of osmoconditioned seeds, neither osmoticum (CA and W only) nor storage time had an impact on phytate content (Table 3). However, sprouting had a significant impact on phytate content, with soybean increasing 40% and wheat 10%.

Chavan and Kadam (1989) and Salunkhe and Kadam (1989) reported that several crops exhibited a reduction in phytate as a consequence of sprouting. However, several of the studies that reported reductions in phytate cited lengthy sprouting times, such as 8 days for peas (Beal et al., 1984), 6 days for lupin (Dagnia et al., 1992), and 6 days for lentils (Vidal-Valverde, 1994). In contrast, typical sprouting times for fresh sprouts are 2 days for wheat, 3-4 days for soybeans, lentils, and peas, and 2-5 days for mungbeans, because longer periods have a negative impact on palatability (Larimore, 1975; and Blanchard, 1975). Several studies reported the occurrence of phytate synthesis during the early stages of hydration and sprouting (Sutardi and Buckle, 1985; Mandal and Biswas, 1970; and Organ et al., 1988). Since their timeframe corresponded with the 48-hour sprouting period employed in this study, phytate synthesis may be responsible for the increase in phytate reported in this study.

In addition, several of the studies citing reductions measured the phytate in the cotyledons only, despite the fact that phytate is present in other parts of the seedling and the entire seedling is usually

consumed when sprouts are used for foodstuffs.

In conclusion, an accurate assessment of phytate content in sprouted seeds requires consideration of the sprouting time and the anatomical components of the seed or seedling. Shorter sprouting times may contribute to improved palatability, but the lack of a decrease in phytate and the potential for increased phytate content poses a nutritional problem.

Sensory

When analyzed by the trained panel, sensory properties did not differ among treatments (Tables 4 and 5). Panelists commented on the highly palatable nature of the soybean sprouts, and they perceived the "nutty flavor" of the sprouts to be desirable. Several panelists commented that they would consider using the wheat sprouts in salads or casseroles, while several other panelists disliked the intense "saccharin-like sweetness" of the wheat sprouts.

Conclusions

The technique of seed osmoconditioning, which enhances sprouting, also can be utilized to reduce microbial counts in dehydrated seeds if seeds are surface-sterilized with NaOCl or hydrated with PEG and CA. However, microbial control in sprouted seeds may require a combination of osmoconditioning treatments and storage in a modified atmosphere. Since panelists did not rate osmoconditioned and untreated seeds differently, undesired sensory changes would not be expected with any of the treatments employed in this study. The increase in the antinutrient

phytate indicates a need for an enzymatic assessment of phytate metabolism in sprouted seeds.

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Table 1. Effect of treatments on aerobic plate counts (APC), total coliforms (TC), and yeasts and molds (YM) in soybean and wheat seeds and sprouts before storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed					
	SOYBEAN			WHEAT		
	APC	TC	YM	APC	TC	YM
Osmoticum	ns[b]	ns	ns	ns	ns	ns
Polyethylene glycol	7.19[c]	6.01	6.84	6.90	5.71	6.58
Citric acid	7.53	5.91	6.73	6.79	5.30	6.16
Water	7.44	6.71	7.19	7.02	5.55	6.55
Surface Sterilant			ns	ns		
NaOCl (NaOCl)	6.61 y	5.18 y	6.50	6.59	4.81 y	5.97 y
Ethanol (EtOH)	7.52 z	7.00 z	7.02	7.02	5.82 z	6.48yz
Untreated (UT)	8.03 z	6.48 z	7.24	7.09	5.98 z	6.83 z
Calcium Propionate	ns	ns	ns	ns		ns
Present	7.47	6.36	6.88	6.80	5.04 y	6.40
Absent	7.30	6.07	6.96	7.00	6.02 z	6.45
Sprouting Stage						
Unsprouted (US)	5.63 y	3.39 y	5.26 y	4.94 y	2.52 y	4.95 y
Sprouted (S)	9.21 z	8.98 z	8.58 z	8.86 z	8.55 z	7.91 z
Surface Sterilant x Sprouting Stage			ns	ns	ns	ns
NaOCl x US	4.24[d]x	1.33 x	4.26	4.43	1.21	4.05
NaOCl x S	9.12 z	9.02 z	8.75	8.75	8.41	7.88
EtOH x US	5.73 xy	4.68 y	5.56	5.15	3.33	5.08
EtOH x S	9.32 z	9.20 z	8.48	8.90	8.62	7.89
UT x US	6.93 y	4.24 y	5.97	5.24	3.02	5.70
UT x S	9.19 z	8.73 z	8.52	8.94	8.62	7.95

[a]General linear models procedure analysis of variance, SAS.
[b]ns = not significant at the 5% level.
[c]Values for main effects are means; different letters (x,y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.
[d]Values for interactions are least squares means; different letters indicate statistically significant differences at the 5% level.

Table 2. Effect of treatments on aerobic plate counts (APC), total coliforms (TC), and yeasts and molds (YM) in soybean and wheat seeds and sprouts before and after storage.

Source of Variation[a]	Log_{10} Means of CFU/g seed					
	SOYBEAN			WHEAT		
	APC	TC	YM	APC	TC	YM
Osmoticum	ns[b]		ns	ns	ns	ns
Citric acid (CA)	7.00	5.13[c]y	5.98	6.62	4.62	6.56
Water (W)	7.31	7.18 z	7.18	6.16	5.11	6.66
Untreated (UT)	5.04	3.39 x	5.46	6.23	3.88	5.90
Storage Time		ns	ns	ns		ns
0 Weeks	7.96 z	6.25	7.01	6.70	5.40 z	6.51
16 Weeks	5.80 y	5.24	5.86	6.06	4.09 y	6.49
Sprouting Stage						
Unsprouted	5.84 y	3.51 y	4.90 y	5.14 y	2.48 y	5.31 y
Sprouted	7.91 z	8.17 z	8.07 z	7.72 z	7.20 z	7.83 z
Osmoticum x Sprouting Stage	ns		ns	ns	ns	ns
CA x Unsprouted	5.83[d]	2.01 x	3.80	5.32	2.68	5.14
CA x Sprouted	8.17	8.26 z	8.15	7.91	6.57	7.99
W x Unsprouted	6.55	6.14 y	6.29	4.86	2.53	5.63
W x Sprouted	8.07	8.20 z	8.07	7.46	7.69	7.69
UT x Unsprouted	4.44	1.23 x	4.34	5.34	1.96	4.99
UT x Sprouted	6.25	7.71 z	7.71	8.00	7.70	7.71
Osmoticum x Storage Time	ns	ns	ns	ns		ns
CA x 0 Weeks	8.23	5.55	6.64	6.83	6.62 y	6.87
CA x 16 Weeks	5.77	4.72	5.32	6.40	2.63 x	6.26
W x 0 Weeks	8.42	7.90	7.89	7.00	4.99 y	6.63
W x 16 Weeks	6.20	6.45	6.46	5.34	5.23 y	6.70
UT x 0 Weeks	5.00	2.46	4.94	5.02	2.16 x	4.58
UT x 16 Weeks	5.06	3.86	5.72	6.83	4.74 y	6.56

[a]General linear models procedure analysis of variance, SAS.

[b]ns = not significant at the 5% level.

[c]Values for main effects are means; different letters (w,y,x) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.

[d]Values for interactions are least squares means; different letters indicate statistically significant differences at the 5% level.

Table 3. Effect of osmoconditioning and sprouting on phytate concentrations in soybean and wheat seeds and sprouts.

Source of Variation[a]	Phytate Concentration (g/100g dry seed) Soybean	Wheat
Osmoticum	ns[b]	ns
Citric acid	1.23	0.77
Water	1.23	0.72
Storage Time	ns	ns
0 Weeks	1.24	0.76
4 Weeks	1.23	0.74
16 Weeks	1.22	0.73
Sprouting Stage		
Unsprouted	1.05[c]y	0.71 y
Sprouted (48 hrs)	1.43 z	0.78 z

[a]General linear models procedure, SAS.
[b]ns=not significant at the 5% level.
[c]Values are means of two osmotica, 3 storage times, and 3 replications; different letters (y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls test.

Table 4. Effect of surface-sterilization and osmoconditioning on sensory attributes of sprouted soybeans.

Source of Variation[b]	Mean Scores[a] Color[c]	Beany Flavor[d]	Nutty Flavor[e]
Surface Sterilant	ns[f]	ns	ns
NaOCl	7.21	4.76	7.22
Untreated	6.80	4.49	7.45
Osmoticum	ns	ns	ns
Citric acid	7.20	4.68	7.65
Water	6.73	4.34	7.09
Untreated	6.95	4.97	7.30

[a]Values are means of three replications, as measured on a 15 cm scale.
[b]General linear models analysis of variance, SAS.
[c]Color scale ranged from light yellow (1 cm) to beige (14 cm).
[d]Beany flavor scale ranged from none (0 cm) to strong (14 cm).
[e]Nutty flavor scale ranged from none (0 cm) to strong (14 cm).
[f]ns = not significant at the 5% level.

Table 5. Effect of surface-sterilization and osmoconditioning on sensory attributes of sprouted wheat.

Source of Variation[b]	Mean Scores[a] Firmness[c]	Sweetness[d]	Chewiness[e]
Surface Sterilant	ns[f]	ns	ns
NaOCl	7.58	6.70	8.33
Untreated	8.43	6.98	8.52
Osmoticum	ns	ns	ns
Citric acid	7.60	6.37	8.47
Water	8.19	7.40	8.64
Untreated	8.87	6.82	8.02

[a]Values are means of three replications, as measured on a 15 cm scale.
[b]General linear models analysis of variance, SAS.
[c]Firmness scale ranged from soft (1 cm) to firm (14 cm).
[d]Sweetness scale ranged from none (0 cm) to strong (14 cm).
[e]Chewiness scale ranged from very little remaining in mouth (0 cm) to almost all remaining in mouth (14 cm).
[f]ns = not significant at the 5% level.

OSMOCONDITIONED SEEDS AS A FOODSTUFF: PHYTATE CONTENT AND PHYTASE ACTIVITY IN THE SEEDS AND SPROUTS OF SOYBEANS AND WHEAT

A paper to be submitted to The Journal of Food Science

Julie M. Goldman[1] and Mark H. Love[2]

Abstract

The phytate content of soybean and wheat seeds and sprouts was determined following an osmoconditioning process (hydration followed by dehydration to original moisture content) and during a subsequent 144-192 hr sprouting period. Untreated seeds were also sprouted, and phytate content determined. Phytase activity was determined at corresponding sampling times. Phytate in wheat seeds and sprouts remained fairly constant during osmoconditioning and sprouting, whereas phytase activity increased throughout the sprouting period. Phytate in soybean seeds reached a maximum content after 8 hr of sprouting, and did not decrease to a lower content until 192 hr of sprouting. This decrease corresponded with a significant increase in phytase activity. Osmoconditioned and untreated sprouts did not differ with regard to phytate content or phytase activity.

Introduction

Seed osmoconditioning is a technique traditionally used to enhance field production of crops. The controlled hydration and dehydration

[1]Graduate Research Assistant, Iowa State University, Ames, IA

[2]Associate Professor, Iowa State University, Ames, IA

process yields a product that germinates more uniformly and more quickly when subsequently exposed to the sprouting environment (Bradford, 1986). In a study of the impact of osmoconditioning on foodstuffs, it was determined that for seeds of soybean, wheat, mung bean, and sesame, the time to achieve 50% germination decreased and osmoticum and moisture status impacted microbial levels. Phytate content in soybean, mung bean, and sesame increased with hydration but returned to near the original content following dehydration (Goldman et al., unpublished).

The assessment of phytate content was deemed important because phytate binds divalent cations such as iron, zinc, and calcium, and thus has an antinutritional impact. Sprouting of seeds is reported to result in decreased levels of phytate (Salunkhe and Kadam, 1989; Vidal-Valverde, 1994; Kumar and Chauhan, 1993; Sattar et al., 1990; Dagnia et al., 1992; and Bartnik and Szafranska, 1987), presumably because of the increased activity of the hydrolytic enzyme phytase (myoinositol hexaphosphate phosphohydrolase E.C. 3.1.3.8). Phytase activity has been reported to increase concomitantly with decreases in phytate (Eskin and Wiebe, 1983; and Bartnik and Szafranska, 1987).

However, phytate synthesis has been noted during the soaking of soybeans for tempeh production (Sutardi and Buckle, 1985) and during the early stages of sprouting of mung beans (Mandal and Biswas, 1970), suggesting that both synthesis and hydrolysis occur in sprouting seeds, depending on the crop and the extent of sprouting. Furthermore, in a follow-up to the original study of osmoconditioning for foodstuff

production, it was determined that phytate content in osmoconditioned seeds increased by 40% in soybean and 10% in wheat following a 48-hour sprouting period (Goldman et al., unpublished).

In this continuing study of osmoconditioning, an assessment of phytate content and phytase activity was conducted with the following objectives: 1) determine phytate content of seeds during the osmoconditioning process; 2) determine phytate content at intervals throughout a lengthy sprouting period; 3) compare osmoconditioned and untreated seeds during the sprouting process; and 4) measure enzymatic hydrolytic activity (phytase) during the the entire timecourse of osmoconditioning and sprouting.

Materials and Methods

Seed Treatment

Hard red winter high protein wheat seeds were obtained from Arrowhead Mills (Hereford, TX) in May 1994 and stored at room temperature until treatments were applied in February 1995. 'Vinton 81' soybean seeds, grown in Iowa and harvested in the fall of 1992, were stored in sealed plastic containers at 4°C until used.

All seeds were surface-sterilized in 2000 ppm NaOCl (Clorox, Oakland, CA) by immersion of seeds for 10 minutes, followed by three 30 s rinses in deionized water. Seeds to be osmoconditioned were surface-sterilized immediately prior to hydration. Untreated seeds were surface-sterilized immediately prior to the sprouting procedure.

Six 300 g samples from each crop were randomly given treatment (osmoconditioned or untreated) and replication (3) assignments. Samples

were withdrawn, placed in sterile 60 ml Whirlpak bags (Nasco, Fort Atkinson, WI), and frozen. After surface-sterilization, seeds receiving the osmoconditioning treatment were placed in Ziploc (Dow Corning, Midland, MI) 2000 ml storage bags of 1.75 mil thickness. Six hundred ml of the osmoticum (deionized water) was added to each bag, and bags were randomly arranged in a dark fermentation cabinet (Model 505-SS, National Manufacturing Company, Lincoln, NB) at 25°C. After 5 hr (soybean) or 6 hr (wheat), the bags were drained of water and the seeds were rinsed once with 600 ml deionized water. Samples were taken at this time.

Hydrated seeds were placed on absorbent toweling for up to 30 min to surface-dry. Seeds were dehydrated in a food dehydrator (Harvest Maid Model 2400, Alternative Pioneering Systems, Chaska, MN) at 40°C until the seed moisture content was approximately equal to original moisture content, a process that required 9 hr for soybean and 4 to 5 hr for wheat. After the seeds cooled to room temperature, samples were taken.

Moisture Content

To verify the degree of hydration and dehydration of seeds, moisture content was determined. Samples of 10 seeds were placed on Kimwipes (Kimberly-Clark, Roswell, GA) until surface-dry, then weighed to determine fresh weight. After being dried to constant weight at 103°C, samples were weighed again to determine dry weight. Moisture content was computed as a percentage, on a fresh weight basis.

Sprouting

Quantities of approximately 150 g seeds (dry weight basis) were placed in sterile 1000 ml glass jars (Ball Co., Muncie, IN) covered with cheesecloth secured by the screw bands. Seeds were soaked in deionized water for 8 hr (soybeans) and 10 hr (wheat) at 25°C in a fermentation cabinet (Model 505-SS, National Manufacturing Company, Lincoln, NB) from which light was excluded. Following the soaking period, the water was drained and seeds were rinsed once with deionized water. Jars were placed on their sides in the chamber. Sprouts were thereafter rinsed and drained at 8 a.m., 12 p.m., and 4 p.m.

Samples of untreated seeds were taken at 0 hr (prior to any hydration), and for comparison purposes the 0 hr sampling time for osmoconditioned seeds was the sampling that occurred following dehydration. Untreated and osmoconditioned seeds were both sampled at 8 or 10 hr (following the soak period), and at 24, 48, 96, 144, and 192 hours of the sprouting period. Wheat samples were only taken through 144 hr due to visible microbial infestation at 192 hr.

Phytate

Samples sufficient to supply 10 g dry matter were placed in 60 ml sterile Whirlpak bags, frozen, and stored at -18 C. Following freeze-drying (FreezeDry-5, Labconco, Kansas City, MO), samples were ground in a coffee grinder (Braun, Inc., Lynnfield, MA) for 20 s and sieved through a 20 mesh screen. Phytate analyses were performed according to the colorimetric method of Vaintraub and Lapteva (1988), which is a modification of the method of Latta and Eskin (1980).

Statistical Analysis

Statistical analyses for phytate data were performed using ANOVA (analysis of variance) produced by the GLM (general linear models) procedure in SAS, version 6.07. Soybeans and wheat were analyzed separately. In experiment #1, phytate content and phytase activity of seeds and sprouts were analyzed throughout the full timecourse, including the osmoconditioning. The experimental design was completely randomized, with sampling time as the factor and replications within sampling time as the error term. In experiment #2, osmoconditioned and untreated seeds were compared, beginning at 0 hr of the sprouting process. The design was a two-way factorial, with sampling time and osmoticum as factors. Factors and/or interactions with a p value of $\leq$ 0.05 were considered significant.

Phytase

The starting material for both soybeans and wheat was the freeze-dried, ground wholemeal prepared for the phytate analyses.

For wheat, 20 ml of 0.2 M acetate buffer at pH 5.1 was added to 1 g of wholemeal sample. The mixture was stirred with a glass rod to insure moistening of all dry material, centrifuged for 15 min at 5000 rpm, and filtered through Whatman #1 filter paper (Whatman International Ltd., England). For the enzyme reaction mixture, 4 ml of filtrate was added to 4 ml of pre-warmed substrate, which was 1.3 g sodium phytate in 1000 ml 0.2 M acetate buffer. This concentration approximates 1.3 mM phytate, taking into account the approximately 0.8 g phytate present in every 100 g of wholemeal. For the blank, 4 ml of the same filtrate was added to 4

ml of pre-warmed 0.2 M acetate buffer. Samples were withdrawn at time zero, and reaction tubes and blank tubes were incubated in a 55°C water bath for 1 hr, at which time aliquots were again withdrawn for phosphorus determination. A timecourse test was conducted, and it confirmed that the reaction was linear over the 1 hr test period.

Phosphorus was determined by a modification of the ammonium molybdate method of Lowry and Lopez (1946). The standard curve was prepared with 0 to 6 mg/L of P as KH_2PO_4. Sample aliquots were appropriately diluted to 8 ml with 0.5 M acetate buffer at pH 4.0. Two ml of 3% ammonium molybdate (3 g in 100 ml 0.5 M acetate buffer) and 1 ml of 1% ascorbic acid were added and tubes were inverted to mix the contents thoroughly. After 45 min, two readings per tube were made at 700 nm on a Spectronic 20. Protein was determined on the initial filtrate using the Biuret method (Bailey, 1967).

For soybean, the wholemeal sample was partially purified prior to the assay. Ten ml of 2% $CaCl_2$ dihydrate was stirred into each 1 g of wholemeal sample. Samples were mechanically shaken for 30 min, followed by centrifugation for 15 min at 3440 g and filtration through Whatman #1 filter paper. Five ml of filtrate from each sample was taken to 30% ammonium sulfate concentration with constant stirring. The sample was cooled for 30 min at 4°C, and centrifuged at 4°C for 20 min at 7710 g. Four ml of supernatant was extracted for each sample, and the ammonium sulfate concentration was increased to 85% saturation. Following centrifugation at 4°C for 20 min at 7710 g, the precipitate was retained and resuspended in 5 ml of 0.2 M tris-maleate buffer at pH 6.5.

This enzyme extract was refrigerated until used, a period of no more than 1.5 hr. Protein was determined on this extract.

For the enzyme assay, several modifications were made to the procedure described for wheat. The assay mixture consisted of 2 ml 0.1 M acetate buffer at pH 4.8, 3 ml 10 mM phytate (sodium phytate dissolved in 0.1 M acetate buffer) to give a final concentration of 5 mM, and 1 ml enzyme extract. The blank mixture consisted of 5 ml acetate buffer plus 1 ml enzyme extract. Tubes were incubated in a water bath at 55°C. Results of a timecourse test indicated that the reaction was linear for up to 0.5 hr. At zero time and after 0.5 hr, aliquots of 2 ml were withdrawn and added to tubes that contained 8 ml of 0.5 M acetate buffer at pH 4.3 and 2 ml 5% trichloroacetic acid. After 1 hr, tubes were centrifuged for 15 min at 3440 g. Eight ml of the supernatant was added to 2 ml ammonium molybdate solution and 1 ml ascorbic acid. Readings were made as for the wheat samples.

Phytase activity is reported as micromoles of phosphorus/min/100 g of dry plant material under the conditions specified in materials and methods.

Results and Discussion

As illustrated in Table 1, phytate content in soybeans increased after 8 hr of sprouting. Phytate content did not decrease to a lower content until 192 hours of sprouting. Phytate in wheat did not differ among sampling stages. When osmoconditioned and untreated sprouts were compared (Table 2), the two groups did not differ. The differences among sprouting stages in soybean were comparable to those noted for

Table 1.

Phytase activity differed among sampling stages for both soybean and wheat (Table 3). However, while activity in soybean did not increase until the latter stages of sprouting (144 and 192 hours), in wheat the activity increased with the initial hydration, decreased when seeds were dehydrated, and increased throughout the sprouting period. Osmoconditioned and untreated sprouts did not differ in activity (Table 4).

The phytase activity of wheat seeds and sprouts increased in a stepwise manner during the sprouting period achieving a ten-fold increase by 144 hr. Bartnik and Szafranska (1987) reported similar increases in phytase activity in wheat at successive stages of sprouting up to 96 hr. They reported concomitant decreases in phytate, with a 22% reduction achieved after 96 hr. The data presented here (Table 1) indicate a decrease of approximately 17% at 96 hr, but the experimental error was sufficiently high to prevent that decrease from being statistically significant.

For soybean, the increase in phytase activity at 144 to 192 hr corresponds with the decrease in phytate content, suggesting that phytase was responsible for phytate hydrolysis. Gibson and Ullah (1988) reported maximal phytase activity in soybean cotyledons at 8-12 days (192-288 hr), but they did not measure concurrent phytate content. The increase in phytate at 8 hr occurred when phytase activity was low, lending support to the earlier cited reports of phytate synthesis occurring during the early stages of hydration and sprouting.

When phytase activity was calculated per unit of protein rather than per unit of dry plant material (data not shown), the statistical analysis produced the same significant factors and the same separation of means.

Although there are numerous research reports on phytate, the interplay of synthesis and hydrolysis in sprouting seeds is not well understood. Williams (1970) pointed out that phytate, in addition to serving as a phosphorus store, is a strong chelator and therefore plays a significant role in controlling the many cellular processes dependent on multivalent cations. Such a role dictates the necessity of regulating phytate metabolism during seed sprouting and seedling establishment. Strother (1980) suggested that a homeostatic mechanism exists with regard to phytate metabolism, based on his finding that phosphate in seedlings tends to remain constant during germination when expressed on a fresh weight basis.

Conclusions

The crop and the stage of hydration and sprouting play a role in the phytase activity and the phytate content in sprouted seeds. In wheat, phytate content remained relatively constant during sprouting while the phytase activity increased steadily during a full 144 hr of sprouting. In contrast, soybean phytate content increased in the early stages of sprouting and did not decrease until phytase activity increased. Phytase activity was quite low in soybean, and did not increase until 144 to 192 hr of sprouting. A thorough study of phytate synthesis in a variety of crops is essential if the changes in phytate

content are to be understandable and predictable.

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Table 1. Phytate content of soybean and wheat seeds and sprouts during hydration, dehydration, and sprouting.

Source of Variation[a]	Phytate Concentration (g/100g dry material) Soybean	Wheat
Sampling Stage		ns[b]
Untreated	1.49[c]y	0.97
Hydrated	1.81 yz	0.98
Dehydrated	1.60 yz	0.90
8/10 Hr Sprouting	2.24 z	1.05
24 Hr Sprouting	1.88 yz	0.88
48 Hr Sprouting	1.98 yz	0.72
96 Hr Sprouting	1.72 yz	0.80
144 Hr Sprouting	1.96 yz	0.85
192 Hr Sprouting	1.42 y	nd[c]

[a]General linear models procedure, SAS.
[b]ns=not significant at the 5% level.
[c]Values are means of 3 replications; different letters (y,z) indicate statistically significant differences at the 5% level, Student-Newman-Keuls (SNK) test.
[d]nd=not determined.

Table 2. Phytate content of osmoconditioned and untreated soybean and wheat seeds and sprouts during a 192 hr sprouting period.

Source of Variation[a]	Phytate Concentration (g/100g dry material) Soybean	Wheat
Hydration Treatment	ns[b]	ns
Osmoconditioned	1.83	0.86
Untreated	1.87	0.90
Sprouting Stage		ns
0 Hr	1.57[c]x	0.90
8/10 Hr	2.13 z	1.01
24 Hr	1.85 xyz	0.94
48 Hr	2.01 yz	0.80
96 Hr	1.78 xyz	0.83
144 Hr	1.94 xyz	0.86
192 Hr	1.66 xy	nd[d]

[a]General linear models procedure, SAS.
[b]ns=not significant at the 5% level.
[c]Values are means of 2 hydration treatments and 3 replications; different letters (x,y,z) indicate statistically significant differences at the 5% level, SNK test.
[d]nd=not determined.

Table 3. Phytase activity in soybean and wheat seeds and sprouts during hydration, dehydration, and sprouting.

Source of Variation[a]	um P/min/100 g dry material Soybean	Wheat
Sampling Stage		
Untreated	4.46[b]x	16.24 v
Hydrated	4.52 x	35.41 w
Dehydrated	3.50 x	19.36 v
8/10 Hr Sprouting	5.10 x	38.28 w
24 Hr Sprouting	3.92 x	40.28 w
48 Hr Sprouting	5.10 x	55.05 x
96 Hr Sprouting	7.90 x	123.73 y
144 Hr Sprouting	16.76 y	160.43 z
192 Hr Sprouting	23.77 z	nd[c]

[a]General linear models procedure, SAS.
[b]Values are means of 3 replications; different letters (v,w,x,y,z) indicate statistically significant differences at the 5% level, SNK test.
[c]nd=not determined.

Table 4. Phytase activity in soybean and wheat seeds and sprouts during a 192 hr sprouting period.

Source of Variation[a]	um P/min/100 g dry material Soybean	Wheat
Hydration Treatment	ns[b]	ns
Osmoconditioned	9.71	75.63
Untreated	8.57	74.82
Sprouting Stage		
O Hr	2.93[c]x	9.76 v
8/10 Hr	4.62 x	39.50 w
24 Hr	4.78 x	38.58 w
48 Hr	5.86 x	57.99 x
96 Hr	8.06 x	138.57 y
144 Hr	15.74 y	155.99 z
192 Hr	20.55 z	nd[d]

[a]General linear models procedure, SAS.
[b]ns=not significant at the 5% level.
[c]Values are means of 2 hydration treatments and 3 replications; different letters (v,w,x,y,z) indicate statistically significant differences at the 5% level, SNK test.
[d]nd=not determined.

GENERAL CONCLUSIONS

For foodstuff production, the osmoconditioning of seeds is a novel and useful means of enhancing sprout production. As expected, osmoconditioned seeds sprouted more quickly than untreated seeds, even following storage. Future research should more fully address suboptimal storage and sprouting conditions, since agronomic research indicates that the greatest benefits of osmoconditioning are observed when seeds are subjected to environmental stress.

Osmoconditioning (hydration followed by dehydration) did not create any microbial problems, but rather provided a means for reducing microbial counts on dry seeds through the use of antimicrobial osmotica. By combining osmoconditioning with extended dry storage in sealed containers, it may be possible to further reduce microbial counts, thus enhancing the safety, shelf-life, and palatability of seeds and sprouts. However, while citric acid is a suitable osmotica for foodstuff production, polyethylene glycol is not currently considered a food-grade material for osmoconditioning treatment.

Sensory attributes were not impacted by osmoconditioning, so there are no drawbacks to osmoconditioning in this regard. Testing of additional crops, different sprouting periods, and longer shelf-life periods may reveal some advantages for using osmoconditioned seeds.

Osmoconditioned seeds did not differ from untreated seeds with regard to changes in phytate content during the sprouting period. The increased phytate content in several crops, noted in the early stages of sprouting, contradicts the generally accepted belief that sprouts have

reduced phytate content. Timecourse studies on a wide range of crops would be useful in determining at what stages and in what crops phytate is relatively higher and lower.

In conclusion, osmoconditioning is a safe and simple means of enhancing sprout production. The minimal input of time and materials would be especially justifiable when rapid production of a microbially clean foodstuff is required, as in space travel and colonization and commercial preparation of weaning foods from sprouted seeds.

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