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Processing: Calcium Sulfate TAP Review

Compiled by OMRI for March 2001 NOSB Meeting

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Calcium Sulfate
Processing

Identification

Chemical Name(s): calcium sulfate

CAS Number: 7778-18-9 (calcium sulfate)
10101-41-4 (calcium sulfate dihydrate)

Other Names:
alabaster, gypsum, plaster of paris, light spar, terra alba

Other Codes: INS 516,
RTECS: EW4150000

This TAP review is based on information available as of the date of this review.

Summary of Advised Recommendation

Synthetic / Non-Synthetic:	Allowed or Prohibited:	Suggested Annotation:
Non-synthetic (Dihydrate form, mechanically extrated and physically processed uncalcined from mined sources only). (reviewers unanimous)	*Allowed [Food grade (FCC) only] (reviewers unanimous)*	*None* (2 reviewers) *OR* *For use only as a coagulant in bean curd (tofu and similar products).* (1 reviewer)

Characterization

Composition:
$CaSO_4$ (anhydrous), $2CaSO_4 \bullet H_2O$ (hemihydrate), $CaSO_4 \bullet 2H_2O$ (dihydrate)

Properties:
A light, porous crystalline structure, 3.0-3.5 on Moh's hardness scale. Usually white, but some ores have a blue, gray, or reddish tinge. In some cases, the ores will be brick red (Budavari, 1996). Melting point: 1,450°C. (Lewis, 1989).

How Made:
Calcium sulfate may be obtained from natural sources or chemically synthesized, with mined gypsum being the primary natural source (Petersen, Kaleta, and Kingston, 1992). Gypsum is one of the most widely used minerals in the world, with the US being the largest producer of any country (Olson, 1999). The petition is regarding only the mined form, so the review will not consider the manufacture of other sources. Mined sources still account for most production of crude gypsum. According to the US Geological Survey, the number of producers and production level is increasing. In 1999, 35 companies that operated 60 mines in 20 states produced record levels of gypsum (Olson, 1999). In 1996, 30 companies operating 61 mines produced 73% of all domestic output (Balazik, 1998). Gypsum deposits occur in many parts of the US and Canada. The top producing states in 1999 were Oklahoma, Iowa, Texas, Michigan, California, Nevada, and Indiana—together they accounted for 72% of total output (USGS, 2000). Most mined sources are from gypsum ore in the dihydrate form, with some anhydrite deposits also naturally occurring (Petersen, Kaleta, and Kingston, 1992). Calcium sulfate dihydrate is obtained by grinding and separating gypsum that contains about 20% water of crystallization (Igoe, 1983).

Specific Uses:
The TAP review will focus on use as a tofu coagulant, as per the petition's example, but other uses will be discussed to illustrate some specific functions and other characteristics of the material relative to the OFPA and NOSB criteria.

Calcium sulfate has been used in China for over 2,000 years to coagulate soy milk to make tofu (Shurtleff and Aoyagi, 1975). FDA GRAS uses are listed in Table 1. One supplier estimated that there are over 100 uses as a direct food additive (Dichter, 2001). Other uses in food processing include: nutrient; dietary supplement; yeast food; dough conditioner; firming agent; sequestrant (Food Chemicals Codex, 1996); jelling ingredient (Ockerman, 1991); baking powder (Igoe, 1983); carrier; filler; pH buffer; and abrasive agent. The primary food processing use is in baked goods (Dichter, 2001). It is used as a firming agent with canned potatoes, tomatoes, carrots, lima beans, and peppers (Igoe, 1983). Calcium sulfate is

an ingredient in confections, frostings, gelatins, soft-serve ice cream, and other frozen desserts (Lewis, 1989). It is used as a carrier, filler, or standardizing agent with many different minor ingredients. The TAP review does not extend to these other ingredients. It is also used to process malt and increase the calcium content of water used for brewing beer (Ash and Ash, 1995). It is used as an abrasive in some scouring cleaning agents that are used on food contact surfaces. Calcium sulfate is also used in cosmetics and toothpaste (Winter, 1989).

Action:

Coagulation of soymilk is a complex interaction of several variables (Hou et al., 1997). The calcium and sulfate ions combine with the soluble proteins in soymilk to denature and take them out of solution.

Calcium sulfate helps fruits and vegetables retain firmness by binding with pectin and increasing the water-holding capacity. The calcium bonds to the negatively charged carboxylic groups in pectin (Gordon and Klimek, 2000). Flour that is low in calcium tends to produce dough that is soft and sticky (Igoe, 1983), so calcium sulfate is used to stiffen dough.

Combinations:

Food grade calcium sulfate is usually sold as pure terra alba. Impurities may include limestone (calcium carbonate) and various naturally occuring forms of silica. It may be combined with magnesium sulfate or calcium chloride as a tofu coagulant (Shurtleff and Aoyagi, 1975) and is also used in combination with carrageenan to produce a gelatinous tofu (Abd Karim, Sulebele, Azhar, and Ping, 1999). A product similar to tofu can be made from cooked field peas and calcium sulfate (Gebre-Egziabher and Sumner, 1983). It may also be used with rennet and cow's milk along with soymilk to make a soy-extended cheese food (Del Valle, et al., 1984).

Status

OFPA

The substance is used in handling and is non-synthetic but not organically produced [7 USC 6517(c)(1)(B)(3)].

Regulatory

FDA GRAS for human (21 CFR 184.1230) and livestock (21 CFR 582.5230). See table 1 for more information.
Bureau of Alcohol, Tobacco, and Firearms (BATF) 27 CFR 240.1051.

EPA/NIEHS/Other Appropriate Sources

EPA - No information on the Envirofacts Master Chemical Integrator (EMCI) or Toxics Release Inventory (TRI) as of January 12, 2001.

NIEHS - No information in the National Toxicology Program (NTP) database for either calcium sulfate or calcium sulfate dihydrate as of January 12, 2001.

Other sources -
Illinois Right-to-Know Toxic Substances List, Illinois Register, Section 205, Table A, Toxic Substances List (1991).
Massachusetts Substance List for Right-to Know Law (11 April 94); General Law C.111F, Chapter 30A (28 Jun 84); 105 CMR 670.000; Appendix A.
Pennsylvania Right-to-Know, Pennsylvania Department of Labor and Industry Hazardous Substance List (1989).

Status Among U.S. Certifiers

California Certified Organic Farmers – Allowed from non-synthetic sources only (CCOF, 2000).
Oregon Tilth Certified Organic – Allowed without restriction as to source or use (Coody, 1999).
OCIA International – calcium sulfate, natural is listed as Allowed as a non-organic ingredient (OCIA, 2000).
Texas Department of Agriculture – Listed as "Regulated: may be used to adjust pH for dyeing organic fiber products. Allowed as a non-organic ingredient in processing. Synthetic sources from chemical industrial byproducts, drywall rejects or sulfuric acid treatment anhydrite are prohibited" (February 2000).

International

CODEX – Allowed for cakes and biscuits, soy bean products, bakers yeast, and as a carrier (FAO/WHO, 1999).
EU 2092/91 – Allowed as a carrier and as a coagulation agent (EU 2092/91 Annex VI). This has been interpreted as prohibiting all direct uses other than as a coagulation agent for soymilk (Haccius and Schmidt, 1998).
IFOAM – Allowed for use in cakes and biscuits (baked goods) and soybean products (IFOAM, 2000).
Canada – Does not appear on the permitted substances list (CGSB, Appendix C1, 1999). Certified Organic Association of British Columbia (COABC) – Allowed as a non-organic ingredient (1999).
KRAV (Sweden) – Allowed for beer (KRAV, 1999).

Japan – Allowed, limited to use as a coagulant, or in confectionary, prepared products of beans, and baker's yeast (Woolsey, 2000).

OFPA 2119(m) Criteria

(1) *The potential of such substances for detrimental chemical interactions with other materials used in organic farming systems.* This is being considered as a processing material.
(2) *The toxicity and mode of action of the substance and of its breakdown products or any contaminants, and their persistence and areas of concentration in the environment.* See processor criteria 3, below.
(3) *The probability of environmental contamination during manufacture, use, misuse or disposal of such substance.* This is considered below under item 2.
(4) *The effect of the substance on human health.* This is considered in the context of the effect on nutrition in 3, below, as well as the consideration of GRAS and residues in 5, below.
(5) *The effects of the substance on biological and chemical interactions in the agroecosystem, including the physiological effects of the substance on soil organisms (including the salt index and solubility of the soil), crops and livestock.* As this is not released into the agroecosystem, there is no direct effect.
(6) *The alternatives to using the substance in terms of practices or other available materials.* See discussion of alternatives in 1, below.
(7) *Its compatibility with a system of sustainable agriculture.* This is considered more specifically below in the context of organic handling in 6, below.

Criteria from the February 10, 1999 NOSB Meeting

A PROCESSING AID OR ADJUVANT may be used if;

1. *It cannot be produced from a natural source and has no organic ingredients as substitutes.*
Food-grade calcium sulfate dihydrate can be produced from natural sources and it can also be synthesized. The review is limited only to the natural mined source. Tofu can be prepared by a number of different recipes, but each has its own distinct flavor, texture, and functionality in various dishes and cuisines (Shurtleff, 1975). It is possible to coagulate tofu by the use of various acidic preparations, such as organic vinegar or organic lemon juice. However, this results in a tart or sour tofu that is generally used as an intermediate for other products and is seldom marketed retail by itself, instead it is primarily used in home recipes (Soyfoods Association, 1986). The NOSB has reviewed magnesium chloride and magnesium sulfate (these two ingredients make up nigari) as an alternative (NOSB, 1995 and 1999). The NOSB discussed various tofu coagulation agents, and issued Recommendation Addendum 16 regarding synthetic magnesium chloride. Calcium chloride is also on the National List. Calcium sulfate has been the traditional coagulation agent in Chinese-style tofu preparations (Shurtleff and Aoyagi, 1975).

   Glucono delta lactone (GDL) was also petitioned for use in soy products in 1995, but the NOSB has not referred that substance to the TAP (NOSB, 1995). GDL is not considered appropriate by itself to make Chinese-style tofu (Tsai, Lan, Kao, and Chen, 1981). It is possible to obtain a tofu with somewhat similar characteristics by the addition of carageenan (Abd Karim, Sulebele, Azhar, and Ping, 1999). Industry standards for grades and quality of tofu are described in a publication from the Soyfoods Association (1986).

   Calcium sulfate is essential for certain kinds of tofu. Tofu made from calcium sulfate will be softer and smoother with a mild, bland flavor profile (Shurtleff and Aoyagi, 1975). One can make tofu without calcium sulfate, but calcium sulfate is essential to make tofu with certain characteristics (Wang and Hesseltine, 1982; DeMan, DeMan, and Gupta, 1986).

2. *Its manufacture, use, and disposal do not have adverse effects on the environment and are done in a manner compatible with organic handling as described in section 6513 of the OFPA.*
Food-grade calcium sulfate is produced from quarries or pit mines of high-grade gypsum deposits (Petersen, 1998). Drilling or blasting, with quarrying by heavy equipment, often opens these pits. Higher-grade veins are selected for food grade, and these deposits are taken to food grade facilities where the crude gypsum is crushed, screened, milled, graded, and packaged. All steps are mechanical for the dihydrate form. The anhydrous form is calcined at temperatures in excess of 115° C., with the highest temperatures used being 215°C. (Petersen, Kaleta, and Kingston, 1992).

The mining process produces dust, which causes air pollution and physical irritation (Material Safety Data Sheet). Persons handling and mixing products may also be exposed to dust.

Exposure Limits

Expressed as Time Weighted Averages (TWAs) over 8 hour working shifts.

NIOSH Recommended Exposure Limit (REL) (guideline)
Total dust: 10 mg/m^3
Respirable fraction: 5 mg/m^3
Source: NIOSH, 1992

OSHA Permissable Exposure Levels (PEL) (regulation)
Total Dust: 15 ppm
Respirable fraction: 5 mg/m^3
No limits on skin exposure
Source: 29 CFR 1910.1000 (2000)

It reacts violently with aluminum when heated; mixtures with phosphorous may ignite. When heated to decomposition it emits toxic fumes of SO_x. It reacts exothermically with the methylating agent diazomethane to the point of exploding (Lewis, 1989).

3. *If the nutritional quality of the food is maintained and the material itself or its breakdown products do not have adverse effects on human health as defined by applicable Federal regulations.*
The nutritional profiles of tofu made from various coagulation agents compares favorably for some nutrients and has lower nutritional value for others. Tofu made from calcium sulfate as a coagulant will have a higher calcium content and a lower magnesium content than tofu made with nigari. As a rule of thumb, tofu made with calcium sulfate has 3.5 times as much dietary calcium as nigari tofu (Soyfoods Association, 1986). The difference may be as great as four times as much per serving (Toyo Shinpo, 1980). The protein quality of tofu produced by calcium sulfate precipitation is comparable to that produced by acid treatment (Schroder, Elliot, and Jackson, 1973). Tofu made with calcium sulfate consistently produces higher yields of tofu per pound of soybeans than nigari (Hou, Chang, and Shih, 1997).

Protein concentration and yield have both been shown to decrease as the amount of calcium sulfate increases. This response was found to be consistent across different varieties (Sun and Breene, 1991). Rats fed tofu made with calcium sulfate retained more calcium than rats fed non-fat dry milk. Nigari treated tofu has one-tenth the bioavailable calcium of calcium sulfate coagulated tofu (Porteros and Erdman, 1988).

4. *Its primary purpose is not as a preservative or used only to recreate/improve flavors, colors, textures, or nutritive value lost during processing except in the latter case as required by law.*
The primary purpose used in tofu making is as a coagulant, and as such it is not used as a preservative. While it provides a distinctively different set of flavors and textures from other coagulants, coagulation is an essential step that defines tofu as different from soymilk. The addition of calcium sulfate increases the calcium content of tofu, but it is not used strictly as a nutritional supplement. Uses in products other than tofu may be used to recreate or improve flavors.

Calcium sulfate is used three ways in beer making: as a yeast food; to increase the yield in malt production from barley; and to add calcium to soft water used in brewing.

5. *Is Generally Recognized as Safe (GRAS) by FDA when used in accordance with Good Manufacturing Practices (GMP), and contains no residues of heavy metals or other contaminants in excess of FDA tolerances.*
Calcium sulfate is Generally Recognized As Safe under 21 CFR 184.1230.

The FDA permits the uses described in Table 1.

Table 1 FDA Approved Uses of Calcium Sulfate	
Use	**21 CFR**
anti-caking agent	170.3(o)(1)
color and coloring adjunct	170.3(o)(4)
dough strengthener	170.3(o)(6)
drying agent	170.3(o)(7)
firming agent	170.3(o)(10)
flour treating agent	170.3(o)(13)
formulation aid	170.3(o)(14)
leavening agent	170.3(o)(17)
nutrient supplement	170.3(o)(20)
pH control agent	170.3(o)(23)
processing aid	170.3(o)(24)
stabilizer and thickener	170.3(o)(28)
synergist	170.3(o)(31)
texturizer	170.3(o)(32)
Source: 21 CFR 184.1230(c)	

Limits for Good Manufacturing Practices use of calcium sulfate are contained in Table 2.

Table 2 Maximum Levels of Calcium Sulfate Allowed Under Current Good Manufacturing Practices (As Served)		
Product Category	**Limit (%)**	**21 CFR**
baked goods	1.3%	170.3(n)(1)
confections and frostings	3.0%	170.3(n)(9)
frozen dairy desserts and mixes	0.5%	170.3(n)(20)
gelatins and puddings	0.4%	170.3(n)(22)
grain products and pastas	0.5%	170.3(n)(23)
processed vegetables	0.35%	170.3(n)(36)
all other food categories	0.07%	184.1230(d)
Source: 21 CFR 184.1230(d)		

Table 3 Specific Food and Beverage References	
Food / Beverage	**Regulation**
Cheeses and related cheese products	21 CFR 133
Asiago fresh and Asiago soft	133.102(c)(2)
Blue	133.106(b)(3)(v)
Caciocavello siciliano	133.111(c)(2)
Gorgonzola	133.141(b)(3)(v)
Parmesan and reggiano	133.165(c)(2)
Provolone	133.181(b)(3)(v)
Romano	133.183(c)(2)
Swiss and emmentaler cheese	133.195(b)(3)(v)
Cereal flours and related products	21 CFR 137
Flour	21 CFR 137.105(a)(5)
Fruit butters, jellies, preserves, and related products	21 CFR 150
Artificially sweetened fruit jelly	150.141(a)(5)
Artificially sweetened fruit preserves and jams	150.161(a)(5)
Colorants for food-contact packaging	
Paper and cardboard	21 CFR 176.170(b)(2)
Polymers	21 CFR 178.3297(e)
Source: EAFUS, 2001	

BATF also permits it to lower pH in sherry wine, provided that the sulfate content of the finished wine does not exceed 2.0g/L, expressed as potassium sulfate (27 CFR 24.246). Use in alcoholic beverages is limited to 16.69 pounds per 1,000 gallons (Lewis, 1989).

The Food Chemicals Codex specifications for food grade calcium sulfate are:
Assay: Not less than 98% $CaSO_4$ calculated on a dry weight basis.
Fluoride: Not more than 0.003%.
Heavy metals: not less than 10 mg/kg expressed as lead (Pb).
Loss on Drying: $CaSO_4$ (anhydrous): not more than 1.5%;
$CaSO_4 \cdot 2H_2O$ (dihydrate): between 19.0% and 23.0%.
Selenium: Not more than 0.003%.

Lead content is a primary contaminant of concern (Shurtleff and Aoyagi, 1975).

6. *Its use is compatible with the principles of organic handling.*
The natural product is allowed and widely used in organic crop production, and used in organic livestock production. It has long been used by certified organic tofu makers. Organic food processors surveyed considered the use of 'mined minerals' as tofu coagulants to be more compatible than the use of 'acidic solutions' but less compatible with organic than the use of nigari (Raj, 1991). Some of its uses are not relevant to organic, e.g., use with artificial sweeteners. Use as a flour ingredient and as a dough conditioner has been questioned because of its historical linkage as a carrier and stabilizer with bleaching agents (Fennema, 1996), rodent control (Winter, 1989), and as an adulterant. Calcium sulfate, by itself, is not a bleaching agent. Its use as a coloring agent is generally restricted to use on packaging.

7. *There is no other way to produce a similar product without its use and it is used in the minimum quantity required to achieve the process.*
The petitioner states that calcium sulfate provides a qualitatively different product from that made by magnesium chloride and magnesium sulfate. Also see question 1, above. The amount used in most recipes amounts to 0.3% wet weight, and generally will not exceed 1% dry weight in most tofu preparations. (See Table 2.)

TAP Reviewer Discussion

TAP Reviewer Comments

OMRI's information is enclosed in square brackets in italics. Where a reviewer corrected a technical point (e.g., the word should be "intravenous" rather than "subcutaneous"), these corrections were made in this document and are not listed here in the Reviewer Comments. The rest of the TAP Reviewer's comments are listed here minus any identifying comments and with corrections of typos. The reviewers were asked the following questions; those reviewers who provided answers that could not be incorporated into the database above have their answers identified by 'A' and the question number (e.g. 'A1' for the answer to question 1).

1) What are the qualitative differences between tofu made from natural nigari, natural terra alba (calcium sulfate), (organic) vinegar, (organic) lemon juice, natural calcium chloride, synthetic (bleached) nigari, and synthetic calcium sulfate?

2) What are the nutritional differences?

3) It was claimed by several users and suppliers that all food-grade sources in the US are mined, or at least all food grade sources from their company. Is this easy to verify?

4) Are you aware of any synthetic steps used to purify or beneficiate mined gypsum to make it food grade?

5) Are the levels of lead, other heavy metals, and other contaminants set by Food Chemicals Codex appropriate for organic food?

6) Should the TAP review, recommendations, and annotations be unconditional and inclusive of all (otherwise FDA- or BATF-approved) uses, should they be limited to use in soy products, or are there other annotations that should be made? (e.g., not for use as a coloring agent, etc.).

7) How easy is it for certifiers and processors to distinguish the mined form of calcium sulfate from the synthetic form?

8) If you support an annotation that allows for additional uses, or no annotations (other than source) and no limitations on use, please provide a review that addresses the criteria for all requested or at least the major uses.

9) We have been asked specifically to address beer. Do you have any information on the use of calcium sulfate in the malting or brewing process? There appears to be more information on use as a yeast food, but I did not pull any references. Beer additives do not appear to be listed in 27 CFR. According to Lewis, it is at 27 CFR 240.1051, but that is a 1989 reference. Approved for wine & sherry uses are listed, but not beer.

Reviewer 1

[Consultant to organic certifiers]

Natural and Synthetic Forms

Calcium Sulfate may be obtained from natural, mined sources, as well as be synthesized from other materials. Thus, it can exist as both a synthetic and a non-synthetic material, under the definitions provided under OFPA and the NOP Final Rule (7 CFR 205).

This review will only consider the non-synthetic forms of calcium sulfate, per guidance given in the petition and the TAP database provided to this reviewer. As there are abundant non-synthetic supplies of calcium sulfate widely available to organic producers (USGS, 2000), there is no need to consider the synthetic forms, so the guidance of the petition and TAP database are appropriate in this regard. Thus, in any case, synthetic calcium sulfate should be prohibited in organic production systems.

Calcium sulfate as a dihydrate crystal may be obtained from mined sources using purely physical processes. While some sources claim to provide relatively pure supplies of the dihydrate form (US Gypsum, 1999) it is often found together with the anhydrous form, along with other impurities (Peterson, Kaleta, and Kingston, 1992). There are pertinent

considerations for this review regarding impurities in the naturally mined material – see below under AFFECTS ON HUMAN HEALTH AND NUTRITION. For that part of the discussion, the dihydrate, anhydrate, and hemihydrate forms will not be considered as impurities, as long as they are results of the natural mining process, derived without chemical or heat processes.

Hydrated forms may be changed to the anhydrous form by subjecting them to heat to drive off the water portion of the original material. This latter step could technically be deemed to be a chemical alteration of the original material, thus making it closer to a synthetic material under the OFPA guidelines, although the functionality of the calcium sulfate is presumably not significantly affected by the calcination as such. Nonetheless, as the naturally occurring form is completely acceptable for the purposes petitioned, this reviewer concludes that the only acceptable form of calcium sulfate for addition to the National List should be the naturally mined form, derived solely by physical methods.

Environmental Considerations:

Non-synthetic calcium sulfate has long been recognized and used in organic crop production, with beneficial results.

The manufacture of the material can be by solely physical processes, where the crude gypsum is crushed, screened, milled, graded, and packaged.

Calcium sulfate derived from natural sources impacts the environment in that mining operations are needed to obtain it. This involves quarrying or blasting, and the use of heavy equipment. In addition to the direct impact of the mining operations on the Earth, there is a negative impact caused by the generation of gypsum dust in the process. This dust can affect air quality, and can be a potential exposure hazard to humans and other animals. There are no other known negative affects of toxicity and/or persistence in the environment caused by production of calcium sulfate from these methods, as long as standard regulations for proper mining activities are followed.

It is the opinion of this reviewer that there is no particular reason to believe that use of non-synthetic calcium sulfate in organic systems would contribute undue adversity to the environment, particularly when compared to many other non-organic materials that are already approved for organic production systems.

USES, ESSENTIALITY:

Potential substitutes for calcium sulfate in processing systems depend on the specific purpose for using the material. There are a wide variety of uses known (see chart below for specifics) (Winter, 1994), and are listed in 21 CFR (EAFUS, 2001) and 27 CFR (27 CFR 24.246). Of the uses mentioned, calcium sulfate should only be considered for some, as the others would be disqualified under criteria used by the TAP. . . . [See Table 4].

The uses and potential substitutes for calcium sulfate in processing systems depend on the specific purpose for using the material. Calcium sulfate has a wide variety of food uses, with the main functions and products summarized in Table 4. (EAFUS, 2001 and 27 CFR 24.246). Of the uses mentioned, calcium sulfate should only be considered for some, as the others would be disqualified under criteria used by the TAP. Each of these uses/functions will be considered individually in this section, as its necessity/essentiality and compatibility in organic production, and potential alternatives (organic or otherwise):

Table 4
Reviewer 1 Commentary on Uses

Use/function	Products	Reviewer 1 Comments:	Alternatives
Firming agent	mainly canned fruits and vegetables	Not compatible, as the calcium sulfate would only be used to recreate texture lost during processing, or "improve" texture not had in the first place.	Grape leaves, cherry leaves, other fruit or oak leaves, for certain applications such as pickling. Salts already included on the National List.
Dough conditioner	Breads, crackers, other baked items	Not compatible or essential, as calcium sulfate is being used to improve a texture that could be otherwise achieved via alternative baking techniques. Not a necessary ingredient for any baked goods formulation.	Salt; skillful baking techniques.
Yeast food	Beer and other fermentation products	Not necessary, as there are a variety of organic ingredients which can be substrates for this purpose.	Organic foodstuffs as substrates
pH adjuster, flocculating agent, calcium source	Beer brewing, winemaking	1) As an aid in beer brewing, use of calcium sulfate purportedly increases yield when it is added to the mash tun. Supposedly it also increases yield by promoting proper gelatinization of the starch in the cooker mash, as well as protein degradation and starch conversion. Although the increase in yield is favorable to the brewer, it is not essential to the process. 2) Water adjustment is said by some brewers to often be necessary to provide a flavor and finish that is needed, particularly with top-fermented yeast. Calcium stimulates enzyme activity and improves protein digestion, stabilizes the alpha amylase, helps gelatinize starch and improves lauter runoff. While sulfates can impart off-flavors, so can chloride (salty) and carbonate (chalky). Calcium also extracts fine bittering principles of the hop and reduces wort color.	Various, depending on the process and product.
Coloring/ bleaching agent	Cheeses, flours	Not compatible, as calcium sulfate is being used to alter a color without any other purpose. Not a necessary ingredient.	Food without colorants
Carrier for bleaching agent	Cereal flours	Not compatible, as color alteration is not a valid use for organic.	Unbleached flour
Jelling agent	fruit jellies	Not compatible, as other more suitable alternatives exist., and it is most often used with artificially sweetened jellies and preserves.	Pectins
Abrasive	Toothpaste, tooth powders	Not really applicable at this time for organic considerations. Alternatives exist.	Calcium carbonate

Effects on Human Health and Nutrition:
Calcium sulfate absorbs water and hardens quickly, and as such, when ingested in high concentration can result in intestinal obstruction (Winter, 1994).

Calcium sulfate added to foods can result in an increased dietary intake of calcium, and as such, can be considered a dietary supplement (Food Chemicals Codex, 1996). There is no evidence that ingestion as such does has deleterious affects on human health and nutrition, and may in fact have positive affects. Tofu made with calcium sulfate has been shown to have more than 3.5 times as much dietary calcium as tofu made with nigari (Soyfoods Association, 1986). However, there is a general pattern of decreasing protein yield in tofu with increasing concentration of calcium sulfate used as a coagulant (Sun and Breene, 1991). Nonetheless, overall protein yield is still comparable to tofu made with other coagulants, and total yield by weight of finished tofu is generally greater using calcium sulfate (Wang and Hesseltine, 1982; Hou, Chang, and Shih, 1997).

It is the opinion of this reviewer that these levels as set by the Food Chemicals Codex are appropriate for organic foods. The only alternative to such standards which occurs to this reviewer is to require synthetic calcium sulfate, which might, but not absolutely be guaranteed to be a more pure form. Given the conceptual inconsistency of using the synthetic rather than the naturally occurring material, this latter option does not seem as reasonable as relying on the FCC guidelines.

Organic certifiers should require that all handlers using calcium sulfate present documentation from the supplier (such as Certificates of Analysis, and other statements) which state that the purity of the product, as well as the source./method from which it was obtained.

SUMMARY AND RECOMMENDATION:
List calcium sulfate, as follows:

Synthetic/Non-synthetic	*Allowed/Prohibited*	*Annotation*
Non-synthetic	Allowed	For use only as a coagulant in bean curd (tofu and similar products). Mined sources only; must be derived and purified using only physical methods; must conform to the Food Chemicals Codex guidelines for impurities.

Reviewer 2

[Ph.D. Biochemistry with food industry experience]

TAP Reviewer Vote:

The material Calcium Sulfate, FCC, is NATURAL.
The material Calcium Sulfate, FCC, should be allowed without annotation.

General Comments

Specific Uses: Given the concerns of the organic community, one misstatement must be corrected to avoid generating an indelible bad impression. Calcium sulfate is **NOT** a "bleaching agent." It is a **carrier** for bleaching agents used to bleach flour and milk. Note that the Foods Chemical Codex description does **not** include "bleaching agent" among the purposes for calcium sulfate use in food. See the following sections in Title 21 CFR for details and corroboration.

133.102(c)(2)	Asiago fresh and Asiago soft cheese
133.106(b)(3)(v)	Blue cheese
133.111(c)(2)	Caciocavello siciliano cheese
133.141(b)(3)(v)	Gorgonzola cheese
133.165(c)(2)	Parmesan and reggiano cheeses
133.181(b)(3)(v)	Provolone cheese
133.183(c)(2)	Romano cheese
137.105(a)(5)	Flour

Similarly, calcium sulfate is used as a "gelling agent" to 'set' a pectin gel, but this usage is specifically allowed only in artificially sweetened fruit jelly [21CFR150.141(a)(5)] and in artificially sweetened fruit preserves and jams [21CFR150.161(a)(5)].

The fact is that artificially sweetened fruit products, cheese made with bleached milk and bleached flour have 'lost any organic integrity' they ever had. Consequently, universal acceptance of calcium sulfate as an ingredient in organic food will not create a problematic 'enablement' of these particular applications.

"The petitioner states that calcium sulfate provides a qualitatively different product from that made by magnesium chloride and magnesium sulfate."

This position is supported by the food science literature, in that calcium sulfate creates tofu with a unique soft, silky texture.

Production Method: The excerpt from the Kirk-Othmer Encyclopedia of Chemical Technology deals entirely with gypsum in its important role as a construction material (stucco, the mineral in wallboard, an ingredient in Portland cement) and does not describe the process for creating food grade calcium sulfate. The Quality procedure scheme for Terra Alba provided in the packet notes "outside piles" of the mineral, which is not reassuring. Overall, the evidence in the packet indicates that "terra alba" calcium sulfate used in food is natural gypsum of sufficient purity to satisfy Food Chemicals Codex quality standards. The enclosed description of the mining procedure for the food and pharmaceutical grade Terra Alba produced by Diamond K Gypsum supports this conclusion.

OFPA 2119(m) Criteria: Calcium sulfate is used as a soil conditioner to treat clay soils, so the small amounts of calcium sulfate used in foods would not represent a significant hazard to soil microorganisms or crops.

[Answers to Questions for TAP Reviewers]

A1a. Given the information provided (which appears reliable), synthetic calcium sulfate is not used to make Food Grade calcium sulfate. The synthetic material has too many impurities (and questions); the natural material is cheap enough so there is no incentive in the U.S. and most of the world to use anything else. One would expect no difference between synthetic calcium sulfate and native/mined calcium sulfate.

A1b. The literature citations, specifically DeMan, DeMan and Gupta (1986) and Wang and Hesseltine (1982), provide the answer to this question and make the point that each coagulant creates a organoleptically different product, thus confirming the petitioner's argument.

A2. The data provided in the report answer this question adequately. Coagulating with a calcium salt adds calcium; coagulating with nigari, a magnesium salt, adds magnesium; and so forth. The mineral elements in this case are all essential nutrients.

A3. . . . Information available on the internet supports the claim that all food grade calcium sulfate available in the U.S. is mined. . .

A5. The maximum levels of heavy metals, including lead, in substances allowed as direct food ingredients are established with safety margins to protect all consumers. These maxima are reviewed from time to time to ensure a safe food supply for the U.S. population. The Food Chemicals Codex standards are appropriate for all food. Note that "organic" does not mean "safer."

A6. The "organically unacceptable" uses of calcium sulfate are as a carrier or a diluent of undesirable "anti-organic" food ingredients, such as the bleaching agents used to bleach flour and milk, and as a gelling agent in fruit spreads (jelly, jam and preserves) artificially sweetened with an unnatural non-nutritive substance. The undesirable and organically unacceptable food ingredient would effectively prevent the use of the term "organic" on these foods containing calcium sulfate.

Consequently, the TAP review, recommendations, and annotations should be unconditional and inclusive of all (otherwise FDA- or BATF-approved) uses.

A7. In the United States, it appears that the synthetic form is not used for food. OMRI, an organic certifying organization, or the tofu processing industry itself should consider site visits and inspections to verify manufacturers' claims of a mined origin and no chemical benefication to remove impurities.

A8. Applications that technically allow interchangeable forms of calcium (yeast food, dough thickener, processed vegetables), for example, should not be restricted with respect to using a food ingredient such as calcium sulfate found acceptable for use in another organic food, such as tofu.

A9. [Attached are] excerpts from the Internet regarding calcium sulfate in the brewing process.

Reviewer 3

[Food Science and Nutrition Professor with inspection and certification experience]

Review of the Food Science literature is replete with the uses of calcium sulfate in foods. The primary use of calcium sulfate as a food additive is in the production of traditional forms of tofu. Sun and Breene (1991) indicate that calcium sulfate is the coagulant of choice among tofu makers. At concentrations of between 0.02 and 0.05 normal, optimum yields and textural quality were obtained using Minnesota grown soybeans. Generally tofu made with coagulants such as magnesium chloride or calcium chloride provide a coarse, granular and hard textured product much less in overall quality than tofu made with calcium sulfate. Historically calcium sulfate has been used for over 2,000 years to produce tofu that has been show to contain 3.5 to 4 times as much dietary calcium as tofu produced using other coagulants such as acid, glucono delta lactone, Nigari and magnesium chloride.

Calcium sulfate is readily obtained as a mined source and is purified by heating the hemihydrate form to the dihydrate by which 1.5 moles of water is removed as water vapor. The dihydrate form of calcium sulfate is then further heat treated to remove the 2 moles of water forming anhydrous calcium sulfate which generally contains approximately 98% $CaSO_4$ and less than 2% of calcium carbonate, calcium oxide, magnesium carbonate and silicon dioxide (United States Gypsum Company and Peterson et al). Generally all US supplies of calcium sulfate are mined in the US and North America. However, calcium sulfate can be manufactured as a by-product of various chemical processes especially in the scrubbing of gasses evolved in burning fuels that contain sulfur such as coal and the chemical industry where sulfuric acid is a by-product. However, the cost of processing and purifying calcium sulfate from these non-mined sources greatly exceeds the cost of the mined form of calcium sulfate. Additionally, I would suggest that calcium sulfate used for organic products processing specify that it is obtained from a mined source either on a certificate of analysis or in product information sheets. Additionally, due to cost considerations non-mined calcium sulfate simply may not be competitive.

With respect to the levels of heavy metals set by Food Chemical Codex, these levels are set by scientific studies that provide the FDA the recommended usage levels. Therefore, as a natural mined product, natural levels of heavy metals may be present with tolerances provided by the Food Chemical Codes. I do not see this as presenting any degree of difficulty or becoming problematic at the usage levels set by the FDA.

Overall. I would recommend on the basis of the scientific literature, that calcium sulfate be considered as non-synthetic and allowed for use in all organic foods as long as it be verified as obtained from a mined source. I would recommend an annotation that governed its use according to FDA approved uses and at the levels as mandated in 21CFR according to Tables 1 and 2 in the NOSB TAP review.

My reasoning is as follows. If calcium sulfate is allowed for use in only tofu processing and this determination is based on sound scientific and organic principles reasoning, then how could it be excluded for use in other foods as a functional, non-synthetic allowed ingredient? Obviously there is a cost benefit, functional and product quality considerations. I feel there is no compelling reason to exclude the use of mined calcium sulfate from organic baked goods, confectionery products, frozen deserts, puddings, grains and pasta, vegetables and other food categories such as beer if it is approved in 27CFR.

I am very much concerned about limiting the use of approved non-synthetic food ingredients since there is no strong scientific reasoning to support its inclusion only for tofu and exclusion for all other products.

It would be very simple for certifiers to determine whether calcium sulfate is from a mined source by mandating that a certificate of analysis or product information bulletin be provided or a letter from the supplier indicating its source. Also, I agree with the positions taken by CCOF, Oregon Tilth, OCIA and the Texas Department of Agriculture on the unconditional use of calcium sulfate but I would add "use be consistent with FDA approved uses at levels mandated by 21CFR."

My position and recommendation regarding the use of calcium sulfate in beer brewing is allowed as documented in 27 CFR, since BATF has approved its usage to lower the pH of sherry wine. Use in beer fermentation may be as a component in a mineral mix to enhance or stabilize the rate of yeast cell growth and therefore the rate of the fermentation process.

In summary, I feel the use of mined calcium sulfate, which has been produced as a function of heat processing, is compatible with both the spirit and intent of organic integrity. Even though it may be deemed a food additive, it is really an ingredient because the calcium ions are the functional chemical components that govern its usage or the sulfate anions, which are responsible for pH adjustment. Scientifically, I see no compelling justification to restrict its usage in organic foods or process operations.

Recommendations to OMRI

1. Non-synthetic
2. Allowed

Suggested Annotation

Mined sources only and use must be consistent with FDA approved uses at levels mandated by 21CFR.

Conclusion

Given the historical use and nutritional value of calcium sulfate used as a coagulant for tofu, it appears that the substance is compatible with organic principles. Similarly, its use in a variety of other applications appear to be compatible with organic principles, but a number may not be. These merit discussion in the overall context of the appropriate use of additives in organic food production. Two of the three TAP reviewers advise the NOSB to list Calcium Sulfate from Natural Mined Sources only as an Allowed Non-synthetic, Non-organic, Ingredient without any additional annotations.

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Simultaneous Curdling of Soy/Cow's Milk Blends with Rennet and Calcium or Magnesium Sulfate, Utilizing Soymilk Prepared from Soybeans or Full-Fat Soy Flour

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ABSTRACT

Mixed soy/cow's milk curds were prepared by simultaneous curdling of soy/cow's milk blends, utilizing rennet as cow's milk coagulant and calcium or magnesium sulfate as soymilk coagulant. The method produced curds of similar characteristics (compactness and yield), whether soymilk prepared from soybeans or full-fat soy flour was used. The effect of a number of process variables on curd characteristics was studied, utilizing a fractional factorial design. Generally, large changes in process variables (23 - 230%) produced relatively small charges in curd characteristics (1 - 16%). Protein contents of raw materials and proximate chemical analyses of pure and mixed milk curds, prepared under conditions yielding maximum curd compactness were determined. Protein recoveries in curd preparation were calculated.

INTRODUCTION

CHEESES of different types are much favored throughout the world, including developing countries. Although these products are known to possess high contents of good quality protein (FAO, 1970), they nevertheless have one important disadvantage as far as their use in developing countries is concerned: their high cost. Soybean products, on the other hand, represent an inexpensive and abundant source of protein, also of good quality (Wolf and Cowan, 1971).

For the above reasons, it appeared desirable to study extension of cheeses with soy proteins. Two possibilities were apparent for doing so: (1) curdle soy and cow's milk separately and mix the curds; and (2) mix soy and cow's milk and curdle the mixture simultaneously. Preliminary work on mixing separately prepared soy and cow's milk curds gave poor cheese because the curds had different structures, poor cohesion and broke on mixing. As a result of these tests, possibility (1) was excluded, leaving possibility (2) as the remaining option for preparing soy-extended cheeses of acceptable quality.

Previous reports on soy-extended cheeses include the work of Hang and Jackson (1967), who inoculated a 15/85 w/w mixture of skimmilk/soymilk with *S. thermophilus;* after incubation, the mixture was curdled by lactic acid produced by fermentation. Added rennet reduced the time from inoculation with starter to cutting the curd. Subsequently, Schroeder and Jackson (1971) applied a similar procedure to 25/75, 50/50 and 75/25 w/w mixtures of skimmilk/soymilk, again with addition of rennet. In all cases, cheeses of satisfactory quality were obtained, although it was found that the amount of skimmilk added had little effect on finished product texture and flavor, the latter being due to the strong flavor of soymilk. It might be added that soymilk obtained from soybeans by the traditional process (Smith and Circle, 1978) was used in both works, and that formation of soy and cow's milk curds was due mainly to isoelectric precipitation of casein and soy proteins by acid produced by fermentation, with added rennet only playing a minor role.

In this work, simultaneous curdling of soy/cow's milk blends of different proportions was studied, utilizing rennet for the cow's milk component and calcium or magnesium sulfate for the soymilk component.

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MATERIALS & METHODS

Preparation of soymilk from soybeans by the traditional process

Davis variety soybeans were soaked 24 hr in water at room temperature, using a water/bean ratio (w/w) of 1:4, the soak water was decanted and the beans washed. The soaked beans were mixed with water (1:9 w/w), ground 5 min in a Waring Blendor (high speed), and the resulting suspension filtered through cheesecloth. The residue was discarded; the filtrate, soymilk, was heated to 95°C and cooked at that temperature for 7 min. The final product was cooled and stored at 5°C until used. Protein content of the soymilk was determined using standard procedures (AOAC, 1970).

Preparation of soymilk from full-fat soy flour

Commercial full-fat soy flour was mixed with water (1:20, w/w) and the resulting suspension heated to 95°C and held at that temperature for 10 min, all with continuous stirring. The hot suspension was mixed vigorously (Waring Blendor jar, high speed) for 3 min. The resulting soymilk was cooled and stored at 5°C until used, and its protein content was determined by standard methods (AOAC, 1970).

Other materials

Raw cow's milk was obtained from a local dairy and stored at 5°C until used; its protein content was measured according to AOAC (1970) methods. Rennet was a commercial product in tablet form, of unspecified activity. Calcium and magnesium sulfates were chemically pure, laboratory-grade reagents, and did not contain water of crystallization.

Procedure for curd preparation

Each run was carried out as follows. Soy and cow's milks were mixed to obtain 2L of blend of the desired proportion (25/75, 50/50 and 75/25 soy/cow's milk, v/v) and blend acidity was adjusted to the required value using a 10% aqueous solution of lactic acid. The blend was heated to the required temperature and the curdling reagents, calcium or magnesium sulfate and rennet, were added in the required amounts, followed by thorough mixing to obtain uniform dispersion. The blend was allowed to repose at the required temperature (30 or 37°C) for the required length of time to allow curdling to occur. After the repose period, blend temperature was raised to 40 - 45°C to cook the curd. The curd was cut with a curd knife and transferred to a perforated wooden box containing 4 - 5 layers of cheesecloth. The whey was allowed to drain, then the warm curd, wrapped in cheesecloth, was placed in a hydraulic press and subjected to a pressure of 1000 psi for 24 hr. The curd was removed and evaluated.

Evaluation of curd characteristics

Two curd characteristics were measured: (1) consistency, determined utilizing a Koehler penetrometer with a universal ASTM

grease penetration cone; and (2) yield, calculated as weight of wet pressed curd obtained from 100L of milk blend.

Choice of independent variables

The following factors, which were believed to affect curd characteristics were chosen as independent variables and coded as follows: blend titratable acidity (A); amount of rennet added (B); type of soymilk coagulant employed (K); amount of soymilk coagulant added ($CaSO_4$ – C_a; $MgSO_4$ – C_b); curdling temperature (D); curdling time (E); and proportion of soy to cow's milk (P). Since a 2^n factorial experimental design was selected and since this requires use of each factor at two levels (low and high), corresponding levels chosen for the above factors were those considered to lie within current practice for manufacture of cheese from cow's milk and soybean curd from soymilk (Webb et al., 1974; Kosikowski, 1977; Davis, 1965; Smith and Circle, 1978; Schroeder and Jackson, 1971). It should be noted that in runs in which soymilk prepared from full-fat soy flour was utilized, one factor – proportion of soy to cow's milk – was studied at three levels.

Experimental design

A 2^n factorial design was selected. Since the complete design would have required performance of a larger number of experiments, which was considered impractical, it was decided to utilize a fractional factorial design instead. In this case, only part of the experiments required by the complete design are performed. A detailed description of the method is given by Fedener (1955).

Experimental design in runs utilizing soymilk prepared from soybeans

The experiment was carried out in 40 runs, with eight of the treatment combinations replicated to better ascertain experimental error. Table 1 lists factors used with corresponding levels. In the experimental design selected (Fedener, 1955), besides main effects, the following interactions were determined because they were believed to be important (refer to Table 1): A*B, A*C, B*K, and E*K. Higher order and the remaining two factor interactions were considered not significant. Due to the fractional design, other interactions were confounded with main effects (Fedener, 1955). In the design employed, C was nested within K. On each run, measurements were made of penetration and yield. The design matrix is given in Table 2, where the low level has been coded as –1 and the high level as +1. Replicate runs are shown in the table as "A" and "B". Results obtained were analyzed by Analysis of Variance techniques (Fedener, 1955).

Table 1—List of independent variables used in runs utilizing blends containing soymilk prepared from soybeans

Code	Factor	Low level	High level
A	Acidity	3.5% as lactic acid	5.0% as lactic acid
B	Rennet concentration	0.021 g/L of cow's milk in blend	0.042 g/L of cow's milk in blend
K	Type of soymilk coagulant[a]	Magnesium sulfate	Calcium sulfate
C	Amount of soymilk coagulant		
(a) $CaSO_4$		3.3 g/L of soymilk in blend	16.6 g/L of soymilk in blend
(b) $MgSO_4$		4.8 g/L of soymilk in blend	15.9 g/L of soymilk in blend
D	Curdling temp	30°C	37°C
E	Curdling time	1.5 hr	3.0 hr
P	Blend proportion of soymilk, v/v	25/75	50/50

[a] For this factor, $MgSO_4$ was coded as "low level" and $CaSO_4$ as "high level."

Experimental design in runs utilizing soymilk prepared from full-fat soy flour

The experiment was set up as a fractional factorial design with six factors. Of these, five were at each of two levels. The sixth factor consisted of three different soy/cow's milk proportions. The experiment was carried out in 48 runs, all replicated. Readings, at each factor combination, were made on penetration and yield. The same soymilk coagulant (magnesium sulfate) was used in all runs. The factors and their levels are given in Table 3, while the corresponding design matrix is listed in Table 4. Note that in the latter table, for the first five factors, low level has been coded as –1 and high level as +1; the sixth factor (P) was defined as follows: soymilk/cow's milk proportion 75/25, +1; 50/50, 0; 25/75, –1. Replicate runs are shown in Table 4 as "A" and "B". Besides main effects, the selected experimental design (Fedener, 1955) permitted calculations of the following interactions which were believed to be important: A*B, A*C, A*P, B*P, C*P, D*P, and E*P. Higher order and remaining second order interactions were assumed to be not significant. Due to the fractional nature of the design, other interactions were confounded with main effects. Results obtained were analyzed by Analysis of Variance techniques (Fedener, 1955).

Proximate analysis and protein balance calculations of pure and mixed milk curds

Proximate analyses of curds with minimum penetration, ob-

Table 2—Design matrix with corresponding results in runs utilizing blends containing soymilk prepared from soybeans

Run	A	B	C	D	E	P	K	Penetration[a]	Yield[b]
1	+1[c]	–1[c]	–1	+1	+1	+1	+1	140	12.00
2	+1	+1	–1	–1	–1	+1	+1	183	11.55
3	+1	–1	+1	–1	–1	+1	+1	103	10.65
4	+1	–1	–1	+1	+1	+1	–1	202	12.95
5	+1	–1	+1	–1	–1	+1	–1	174	12.30
6	+1	+1	+1	+1	+1	+1	–1	113	7.50
7	+1	–1	–1	+1	+1	–1	+1	170	11.55
8	+1	+1	–1	–1	–1	–1	+1	192	12.95
9	+1	–1	+1	–1	–1	–1	+1	197	22.40
10	+1	–1	–1	+1	+1	–	–1	203	12.10
11	+1	–1	+1	–1	–1	–1	–1	201	12.70
12	+1	+1	+1	+1	+1	–1	–1	204	9.50
13A[d]	+1	+1	+1	+1	+1	+1	+1	103	9.90
13B[d]	+1	+1	+1	+1	+1	+1	+1	168	10.65
14A	+1	+1	–1	–1	–1	+1	–1	194	11.30
14B	+1	+1	–1	–1	–1	+1	–1	183	9.50
15A	+1	+1	+1	+1	+1	–1	+1	142	11.75
15B	+1	+1	+1	+1	+1	–1	+1	150	12.00
16A	+1	+1	–1	–1	–1	–1	–1	195	12.75
16B	+1	+1	–1	–1	–1	–1	–1	203	12.75
17	–1	+1	+1	–1	–1	+1	+1	144	11.80
18	–1	+1	+1	–1	+1	+1	+1	213	14.70
19	–1	–1	+1	+1	–1	+1	–1	290	8.00
20	–1	+1	–1	+1	–1	+1	–1	158	12.35
21	–1	+1	+1	–1	+1	+1	–1	193	11.75
22	–1	–1	–1	–1	+1	+1	–1	194	11.50
23	–1	–1	–1	–1	+1	–1	–1	135	11;15
24	–1	+1	–1	+1	–1	–1	–1	173	11.90
25	–1	+1	+1	–1	+1	–1	+1	240	12.30
26	–1	+1	–1	+1	–1	–1	–1	138	9.85
27	–1	–1	+1	+1	–1	–1	–1	182	12.15
28	–1	+1	+1	–1	+1	+1	–1	180	12.20
29A	–1	–1	–1	–1	+1	+1	+1	156	12.95
29B	–1	–1	–1	–1	+1	+1	+1	196	12.95
30A	–1	–1	+1	+1	–1	+1	+1	194	14.05
30B	–1	–1	+1	+1	–1	+1	+1	108	10.65
31A	–1	–1	+1	+1	–1	–1	+1	145	12.00
31B	–1	–1	+1	+1	–1	–1	+1	165	13.80
32A	–1	–1	–1	–1	+1	–1	–1	124	10.20
32B	–1	–1	–1	–1	+1	–1	–1	135	11.50

[a] Penetration reported as penetrometer reading.
[b] Yield reported as percent wet pressed curc with respect to milk blend.
[c] High level coded as +1, low level coded a –1.
[d] Replicate runs coded as "A" and "B".

tained in both series of runs (i.e., utilizing soymilk from soybeans and from full-fat soy flour) were determined (AOAC, 1970). Considering these data, as well as protein contents of pure soy and cow's milks previously obtained (AOAC, 1970), protein balance calculations were carried out in order to determine protein recovery in curds.

RESULTS & DISCUSSION

Soymilk prepared from soybeans

Results of analysis of variance calculations are given in Tables 5 and 6 for penetration and yield, respectively. Due to unequal numbers of observations per cell, the design was not orthogonal, and hence different sums of squares do not add up to the total.

Factors found to be significant were K ($p < 0.05$), A*C ($p < 0.01$), A*P ($p < 0.05$) and B*K ($p < 0.05$) in the case of penetration, and D ($p < 0.05$), K ($p < 0.05$), A*B ($p < 0.10$), A*P ($p < 0.05$) and E*K ($p < 0.05$) in the case of yield. Most factors were significant at the $p < 0.05$ level; also, only two main effects – K, coagulant type and D, curdling temperature – were significant in these runs, both at the $p < 0.05$ level.

Effects of significant interactions on penetration and yield were calculated (Fedener, 1955) and found to be less than 1%.

Average penetration and yield values calculated, utilizing data for all runs, corresponding to low and high levels of significant main effects are shown in Table 7. Use of calcium sulfate as coagulant gave softer curds with higher average penetration and yield values (194 and 12.05%, respectively) than use of magnesium sulfate, for which corresponding values were 184 and 11.25%. On the other hand, an increase in curdling temperature from 30 to 37°C decreased average yield from 12.00 to 11.35%.

Nevertheless, Table 7 shows that overall percentage changes in both penetration and yield, caused by variation of type of coagulant and curdling temperature, were only of the order of 5 – 7%. Thus, variations in individual values reported in Table 2 were probably due more to experimental error than to significant main effects and interactions. It also means that it was possible to produce mixed soy/cow's milk curds by the method described, but that characteristics of these curds were not too sensitive to changes in experimental conditions, within limits of the experimental design employed. Given the small magnitude of these significant main effects, therefore, they will not be discussed further.

When studying main effects (an also interactions), it was found that in some cases, increasing penetration coincided with increasing yield, suggesting the possibility that the two parameters were correlated. To test this hypothesis, the correlation coefficient (r) was calculated (Snedecor and Cochran, 1967), utilizing data for all runs as reported in Table 2. The corresponding value calculated as 0.23, which indicated that the hypothesis was incorrect, and that penetration and yield were not correlated.

Table 3—List of independent variables used in runs utilizing blends containing soymilk prepared from full-fat soy flour

Code	Factor	Low level	High level
A	Acidity	3.5% as lactic acid	5.0% as lactic acid
B	Rennet Concentration	0.021 g/L of cow's milk in blend	0.042 g/L of cow's milk in blend
C	Amount of soymilk coagulant	4.8 gYL of soymilk in blend	15.9 g/L of soymilk in blend
D	Curdling temperature	30°C	37°C
E	Curdling time	1.5 hr	3.0 hr
P	Blend proportion of soymilk[a]	25/75 50/50	75/25

[a] Note that variable P, blend proportion of soymilk, was studied at three levels.

Soymilk prepared from full-fat soy flour

Analysis of variance calculations for the fractional factorial design employed in these runs are shown in Tables 8 and 9 for penetration and yield, respectively.

Factors found to be significant were A ($p < 0.01$), B ($p < 0.05$), O ($p < 0.01$), D ($p < 0.05$), E ($p < 0.05$), P ($p < 0.05$), A*C ($p < 0.10$), A*P ($p < 0.01$), B*P ($p < 0.05$), C*P ($p < 0.10$), D*P ($p < 0.01$) and E*P ($p < 0.01$)

Table 4—Design matrix with corresponding results in runs utilizing blends containing soymilk prepared from full-fat soy flour

Run	A	B	C	D	E	P	Penetration[a]	Yield[b]
1A[d]	+1[c]	−1[c]	−1	+1	+1	+1	188	12.70
1B[d]	+1	−1	−1	+1	+1	+1	122	12.45
2A	+1	+1	−1	−1	−1	+1	165	10.80
2B	+1	+1	−1	−1	−1	+1	112	12.20
3A	+1	−1	+1	−1	−1	+1	154	11.30
3B	+1	−1	+1	−1	−1	+1	124	11.15
4A	+1	+1	+1	+1	+1	+1	140	11.20
4B	+1	+1	+1	+1	+1	+1	112	11.80
5A	+1	−1	−1	+1	+1	0	95	11.35
5B	+1	−1	−1	+1	+1	0	163	10.55
6A	+1	+1	−1	−1	−1	0	120	11.25
6B	+1	+1	−1	−1	−1	0	150	10.35
7A	+1	−1	+1	−1	−1	0	211	11.50
7B	+1	−1	+1	−1	−1	0	193	10.75
8A	+1	+1	+1	+1	+1	0	98	11.00
8B	+1	+1	+1	+1	+1	0	118	11.50
9A	+1	−1	−1	+1	+1	−1	215	11.35
9B	+1	−1	−1	+1	+1	−1	193	11.05
10A	+1	+1	−1	−1	−1	−1	156	11.75
10B	+1	+1	−1	−1	−1	−1	133	11.65
11A	+1	−1	+1	−1	−1	−1	156	11.60
11B	+1	−1	+1	−1	−1	−1	210	12.00
12A	+1	+1	+1	+1	+1	−1	209	11.25
12B	+1	+1	+1	+1	+1	−1	217	11.60
13A	−1	−1	−1	−1	+1	+1	124	10.80
13B	−1	−1	−1	−1	+1	+1	103	11.20
14A	−1	+1	−1	+1	−1	+1	142	11.40
14B	−1	+1	−1	+1	−1	+1	102	11.75
15A	−1	−1	+1	+1	−1	+1	170	12.50
15B	−1	−1	+1	+1	−1	+1	126	12.15
16A	−1	+1	+1	−1	+1	+1	130	10.85
16B	−1	+1	+1	−1	+1	+1	109	10.90
17A	−1	−1	−1	−1	+1	0	161	12.70
17B	−1	−1	−1	−1	+1	0	212	12.95
18A	−1	+1	−1	+1	−1	0	82	11.80
18B	−1	+1	−1	+1	−1	0	93	11.05
19A	−1	−1	+1	+1	−1	0	169	12.00
19B	−1	−1	+1	+1	−1	0	195	12.10
20A	−1	+1	+1	−1	+1	0	194	12.10
20B	−1	+1	+1	−1	+1	0	175	12.65
21A	−1	−1	−1	−1	+1	0	107	12.65
21B	−1	−1	−1	−1	+1	−1	128	12.45
22A	−1	+1	−1	+1	−1	−1	78	11.85
22B	−1	+1	−1	+1	−1	−1	95	12.20
23A	−1	−1	+1	+1	−1	−1	97	12.50
23B	−1	−1	+1	+1	−1	−1	106	12.60
24A	−1	+1	+1	−1	+1	−1	184	12.45
24B	−1	+1	+1	−1	+1	−1	205	12.55

[a] Penetration reported as penetrometer reading.
[b] Yield reported as percent wet pressed curd with respect to milk blend.
[c] High level coded as +1, low level coded as −1.
[d] Replicate runs coded as "A" and "B".

for penetration; and A ($p < 0.01$), B ($p < 0.05$), P ($p < 0.01$), A*P ($p < 0.01$), D*P ($p < 0.01$) and E*P ($p < 0.05$) for yield. It is interesting to note that many more factors were found to be significant in these runs than in those utilizing soymilk from soybeans; also, most of these factors were significant at the $p < 0.01$ and $p < 0.05$ levels.

All main effects were found to be significant in the case of penetration, at least at the $p < 0.05$ level, while three of these – A, acidity, B, rennet dose and P, blend proportion of soymilk – were significant at the $p < 0.01$ level.

As in the previous runs, effects of significant interactions on penetration and yield were calculated (Fedener, 1955) and found to be less than 1% so that consequently, these will not be discussed further.

Average penetration and yield values calculated, utilizing data for all runs, corresponding to low and high levels of significant main effects are shown in Table 10. It is apparent that in all cases, large changes in independent variables (i.e., acidity, rennet dose, etc.; of the order of 23 - 230%) produced relatively small changes in both penetration and yield (1 - 16%). This same effect was observed in the previous runs utilizing soymilk prepared from soybeans. Also, average yield and penetration values obtained in the full-fat soy flour runs (Tables 4 and 10) were not much different from those obtained in the soybean runs (Tables 2 and 7).

These observations indicate that soymilk prepared from full-fat soy flour behaved similarly to that prepared from soybeans, at least as far as behavior in preparation of mixed curds by the present method is concerned. The proposed method, therefore, yielded curds of similar characteristics, regardless of soymilk origin.

Increase in acidity, magnesium sulfate concentration and curdling time increased penetration, resulting in softer curds, while increases in rennet concentration, curdling temperature and blend proportion of soymilk had the opposite effect, producing harder curds (Table 10). On the other hand, increases in acidity, rennet concentration and blend proportion of soymilk all resulted in a decrease in yield. Since they had little effect on both penetration and yield, as noted previosuly, however, these factors will not be discussed further.

The correlation coefficient between penetration and yield was calculated, using data obtained in all runs in which soymilk prepared from full-fat soy flour was utilized (Table 4; Snedecor and Cochran, 1967) and found to be 0.10. It was concluded that in these runs, as in those in which soymilk prepared from soybeans was used, penetration and yield were not correlated.

Proximate analysis and protein balance calculations of pure and mixed milk curds

Proximate analysis determinations of pure and mixed milk curds prepared under conditions giving minimum penetration, including protein contents of pure milks (experimentally determined) and milk blends (calculated) are given in Table 11. Soymilk prepared from soybeans had more than twice the protein content of that prepared from full-fat soy flour (5.3% and 2.0%, respectively). This difference may be explained as follows. In preparing soymilk from soybeans by the method described in this paper, raw soybeans containing native proteins were extracted with water; since native proteins possess high solubility, depending upon the water/bean ratio, high soymilk protein con-

Table 5—Results of analysis of variance calculations for penetration, in runs utilizing blends containing soymilk prepared from soybeans

Source of variation[a]	Degrees of freedom	Sum of squares	F Value[b]
A	1	164.1	0.17
B	1	91.6	0.09
C	2	1085.7	0.55
D	1	1283.1	1.29
E	1	364.6	0.37
P	1	68.6	0.07
K	1	4466.1	4.50**
A*B	1	528.5	0.53
A*C	2	12809.3	6.45***
A*P	1	7171.6	7.22**
B*K	1	5705.7	5.75**
E*K	1	54.8	0.06
Model	14	32254.8	2.32**
Error[c]	25	24817.7	
Total (corrected)	39		

[a] Please refer to Table 1 for coding.
[b] Significance level indicated as follows: *p < 0.10; **p < 0.05; ***p < 0.01.
[c] Error standard deviation = ± 31.5.

Table 6—Results of analysis of variance calculations for yield, in runs utilizing blends containing soymilk prepared from soybeans

Source of variation[a]	Degrees of freedom	Sum of squares	F Value[b]
A	1	392.2	0.64
B	1	334.0	0.55
C	2	1537.6	1.26
D	1	2635.4	4.31**
E	1	110.0	0.18
P	1	846.4	1.38
K	1	3760.0	6.15**
A*B	1	2405.4	3.93*
A*C	2	2931.0	2.40
A*P	1	2592.1	4.24**
B*K	1	874.3	1.43
E*K	1	1960.0	3.20**
Model	14	19018.1	2.22**
Error[c]	25	15293.5	
Total (corrected)	39		

[a] Please refer to Table 1 for coding.
[b] Significance level indicated as follows: *p < 0.10; **p < 0.05; ***p < 0.01.
[c] Error standard deviation = ± 1.24.

Table 7—Average yield and penetration values corresponding to low and high levels of significant main effects, in runs utilizing blends containing soymilk prepared from soybeans

Parameter	Average value at level −1[e] Penetration[a]	−1[e] Yield[b]	+1[f] Penetration[a]	+1[f] Yield[b]	Percent variation in Penetration	Percent variation in Yield
Coagulant type (K)[c]	184	11.25	194	12.05	+5.4%	+7.1%
Temperature (D)[d]	–	12.00	–	11.35	–	−5.4%

[a] Penetration reported as pentrometer reading.
[b] Yield reported as percent wet pressed curd with respect to milk blend.
[c] Percent variation in coagulant type not calcuable.
[d] Percent variation in temperature, +23.3%
[e] Low level coded as −1.
[f] High level coded as +1.

centrations are possible. Full-fat soy flour, on the other hand, has been considerably heat treated in order to inactivate enzymes and antinutritional factors, with a consequent sharp drop in protein solubility. Proteins present in full-fat soy flour soymilk therefore, were mainly those contained in insoluble flour particles suspended in water. Since a limit exists on the maximum amount of flour that may be suspended in order to yield a stable dispersion, a limit also existed on the maximum protein concentration of full-fat soy flour soymilk.

Milk curd protein content paralleled milk protein level (Table 11). Thus both soymilk curds ($CaSO_4$ and $MgSO_4$ precipitated) possessed higher protein contents than did the cow's milk curd. The cow's milk curd contained more fat (20.4%) than both soymilk curds (7.6% and 6.6% for the $MgSO_4$ and $CaSO_4$ precipitated curds, respectively), probably reflecting a similar difference in fat levels between soy and cow's milks (fat contents of these milks were not determined). Curd moisture contents were inversely proportional to total solids; due to its appreciably higher fat content, the cow's milk curd contained less moisture than either soymilk curd. The type of soymilk coagulant employed appeared to have little effect on curd proximate analysis.

Mixed milk curds prepared from soybean soymilk blends possessed higher protein contents than did those made from full-fat soy flour soymilk blends (Table 11). This is as m have been expected, in view of the higher protein con of the former with respect to the latter soymilk. Gener curd protein content paralleled milk blend protein cont Thus, in the case of blends containing soymilk prep from soybeans, protein content of both milk blends curds increased with increasing soy proportion, w the opposite was true with blends containing soymilk pared from full-fat soy flour. As in the case of pure curds, type of soymilk coagulant employed had little e on mixed curd proximate analysis. As a whole, soy soymilk curds exhibited lower fat contents than fu soy flour soymilk curds, probably reflecting parallel d ences between raw material fat contents.

An interesting observation regarding mixed soy soymilk curds is that all had lower protein contents either 100% cow's or soymilk curds. This is impo because, in view of what was noted in the previous graph, mixed curd protein levels should have beer pected to lie between, and not below, those for corresp ing pure milk curds. Since all mixed curds had lowe contents than the pure cow's milk curd, this observ is probably best explained by the fact that protein rec in mixed curds was lower than in pure ones (Table 12). difference, in turn, indicates that curdling subst worked better when used with pure milks than when

Table 8—Results of analysis of variance calculations for penetration, in runs utilizing blends containing soymilk prepared from full-fat soy flour

Source of variation[a]	Degrees of freedom	Sum of squares	F Value[b]
A	1	4508.6	8.04***
B	1	3383.5	6.03**
C	1	6594.1	11.75***
D	1	3162.3	5.64**
E	1	2697.0	4.81**
P	1	4735.9	4.22**
A*B	1	714.6	1.27
A*C	2	1930.4	3.44*
A*P	1	12315.0	10.97***
B*P	1	6076.5	5.42**
C*P	2	3029.1	2.70*
D*P	2	7679.3	6.84***
E*P	2	8964.5	7.99***
Error[c]	28	15710.2	
Total	47		

[a] Please refer to Table 3 for coding.
[b] Significance level indicated as follows: *p < 0.10; **p < 0.05; ***p < 0.01.
[c] Error standard deviation = ± 23.7.

Table 9—Results of analysis of variance calculations for p in runs utilizing blends containing soymilk prepared from fu soy flour

Source of variation[a]	Degrees of freedom	Sum of squares	F Va
A	1	1728.0	30
B	1	377.4	6
C	1	28.8	0
D	1	12.6	0
E	1	33.0	0
P	2	665.0	5
A*B	1	95.8	1
A*C	1	91.8	1
A*P	2	1676.7	15
B*P	2	82.6	0
C*P	2	131.1	1
D*P	2	1579.9	14
E*P	2	418.4	3
Error[c]	28	1563.0	
Total		8484.2	

[a] Please refer to Table 3 for coding.
[b] Significance level indicated as follows: *p < 0.10; **p < ***p < 0.01.
[c] Error standard deviation = ± 0.37.

Table 10—Average penetration and yield values corresponding to low, intermediate and high levels of significant main effects in runs ut blends containing soymilk prepared from full-fat soy flour

Parameter	Average value at level						Percent variatic
	−1[h]		0[h]		+1[h]		
	Penetration[a]	Yield[b]	Penetration[a]	Yield[b]	Penetration[a]	Yield[b]	Penetratio
Acidity (A)[c]	137	12.00	–	–	156	11.90	+13.9%
Rennet dose (B)[d]	155	11.85	–	–	138	11.60	−11.0%
Magnesium sulfate dose (C)[e]	135	–	–	–	156	–	+15.6%
Temperature (D)[f]	156	–	–	–	135	–	−13.5%
Soymilk proportion (P)[g]	156	11.95	152	11.65	133	11.50	−14.7%

[a] Penetration reported as penetrometer reading.
[b] Yield reported as percent wet pressed curd with respect to milk blend.
[c] Percent variation in acidity, +42.9%.
[d] Percent variation in rennet does, +100.0%.
[e] Percent variation in amount of magnesium sulfate, +31.3%.
[f] Percent variation in temperature, +23.3%.
[g] Percent variation in soymilk proportion, overall, +200.0%.
[h] Low level coded as −1; intermediate level coded as 0 (only for P, soymilk proportion); high level coded as ±1.

in blends, probably because, according to the manner in which they were added (grams coagulant per liter of soy or cow's milk in blend), concentrations of these substances in blends were lower than in pure milks, in proportion to blend composition (e.g., in 50/50 blends, coagulant concentrations were 50% of those in pure milks). Presence of the opposite milk component probably also hindered action of a given milk coagulant, especially rennet.

Table 12 is interesting for a number of other reasons. It shows that protein recovery was the same, regardless of whether soymilk was curdled with calcium or magnesium sulfate, or cow's milk was curdled with rennet. With all mixed curds (soybean or full-fat soy flour soymilk blends), curd protein recovery decreased with increasing proportion of cow's milk; this probably indicated that cow's milk was more difficult to curdle than soymilk in blends. This may be so since curdling of cow's milk with rennet, being an enzymatic reaction, was probably more sensitive to curdling conditions than curdling of soymilk with calcium or magnesium sulfate, which is essentially a chemical reaction. For equal blend soymilk levels, higher protein recoveries were obtained with full-fat soy flour soymilk than with soybean soymilk. This observation is probably explained by the fact that full-fat soy flour soymilk, as previously noted, consisted of insoluble suspended particles, which were, therefore, quite easy to precipitate, while soybean soymilk had a much higher content of soluble protein which had to be precipitated by a chemical reaction.

Curds prepared using magnesium sulfate as soymilk coagulant exhibited somewhat higher protein recovery than those prepared using calcium sulfate. The former salt gave better and more thorough precipitation of proteins than did the latter. Interestingly, however, and as was previously noted, no effect of type of coagulant on protei recovery was found in the case of pure curds. Excludin experimental error, no explanation for this discrepancy apparent at this time.

Table 11—Results of proximate analysis determinations for pure milks, milk blends and curds, in preparation of pure and mixed milk curds

System[a]	% Protein	% Fat	% Moisture
Pure milks and milk blends			
100% Soybean soymilk	5.3	–	–
100% Full-fat soy flour soymilk	2.0	–	–
100% Cow's milk	3.4	–	–
50/50 Soybean soymilk	4.4[b]	–	–
25/75 Soybean soymilk	3.9[b]	–	–
25/25 Soy flour soymilk	2.4[b]	–	–
50/50 Soy flour soymilk	2.7[b]	–	–
75/25 Soy flour soymilk	3.1[b]	–	–
Pure milk curds[c]			
100% Soybean soymilk, $MgSO_4$	34,9	7.6	54.3
100% Soybean soymilk, $CaSO_4$	35.3	6.6	53.8
100% Cow's milk, Rennet	29.9	20.4	45.6
Curds from blends containing soybean soymilk[c]			
50/50 Blend, $CaSO_4$	26.7	9.8	54.3
50/50 Blend, $MgSO_4$	26.6	9.4	54.7
25/75 Blend, $CaSO_4$	22.8	17.4	54.3
25/75 Blend, $MgSO_4$	20.4	17.4	55.9
Curds from blends containing full-fat flour soymilk[c]			
75/25 Blend, $MgSO_4$	16.9	23.0	57.0
50/50 Blend, $MgSO_4$	18.4	26.1	53.6
25/75 Blend, $MgSO_4$	19.6	28.4	50.2

[a] Key for blends: first number ferers to soymilk proportion.
[b] Calculated value, from blend composition and protein content of pure components.
[c] Type of coagulant used Indicated after blend composition. In the case of blends, the other coagulant used was rennet, as noted in text.

SUMMARY & CONCLUSIONS

MIXED SOY/COW'S MILK curds can be prepared utilizin the method described in this paper. The method appears t work whether soymilk prepared from soybeans or ful fat soy flour is used, and curd characteristics (penetratio and yield) are approximately the same in both case

In curds prepared from blends containing soybean soy milk, curd consistency increased (penetration decreasec by using magnesium sulfate as soymilk coagulant; in curc from blends containing full-fat soy flour soymilk, cur consistency increased with increasing rennet concentratio curdling temperature and blend soymilk proportion, an decreased with increasing acidity, magnesium sulfate dos and curdling time. In curds prepared from blends coi taining soybean soymilk, yield decreased when using ma nesium sulfate as soymilk coagulant, and also decrease with increasing curdling temperature; in curds made fro blends containing full-fat soy flour soymilk, yield decrease with increasing acidity, rennet concentration and blen soymilk proportion. In all of these cases, however, larg changes in independent variables (23 - 230%) produce small changes in dependent ones (1 - 16%).

Due to their good consistency and compactness, bes curds were judged by the researchers to be those with min mum penetration, because of ability of converting them t hard cheese. Conditions for obtaining these curds, from th above results, were as follows: titratable acidity, 3.5% rennet concentration, 0.042 g/L of cow's milk in blen magnesium sulfate concentration, 4.8 g/L of soymilk i blend; curdling tmperature, 37°C; curdling time, 1.5 h maximum proportion of soymilk in blend. Similarly, coi ditions for obtaining maximum curd yield were: acidity 3.5%; rennet concentration, 0.021 g/L of cow's milk i blend; use of calcium sulfate as soymilk coagulant, inde pendent of concentration; and minimum blend proportio of soymilk. Characteristics of best curds were: blends cor taining soymilk prepared from soybeans, penetration 138 and yield = 11.51%; blends containing soymilk pre pared from full-fat soy flour, penetration = 122 and yield

Table 12—Results of protein balance calculations in preparation o pure and mixed milk curds

System[a]	Percent total protein recovered in curd
Pure milk curds[b]	
100% Soybean soymilk, $MgSO_4$	86.4
100% Soybean soymilk, $CaSO_4$	86.2
100% Cow's milk, rennet	87.1
Curds from blends containing soybean soymilk[b]	
50%50 Blend, $CaSO_4$	71.9
50%60 Blend, $MgSO_4$	73.6
25/75 Blend, $CaSO_4$	61.7
25/75 Blend, $MgSO_4$	69.0
Curds from blends containing full-fat soy flour soymilk[b]	
75/25 Blend, $MgSO_4$	83.1
50/50 Blend, $MgSO_4$	81.4
25/75 Blend, $MgSO_4$	76.9

[a] Key for blends: first number refers to soymilk proportion in blend.
[b] Type of coagulant employed indicated next to system composition. In the case of blends, the other coagulant used was rennet, as noted in text.

11.55%. Unfortunately, these characteristics could not be compared with those of pure (i.e., 100% soy or cow's) milk curds, since the latter were not prepared.

In runs utilizing soymilk prepared from soybeans, as in those in which soymilk manufactured from full-fat soy flour was used, curd penetration and yield were not correlated.

The order of decreasing protein content, in the case of pure milks, was as follows: soybean soymilk, cow's milk and full-fat soy flour soymilk. Curds prepared from these milks reflected the following characteristics: curd protein content paralleled milk protein content; curds prepared from soybean soymilk had higher protein, lower fat and somewhat higher moisture content than the cow's milk curd; and type of soymilk coagulant employed had little effect on curd proximate analysis.

On the other hand, the following was true concerning curds prepared from soy/cow's milk blends: curd protein content paralleled blend protein content; curds made from blends containing full-fat soy flour soymilk had lower protein, higher fat, and approximately the same moisture content as those prepared from soybean soymilk blends; all mixed curds had lower protein content than pure milk curds; and type of soymilk coagulant employed had no effect on curd protein content.

Protein recoveries in pure milk curds were approximately equal, whether cow's milk was curdled with rennet or soymilk was curdled with calcium or magnesium sulfates. Protein recovery in mixed curds was lower than in pure milk curds, decreasing with increasing blend proportion of cow's milk, and was higher in those prepared from full-fat soy flour soymilk. Type of soymilk coagulant had no effect on pure milk protein recovery; on the other hand, in mixed curds, higher protein recoveries were obtained when utilizing magnesium sulfate for this purpose.

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was greater for polyphosphate at the 0.5% level. For acetic acid and ascorbic acid, H_2S formation prevention was more prominent than chelating. Ascorbic acid enhanced H_2S production in the liquid egg mixtures during cooking, while a H_2S reducing effect was observed for the cysteine solution.

Adjusting pH of the cysteine solutions between 2.5 and 5.5 did not affect their H_2S production during heating ($P > 0.05$). A further pH increase of the cysteine solutions to 7.5 significantly ($P < 0.05$) increased the H_2S content of heated solutions (Fig. 3).

At lower concentrations, H_2S probably contributes to the flavor of all heated proteinaceious foods; while at high levels, the objectionable odor of H_2S is detrimental to the flavor of such foods (Johnson and Vickery, 1964). Results reported in this study have shown that the H_2S content of egg mixtures can be altered through pH adjustment or the addition of additives at the proper levels.

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11.55%. Unfortunately, these characteristics could not be compared with those of pure (i.e., 100% soy or cow's) milk curds, since the latter were not prepared.

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heat in a water bath at 90° for exactly 5 min. Cool immediately to below 20° in an ice bath for 5 min, add 3 drops of *p*-phenylphenol TS, shake immediately, and heat in a water bath at 30° for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 s, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1-cm cell, at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in μg, of lactic acid in the portion of the *Test Preparation* taken for the *Procedure* by means of the *Standard Curve*.

Packaging and Storage Store in tight containers in a cool, dry place.

Calcium Sulfate

$CaSO_4.xH_2O$ Formula wt, anhydrous 136.14

INS: 516 CAS: anhydrous [7778-18-9]

DESCRIPTION

Calcium Sulfate is anhydrous or contains two molecules of water of hydration. It occurs as a fine, white to slightly yellow-white, odorless powder.

Functional Use in Foods Nutrient; dietary supplement; yeast food; dough conditioner; firming agent; sequestrant.

REQUIREMENTS

Identification Dissolve about 200 mg by warming with a mixture of 4 mL of 2.7 *N* hydrochloric acid and 16 mL of water. A white precipitate forms when 5 mL of ammonium oxalate TS is added to 10 mL of the solution. Upon the addition of barium chloride TS to the remaining 10 mL, a white precipitate forms that is insoluble in hydrochloric and nitric acids.
Assay Not less than 98.0% of $CaSO_4$, calculated on the dried basis.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 10 mg/kg.
Loss on Drying $CaSO_4$ (anhydrous): not more than 1.5%; $CaSO_4.2H_2O$ (dihydrate): between 19.0% and 23.0%.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve 250 mg, accurately weighed, in 100 mL of water and 4 mL of 2.7 *N* hydrochloric acid, boil to effect solution, and cool. While stirring, preferably with a magnetic stirrer, add about 30 mL of 0.05 *M* disodium EDTA from a 50-mL buret, then add 25 mL of 1 *N* sodium hydroxide and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each mL of 0.05 *M* disodium EDTA is equivalent to 6.807 mg of $CaSO_4$.

Fluoride Weigh accurately 1.67 g, and proceed as directed in the *Fluoride Limit Test*, Appendix IIIB.
Heavy Metals Mix 2 g with 20 mL of water, add 25 mL of 2.7 *N* hydrochloric acid, and heat to boiling to dissolve the sample. Cool, and add ammonium hydroxide to a pH of 7. Filter, evaporate to a volume of about 25 mL, and refilter if necessary to obtain a clear solution. This solution meets the requirements of the *Heavy Metals Test*, Appendix IIIB, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, Appendix IIC Dry at 250° to constant weight.
Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, Appendix IIIB, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Cananga Oil

CAS: [68606-83-7]

DESCRIPTION

The oil obtained by distillation from the flowers of the tree *Cananga odorata* Hook f. et Thoms., (Fam. *Anonaceae*). It is a light to deep yellow liquid having a harsh floral odor suggestive of ylang ylang. It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

Functional Use in Foods Flavoring agent.

REQUIREMENTS

Identification The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum in the section on *Infrared Spectra, (Series A: Essential Oils)*, using the same test conditions as specified therein.
Angular Rotation Between −15° and −30°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.495 and 1.505 at 20°.
Saponification Value Between 10 and 40.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.904 and 0.920.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical (Specific) Rotation*, Appendix IIB.
Heavy Metals Shake 10 mL of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, Appendix IIB Determine with an Abbé or other refractometer of equal or greater accuracy.

Preparation of High Protein Curd from Field Peas

A. GEBRE-EGZIABHER and A. K. SUMNER

ABSTRACT

Field peas were investigated as an alternative to soybeans to produce a high protein curd which resembles tofu. Yield, texture, color, proximate composition and sensory evaluation of both curds were compared. The yields of total curd from pea flour and soybeans were 13.6% and 39.8% respectively with protein yields of 43.0% and 55.5%. Amino acid composition of the pea curd compared quite closely to that of the soybean curd. Addition of gluten improved the sulfur amino acid profile but reduced the lysine content. Flavor of the pea and soybean curds was rated similar but texture and color of the pea curd was scored lower ($p < 0.05$). Gluten modified the texture and the color of the curds.

INTRODUCTION

EXPANDING world food requirements have increased interest in the use of leguminous seeds for high protein foods. Soybeans and their products have played an important role for many centuries as a source of protein in the diet of Oriental people (Smith and Circle, 1972). Field peas (*Pisum sativum*) are being evaluated as a high protein crop for foods and feed in some areas where soybeans cannot be grown.

The market for field peas is being expanded by the development of new products such as fortified bread, meat extenders, snacks and beverages (Youngs, 1975; Nielsen et al., 1980; Sumner et al., 1981). It is possible that field peas may be suitable for the production of high protein food curd similar to tofu.

In recent years soy foods in general and tofu in particular have been receiving considerable attention in North America. Tofu is one of the most important soy foods which consists of a bland, cheese-like curd. It is prepared by adding a precipitant to the water extract of soybeans and molding the resulting high-protein curd into cakes (Smith et al., 1960; Lu et al., 1980). Tofu is highly digestible and relatively inexpensive and is used in a variety of Chinese dishes (Miller et al., 1952). Fresh tofu contains about 6–8% protein, 3.5% oil, 1.9% carbohydrate, 0.6% ash and 88.0% water (Smith and Circle, 1972).

The objective of this study was to investigate whether field peas could produce a curd similar to tofu. Also, the effect of wheat gluten on soybean and pea curds was investigated on the basis of nutritional and physical properties. All products were compared on the basis of yield, composition, texture, color and sensory evaluation.

MATERIALS & METHODS

Materials

Pea flour and pea protein concentrate were prepared by the Prairie Regional Laboratory of the National Research Council, from field peas (*Pisum sativum* var. Trapper) by the procedure of Youngs (1975). Dry whole soybeans (Grade No. 1) were purchased from Early Seed and Feed Ltd., Saskatoon, Canada. Vicrum vital gluten containing 80.0% protein was provided by J.R. Short Canadian Mills Ltd., Toronto, Canada.

Preparation of curds

To determine the effect of coagulant type and concentration on pea curd properties, preliminary trials were carried out at coagulant concentrations in the extract of 0.15–0.54%. The coagulants evaluated included reagent grade calcium sulfate, calcium chloride and acetic acid obtained from Fisher Scientific Co.

Preparation of the pea flour extract was carried out as described by Sumner et al. (1981) and consisted of extracting 100g of flour with 500 ml of distilled water adjusted between pH 8.8–9.0 with 0.2% calcium oxide while stirring for 20 min. The extract was separated by centrifuging at 1000 x *g* for 20 min. The resulting solution was heated for 20 min at 95–100°C and then filtered through a double layer of cheesecloth. The pea extract was cooled to 75–80°C, stirred vigorously for about 20 sec; agitation was stopped and the curd was then precipitated by 0.54% coagulant concentration using the procedure described by Lu et al. (1980). When formed, the curd was then transferred into Tyler 20-mesh screens (12.5 cm diam and 6.5 cm deep) lined with cheesecloth. A circular plywood disc was placed on the top of the curd and pressed with a 275g weight for 1–2 hr until draining almost stopped.

When preparing the soybean curd, 50g of dry whole soybeans were washed and soaked in water at room temperature overnight. Next day the water was discarded and the soaked beans were transferred to a Waring blendor and water was added in the ratio of 10:1 (water:dry soybeans). The curd was then precipitated, separated and drained using the procedure previously described.

Yield

Curd yield was expressed as the percentage of the original pea flour or soybeans recovered in the final curd (dry basis). The protein yield was expressed as the percentage of the original pea flour or soybean protein recovered in the curd (dry basis).

Proximate analysis

Moisture content was determined by drying a 5–10g sample of curd at 100 ± 1°C to constant weight.

The proximate composition was determined by standard AACC (1976) procedures for crude protein (method 46-11), crude fat (method 30-25), crude fiber (method 32-15) and ash (method 08-11). Protein calculation was based on nitrogen factors of 5.7 for wheat and 6.25 for pea flour and soybeans. Nitrogen factors for blends were weighted on the basis of the relative proportion of the proteins in the ingredients. All analyses were carried out in duplicate.

Amino acid analysis of the curds was carried out on a Beckman Model 120c analyzer using the procedure described by Sosulski and Sarwar (1973). Results were corrected to 100% nitrogen recovery and expressed as grams amino acid per 16 g nitrogen. The chemical score for these products reflects the nutritional value and was calculated as described by Block and Mitchell (1946). The FAO/WHO (1973) provisional pattern of essential amino acids was used as the reference protein.

Texture

Textural quality was measured by a Texturecorder Model T-2100 using the CE-1 Universal cell with a parallel extrusion grid base and the following conditions: 300 lb ring, ram speed 0.7 cm/sec, and ram force 100 lb. Replicate curd samples were 5 cm diam x 1 cm thick. Maximum shear stress is reported in Newtons (N)/cm^2.

–Continued on next page

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The color of the curd was measured with the Hunterlab Model D25 D2M Color and Color Difference Meter. The L a b values were an average of four readings obtained while rotating samples 90° between readings.

Sensory evaluation

The sensory evaluation panel was composed of 25 adult males and females who were not familiar with tofu. Curd samples measuring 2.5 x 2.5 x 1 cm were fried on each side for 2 min in an all purpose, deep frying vegetable oil, in a Hoover stainless steel frying pan set at 375°F (190.5°C). Panel members evaluated the flavor, texture, color and acceptability of the pea and soybean curds alone and in combination with gluten using a 7-point scale in which 1 = extremely good, 2 = very good, 3 = slightly good, 4 = average, 5 = slightly poor, 6 = poor, 7 = extremely poor. Scores were subjected to analysis of variance and Tukey's least significant difference test (Larmond, 1977).

RESULTS & DISCUSSION

Curd coagulants

Preliminary trials were carried out to determine the most suitable coagulant and concentration for producing pea curd. Calcium sulfate, calcium chloride and acetic acid in concentrations ranging from 0.15–0.54% were used to precipitate pea flour protein. Curd produced by acetic acid had a firmer texture than when calcium salt coagulants were used, but it was easily broken and had a sour taste when the coagulant concentration exceeded 0.27%. For subsequent trials, calcium sulfate was used to precipitate the protein curd because it appeared to be the most commonly used salt in the production of traditional tofu (Wang, 1967; Schroder et al., 1973; Tsai et al., 1981).

Table 1 summarized the relationship between the concentration of calcium sulfate used and the characteristics of the resulting pea curd. The moisture content of the curd decreased from 86.4% to 83.8% with increasing concentration of the coagulant from 0.15% to 0.54%. On the other hand, protein yield increased from 36.8% to 43.0% and curd yield increased from 9.8 to 13.6% as the concentration of the coagulant increased over the range investigated. The lower yield and the turbid filtrate that appeared, when 0.15% calcium sulfate was used, indicated incomplete coagulation of the protein. The protein yield was slightly lower than yields reported for soybean curds by Hang and Jackson (1967).

Table 1—Effect of calcium sulfate concentration on pea flour curd properties

Coagulant conc (%)	Moisture (%)	Protein yield (%)	Curd yield (%)	Shear stress (N/cm^2)
0.15	86.4 ± 1.0[a]	36.8 ± 1.4	9.8 ± 2.1	1.11 ± 0.15
0.27	85.8 ± 0.3	37.1 ± 1.8	11.8 ± 1.8	1.48 ± 0.25
0.40	85.0 ± 0.4	40.0 ± 2.0	13.3 ± 2.5	1.74 ± 0.19
0.54	83.8 ± 0.8	43.0 ± 1.4	13.6 ± 1.3	2.09 ± 0.08

[a] Mean ± standard deviation of four batches.

The highest moisture curd resulted in a soft texture. An increase in the amount of coagulant concentration from 0.15% to 0.54% led to an increase in curd shear stress from 1.11 to 2.09 N/cm^2. Lu et al. (1980) in their studies of soybeans, reported that calcium salt concentrations ranging from 0.10–0.50% were suitable for soybean curd preparation.

From the trial data, it was found that best curd on the basis of curd yield, protein yield, firmness and smooth texture, was formed when the pea protein was coagulated at 75–80°C by the addition of a 2% calcium sulfate solution until it reached a concentration of 0.54% in the extract. These conditions were used to evaluate field pea and soybean curds in subsequent studies.

Comparison of pea and soybean curds

Pea flour containing 24% protein was compared to pea protein concentrate with about 60% protein for producing high protein curd. As expected, the curd yield from pea protein concentrate was about three times greater than from pea flour and was quite similar to the soybean curd yield. Protein yield, texture and other properties of the two pea curds were similar. Remaining trials were carried out with pea flour rather than pea protein concentrate which is more expensive to produce.

Table 2 compares the yield and properties of curds prepared from the pea flour and soybeans. The moisture contents of the pea and soybean curds were 83.8% and 79.5%, respectively. Curd made from peas was softer in texture than soybean curd as shown by shear stress values of 2.09 and 5.40 N/cm^2 respectively.

Although there is no recognized standard of identity for tofu, generally commercially available hard tofu contains from 79–75% moisture while the water content of soft tofu ranges from 88–82% (Shurtleff and Aoyagi, 1979). Moisture contents of 84.7% and 85.9% for tofu products reported by Tsai et al. (1981) and Smith et al. (1960), were similar to the pea curd moisture.

The yields of curds from the pea flour and soybeans were 13.6% and 39.8% respectively with protein yields of 43.0% and 55.5%. The lower curd yield from pea flour was due to the lower protein content and also to the large amount of carbohydrate residue removed during the curd preparation. Schroder and Jackson (1972), in their study of soybeans, obtained a curd yield of 31.7%.

Results of the curd color measurements (Table 2) showed that soybean curd had a light yellow color whereas the pea curd was gray in color. The undesirable gray color may have been caused by polyphenols which oxidize readily at a high pH. A better color might be achieved at a lower pH.

Effect of gluten on curd properties

Preliminary trials were carried out to determine the best concentration of gluten to be added for texture modification by incorporating 7.5, 15.0 and 30.0g of gluten into the pea and soybean extracts equivalent to concentrations of 2.0, 4.0 and 8.0% (w/v). The data showed that firmer and smoother textured curd was formed when 4.0% gluten was

Table 2—Comparison of curds prepared from pea flour and soybean alone and in combination with wheat gluten

Sample	Moisture (%)	Curd yield (%)	Shear stress (N/cm^2)	Color[a]		
				L	a	b
Pea curd	83.8 ± 0.8[b]	13.6 ± 0.5	2.09 ± 0.08	51.9	−1.3	8.0
Pea/gluten curd	78.7 ± 1.0	27.7 ± 2.8	3.79 ± 0.32	61.3	−1.5	12.2
Soybean curd	79.5 ± 0.9	39.8 ± 1.0	5.40 ± 0.18	70.6	−0.4	18.0
Soybean/gluten curd	72.7 ± 0.5	50.5 ± 2.1	5.66 ± 0.41	71.0	−0.3	13.1

[a] L (100 white, 0 black); a (+ red, − green); b (+ yellow, − blue).
[b] Mean ± standard deviation of four batches.

Table 3—Compositions of pea flour, soybeans and curds

Constituents (dry basis)	Pea flour	Pea curd	Soybeans (dry, whole)	Soybean curd
Crude protein %	23.9	81.4	41.3	57.3
Crude fat %	1.5	3.7	19.6	29.0
Crude fiber %	3.8	0.3	5.1	0.0
Ash %	2.7	5.3	4.6	5.6
Nitrogen free extract %	68.1	9.3	29.4	8.1

added to the soybean and pea extracts. When 2.0 and 8.0% gluten were incorporated, the resulting curds were softer and lacked elasticity.

Table 2 also compares some properties of the pea and soybean curds when 4.0% gluten was incorporated into the extracts. Gluten decreased the moisture in these curds by 5.1 and 6.8 percentage points respectively. This increased the firmness of pea curd from 2.09 to 3.79 N/cm^2 but had little effect on the soybean curd. The higher gluten recovery compared to pea flour increased the pea/gluten curd yield to 27.7% compared to 13.6% for pea curd. The corresponding increase for soybean/gluten curd was from 39.8% to 50.5%. Gluten increased the lightness and yellow appearance of pea curd so that it resembled more closely the soybean curd.

Chemical composition

Proximate analysis of pea flour, soybeans and the curds produced from them are shown in Table 3. There was a concentration of protein in the pea curd to 81.4% compared to 23.9% in pea flour because the carbohydrates and crude fiber were largely removed during the preparation of the curd. Pea curd contained 3.7% crude fat which indicated most of the lipids remained with the protein. The soybean curd, on a moisture-free basis, contained 57.8% crude protein, 29.0% crude fat and no crude fiber. These values were similar to those reported by Smith et al. (1960) and Schroder and Jackson (1972). The major differences between the pea and soybean curd were the higher protein and lower crude fat content in the pea curd. However, it was found that the crude fat content of pea curd could be increased to about the level in soybean curd, with a corresponding decrease in protein, by the addition of Canola (rapeseed) oil to the extract prior to coagulation.

The high ash content in both curds resulted from the calcium sulfate used for the precipitation. Soybean curd prepared in this way is considered to be a good source of calcium in countries where the milk supply is low or expensive (Miller et al., 1952; Chiu and Van Duyne, 1961).

Table 4 presents the essential amino acid composition and chemical scores of the curds prepared from pea flour and soybeans alone and in combination with gluten. The essential amino acid profile of the FAO/WHO reference protein is also shown. In general, the amino acid distribution of the pea curd was quite similar to soybean curd. The combined sulfur amino acids methionine and cystine were the first limiting amino acids for the curds which did not contain gluten. Pea curd was the most deficient with a sulfur amino acid content of 2.1 g/16g nitrogen and soybean curd contained 2.6 g/16g nitrogen compared to the recommended reference protein at 3.5 g/16g nitrogen. This resulted in chemical scores of 60 and 75 respectively for the two curds. Threonine was limiting also in the pea curd. Unlike cereal proteins, the pea and soybean curds with lysine contents of 7.2 and 6.3 g/16g nitrogen respectively, surpassed the reference protein requirements. The data on amino acid analysis of the soybean curds was in general agreement with those reported by Hackler and Stillings (1967) and Schroder and Jackson (1972).

Table 4—Essential amino acid distribution and chemical score of curds

Sample	Amino acids (g/16 g N)[b] Lys	Met + Cys	Thr	Iso	Leu	Tyr + Phe	Val	Chem. score
Reference[a] protein	5.5	3.5	4.0	4.0	7.0	6.0	5.0	100
Pea curd	7.2	2.1	3.7	4.5	8.2	9.5	5.0	60
Pea/gluten curd	3.5	3.0	2.7	4.0	7.0	8.4	4.3	64
Soybean curd	6.3	2.6	4.1	4.8	8.0	9.1	5.1	74
Soybean/gluten curd	3.9	3.1	3.0	4.0	7.3	9.1	4.3	71

[a] FAO/WHO (1973).
[b] First limiting amino acid underlined.

Table 5—Sensory evaluation of curds prepared from pea flour and soybean alone and in combination with gluten

Sensory property	Rating scores[ab] Pea curd	Pea/gluten curd	Soybean curd	Soybean/gluten curd
Flavor	2.72a	2.64a	2.12a	2.12a
Texture	3.20b	3.12b	2.00a	1.96a
Color	2.96b	2.76b	1.44a	1.76a
Ranking score	3.08b	3.04b	1.88a	2.00a

[a] 1 = Extremely good, 7 = Extremely poor.
[b] Means in the same row followed by different postscripts differ significantly ($p < 0.05$).

Amino acid analysis of curds containing gluten was also carried out to determine if a better essential amino acid balance was obtained. These curds were prepared by the addition of 8.0% (30g) gluten to the pea and soybean extracts which provided an initial gluten protein concentration similar to the protein contents in the legume ingredients. Gluten improved the sulfur amino acid balance from 2.1 to 3.0 g/16g nitrogen for the pea/gluten blend and from 2.6 to 3.1 g/16g nitrogen for the soybean blend (Table 4). However, because of the low lysine content in gluten, lysine became the limiting amino acid for both of the blended curds while the chemical scores remained almost unchanged. The essential amino acid profiles and chemical scores would be improved if less gluten was added. On the basis of the values in Table 4, it was estimated that 4% (15g) gluten added to the extracts would increase the chemical score of the pea/gluten curd to 73% and the soybean gluten curd to 81%.

Sensory evaluation

Sensory evaluations of freshly prepared pea and soybean curds, with and without gluten, are summarized in Table 5. In general, judgements for the sensory properties of all samples ranged from a mean of about 2 (very good) to 3 (slightly good). Addition of gluten did not result in any significant ($p < 0.05$) change. The panelists did not detect any significant ($p < 0.05$) difference in the flavor of any of the four samples, but the soybean curds received a slightly better mean score. Texture and color of both soybean curds were judged to be significantly ($p < 0.05$) better than the corresponding pea curds. When the 25 panel members were asked if any of the curd samples were unacceptable, three judged the pea and two judged the soybean curds to be unacceptable, but this was not significant.

—Continued on page 388

Yield and Textural Properties of Soft Tofu as Affected by Coagulation Method

H.J. HOU, K.C. CHANG, and M.C. SHIH

ABSTRACT

Soft tofu was made using two coagulants (calcium sulfate and modified nigari), three stirring speeds (137, 207, and 285 rpm), and six stirring times (5, 10, 15, 20, 25, and 30 sec). The lowest stirring speed, 137 rpm, did not coagulate the soymilk. Tofu made by the highest stirring speed (285 rpm) had a lower yield, but higher brittleness force, hardness and elasticity than tofu made at 207 rpm. Tofu made from modified nigari had lower textural parameter values than those made from calcium sulfate. Yield of tofu made from both coagulants stirred at 207 or 285 rpm decreased as stirring time increased to 30 sec. Textural properties were related to stirring time. Stirring time < 25 sec was appropriate for soft tofu making.

Key Words: soybean, soft-tofu, coagulation

INTRODUCTION

TOFU has been an important source of protein in Asia for many years. Major steps in tofu making have changed little for more than 2000 years (Wang and Hesseltine, 1982). There are many types of fresh tofu and tofu derivatives with different textures, compositions, processing, and packaging methods.

Soft tofu is a type of fresh tofu, which contains about 89% moisture, 6% protein, and 2–3% lipid (Saio, 1979). Soft tofu is normally processed by pressing unbroken bean curd to remove whey, which has a soft texture but is firm enough to be cut into small pieces and packed in containers with water. Soft tofu is usually eaten directly, topped with a little soy sauce, or is prepared for soup (Tsai et al., 1981).

Generally, tofu making procedures include soaking, grinding beans in water, filtering, boiling, coagulation, and pressing. Researchers have investigated effects of processing conditions, including water to bean ratio (Beddows and Wong, 1987a), heat processing (Beddows and Wong, 1987b), type and concentration of coagulants (Tsai et al., 1981; deMan et al., 1986; Lim et al., 1990; Sun and Breene, 1991; Shen et al., 1991), stirring speed and time of coagulation (Beddows and Wong, 1987a; Wang and Hesseltine, 1982), and pressing time and pressure (Gandhi and Bourne, 1988).

The quality of tofu products was significantly influenced by type of coagulant (Tsai et al., 1981). Calcium sulfate and bittern (nigari, in Japan) were suitable coagulants for making Chinese-style tofu (regular tofu), but glucono-delta-lactone (GDL) was not. Bean curd made with $CaCl_2$ and $MgCl_2$ was coarse, granular, and hard, whereas calcium sulfate and GDL gave a smooth, soft, and uniform curd (deMan et al., 1986).

Coagulation of soymilk is the most important step in tofu-making and the most difficult to control because it depends on complex interrelationships of many variables. Increasing coagulation temperature increases hardness, and increasing the rate of stirring immediately after adding coagulant also increases hardness (Saio, 1979). Stirring method and mixing speed and time had a significant effect on tofu yield and quality (Shurtleff and Aoyagi, 1990). Traditionally, coagulant is added to soymilk, which is stirred vigorously by hand with a paddle and the stirring is continued, vigorously six to eight more times (Shurtleff and Aoyagi, 1990). Alternatively, coagulant and soymilk are poured simultaneously into a container from a height of ~1 meter for ensuring mixing (Lim et al., 1990).

A complex interaction of several chemical factors take place in making regular, soft, and other tofu products. Most tofu studies (Wang and Hesseltine, 1982; Beddows and Wong, 1987a, b, c; Shen et al., 1991) have focused on regular tofu and have used small laboratory-scale conditions. In general, laboratory scale methods using small amounts of beans (80g to 150g) are difficult to reproduce due to the complex factors involved. Generally, small scale methods for evaluating tofu have not described processing steps in detail. Variations in tofu making procedures and new soybean materials have caused difficulties in comparing inter-laboratory results. Scale-up methods, using larger amounts of beans and motorized stirrers for coagulation may be more reproducible for evaluation tofu making. The objectives of this study were to investigate how yield and texture characteristics of soft tofu made by a pilot-plant scale method were affected by types of coagulants, stirring speeds, and stirring times.

MATERIALS & METHODS

Materials

Soybeans of the Proto cultivar were obtained from Sinner Brothers & Bresnahan Company (Casselton, ND). The coagulants used were food grade modified nigari (Ca^{++} 14.73g/100g dry basis; Mg 0.22g/100g dry basis) obtained from Taiwan Salt Workers (Tainan, Taiwan) and food grade purified calcium sulfate (Ca^{++} and Mg^{++} 16.53, 0.01g/100g dry basis, respectively) from Koah Co. (Wakayama, Japan). Antifoaming agent, containing 89.5% glycerol fatty acid ester, 8% lecithin, 2% $MgCO_3$, and 0.5% silicon resin, was obtained from Koah Co. (Wakayama, Japan).

Preparation of soymilk

Proto soybeans (1992) (900g) were washed and soaked in tap water for 8 hr at room temperature (20–22°C). The hydrated beans were drained and ground with tap water (five times dry bean weight), using a soymilk grinder/extractor (Chang-Seng Mechanical Company, Taoyuan, Taiwan). The grinder/extractor, equipped with a 0.15 mm screen, could separate soymilk automatically from the residues. The volume and solids content of the collected soymilk were measured.

Preparation of soft tofu

The solids content of soymilk was adjusted to 12°Brix with a refractometer (Auto Abbe Refractometer, Model 10500, Leica Company, Buffalo, NY). A 4.5L sample of soymilk was placed in a stainless steel pot (diam 21.5 cm × ht 22 cm) and heated on an electric stove to 95°C in 20 min with constant stirring and maintained at 95°C for 5 min. After heating, soymilk was cooled to 82°C with constant stirring at room temperature. A stirrer (Model RZR1, rpm 35-250/280-2200, Caframo LTD, Wiarton, Ontario, Canada), equipped with a paddle (7 cm × 7 cm) fixed at 4 cm from the bottom of the pot, was used for stirring. In order to increase turbulence of the mixture, a baffle (1 5 cm × w 6 cm) was placed into the soymilk against the pot.

A coagulant suspension of 13.5g (~0.3% soymilk weight) calcium sulfate ($CaSO_4 \cdot 2H_2O$) or modified nigari in 135 mL distilled water was prepared. The coagulant suspension was poured into soymilk while it

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was being stirred at speed settings 3, 6 or 9 (137 rpm, 207 rpm, or 285 rpm, respectively).

After addition, stirring was continued for 5, 10, 15, 20, 25, or 30 sec, and the mixture was poured immediately into a muslin-cloth-lined wooden mold (25 × 25 × 7 cm), which was lined with a plastic sheet. The curd was left for 10 min at 75 to 80°C for coagulation. After coagulation, the plastic sheet was removed from the mold, and the cloth was folded over the top. The curd was covered with a piece of wooden board, and pressed by placing a weight of 21.8 g/cm² for 10 min, adding to 43.6 g/cm² another 10 min, and adding to 65.4 g/cm² for 30 min to separate whey from curd. The weight of freshly formed tofu was recorded. The tofu was cooled in water to about 20°C in 1 hr, then stored at 5°C for 24 hr, and analyzed for textural properties. The tofu yield was expressed as g tofu/100g raw soybeans.

Determination of textural properties

The textural properties were measured using an Instron Universal Testing Machine (Model 1011, Instron Corporation). Cylindrical samples (5 cm diam × 1.5 cm ht) were cut from the central portion of tofu cake with a stainless steel cylindrical cutter. Three samples were taken from the center of one curd of tofu, and each cylindrical sample was cut into two parts: top and middle. A cylindrical plunger with 5 cm diameter and a weight beam of 5 kg was used. The speed of the crosshead and the recording chart was set at 20 mm/min. The plunger traveled 75% depth into the tofu sample. Textural properties including hardness, brittleness, and elasticity were calculated from the curve according to Bourne (1978).

Statistical analysis

All treatments were evaluated in duplicate. Data were evaluated using the Statistical Analysis System program (SAS Institute, Inc., 1985). Analysis of variance (ANOVA) was conducted, and differences between group means were analyzed by the Duncan Multiple Range Test. Statistical significance was established at $p \leq 0.05$. Linear regression analysis was used to determine apparent correlations among measurements.

RESULTS & DISCUSSION

Yield of soft tofu

At the lowest stirring speed (137 rpm), soymilk did not coagulate completely after mixing with coagulant suspension, even when stirring time was increased to 30 sec (Table 1). Therefore, tofu could not be produced at this low speed. Beddows and Wong (1987c) reported that there were more soluble proteins uncoagulated when coagulant was added at slower stirring speeds. Because the solubility of the coagulants was very low, the stirring was necessary to keep the coagulant suspended. Speed of stirring had to be sufficient to maintain uniform distribution of coagulant in the soymilk.

However, it is very important to mix soymilk with coagulant in a short period of time to homogeneity to produce a high yield of tofu, because prolonged stirring might break the curd. Bean curd still did not form at stirring speed 137 rpm even with stirring times increased to 30 sec. High yields of tofu were obtained when stirring time was within 25 sec for 207 rpm and within 15 sec for 285 rpm. A notable decrease of yield was found as stirring time increased to 30 sec for both stirring speeds (Table 1). Thus, higher stirring speeds and shorter stirring times were better for making high yields of tofu.

The yields of soft tofu made from $CaSO_4$ ranged from 535 to 469g whereas yields from modified nigari ranged from 541 to 461 g/100g beans (Table 1). These values were higher than reported values. Beddows and Wong (1987c) reported yields of silken tofu from $CaSO_4$ ranged from 3.01 to 4.09 (g/g). Shurtleff and Aoyagi (1990) stated that bulk yield for soft tofu made by $CaSO_4$ ranged from 3.9 to 4.5 (g/g). However, Lim et al. (1990) reported that fresh yield of tofu from different soybean varieties by $CaSO_4$ ranged from 4.46 to 5.26 (kg/kg).

At 207 rpm, yields of coagulants were not significantly different at stirring times ranging from 5 to 25 sec, but decreased after stirring 30 sec (Table 1). At 285 rpm, yields were not affected by stirring for 5 to 15 sec, but decreased after stirring 25 sec. Tofu made with stirring time 30 sec at both speeds had coarse and grainy texture by visual examination. However, tofu texture appeared smooth when made with stirring times from 5 to 20 sec.

Table 1—Yield (g/100g bean) of fresh soft tofu

Stirring time (sec)	Calcium sulfate			Modified nigari		
	137 rpm	207 rpm	285 rpm	137 rpm	207 rpm	285 rpm
5	—[e]	529.3[a] (10.0)	527.0[a] (5.6)	—	538.7[a] (11.7)	534.7[a] (7.8)
10	—	527.3[a] (13.1)	533.0[a] (7.5)	—	533.0[a] (0)	534.7[a] (11.7)
15	—	535.0[a] (8.7)	522.0[ab] (13.5)	—	538.7[a] (7.8)	535.0[a] (8.7)
20	—	519.7[a] (11.7)	500.7[bc] (11.7)	—	541.0[a] (8.0)	532.0[a] (9.5)
25	—	512.0[a] (11.8)	488.3[cd] (17.6)	—	540.0[a] (9.5)	511.0[a] (13.5)
30	—	474.0[b] (15.6)	469.0[d] (21.2)	—	513.3[b] (9.6)	461.7[b] (38.1)

[a-d] Means of three replicates, wet weight basis, expressed as means (SD). Means within same column not followed by same letters significantly different ($p<0.05$).
[e] No tofu produced.

The effects of coagulant type on yields was significant (Table 1). The yields of tofu from modified nigari were higher than those from $CaSO_4$, when the stirring time was beyond 20 sec . Tsai et al. (1981) reported that tofu from calcium sulfate had the highest yield among calcium ion coagulants including calcium chloride, calcium acetate, and calcium gluconate. Nigari-type coagulants for tofu making require more care, attention, and time to use, but they give solid yields equal to those using $CaSO_4$, and produce tofu with the finest flavor. Calcium sulfate gave 15 to 20% higher tofu yield than pure nigari (Shutleff and Aoyagi, 1990). Therefore, most tofu makers use nigari in combination with calcium sulfate. The modified nigari we used was a combination with calcium sulfate (Li, 1996).

At 207 rpm stirring, yield of tofu using modified nigari was higher than that using $CaSO_4$ ($p < 0.05$). However, no difference of yield was found between the coagulant types at 285 rpm stirring speed. Results indicated a significant difference of fresh yield between 207 and 285 rpm (Table 2). The higher stirring speed (285 rpm) led to lower yields particularly with stirring time 20 sec or longer. Beddows and Wong (1987c) fixed the stirring time at 30 sec and used only 250 mL soymilk for making tofu. Stirring speed from 240 to 280 rpm was the optimum for solid and protein recovery, yield, and texture. At higher stirring speed (>350 rpm), the yield decreased. Similarly, Watanabe et al. (1964) found that volume of tofu decreased as mixing speed increased from 60 to 120 rpm. Wang and Hesseltine (1982) also reported that stronger mixing resulted in higher hardness of tofu, lower yields and lower moisture content of tofu. Both the studies of Watanabe et al. (1964) and Wang and Hesseltine (1982) only used small amounts (25 and 50 mL, respectively) of soymilk for tofu making. A high stirring speed would lead to overmixing, break the gel matrix of soybean curd and cause partial loss of water-holding capacity during pressing. Although the production scales among the studies were different, the conclusions of the effects due to stirring speed were consistent. Various speeds in rpm were reported, but the dimension of soymilk containers and mixers were not reported.

Textural properties of middle part

Brittleness (fracturability) was defined as the force of the significant break on the first compression cycle, that was the force at which the sample fractured (Bourne, 1978). Hardness was defined as the height of the peak force on first bite, which was the force necessary to attain a given deformation. Elasticity was measured by drawing a perpendicular line from the peak of the second bite and measuring the distance along the baseline from that point back to the point where the plunger contacted the tofu

Table 2—ANOVA for yield of fresh soft tofu

Source	DF	Sum of square	Pr>F[a]
Model	23	35747.7	0.0001
Coagulant	1	3211.9	0.0001
RPM	1	2418.7	0.0006
Time	5	24508.2	0.0001
Coagulant * rpm	1	178.3	0.3254
Coagulant * Time	5	1366.9	0.2033
RPM * Time	5	2576.9	0.0248
Coagulant * rpm * Time	5	1486.9	0.1660

[a] Probability level.

in the second bite. Elasticity was expressed as distance/original height × 100%.

When samples were cut into top and middle parts, the top had a hard surface layer because of pressing during processing. The middle had a homogeneous texture. The values for the middle part texture were lower than those for the top part.

Brittleness force of tofu from $CaSO_4$ decreased as stirring time increased from 5 to 30 sec. A similar tendency was found for tofu from modified nigari (Table 3). The lowest brittleness force was at stirring time 30 sec which also produced the lowest yield. There was no difference of brittleness force between 207 and 285 rpm for either coagulant. In addition, the effect of coagulant types on brittleness force was not significant.

Hardness of $CaSO_4$ tofu made at 207 rpm increased as stirring time increased from 5 to 30 sec. A similar tendency was observed for nigari tofu made at 285 rpm (Table 3). The highest hardness for all treatments appeared with stirring time 30 sec. Tofu from modified nigari, stirring at 207 rpm had the lowest hardness, but the highest yield (Table 1). A difference in hardness ($p < 0.05$) was found between 207 and 285 rpm for both coagulants. Tofu made at 285 rpm had higher hardness than that made at 207 rpm. The effects of coagulant types on hardness was significant ($p < 0.05$). Tofu of $CaSO_4$ was harder than that of modified nigari.

Elasticity of tofu made using $CaSO_4$ ranged from 19.3 to 31.5% with stirring time within 20 sec and increased with stirring time 30 sec (Table 3). There was no difference in elasticity for nigari tofu made by 207 rpm between stirring times from 5 to 25 sec. Nigari tofu made at 285 rpm had consistent elasticity with stirring time 20 sec. The highest elasticity appeared with stirring time 30 sec. High stirring speed increased elasticity of tofu ($p < 0.05$). The effect of coagulant types was significant ($p < 0.05$). In general, tofu made from $CaSO_4$ and made at 285 rpm had higher elasticity.

A positive relationship was found for the middle part of tofu between the following parameters: yield and brittleness force of $CaSO_4$ tofu made at 207 rpm ($r = 0.93$); yield and brittleness force of nigari tofu made at 285 rpm ($r = 0.93$). A tofu with higher yield would have higher water-holding capacity and result in a higher brittleness force. Tofu with lower yield contained less water and was more fragile (lower brittleness force). Hardness and elasticity of tofu except that made from $CaSO_4$ at 285 rpm had negative correlation with yield of tofu (Table 4). A tofu with a lower yield would be more fragile, more elastic and harder.

Textural properties of top part

The brittleness force of tofu made with $CaSO_4$ at both stirring speeds and made with modified nigari at 285 rpm was the lowest at stirring time 25 sec and highest at stirring time 30 sec (Table 5). The result was in contrast to those with the middle part. This may be due to the hard surface layer of the top part. However, tofu made from modified nigari and 207 rpm had lower brittleness force at stirring times 25 and 30 sec and the yield of tofu was still high (Table 5). There was no difference in brittleness force of $CaSO_4$ tofu between 207 and 285 rpm, however, the difference was significant ($p < 0.05$) for nigari tofu. The effect

Table 3—Textural properties of the middle part of soft tofu

Stirring time (sec)	Calcium sulfate		Modified nigari	
	207 rpm	285 rpm	207 rpm	285 rpm
	Brittleness force (g)			
5	737[a] (42)	778[a] (68)	571[b] (33)	659[b] (69)
10	728[a] (59)	752[b] (26)	672[a] (80)	749[a] (73)
15	730[a] (98)	452[c] (23)	574[b] (27)	673[b] (77)
20	538[b] (110)	440[c] (50)	572[b] (54)	671[b] (88)
25	478[b] (44)	530[b] (19)	619[b] (15)	419[c] (58)
30	322[c] (49)	342[d] (62)	465[c] (78)	292[d] (13)
	Hardness (g)			
5	632[d] (51)	787[b] (66)	560[b] (44)	553[d] (33)
10	600[d] (86)	682[c] (41)	584[b] (71)	534[d] (36)
15	637[d] (40)	948[a] (177)	549[b] (43)	607[cd] (35)
20	722[c] (69)	941[a] (62)	538[b] (30)	678[bc] (33)
25	812[b] (132)	738[bc] (12)	560[b] (41)	772[b] (111)
30	1024[a] (97)	1013[a] (50)	646[a] (86)	1255[a] (213)
	Elasticity (%)			
5	20.0[c] (0)	20.0[c] (4.7)	15.6[b] (2.4)	18.2[c] (2.4)
10	21.5[bc] (3.0)	22.6[c] (3.6)	16.7[b] (2.9)	17.8[c] (2.9)
15	19.3[c] (2.2)	28.9[b] (3.4)	15.6[b] (2.4)	18.9[c] (2.3)
20	24.4[b] (4.4)	31.5[b] (2.9)	18.1[b] (3.0)	18.5[c] (4.1)
25	25.2[b] (1.8)	21.1[c] (2.7)	17.1[b] (2.0)	27.0[b] (5.9)
30	37.0[a] (8.9)	52.2[a] (5.0)	21.5[a] (4.1)	44.5[a] (3.5)

[a-d] Means of three replicates, expressed as means (SD). Means within same column not followed by same letters significantly different ($p<0.05$). Higher brittleness value indicates sample less fragile, more force is needed to break the tofu. The highest elasticity is 75%.

Table 4—Correlation coefficients (r) between yield and textural properties of the middle part of tofu[a]

Textural properties	Calcium sulfate		Modified nigari	
	207 rpm	285 rpm	207 rpm	285 rpm
Brittleness force	0.93**	0.78	0.67	0.93**
Hardness	−0.97**	−0.53	−0.98**	−0.99***
Elasticity	−1.00***	−0.73	−0.84*	−1.00***

[a] Significant levels: ***$p<0.001$, **$p<0.01$, *$p<0.05$

of coagulant types on brittleness force was significant ($p < 0.01$). Tofu made with $CaSO_4$ had higher brittleness force than that made with modified nigari.

Hardness of the top part was much higher than that of the middle part (Table 5). Hardness of the $CaSO_4$ tofu (2358g to 3085g) was higher than that of nigari tofu (1838g to 2232g) when stirring time increased from 5 to 20 sec. The lowest hardness appeared at stirring time 25 sec. For $CaSO_4$ tofu, there was no effect of stirring speeds on hardness. However, a difference between stirring speeds was found for tofu made with modified nigari. Coagulant types affected hardness ($p < 0.05$).

Elasticity of $CaSO_4$ tofu was the highest at stirring time 30 sec. There was also no difference in elasticity of $CaSO_4$ tofu between 207 and 285 rpm. However, a significant effect of stirring speeds on elasticity of nigari tofu was found; higher stirring speed (285 rpm) produced tofu with higher elasticity (Table 5). In general, nigari tofu had lower elasticity than $CaSO_4$ tofu. The effect of stirring speed on all textural parameters of the top part of tofu made from $CaSO_4$ was not significant, but it was significant for that made from modified nigari.

soft tofu
dified nigari
285 rpm

659[b]
(69)
749[a]
(73)
673[b]
(77)
671[b]
(88)
419[c]
(58)
292[d]
(13)

553[d]
(33)
534[d]
(36)
607[cd]
(35)
678[bc]
(33)
772[b]
(111)
1255[a]
(213)

18.2[c]
(2.4)
17.8[c]
(2.9)
18.9[c]
(2.3)
18.5[c]
(4.1)
27.0[b]
(5.9)
44.5[a]
(3.5)

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Table 5—Textural properties of the top part of soft tofu

Stirring time (sec)	Calcium sulfate 207 rpm	Calcium sulfate 285 rpm	Modified nigari 207 rpm	Modified nigari 285 rpm
	Brittleness force (g)			
5	1034[b]	1006[bc]	763[b]	870[b]
	(109)	(43)	(42)	(104)
10	1129[b]	1105[b]	919[a]	822[bc]
	(95)	(156)	(99)	(104)
15	1046[b]	862[cd]	767[b]	760[c]
	(115)	(75)	(47)	(21)
20	781[c]	912[c]	790[b]	888[b]
	(128)	(71)	(71)	(101)
25	668[d]	728[d]	789[b]	605[d]
	(59)	(35)	(37)	(18)
30	1955[a]	1770[a]	678[c]	2187[a]
	(101)	(267)	(107)	(92)
	Hardness (g)			
5	2552[a]	2461[c]	2078[a]	2143[a]
	(137)	(176)	(264)	(320)
10	2402[ab]	2382[c]	2005[a]	2120[a]
	(165)	(170)	(186)	(199)
15	2383[ab]	2810[b]	1993[a]	2232[a]
	(163)	(182)	(302)	(211)
20	2359[b]	3085[a]	1838[a]	2016[a]
	(151)	(125)	(109)	(158)
25	1723[d]	1680[d]	1597[b]	1528[b]
	(197)	(127)	(142)	(122)
30	1955[c]	1770[d]	1580[b]	2187[a]
	(101)	(267)	(47)	(92)
	Elasticity (%)			
5	28.2[c]	25.2[c]	30.0[ab]	27.3[c]
	(5.3)	(6.5)	(5.6)	(8.9)
10	34.1[c]	30.8[c]	25.2[bc]	31.8[bc]
	(4.0)	(7.1)	(4.4)	(6.5)
15	24.8[c]	31.5[c]	25.9[bc]	25.6[c]
	(4.4)	(7.7)	(7.6)	(4.1)
20	36.3[b]	42.2[b]	24.5[bc]	25.6[c]
	(3.5)	(5.8)	(4.7)	(5.3)
25	33.3[b]	31.7[c]	23.0[c]	40.7[a]
	(4.7)	(5.9)	(4.8)	(5.2)
30	51.1[a]	53.9[a]	33.3[a]	37.8[ab]
	(3.4)	(3.3)	(4.7)	(8.1)

[a-d] Means of three replicates, expressed as means (SD). Means within same column not followed by same letters significantly different ($p<0.05$). Higher brittleness value indicates sample lass fragile, more force is needed to break the tofu. The highest elasticity is 75%.

Table 6—Textural properties of commercial soft tofu from grocery stores

Product	Top part Brittleness (g)	Top part Hardness (g)	Top part Elasticity (%)	Middle part Brittleness (g)	Middle part Hardness (g)	Middle part Elasticity (%)
Tofu A	1610	1930	46.7	1340	655	26.7
	(14.8)	(15.6)	(7.8)	(16.3)	(4.3)	(2.3)
Tofu B	1070	1600	33.3	820	820	26.7
	(12.8)	(15.6)	(7.8)	(16.3)	(6.3)	(2.3)
Tofu C[a]	1245	1115	33.3	1218	890	26.7
	(18.4)	(9.6)	(0.8)	(22.6)	(1.4)	(0.6)
Tofu D	1670	2000	29.3	1365	1800	21.3
	(4.2)	(35.3)	(2.8)	(96.9)	(26.9)	(0)

[a] Different style of soft tofu which was directly coagulated in the box.

A negative correlation was found only between yield and elasticity of $CaSO_4$ tofu at both stirring speeds of 207 rpm and 285 rpm (−0.95 and −0.81, respectively) and between yield and brittleness force of nigari tofu at 285 rpm (−0.89). The top part had higher textural properties than the middle part. Although differences of textural properties of tofu may be overcome by the hard layer of the top part, this hard surface layer was important for soft tofu to retain shape, and to increase water-holding capacity. Therefore, results indicated that the middle part was more appropriate than the top part to judge quality.

The textural properties of commercial soft tofus made by four companies were compared (Table 6). The brittleness force of the middle part of commercial tofu ranged from 820 to 1365g, hardness ranged from 655 to 1800g, and elasticity from 21.3 to 26.7%. For the top part of commercial tofu, brittleness force ranged from 1070 to 1670g, hardness ranged from 1600 to 2000g, and elasticity from 29.3 to 46.7%. The wide range of brittleness force and hardness indicated that different tofu companies produced various textures of soft tofu. Compared to commercial tofu, that from our study had less homogeneous texture, and had lower brittleness force but higher hardness. This may have been due to tofu containing higher water and harder surface layers in the top part.

CONCLUSION

IN GENERAL, tofu made at 285 rpm stirring speed had lower yield but higher textural properties than tofu made at 207 rpm stirring speed. Tofu made using $CaSO_4$ had lower yield but higher textural properties than that made using modified nigari. Yield of soft tofu decreased with stirring time increased to 30 sec and textural properties were affected as stirring time increased to 25 sec. Stirring time <25 sec was appropriate for soft tofu making to avoid breaking curd. Factors such as soymilk solids, coagulant concentration, and soymilk temperature were also important for tofu quality.

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Effect of carrageenan on yield and properties of tofu

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Abstract

The effects of the polysaccharide carrageenan and three types of coagulants namely, glucono-δ-lactone (GDL), calcium sulphate (CS) and calcium acetate (CA) on yield and physical properties of tofu (soybean curd), were investigated. Moisture content and yield of GDL-coagulated tofu were higher than that coagulated with CS and CA. Addition of carrageenan did not increase yield of GDL-tofu significantly but increased yield of CS-tofu and CA-tofu by 33 and 46.7%, respectively. Texture of CS-tofu was harder than CA- and GDL-tofu. Carrageenan brought about a significant decrease in hardness of CS- and CA-tofu which was more pronounced at the higher gum concentration. Hardness of GDL-tofu was not significantly altered. The tofu gels exhibited syneresis on storage at 4°C for 24 h in the order: CS-tofu < CA-tofu < GDL-tofu. Addition of carrageenan increased the syneresis, which was higher in CA-tofu than CS-tofu. On the other hand, GDL-tofu showed a decrease in syneresis in the presence of carrageenan.

1. Introduction

Tofu (soybean curd) is made by coagulation of heated soya milk with a coagulant, followed by moulding and pressing the curd to draw the whey. The resulting curd or tofu is valued for its flavour and texture. Yield, quality and texture of tofu are influenced by several factors (Sun & Breene, 1991) such as variety of soybeans and storage conditions, time and temperature of soaking the soybeans, extent of heat-treatment of soymilk, type and concentration of coagulant and rate of stirring and coagulation temperature.

Various coagulants have been used in the preparation of tofu, each coagulant resulting in a product with textural characteristics varying from soft to firm and with moisture content ranging from 70 to 90% (de Man, J.M., de Man, L., & Gupta, 1986). Although calcium sulphate and glucono-δ-lactone are the coagulants of choice, other soluble salts of calcium, such as calcium acetate and calcium chloride have also been recommended for the coagulation of soymilk (Lim, de Man, J.M., de Man, L., & Buzzel, 1990; Tsai, Lan, Kao, & Chen, 1981; Shen, de Man, L., Buzzel, & de Man, J.M., 1991; Wang & Hesseltine, 1982; Sun & Breene, 1991).

The rheological and other physicochemical properties of many processed and convenience foods are determined to a large extent by the behaviour of proteins and polysaccharide components (Samant, Singhal, Kulkarni, & Rege, 1993). The protein–polysaccharide interaction has generated considerable research interest (Bernal, Smadja, Smith, & Stanley, 1987; Lin & Hansen, 1970; Stainsby, 1980; Tolstoguzov, 1986) and the industrial significance of these interactions for food texture modification has been documented (Antonov, Grinberg, Zhuravskaya, & Tolstoguzov, 1980). Understanding the mechanisms involved in the interactions between these components is important to exploit their potential to meet new technological requirements.

Polysaccharides play a key role in modifying the textural properties of protein food systems. Carrageenans, a group of sulphated linear polysaccharides of D-galactose, and 3,6 anhydro-D-galactose are used extensively in the food industry for their thickening, stabilizing and gelling properties (Trius & Sebranek, 1996). Being negatively charged polysaccharides over a wide range of pH, carrageenans are capable of forming complexes with proteins in the presence and absence of calcium ions (Bernal, Smadja, Smith, & Stanley, 1987; Glicksman, 1983). There have been several studies on carrageenan-milk protein interaction (Hansen, 1968; Hood & Allen, 1977; Ozawa, Niki, & Arima, 1984; Schmidt & Smith, 1992; Xu, Stanley, Goff, Davidson, & Le Maguer, 1992). The unique

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interaction of carrageenan with the casein micelle has led to its widespread usage in the dairy industry. Addition of κ- or ι-carrageenan to milk has been shown to increase yield of cottage cheese by 10–20% (Kailasapathy, Hourigan, & Nguyen, 1992). Studies on carrageenan-meat protein interaction, and the effect on functional properties, have also been carried out (Foegeding & Ramsey, 1987; DeFreitas, Sebranek, Olson, & Carr, 1997: Shand, Sofos, & Schmidt, 1994; DeFreitas, Sebranek, Olson, & Carr, 1997).

Tofu manufacturers are concerned, firstly with the yield which is of economic importance, and secondly, texture which determines its acceptability. In the course of tofu preparation, proteins in the soymilk are coagulated with a concomitant release of curds and whey; the curds are filtered off and moulded into shape under pressure. A large amount (approximately one third) of the original soybean mass is unavoidably lost in the whey during the first filtration step (van der Riet, Wight, Cilliers, & Datel, 1989). The whey is a byproduct of relatively low nutritive value and has the disadvantage of containing appreciable amounts of flatulence-causing carbohydrates (Liener, 1981). The objective of our study at the outset was to study the feasibility of increasing the yield of tofu by exploiting the carrageenan–protein interaction, and to determine how the yield and physical properties of tofu are influenced by carrageenan addition to soymilk prior to coagulation by calcium sulphate, calcium acetate and glucono-δ-lactone.

2. Materials and methods

2.1. Materials

Green maple leaf brand of Canadian soybean variety was purchased from a local supplier. The seed density was 18.8 g 100 beans.

Food grade calcium sulphate and glucono-δ-lactone were procured from a local chemical supplier. Calcium acetate was purchased from BDH Chemicals Ltd., Poole, England. Food grade carrageenan (Grindsted SL 320), with 26% galactose and 21% sulphate, was obtained through the courtesy of Ms. Danisco Ingredients, Penang.

2.2. Preparation of tofu

Tofu was prepared by a modification of the methods proposed by Escueta, Bourne, and Hood (1986), Lim, de Man, de Man, L., Buzzel (1990) and Sun and Breene (1991). Washed soybeans (approximately 72 g) were soaked in 1 litre beakers at room temperature (28°C) for 16 h in 500 ml tap water. After the stipulated soaking time, the beans were drained and ground with 725 ml tap water in a Waring blender for 2 min at high speed. The mash was strained through a muslin cloth and pressed to obtain soymilk. The soymilk (710 ml) was heated to 95–98°C for 15 min in a steam-jacketed kettle and then cooled rapidly to 30°C in an ice bath and volume made up to 710 ml by adding cooled, boiled water. The required quantity of carrageenan (1 or 2 g l^{-1}) was added to the soymilk and heated to 85°C in a water bath. The hot soymilk (700 ml) was poured into 100 ml water at 70°C containing coagulant at the respective concentration in a 1 litre beaker. Calcium sulphate (CS) and glucono-δ-lactone (GDL) were added at a level of 0.02 mol l^{-1} while calcium acetate (CA) was added at 0.01 mol l^{-1}. The 800 ml soymilk-coagulant suspension was held at 70°C for 15 min in a water bath for 15 min to ensure that coagulation occurred. The curd thus formed was transferred to a specially designed mould (11 cm diameter and 10.4 cm height) lined with cheese cloth. The whey was drained off naturally for 10 min and the curd was pressed for 30 min by placing a 1.3 kg weight on the 86.6 cm^2 plate covering the curd.

2.3. Analyses

2.3.1. Yield, solid recovery and proximate analyses

Yield of tofu was expressed as fresh weight of tofu obtained from 700 ml of soymilk. Moisture content was determined by drying 5 g of fresh tofu at 105°C in an air oven to constant weight (Tsai, Lan, Kao & Chen, 1981). Total solids in whey was determined by drying 10 ml of the whey at 105°C in an air oven for 24 h. Total protein was determined by the micro Kjeldahl method (AOAC, 1975) on air-dried tofu samples and using the factor N × 6.25 to convert nitrogen to protein. pH of the tofu samples was measured with a digital pH meter with a glass electrode (Metrohm AG, Switzerland).

2.3.2. Syneresis

A modified method of Amstrong, Hill, Schrooyen, and Mitchell (1994) was employed. Three pieces of tofu samples of 1.5 cm diameter were weighed and filled into Visking tubing (2.5 cm diameter). The tube was wrapped with plastic wraps and tied to a wire frame placed over a 2 litre beaker in a hanging position for 24 h at 4°C. Percentage syneresis was calculated as the weight of water released from the tofu in 24 h divided by the weight of sample and multiplied by 100.

2.3.3. Texture profile of tofu

Textural properties of tofu were evaluated with TA.XT2 Texture Analyser (Stable Micro Systems, Goldaming, Surrey, UK) fitted with a 5 kg load cell. Cylindrical samples of tofu (20 mm diameter, 16 mm height) were compressed by a flat plate (4×4 cm) to 80% deformation. The parameter setting and operation of the instrument were accomplished through a PC with Texture Expert software version 1.0. The force and

probe calibration procedure for the system were followed before actual tests. The test mode was set to 'Texture Profile in Compression'. The pre-test, test and post-test speeds were set to 2, 2 and 4 mm s^{-1}, respectively. Each test was repeated 10 times at room temperature. Coefficients of variation for all determinations were less than 8%.

2.4. Colour

Colour evaluation was performed on fresh tofu samples using a Hunterlab Model D25 Tristimulus Colorimeter, equipped with a D25 circumferential optical sensor. A standard white tile with reflectance values of $X = 83.24$, $Y = 85.23$ and $Z = 100.92$ was used as a reference. A representative sample was placed into a 6 cm Petri dish and covered to avoid stray light. Hunter L (lightness), +a (red) to −a (green), and +b (yellow) to −b (blue) were then determined for each sample. Each value represented a mean value of five replicate determinations. Coefficient of variations for all measurements were less than 3%.

2.5. Sensory evaluation

Nineteen untrained panellists, composed of adult males and females who were familiar with tofu, were used in each tasting session. Tests on overall acceptability, colour, flavour and mouthfeel were conducted using a 9-point hedonic scale. Samples were steamed before being served in the form of a cube (1.5 cm) in 90 ml vegetable soup. All samples were coded and always presented in a randomized arrangement.

2.5.1. Statistical design and analysis

Experiments were based on a randomised complete block design. Data were analysed using an ANOVA procedure of the MINITAB software version 10 for Windows (MINITAB Inc.). A two-way analysis of variance was conducted, with carrageenan concentrations and coagulant types as factors. When significance was indicated, means were separated using Fisher's Least Square Difference Test. Statistical tests were conducted at the 5% probability level.

3. Results and discussion

3.1. Yield and composition of tofu

One of the critical steps in the preparation of tofu is the addition of salt to precipitate soy protein. Calcium sulphate (CS) and glucono-δ-lactone (GDL) are traditionally used as coagulants in tofu manufacture, yielding tofu of uniform texture with high moisture content. Being practically insoluble (Lu, Carter, & Chung, 1980), use of CS demands skill to avoid variation in quality. Lu et al. (1980) proposed use of soluble salts such as calcium acetate (CA), which yielded tofu of acceptable quality at half the levels of CS and GDL. Hence, in the present study, we decided to use CS and GDL at 0.02 mol l^{-1} and CA at 0.01 mol l^{-1}.

Yield of tofu in this experiment was of the order: GDL tofu > CS-tofu > CA-tofu, which is in agreement with the findings of Tsai et al. (1981) and Shen et al. (1991) (Fig. 1). Moisture content of GDL-tofu was higher than CS- and CA-tofu, which is reflected in the lower yield of whey (Table 1). Moisture retention, on a protein basis, was higher for GDL-tofu, followed by CA-tofu and CS-tofu. The lower pH of GDL-tofu reflects the isoelectric precipitation of soyproteins by the release of protons from GDL (Smith & Circle, 1972). Both CS- and CA-tofu gave a clear whey, indicating that the level of coagulant added was sufficient for complete coagulation of the soyproteins. The solids in whey are most probably soluble sugars and low molecular weight proteins. The variation in the whey volume was most likely due to a change in the water-holding capacity of tofu, which is affected by carrageenan.

Addition of carrageenan to soymilk prior to coagulation resulted in significant increases in yields of tofu ($p < 0.05$). At a concentration of 1 g l^{-1} and 2 g l^{-1}, carrageenan increased the yield of CS-tofu by approximately 9.5 and 33%, respectively. Corresponding increases in yield for CA-tofu were 17.1 and 46.7%, respectively. The increase in yield was reflected by the higher moisture content of CS- and CA-tofu and a corresponding decrease in yield of whey (Table 1). With GDL-tofu, carrageenan at 1 g l^{-1} resulted in a significant increase in yield ($p < 0.05$) and decrease in whey volume, without affecting other parameters. Higher concentration of carrageenan, however, did not sustain the observed changes.

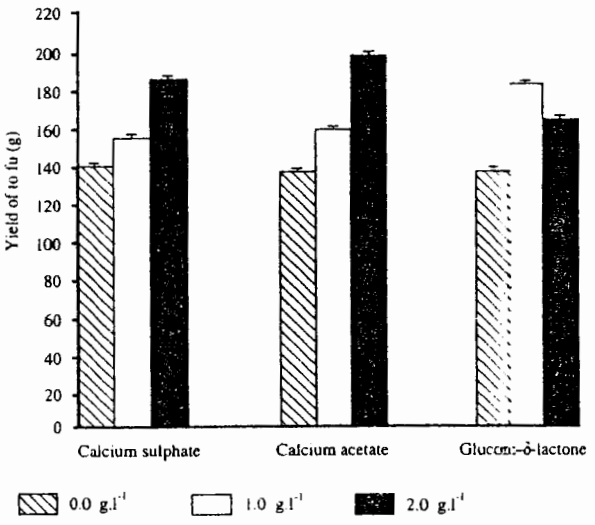

Fig. 1. Effect of coagulants and carrageenan on yield of tofu.

Table 1
Effect of carrageenan and coagulants on the composition of tofu[a]

Coagulant[b]	Carrageenan (g l^{-1})	Yield[c] (g)	Moisture (%)	Protein[d] (%)	Moisture retention[e] (g g^{-1})	pH	Whey (ml)
Calcium sulphate	0	140.5[f]	78.1[f]	11.5[f] (52.30)	6.82[f]	5.57	623[f]
	1.0	153.8[g]	81.3[g]	9.49[f] (50.83)	8.57[g]	5.62	590[g]
	2.0	186.9[h]	82.8[h]	8.77[f] (50.88)	9.44[h]	5.60	558[h]
Calcium acetate	0	136.3[f]	79.0[f]	11.0[f] (52.29)	7.20[f]	5.62	622[f]
	1.0	159.6[g]	82.4[g]	8.93[f] (50.86)	9.23[g]	5.82	596[g]
	2.0	200.0[h]	84.6[g]	7.45[g] (48.46)	11.36[g]	5.76	532[h]
Glucono-δ-lactone	0	161.1[f]	81.3[f]	9.41[f] (50.29)	8.64[f]	4.52	586[f]
	1.0	183.5[g]	83.1[f]	8.29[f] (48.94)	10.02[f]	4.55	561[f]
	2.0	164.2[f]	79.7[f]	10.41[f] (51.4)	7.66[f]	4.52	580[f]

[a] Means of quadruplicates. Coefficient of variation < 5%. For each tofu, means with the same superscripts in the same column are not significantly different ($p > 0.05$).
[b] Calcium sulphate at 0.02 mol l^{-1}; Glucono-δ-lactone at 0.02 mol l^{-1}.
[c] Fresh weight basis from 700 ml soymilk.
[d] Figures in parentheses represent protein content on dry weight basis.
[e] Gram per gram protein basis (dry).

A two-step mechanism for the gelation of tofu proteins has been proposed by Kohyama, Sano, and Doi (1995). The first step is the heat-induced denaturation of the soyprotein which exposes the hydrophobic regions of the protein molecules in the native state to the outside. The denatured soyprotein is negatively charged (Kohyama & Nishinari, 1993) and the release of protons induced by GDL or calcium ions from the coagulant neutralises the net charge of the protein. As a result, the hydrophobic interaction of the neutralised protein molecules becomes more predominant and induces the random aggregation of proteins, leading to gel formation (de Man, de Man, L., & Gupta, 1986).

Being a sulphated polysaccharide, carrageenan can exist as a negatively charged polymer over a wide range of pH. Above the isoelectric point, polyvalent metal ions such as Ca^{2+} can form bridges between the negatively charged carboxyl groups of the protein and the ester sulphate groups of the polysaccharide. In such protein–polysaccharide–calcium systems, both the protein and the polysaccharide can interact independently with the calcium ions (Hughes, Ledward, Mitchell, & Summerlin, 1980). The extensive network structure formed by carrageenan could trap more water in the interstitial spaces of the gel, and reduce syneresis, resulting in the observed yield increase. The higher moisture retention in CA-tofu, compared to CS-tofu, is probably due to the differences in the gel network effected by the ionic strengths of the coagulants and/or the effect of the different anions on the water-holding capacity of the soyprotein gels (Wang & Hesseltine, 1982).

The observed effects of carrageenan in the present study can also be explained on the basis of the two component type II mixed gel networks proposed by Morris (1986). In the first instance, normal aggregated soyprotein gels are formed on the addition of calcium salts or GDL. When calcium salts are employed, the pH being above the isoelectric point, soyprotein molecules will be negatively charged. The protein and the polysaccharide can interact on their own with the metal ions or with each other, with or without the involvement of metal ions. Similar ionic interactions between κ-casein and κ-carrageenan and the resulting mixed coupled gel networks are held responsible for the stabilization of milk products (Lin & Hansen, 1970; Ozawa, Niki & Anna, 1984; Schmidt & Smith, 1992). The formation of a coupled gel network of carrageenan and soyprotein around the aggregated tofu gel could bind more water, increasing yield and reducing hardness. On the other hand, addition of GDL as a coagulant results in a tofu gel at isoelectric point which precludes formation of coupled mixed gel networks of carrageenan and soyprotein. However, weak gels of carrageenan with the calcium or potassium constituents of soymilk would still be possible. This may explain the lack of effect of carrageenan on the yield and hardness of GDL-tofu as compared to CA- and CS-tofu, although syneresis was reduced perhaps due to better moisture retention.

3.2. Textural properties

Textural parameters of the tofu were evaluated according to definitions given by Bourne (1982). Fig. 2 shows a typical force-time curve obtained after the application of 80% uniaxial compression. Similar curves were obtained from tofu made with the addition of carrageenan. As shown in Table 2, hardness, cohesiveness, elasticity, gumminess and chewiness decreased with increasing concentration of carrageenan for CA- and CS-tofu. However, effect of carrageenan on the other two parameters, i.e., adhesiveness and fracturability was not consistent. In most cases, CS-tofu

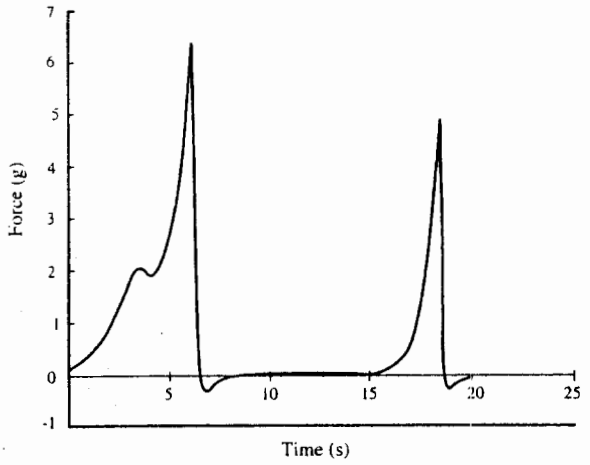

Fig. 2. Typical texture profile curve for tofu subjected to 80% compression.

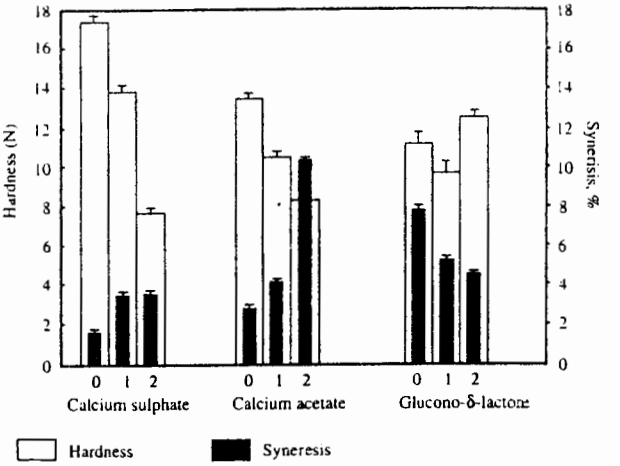

Fig. 3. Effect of coagulants and carrageenan on hardness and syneresis of tofu. The numbers on x-axis represent carrageenan concentrations (g l^{-1}).

(without carrageenan) showed highest values for hardness, gumminess, elasticity and chewiness but, with addition of carrageenan, especially at 2 g l^{-1}, GDL-tofu showed highest values for hardness and gumminess.

Fig. 3 shows the effect of coagulants and carrageenan on hardness and syneresis of tofu. Texture analysis revealed that CS-tofu was harder than CA- and GDL-tofu, which is in contrast to the findings of Shen et al. (1991). It has been reported that coagulant concentration and type of anion affect hardness of tofu (Sun & Breene, 1991; Wang & Hesseltine, 1982). Probably the way protein interacts with calcium and other constituents, e.g. phytic acid, in soy milk and anions to form the microstructure could determine the hardness of tofu (Lim, de Man, de Man, L., & Buzzel, 1990; Wang & Hesseltine, 1982). Addition of carrageenan, at 1 g l^{-1} and 2 g l^{-1} resulted in a significant decrease ($p < 0.05$) in hardness of CS-tofu (Fig. 2) by 21.2 and 55.9% and that of CA-tofu by 21.6 and 38.3%, respectively. However, the effect of carrageenan on hardness of GDL-tofu was insignificant ($p > 0.05$).

Gelation of food protein involves heat denaturation followed by aggregation. If aggregation is relatively slower than denaturation an ordered structure will be promoted, by allowing the denatured molecules to orient themselves in a systematic fashion prior to aggregation (Hermansson, 1978). Conditions that retard intermolecular interaction will result in a more homogeneous and regular network and consequently a stronger gel (Bernal, Smadja, Smith & Stanley, 1987). Recently, soybean protein composition has been related to texture of tofu (Murphy, Chen, Hauck, & Wilson, 1997). Contrary to expectations, interaction between soyprotein and carrageenan resulted in reduction of the hardness of tofu, which is a desirable sensory attribute.

Table 2
Texture profile analysis of tofu[a]

Types of tofu	Carrageenan (g l^{-1})	Texture parameters				
		Hardness (N)	Springiness (mm)	Chewiness (N mm)	Cohesiveness	Gumminess (N)
CS-tofu	0.0	17.59^{b}	0.09^{b}	0.40^{b}	0.18^{b}	3.1^{b}
	1.0	13.86^{c}	0.08^{c}	0.20^{c}	0.07^{c}	2.45^{c}
	2.0	7.75^{d}	0.02^{d}	0.02^{d}	0.00^{d}	0.54^{d}
CA-tofu	0.0	13.49^{b}	0.07^{b}	0.18^{b}	0.18^{b}	2.46^{b}
	1.0	10.58^{c}	0.05^{c}	0.07^{c}	0.13^{c}	1.42^{b}
	2.0	8.33^{c}	0.03^{d}	0.04^{c}	0.08^{c}	0.66^{c}
GDL-tofu	0.0	11.15^{b}	0.06^{b}	0.12^{b}	0.13	1.50^{c}
	1.0	9.72^{b}	0.04^{b}	0.06^{b}	0.11	1.07^{b}
	2.0	12.56^{b}	0.07^{b}	0.16^{b}	0.15	1.59^{b}

[a] Means of ten replicates. Coefficient of variation < 8%. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$).

3.3. Syneresis

On storage at 4°C for 24 h, syneresis was low in CS- and CA-tofu, but higher in GDL-tofu (Fig. 2). Carrageenan enhanced syneresis in CA-tofu compared to CS-tofu. Interestingly, syneresis was reduced by carrageenan in GDL-tofu. Increase of syneresis from the curd could be due to increased bonding occurring during storage, making the protein matrix more dense or compacted (Sun & Breene, 1991). In GDL-tofu, the decrease in syneresis could result from enhanced water retention in the gel microstructure.

3.4. Colour

Tofu of good quality is generally white or light-yellow in colour. All the tofu samples prepared in this study had a light yellow colour. CA-tofu with 2 g l^{-1} carrageenan showed a slightly higher L, 'a' and 'b' values (Table 3). The greenness ('a' value) increased with increasing concentration of carrageenan in CS-tofu. In contrast, 'a' values decreased with increase in carrageenan concentration for GDL-tofu. No significant effect ($p > 0.05$) on 'a' value was noted for CA-tofu. Two-way analysis of variance indicated that both coagulant and carrageenan affected the colour of tofu significantly. The effect of coagulants on colour of tofu was greater than the effect of carrageenan. In addition, an interaction between coagulant and carrageenan was evident.

3.5. Sensory characteristics

Tofu samples were evaluated by a panel for colour, flavour, mouthfeel and overall acceptability on a 9-point scale. Tofu prepared with CA/2 g l^{-1} carrageenan and CA and CS alone (without carrageenan) were evaluated (Table 4). These three representative samples were chosen for sensory evaluation because of the pronounced effect on the hardness of CA-tofu brought about by addition of carrageenan. The acceptability scores ranged from 5.05 to 7.53 which are moderate even though tofu is very much a customary part of the Malaysian diet. Highest scores were given to CA-tofu with 2 g l^{-1} carrageenan, which had a smooth, soft but firm texture.

Table 3
Effect of coagulants and carrageenan on the colour of tofu[a]

Types of tofu	Carrageenan (g l^{-1})	Hunter values		
		L	a	b
CS-tofu	0.0	84.13[b]	−0.27[b]	14.17[b]
	1.0	83.83[b]	−0.73[c]	14.20[b]
	2.0	83.77[b]	−0.87[c]	14.82[b]
CA-tofu	0.0	84.05[b]	0.08[b]	14.87[b]
	1.0	83.73[c]	0.20[b]	14.87[b]
	2.0	84.47[d]	0.12[b]	14.90[b]
GDL-tofu	0.0	82.75[b]	0.33[b]	13.82[b]
	1.0	82.25[b]	0.25[b]	13.82[b]
	2.0	82.53[b]	−0.07[c]	13.60[b]

[a] Means of six replicates. Coefficient of variation < 3%. For each type of tofu, the same superscripts in the same column are not significantly different ($p > 0.05$).

Table 4
Effect of coagulants and carrageenan on sensory characteristics of tofu[a]

Sensory parameters	CA + carrageenan tofu[b]	CA-tofu[c]	CS-tofu[d]
Colour	7.53	7.05	7.00
Flavour	6.74	6.26	5.84
Mouthfeel	6.68	5.95	5.05
Overall acceptability	6.63	6.21	6.05

[a] On the 9-point hedonic scale.
[b] Calcium acetate 0.1 mol l^{-1} and carrageenan at 2 g l^{-1}.
[c] Calcium acetate only at 0.1 mol l^{-1}.
[d] Calcium sulphate only at 0.2 mol l^{-1}.

4. Conclusions

The present study has confirmed the feasibility of replacing calcium sulphate by calcium acetate for the coagulation of soybean milk in the manufacture of tofu. Furthermore, the study has demonstrated the possibility of increasing the yield and moisture content of tofu through use of a plant hydrocolloid, i.e. carrageenan. Carrageenan at relatively low concentration (2 g l^{-1}) has been shown to enhance the water holding capacity of the soybean protein gel without affecting the hardness of the GDL-tofu significantly but brought about a significant decrease in hardness of CS- and CA-tofu. Carrageenan has also been shown to have an interaction with the coagulant. Optimization of conditions for gelation of soyprotein and the carrageenan-induced modifications of the gel network may be useful in modifying the tofu processing technology with tangible commercial advantages.

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CAS: 1344-95-2

SILICATE

ing proportions of CaO and SiO_2.
ler. insol in water.

)OD:

nticaking agent, filter aid.

t: Baking powder, chips (fabricated),

s: FDA - 21CFR 172.410, 182.2227.
h a limitation to 2 percent by weight
alt, 5 percent by weight in baking

PROFILE: A nuisance dust.

M SORBATE

olid. Sltly sol in water.

FOOD:

Mold retardant, preservative.

Used: Cheese, margarine, oleomarga-
kaging materials.

ons: FDA - 21CFR 182.3189. GRAS
d in accordance with good manufactur-
tice. USDA - 9CFR 318.7. Limitation
ercent individually, of if used in combi-
with its salts or benzoic acid or its salts,
ent (expressed as the acids in the weight
nished product. Not allowed in cooked

Y PROFILE: When heated to decompo-
emits acrid smoke and irritating fumes.

AS: 1592-23-0

50
IUM STEARATE

: Variable proportions of calcium stearate
lcium palmitate. Fine white powder; slt
teristic odor. insol in water, alc, ether.

N FOOD:

se: Anticaking agent, binder, emulsifier,
ing agent, lubricant, release agent, stabi
thickener.

e Used: Beet sugar, candy (pressed), garlic
meat tenderizer, molasses (dry), salad
ing mix, vanilla, yeast.

lations: FDA - 21CFR 172.863, 173.3
conform to FDA specifications for
tty acids derived from edible oils. 21C
29. Use at a level not in excess of

Lewis (1989) Food Additive Handbook

amount reasonably required to accomplish the intended effect. 21CFR 184.1229. GRAS when used in accordance with good manufacturing practice.

SAFETY PROFILE: When heated to decomposition it emits acrid smoke and irritating fumes.

CAX375
CALCIUM STEAROYL LACTATE

PROP: Cream-colored powder; caramel odor. Sltly sol in hot water.

SYN: CALCIUM STEAROYL-2-LACTATE

USE IN FOOD:

Purpose: Dough conditioner, stabilizer, whipping agent.

Where Used: Bakery products (yeast-leavened), coffee whiteners, egg white (dried), egg white (liquid and frozen), margarine (low fat), potatoes (dehydrated), puddings, vegetable toppings (whipped).

Regulations: FDA - 21CFR 172.844. Limitation of 0.5 parts for each 100 parts of flour in yeast-leavened bakery products; 0.05 percent in egg white, liquid and frozen, and egg white, dried; 0.3 percent in whipped vegetable topping; 0.5 percent in dehydrated potatoes. Must conform to FDA specifications for fats or fatty acids derived from edible oils.

SAFETY PROFILE: When heated to decomposition it emits acrid smoke and irritating fumes.

CAX500
CALCIUM SULFATE

CAS: 7778-18-9

mf: $CaSO_4$ mw: 136.14

PROP: Pure anhydrous, white powder or odorless crystals. D: 2.964; mp: 1450°.

USE IN FOOD:

Purpose: Anticaking agent, color, coloring agent, dietary supplement, dough conditioner, dough strengthener, drying agent, firming agent, flour treating agent, formulation aid, leavening agent, nutrient supplement, pH control agent, processing aid, sequestrant, stabilizer, synergist, texturizer, thickener, yeast food.

Where Used: Baked goods, canned potatoes, canned tomatoes, carrots (canned), confections, frostings, frozen dairy dessert mixes, frozen dairy desserts, gelatins, grain products, ice cream (soft serve), lima beans (canned), pasta, peas (canned), puddings, wine (sherry).

Regulations: FDA - 21CFR 184.1230. GRAS with a limitation of 1.3 percent in baked goods, 3.0 percent in confections and frostings, 0.5 percent in frozen dairy desserts and mixes, 0.4 percent in gelatins and puddings, 0.5 percent in grain products and pastas, 0.35 percent in processed vegetables, 0.07 percent in all other foods when used in accordance with good manufacturing practice. BATF - 27CFR 240.1051. Limitation of 16.69 pounds/1000 gallons.

SAFETY PROFILE: Reacts violently with aluminum when heated. Mixtures with diazomethane react exothermically and eventually explode. Mixtures with phosphorus ignite at high temperatures. When heated to decomposition it emits toxic fumes of SO_x.

CBA500
CAMPHENE

CAS: 79-92-5

DOT: 9011

mf: $C_{10}H_{16}$ mw: 136.26

PROP: Colorless cubic crystals; oily odor. Mp: 50-51°, bp: 159°, d: 0.842 @ 54°/4°, refr index: 1.452 @ 55°. Sol in alc; misc in fixed oils; insol in water.

SYN: FEMA No. 2229

USE IN FOOD:

Purpose: Flavoring agent.

Where Used: Various.

Regulations: FDA - 21CFR 172.515. Use at a level not in excess of the amount reasonably required to accomplish the intended effect.

DOT Classification: ORM-A; Label: None

SAFETY PROFILE: Mutagenic data. Combustible; yields flammable vapors when heated and can react with oxidizing materials. To fight fire, use water spray, foam, fog, CO_2. When heated to decomposition it emits acrid smoke and irritating fumes.

TOXICITY DATA and CODEN

bfa-rat/sat 2500 mg/kg NUCADQ 1,10,79

CBC100
CANANGA OIL

PROP: From flowers of the tree *Cananga odorata* f. et Thoms., (Fam. *Anonaceae*). Yellow liquid; harsh floral odor. Sol in fixed oils, mineral oil; insol in glycerin, propylene glycol.

Yield and Quality of Tofu as Affected by Soybean and Soymilk Characteristics. Calcium Sulfate Coagulant

B.T. LIM, J.M. DeMAN, L. DeMAN, and R.I. BUZZELL

ABSTRACT

Nine light hilum soybean (*Glycine max* (L.) Merr.) varieties were used to study the characteristics of soybeans and soymilk that affect the yield and quality of tofu coagulated with calcium sulfate. The yield of tofu was not affected by the size of soybeans. Soybean varieties high in protein, fat and phosphorus contents produce tofu with higher protein, fat and phosphorus contents. Two models for predicting the yield of tofu were proposed. According to model one, soymilk with higher pH and total solids gives a higher yield of tofu. According to model two, soybeans high in protein and ash and low in phosphorus give a higher yield of tofu.

INTRODUCTION

TOFU is a high protein product widely consumed in the Orient. In the United States and Canada, production of tofu is increasing due to an increase of Asian immigrants and acceptance by the general population. Both the United States and Canada export soybeans to the Orient for making tofu.

Soybeans used for the production of soymilk and tofu are known as edible soybeans. The light colored hilum varieties are the preferred choice as the resulting product has a better color. Tofu manufacturers prefer soybean varieties that produce tofu with high yield and good textural properties. The texture of tofu must be coherent, smooth and firm but not hard and rubbery. Due to its bland nature, the textural properties of tofu play an important role in influencing quality and consumer acceptability.

Tofu is generally made from a filtered water extract of whole soybeans called soymilk. The curd is obtained by coagulation of hot soymilk with a coagulant, followed by molding and pressing to remove whey. There are currently two popular methods of preparing soymilk, the traditional method (Shurtleff and Aoyagi, 1979) and the hot-grind method (Wilkens et al., 1967). The traditional method which involves soaking and cold-grinding of beans yields soymilk with a beany flavor which oriental people favor. In contrast, the hot-grind method which involves soaking and hot-grinding of beans yields soymilk with reduced beany flavor, which the non-Oriental people prefer. In the hot-grind method, the enzyme lipoxygenase which causes the beany flavor is inactivated. Schroder and Jackson (1972) produced tofu with reduced beany flavor by blending the beans in hot water without any previous soaking other than rinsing.

In recent years, processing conditions such as temperature, type and concentration of coagulants, rate of stirring, and water to bean ratio that affect the quality and yield of tofu have been investigated (Saio, 1979; Tsai et al., 1981; Wang and Hesseltine, 1982; deMan et al., 1986; Beddows and Wong, 1987a, b). Also storage conditions of soybeans affect the composition of soymilk and the properties of tofu (Thomas et al., 1989). It is now well established that there is a complex interaction of many factors that are involved in the making of tofu. The variety of soybeans used may affect the quality of tofu and th is considered to be due to differences in protein content soybeans (Skurray et al, 1980). Wang et al. (1983) studied th effect on the yield of tofu of five U.S.A. and five Japane soybean varieties grown under the same environmental co ditions. They found tofu prepared from different soybean v rieties showed significant differences in fresh yield. Howeve they reported no significant correlation between the prote content of the soybeans and the yield of tofu. Snyder and Kwo (1987) state that soybeans that have large uniform size, lig colored hilum, thin seed coat and high protein are preferr for soymilk and making tofu.

Tofu manufacturers are uncertain about what makes a so bean variety better for tofu production. Therefore, soybe breeders have no guidelines in their selection of edible soybe cultivars. The objectives of this study were: (i) To identify t characteristics of soybeans and soymilk that affect the yie and quality of tofu, and (ii) To propose appropriate mod equations based on soybean and soymilk characteristics for t optimization of tofu yield.

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MATERIALS & METHODS

Materials

Nine soybean varieties with different seed size were used to stu the effect of soybean and soymilk characteristics on the yield a quality of tofu. Seed size (g/100 seeds) was determined on dry weig basis to remove variation of moisture caused by different seed sourc Varieties OX733, Corsoy 79, OXY734, Ryokko, Kurozaya (PI81.8 and PI86.454 were grown at the Harrow Research Station of Ag culture Canada, Ontario. Ryokko, Kurozaya and PI86.454 were J anese varieties. KSX 1768, B220 and Nattoking-K86 were suppl by King Agro Inc., Ontario. Soybeans were grown in 195 . Wh received in the laboratory they were stored at 10°C until used for t research during spring and summer of 1988.

Preparation of samples for analysis

Soybeans were ground in a Wiley mill (Arthur H. Thomas C Philadelphia, PA) to pass through a 20 mesh sieve. Both the soym and tofu samples were freeze-dried by using a Stokes freeze dr (Pennsalt Chemicals Corp., Phila., PA., U.S.A.) and then ground a powder using a porcelain mortar and pestle. The samples w further dried in an air oven at 100 to 110°C to constant weight. of the dried samples were stored in air-tight plastic bags in a des cator.

Preparation of soymilk

Soybeans (150g) were soaked overnight (16 h) in 500 mL disti water at 20°C. The soaked beans were drained, rinsed and blen with 375 mL distilled water in a commercial Waring blender (Wa Products Division, New Hartford, CT) for 2 min at high speed, lowed by the addition of 200 mL boiling water and blending at h speed for another 2 min. The resultant slurry was strained throug small centrifugal juice extractor (Golden Harvest Juicer, model 120 supplied by Natural Sales Co., P.O. Box 25, Pittsburgh, PA. 152 U.S.A.) lined with filter cloth. The final volume of soymilk adjusted to 1000 mL with distilled water.

Preparation of tofu

A total of 300 mL fresh soymilk was heated on a hot plate to boiling with constant stirring. A suspension of 2.7g calcium sulfate ($CaSO_4.1/2H_2O$) in 7.5 mL distilled water was prepared. The hot soymilk and coagulant were poured simultaneously into a 500 mL plastic container ensuring mixing without stirring. The plastic container had a removable lid and the base was cut off so that it could be used in the inverted position. The curd was left at 20°C to coagulate for 15 min before transferring to a perforated plastic container lined with cheese-cloth. During transfer, the open ends of both containers faced each other and transfer without breakage was achieved when the removable lid was lifted. The top of the curd was then covered with cheese-cloth and a weight was applied on the top to give a pressure of 15.7 g/cm^2 for 15 min. The weight of freshly formed tofu and the volume of pressed whey were recorded. Tofu yield was expressed as kg tofu per kg of beans (dry basis). The tofu was transferred into a plastic bag which was then sealed, cooled under running water and stored at 5°C overnight for texture measurement the next day.

Moisture determination

Moisture content of the tofu was determined by drying 5g freshly prepared tofu at 100 to 110°C in an air oven (Fisher laboratory oven, model 30G) to constant weight (Tsai et al., 1981). Moisture content of the soybeans was determined by drying in a forced draft air oven (Blue M Electric Company, Blue Island, IL) at 130 ± 3°C for 3 hr (AOCS, 1981a).

Total solids determination

Total solids of the whey and soymilk were determined according to the AOAC (1984) method. About 5g of the sample in an aluminum dish was heated on a steam bath for 10 to 15 min followed by further heating in an air oven for 3 hr at 98 to 100°C. The sample was then cooled in a desiccator, weighed quickly and the residue was reported as total solids.

pH measurement

pH of the soymilk was measured by using a Fisher Accumet pH meter model 825 mp fitted with Fisher Universal glass pH electrodes. Commercially prepared buffer solutions of pH 4.00 and 7.00 (Fisher Scientific) were used to standardize the pH meter.

Determination of protein

The total nitrogen contents of the soybeans, freeze-dried tofu and freeze-dried soymilk were determined by using a Technicon Auto-Analyzer II system (Technicon Instruments Corp., Tarrytown, NY 10591). The determination of nitrogen was based on a calorimetric method in which an emerald-green color was formed by the reaction of ammonia, sodium salicylate, sodium nitroprusside and sodium hypochlorite in a buffered alkaline medium at a pH of 12.8 to 13.0 (Technicon, 1976). The ammonia-salicylate complex formed was then read at 660 nm. The protein contents were obtained by multiplying the total nitrogen by the factor 6.25.

Determination of fat

Fat content of soybeans was determined by extraction with ether (AOCS, 1981b) using a Tecator extraction unit (RaFaTec). Fat content of freeze-dried soymilk and tofu were determined by the Roese-Gottlieb ether extraction method (APHA, 1972) using a Mojonnier fat and solid tester, model D (Mojonnier Bros. Co., Chicago).

Determination of ash

Ash content of the soybeans and freeze-dried tofu were determined as the residue remaining after incineration in a Lindberg electric muffle furnace at 600 ± 15°C for 2 hr (AOCS, 1981c).

Determination of phosphorus

Phosphorus content of the soybeans, freeze-dried soymilk and tofu were determined by using a Technicon AutoAnalyzer II system. Samples were digested according to the method of Thomas et al. (1967). The determination of phosphorus was based on the colorimetric method in which a blue color was formed by the reaction of ortho-phosphate, molybdate ion and antimony ion followed by reduction with ascorbic acid at an acidic pH (Technicon, 1976). The phosphomolybdenum complex formed was then read at 660nm.

Determination of carbohydrates

Carbohydrate content of the soybeans and freeze-dried tofu were determined by difference (% carbohydrate = 100 − % protein- % fat − % ash).

Texture of tofu

The texture of the tofu was evaluated by using an Instron Universal Testing Machine, model TM (Instron, Corp., Canton, MA) with a Sensotec load cell (cap. 10 lb) and a Daytronic 9000 strain gauge conditioner-indicator (Daytronic Corp., Miamisburg, OH). The signal voltage was fed to an A-D Converter linked to an Apple //e computer. The instrument output was stored on a floppy disk and analyzed using a texture program developed by the Engineering and Statistical Research Centre, Agriculture Canada, Ottawa. Cylindrical samples (20 mm diameter, 20 mm height) were prepared from the tofu with a stainless steel boring tube and wire cutter. Samples were compressed by a cylindrical flat plate (39 mm diameter) to 50% deformation using a crosshead speed of 10 mm/min. Peak force (N) and firmness (N/mm) were measured. The hardness was expressed as the peak force obtained at 50% deformation.

Statistical analysis

The experiment was a completely random design (Steel and Torrie 1980). Seed size was reported as the mean of 3 determinations. All analyses, except yield of tofu, whey volume and texture of tofu were carried out in triplicate. The yield of tofu, whey volume and texture of tofu were the mean of 6 determinations.

Linear regression analysis was used to determine the correlation between the parameters of soybeans, soymilk, whey and tofu. Multiple regression analysis was used to determine the parameters of soybeans and soymilk which affect the yield of tofu. Multiple stepwise regression analysis was used to determine the relative contribution of selected independent variables to the observed variation of dependent variables. Coefficient of determination and coefficient of correlation are expressed as R^2 (multiple) and r (simple), respectively. Regression analyses were performed using the ACTS statistical programs of Agriculture Canada. All tests of significance were carried out at the probability level of $p = 0.05$.

RESULTS & DISCUSSION

Chemical composition and properties of soybeans

The chemical composition and properties of the nine soybean varieties used are shown in Table 1. The size of the soybeans used when expressed on a dry weight basis varied from 8.71 to 41.27 g/100 beans. This range of bean size is much larger than the size of 15.24 to 35.51 g/100 beans used by Wang et al. (1983) and the 17.2 to 23.3 g/100 beans used by deMan et al. (1987). Soybean breeders and tofu manufacturers are uncertain about whether larger beans give a high yield of tofu.

The chemical composition of the soybean varieties had the following range: moisture content 6.62 to 11.02%, protein content 36.87 to 45.10%, fat content 14.45 to 19.13%, carbohydrate content 32.52 to 39.40%, ash content 4.96 to 5.75% and phosphorus content 0.57 to 0.68% (Table 1). Lolas et al. (1976) reported a high correlation (r = 0.98) between phytic acid and the total phosphorus content of soybeans.

Properties of soymilk, whey and tofu

The properties of soymilk, whey and tofu obtained from the different soybean varieties are shown in Table 2. Total solids of the soymilk ranged from 9.09 to 9.90%. The variation in the total solids of soymilk could be due to the different mo-

Table 1—Proximate analyses of the 9 varieties of soybeans used.

Varieties	Seeds g/100	Moisture %	Protein[a] %	Fat[a] %	Ash[a] %	Carbohydrate[a] %	Phosphor %
Ryokko	41.27	10.02	41.60	16.09	4.96	37.35	0.57
KSX1768	25.85	7.40	41.67	18.49	5.58	34.26	0.59
Kurozaya	24.21	10.17	42.20	15.40	5.10	37.30	0.64
OX733	22.56	6.85	45.10	16.71	5.67	32.52	0.61
B220	16.08	6.75	36.87	18.24	5.75	39.14	0.60
Corsoy79	13.74	6.72	38.90	16.07	5.63	39.40	0.59
PI86.454	12.92	11.02	42.20	18.27	5.44	34.09	0.64
Nattoking K86	9.20	7.06	39.10	19.13	5.54	36.23	0.63
OX734	8.71	6.62	42.23	14.45	5.70	37.62	0.68
SD[b]	10.35	1.79	2.42	1.61	0.28	2.36	0.03
CV[c](%)	53.4	22.1	5.9	9.5	5.0	6.5	5.5

[a] Dry basis.
[b] SD = standard deviation.
[c] CV = coefficient of variation.

Table 2—Properties of soymilk, whey and tofu obtained from the 9 varieties of soybeans used.

	Varieties								
	Ryokko	KSX1768	Kurozaya	OX733	B220	Corsoy79	PI86.454	Nattoking K86	O
Soymilk									
pH	6.42	6.50	6.42	6.55	6.49	6.54	6.54	6.48	
Total solids (%)	9.60	9.69	9.46	9.90	9.44	9.37	9.09	9.40	
Protein[a] (%)	51.87	54.40	52.29	52.82	47.93	49.69	52.87	50.43	5
Phosphorus[a] (%)	0.69	0.72	0.77	0.73	0.74	0.74	0.80	0.77	
Whey									
Volume (mL)	82	62	93	59	77	78	89	82	8
Total solids (%)	3.40	3.09	3.19	3.04	3.13	3.19	2.75	2.97	
Tofu									
Fresh yield (kg/kg beans)	4.71	5.11	4.45	5.26	4.72	4.83	4.54	4.67	
Moisture (%)	87.63	88.13	87.59	88.58	87.67	88.37	86.58	87.41	8
Phosphorus (%)	0.70	0.69	0.77	0.70	0.72	0.72	0.76	0.75	
Peak force[b] (N)	2.11	2.41	1.89	2.62	1.95	2.07	1.80	2.24	
Firmness (N/mm)	0.25	0.28	0.23	0.29	0.23	0.26	0.23	0.25	

[a] Dry basis.
[b] At 50% deformation.

ture contents of soybeans or to different moisture solubility and extractability of some of the components.

The protein content of the soymilk varied from 47.93 to 54.40% (dry basis). The pH of the soymilk ranged from 6.42 to 6.55 with a coefficient of variation of 0.7%. The coagulability of soymilk can be affected by changes in pH (Kamel and deMan, 1982). The ionic strength of soymilk can also affect its pH. The phosphorus content of the soymilk ranged from 0.69 to 0.86%.

The volume of the whey varied from 59 to 93 mL. The variation of the whey volume was due to the different water holding capacity of the tofu. The total solids of the whey ranged from 2.75 to 3.40%. Solids in the whey are probably soluble sugars and low molecular weight proteins. All of the soybean varieties gave a clear whey indicating the amount of coagulant added was sufficient for complete coagulation of the soymilk.

The fresh yield of the tofu ranged from 4.45 to 5.26 kg/kg beans. The difference between the maximum and minimum yield of the tofu could be of economic importance in large tofu factories. This difference could result in 18% difference in tofu yield.

The moisture content of the fresh tofu varied from 86.58 to 88.58%. The moisture contents are comparable to the average of 88% moisture of fresh commercial tofu sold in Japan (Standard Tables of Food Composition, 1954). Tofu sold in retail stores is normally packed in water (for sanitation) so that water can be changed to keep the bacterial load to a minimum. The phosphorus content of tofu ranged from 0.69 to 0.83%.

A typical force-deformation plot of tofu when compressed to 50% deformation by the Instron Universal Testing Machine is shown in Fig. 1. The hardness of tofu as measured by peak force (N) is the force necessary to attain a given deformation. The firmness (N/mm) of tofu is defined as the ratio of stress/strain and was measured by the slope of the curve between points a and b in Fig. 1. The hardness and firmness of ranged from 1.80 to 2.62N and 0.23 to 0.29 N/mm, res tively. Different ratios of 7S/11S proteins have been repc to cause variation in the hardness of tofu (Saio et al., 19(

The chemical composition (dry basis) of the tofu made f different soybean varieties is shown in Table 3. The chen composition of the tofu had the following range: protein tent 46.03 to 52.50%, fat content 16.69 to 23.54%, ash cor 11.18 to 12.43% and carbohydrate content 16.47 to 20.0 About half of the dry weight of the tofu was protein.

The protein recovery of soymilk is the percentage of protein of the beans that is recovered in the soymilk. The r of protein recovery in the soymilk and tofu was: 83.0 91.09% from the soybean to the soymilk, 83.37 to 93. from the soymilk to the tofu, 69.39 to 81.39% from the bean to the tofu (Table 3). On average, 13.45% of the soyl protein was lost in the 'okara' (residue) during soymilk p aration and another 10.23% was lost in the whey. The varia of the protein recovery could be the result of differences ir solubility, extractability and coagulability of proteins from ferent soybean varieties.

Relationship between various parameters of soybeans, soymilk, whey, and tofu

Correlation between various parameters of the soybe soymilk, whey and tofu were obtained from data for the soybean varieties. No significant correlations were obta between seed size and the following parameters: (a) Soybe fat, protein, carbohydrate and phosphorus content: (b) S milk: total solids and pH; (c) Tofu: fresh yield, protein, hardness and firmness. There was, however, a significant ative correlation ($r = -0.72$) between the size and ash cor of the soybeans. As smaller beans have more surface area

Table 3 – Chemical composition of tofu and the protein recoveries obtained from the 9 varieties of soybeans used

Varieties	Protein[a] %	Fat[a] %	Ash[a] %	Carbohydrate[a] %	% Protein recovery[b]	% Protein conversion[c]	% Protein recovery[d]
Ryokko	50.83	18.38	11.18	19.61	88.68	86.67	76.84
KSX1768	51.07	21.14	11.28	16.51	91.09	87.78	80.01
Kurozaya	52.50	18.09	11.71	17.70	86.95	85.57	74.39
OX733	51.04	17.73	11.18	20.05	83.00	88.26	73.26
B220	46.03	23.54	11.62	18.80	87.74	91.67	80.41
Corsoy79	49.79	18.65	11.56	19.99	85.92	83.62	71.52
PI86.454	49.53	20.36	12.43	17.68	85.36	93.09	79.70
Nattoking-K86	50.63	21.40	11.49	16.47	86.97	93.57	81.39
OX734	52.29	16.69	11.53	19.48	83.20	83.37	69.39
SD	1.91	2.19	0.38	1.43	2.56	3.85	4.36
CV (%)	3.8	11.2	3.3	7.7	3.0	4.4	5.7

[a] Dry basis.
[b] Soybean to soymilk.
[c] Soymilk to tofu.
[d] Soybean to tofu.

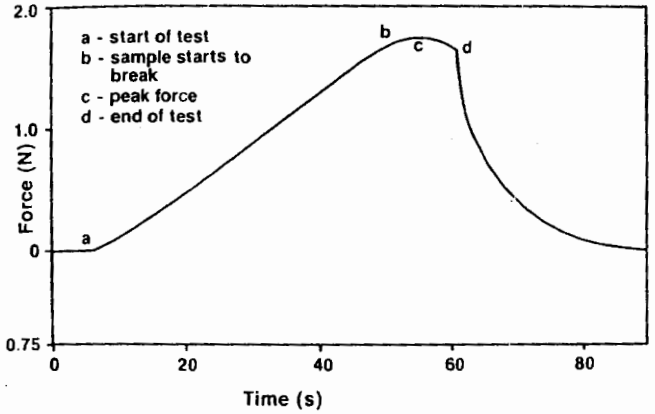

Fig. 1 – Typical force-deformation plot for compression of tofu to 50% deformation.

Table 4 – Linear regression equations[a] of various parameters obtained from 9 soybean varieties under the conditions used in this study

1. Protein of soymilk (%, db) = 0.688 (protein of soybean, %, db) + 23.43
   r = 0.84
2. Protein of tofu (%, db) = 0.712 (protein of soymilk, %, db) + 13.60
   r = 0.74
3. Protein of tofu (%, db) = 0.553 (protein of soybean, %, db) + 27.67
   r = 0.70
4. Fat of tofu (%, db) = 1.174 (fat of soybean, %, db) – 0.38
   r = 0.86
5. Phosphorous of soymilk (%, db) = 1.42 (phosphorus of soybean, %, db) – 0.12
   r = 0.96
6. Volume of whey (mL) = –41.56 (Fresh yield of tofu, kg/kg soybeans) + 276.41
   r = –0.99
7. Hardness of tofu (N) = 0.82 (total solids of soymilk, %) – 5.59
   r = 0.71
8. Hardness of tofu (N) = 0.332 (moisture of tofu, %) – 26.93
   r = 0.73
9. Firmness of tofu (N/mm) = 0.08 (hardness of tofu, N) + 0.08
   r = 0.95

[a] db = dry basis

larger beans on equal weight basis, the seed coat probably contains most of the soybean ash. A significant negative correlation (r = –0.72) was also obtained between the size of beans and the phosphorus content of the soymilk. Soybeans smaller in size produced soymilk which was higher in phosphorus content.

Using 17 varieties of soybeans with size ranging from 17.2 to 23.3 g/100 seeds, deMan et al. (1987) also found no correlation between seed size and protein content of soybeans.

There was no significant correlation (r = –0.37) between the protein and the fat content of the soybeans, but there was a significant negative correlation (r = –0.78) between the protein and the fat content of the tofu. There was also a significant negative correlation (r = –0.75) between the protein and the carbohydrate content of the soybeans.

A significant correlation was obtained between the following parameters: protein of the beans and protein of the soymilk (r = 0.84); protein of the soymilk and protein of the tofu (r = 0.70). Wang et al. (1983) and deMan et al. (1987) also reported a positive correlation between the protein content of beans and the tofu produced. Bourne et al. (1976) reported a positive correlation between protein in soybean and protein in soymilk, but high protein beans did not always give high protein milk.

No significant correlation was found between fresh yield of tofu and the protein content of either the soybeans or the soymilk. Contrary to the report of Wang et al. (1983), there was no significant correlation between the protein content of the soybeans and the dry yield (moisture free) of the tofu. Therefore, the yield of the tofu was not affected by the protein content of the soybeans alone.

There was a significant positive correlation (r = 0.86) between the fat content of the soybeans and the fat content of the tofu. Therefore, a soybean variety which is high in fat will produce tofu with higher fat content. In the process of making soymilk, fat is extracted with the proteins as a stable emulsion.

The ash of the soybeans was found to be positively correlated (r = 0.75) with the pH of the soymilk. There was however, no significant correlation between the ash content of the soybeans and tofu yield.

A significant correlation was obtained between the following parameters: phosphorus content of soybeans and phosphorus content of soymilk (r = 0.96); phosphorus content of soymilk and phosphorus content of tofu (r = 0.95); phosphorus content of soybeans and phosphorus content of tofu (r = 0.93). DeMan et al (1987) found a high correlation between phosphorus content in soybeans and in soymilk (r = 0.92). Recoveries of phosphorus in the soymilk and tofu were very good (mean phosphorus contents as reported in Tables 1 and 2 are as follows: soybean 0.62%, soymilk 0.76% and tofu 0.74%). Therefore, phytic acid (as measured by the phosphorus content) in the soybeans was extracted in the soymilk and became a component of the tofu.

It has been reported that phytic acid (as measured by phosphorus content) can bind both calcium and soy proteins and affects the texture of tofu (Saio, 1979; Prattley and Stanley 1982). We found no significant correlation between phosphorus content of the soybeans and hardness of tofu. There was however, a significant positive correlation (r = 0.71) between total solids of soymilk and hardness of tofu. Therefore, hardness and firmness (correlation between hardness and firmness was 0.95) of the tofu were not affected by protein content in either beans or tofu. Probably the way protein interacts with other structural components to form the microstructure of tofu and not the quantity of protein determines the hardness of tofu

Total solids content of soymilk was the only parameter that showed a significant correlation (r = 0.81) with fresh yield of tofu.

The linear regression equations of some parameters that involve processing of beans to tofu are given in Table 4. These equations can be used to predict the outcome of related parameters during processing of soybeans to tofu, under conditions similar to those used in this study.

Proposed models for the yield of tofu

Multiple stepwise regression and multiple regression analyses were used to develop models for the yield of tofu. Two models were proposed, one based on soybean characteristics and the other on soymilk characteristics. The model for the yield of tofu based on soybean characteristics will be useful to soybean breeders in cultivar selection. The other model for the yield of tofu based on soymilk characteristics will be useful to tofu manufacturers in process control to optimize the yield of tofu.

A proposed model for the yield (kg/kg beans) of tofu based on soymilk characteristics is shown below:

$$\text{Fresh yield of tofu} = 0.99\,(\text{Total solids of soymilk, \%}) + 3.02\,(\text{pH of soymilk}) - 24.23$$

$R^2 = 0.927$; $p = 0.05$; standard error of estimate = 0.087.

According to the above model, the variation in the total solids content and pH of soymilk accounted for 92.7% of the variation in the fresh yield of tofu. As suggested by the model, under similar conditions used in this study soymilk with higher pH and total solids content will give a higher yield of tofu. Because of differences in soybean variety, source and storage conditions, tofu producers are probably obtaining soymilk with a large variation in total solids content despite the use of an identical water:bean ratio. Therefore, in commercial large scale production of tofu, standardization of the total solids content of soymilk can minimise deviation in the yield of tofu. In cases where extraction of solids of the beans is low either because of variety of beans or storage conditions (Thomas et al., 1989) less water should be used in the extraction process.

Smaller soybeans will produce soymilk with a higher pH due to the higher ash content, which according to the above model will give a higher yield of tofu. There was, however, no correlation between soybean size and the total solids content of the soymilk. According to Kroll (1984), the amount of calcium ions bound to soy proteins increased rapidly with a small increase in pH in the range from 3.0 to 7.0. Calcium ions combining with soy proteins are highly sensitive in this pH range, probably due to the competition between the hydrogen ions and calcium ions for the same binding sites on the protein molecules. At higher pH, more calcium ions are possibly bound to the soy proteins to form a more extensive network structure that can trap more water and hence increase the yield of tofu.

A proposed model for the yield (kg/kg beans) of tofu based on soybean characteristics is as follows:

$$\text{Fresh yield of tofu} = 0.07\,(\text{protein of soybeans, \%, db}) + 0.70\,(\text{ash of soybeans, \%, db}) - 7.37\,(\text{phosphorus of soybeans, \%, db}) + 2.38$$

$R^2 = 0.933$; $p = 0.05$; standard error of estimate = 0.091.

According to this model, the variation in the protein, ash and phosphorus content of soybeans accounted for 93.3% of the variation in the fresh yield of tofu. The above model suggests that under the conditions used in this study, soybeans which are high in protein and ash and low in phosphorus will give a higher yield of tofu using $CaSO_4$ as a coagulant.

Since the proteins of soybeans can be extracted into soymilk, soybeans which are high in protein will produce a more extensive tofu protein network structure that probably will result in a higher yield of tofu. Soybeans which are high in ash produce soymilk with a higher pH, which according to the model based on soymilk characteristics will give a higher yield of tofu.

Phytic acid (as measured by the phosphorus content) can bind both calcium and soy proteins. It can then interfere in the coagulation process by reducing the interaction of calcium ions and soy proteins necessary to form the network structure of tofu.

The above model suggests that soybeans with higher ash content give a higher tofu yield, which has previously been shown to be a characteristic of the smaller beans. The model also suggests that a higher yield of tofu can be produced by soybeans low in phosphorus, which has previously been shown to be a characteristic of the larger beans. It is, therefore, not surprising that the size of soybeans has no significant effect on the yield of tofu.

The proposed models for the yield of tofu should next be tested in tofu factories. Such testing is important because in large scale commercial production of tofu the conditions used may vary from the smaller scale or laboratory scale production.

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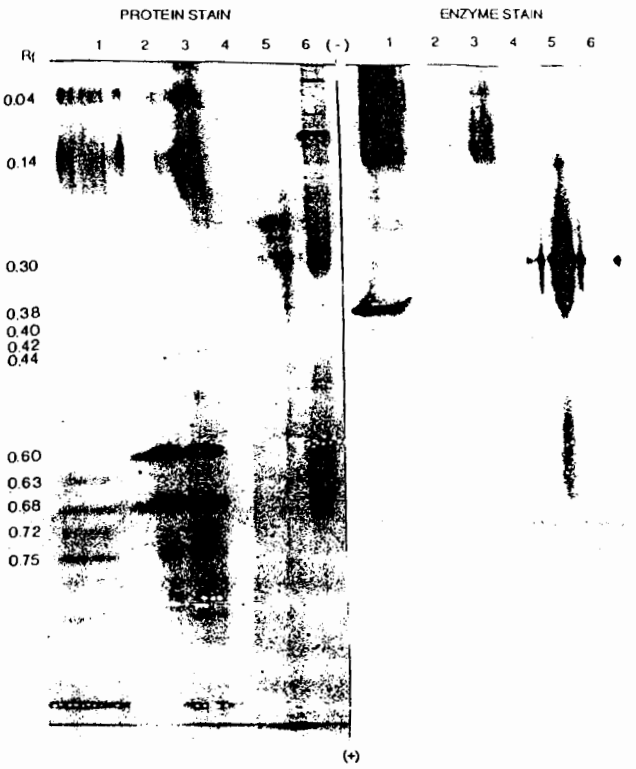

Fig. 3—Polyacrylamide gel electrophoresis of high performance liquid chromatography-hydrophobic interaction chromatography enriched PPO showing stain for protein (left) and enzyme (right) with R_f values of bands. Each lane contains 20 μg protein. Lane 1: PPO-retentate; 2: protein-retentate; 3: crude PPO; 4: blank; 5: standard mushroom PPO; 6: protein standards (bovine serum albumin, β-galactosidase, myosin, ovalbumin, and phosphorylase B).

CONCLUSIONS

IMPROVED ENRICHMENT of PPO can be achieved by increasing the number of HPLC fractions collected at the point of enzyme-protein overlap or with a subsequent separation on the HINT column. Refinement may also be possible by altering the initial salt concentration or the type of solvent for elution. Enrichment of PPO provides a procedure to isolate this enzyme in quantities for further biochemical studies. Analysis of the enzyme's properties would best be performed by purifying each isoform and should establish a better understanding of the role of the enzyme in chocolate flavor and color development during cocoa fermentation and drying. A more complete picture could be achieved through the isolation and determination of PPO activity at each stage of fermentation and drying. Assays with substrates other than (−)-epicathechin, for example (+)-catechin and anthocyanidins, could provide fruitful to determine whether with different substrates activity levels peak at different stages of fermentation.

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YIELD AND QUALITY OF TOFU. . .From page 1092

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Micronization Effects on Composition and Properties of Tofu

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ABSTRACT

ffect of infra-red heating (micronization) was investigated on mposition and textural, biochemical and nutritional properties ymilk and tofu. The yield, protein content and textural properties f made from micronized beans using the common procedures as coagulating temperature and $CaSO_4 2H_2O$ as coagulating agent) ower than those of tofu from unprocessed beans; tofu prepared micronized beans and coagulated at 90°C using a mixture of acid 0.01M) and calcium sulfate (0.03M) showed improved teristics. The microstructure of tofu prepared from micronized lacked the regularity of the honeycomb-like structure shown by from unprocessed beans. The heat treatment by micronization tle effect on the protein components of soymilk and tofu as mined by polyacrylamide disc gel electrophoresis.

INTRODUCTION

BEANS are valued for their high oil and protein content when properly processed, are a source of protein of good ty (Wang and Cavins, 1989). In Asia, soybeans have con- ed to an important part of the human diet for centuries f and Cowan, 1971; Coppock, 1974; Norman, 1978), y as soymilk and associated products (e.g., curd and e) and as fermented products such as soy sauce, miso, , and tempeh (Wolf and Cowan, 1971). In the Western sphere, soybeans have been predominantely for produc- f edible oil and protein concentrates for animal feeding. has been recent increasing interest in the use of whole ans as a food source both in the Western hemisphere and export crop (Kamel and deMan, 1982).

despread utilization of soy protein products offers im- nt nutritional and economic advantages (MacLeod and , 1988). However, there are problems associated with f soybeans for human food. These include the presence bean of various antinutritive factors, such as phytic acid, ase inhibitors, hemagglutinins, flatus factors, saponins, gens, lysinoalanine, and allergenic factors (Rackis, 1978, .b; Liener, 1980; Jaffe, 1981). Some of these are heat- , while others being heat-labile, can be removed by heat- ssing (Rackis, 1981a).

at treatment has been shown (Wolf and Cowan, 1971) to fective for elimination or reduction of antitryspin factor, y flavor" components, and hemagglutinins. However, l control of thermal processing conditions is essential to nt functional and nutritional changes which can result excessive heat treatment of the protein (Kakade et al., . Excessive heat can result in loss of heat-sensitive nu- , such as lysine, cystine, methionine and thiamine (Kouzeh- ni et al., 1981). Oxidative stability may be impaired due heat-induced destruction of natural antioxidants present ybeans. Furthermore, heat treatment can result in off- and off-flavors (Kouzeh-Kanani et al., 1981).

addition to the conventional moist heating process (Smith ircle, 1972), other traditional methods for heat treatment of soybeans include immersion cooking (Alberecht et al., 1967), dry heating or roasting (Cowan, 1979), extrusion (Bookwalter et al., 1971), dielectric heating (Borchers et al., 1972), microwave processing (Wing and Alexander, 1975), and infrared (micronization) cooking (Livingston, 1977). The term "micronization" is often used to refer to a continuous process of heat treatment of cereals, pulses, oilseeds, etc., and is based on relatively short-time processing by infrared radiation (Livingtson, 1977). There has been increasing interest in the application of micronization as a processing technology for raw grains and oilseeds. The objective of our study was to evaluate the effects of processing by micronization (infrared heating) on the properties of soymilk and tofu derived from micronized full-fat soybeans. The effects of micronization on the proximate composition, textural properties, and the biochemical and nutritional properties of soymilk and tofu prepared from incronized soybeans were determined. Polyacrylamide gel electrophoresis was used to determine the effect of the micronization process on protein components and protein interactions.

MATERIALS & METHODS

Materials

Unprocessed and micronized (infrared-heated) full-fat soybeans were obtained from a soybean processing plant (Micrograin Inc., St-Robert, Québec, Canada). The micronization process for the full-fat soybeans involved infrared dry heating for 90 sec during which the soybeans reached an average temperature of 110–115°C.

Preparation of soymilk and tofu

Samples of the unprocessed and micronized soybeans (250g) were rinsed, soaked in tap water (1,500 mL) overnight (16 hr) at 5°C, then homogenized with water in a Waring Blendor (2 min). The soybean slurry was heated to boiling, simmered for 10 min, then filtered hot through several layers of cheesecloth to separate the soluble components (soymilk) from the insoluble material (okara). The soymilk (1250 mL) was heated to boiling and cooled to 70°C. A calcium sulfate dispersion (7.5g $CaSO_4 2H_2O$ in 250 mL hot water) was added to soymilk at 70°C (coagulating temperature) with stirring to coagulate the proteins. This gave a final $CaSO_4$ concentration of 0.03M. The mixture was allowed to stand for another 10 min without agitation in order to obtain complete coagulation. The coagulated soymilk was poured gently into a perforated plastic mold (15×12×5.5 cm) lined with several layers of cheesecloth. The curd which collected in the cheesecloth was pressed with a weight of 0.5 kg for 30 min. The resultant tofu cake was weighed and refrigerated (5°C) until analyzed further. Samples of soymilk and tofu were freeze-dried and stored at 5°C until used for subsequent analysis. The transmission (400 nm) of the whey obtained from the curd was measured immediately after separation using an LKB Biochrom Novaspec 4049 spectrophotometer.

For the study of the effect of coagulating temperature, tofu was prepared from micronized soybeans at coagulating temperatures of 70, 80, 90°C; calcium sulfate was used as the coagulating agent. For the study of the effect of the concentration of coagulating agent (calcium sulfate), tofu was prepared from micronized soybeans at a coagulating temperature of 90°C; calcium sulfate at three different concentrations (0.02M, 0.03M, 0.04M was used for coagulation. To study the effect of the different coagulating agents, tofu was prepared from micronized soybeans at a coagulating temperature of 90°C; calcium sulfate (0.03M), citric acid (0.01M) and a mixture of citric acid and calcium sulfate

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Composition and textural properties of soymilk and tofu from ed and micronized soybeans

nents	Soymilk		Tofu[a]	
	Unprocessed	Micronized	Unprocessed	Micronized
	93.7 ± 1.24[a]	93.9 ± 1.07[a]	81.4 ± 1.11[a]	82.4 ± 1.13[a]
	0.32 ± 0.02[a]	0.28 ± 0.02[a]	1.46 ± 0.14[a]	1.68 ± 0.11[a]
	1.43 ± 0.15[a]	1.73 ± 0.09[b]	5.43 ± 0.34[a]	5.17 ± 0.25[a]
	2.77 ± 0.25[a]	3.66 ± 0.31[b]	9.56 ± 0.90[a]	6.58 ± 0.50[b]
ate[d]	1.75	0.41	2.95	4.14
	–	–	253.6 ± 3.9[a]	179.7 ± 6.8[b]
	–	–	17.4 ± 1.1[a]	17.9 ± 0.5[a]
(N)	–	–	0.67 ± 0.08[a]	0.29 ± 0.1[b]
N/mm	–	–	0.108 ± 0.01[a]	0.058 ± 0.02[b]

triplicates. Means in same row with same superscript are not significantly different ($p < 0.05$).
N × 6.25.
ate by extraction
with $CaSO_4.2H_2O$ at 70°C.

rate/0.03 M calcium sulfate) were used as coagulating agents. experiments, sufficient quantities of soymilk required for iables were prepared at the same time.

e analysis

e content was determined using the procedure of the AOAC eighed quantities (2–3g) of soymilk were oven-dried at 110°C tant weight was obtained. Weighed quantities (approxi-5g) of tofu (which included the whey that separated during d storage) were dried on a steam bath for 15 min then by oven drying at 110°C for 12 hr. Ash content was determined ing weighed quantities (about 2g) of samples before placing heated (600°C) muffle furnance for 2 hr (AOAC, 1984). -Kjeldahl method (AOAC, 1984) was used to determine the ein content (N × 6.25) using a Labconco Rapid Still III Corporation, Kansas City, MO). Petroleum ether extrac- Soxhlet fat extraction; AOAC, 1984) was used to deter- e fat.

nalysis of tofu

characteristics of tofu samples were measured using an niversal Testing Machine. Cubes ($2cm^3$) of tofu were cut nterior of prepared tofu cake (15 × 12 × 5.5 cm). The cubes pressed to 25% of the original height (5 mm) at a probe 0 mm/min. The compression force was recorded at a chart 00 mm/min. The load cell scale was 0-50 kg. Force mea- were converted from kg to N.

cture of tofu

ructure of tofu was determined using a Stereoscan 600 Elec- oscope (Cambridge Scientific Instruments Limited, Cam- A). The tofu samples were fixed in glutaraldehyde solution .2) for 2 hr at room temperature and then rinsed 3x with samples were postfixed in osmium tetroxide (1%) for 1 hr mperature, then washed 3× with water. The fixed samples n in liquid nitrogen and freeze-dried. The dried samples nted on an aluminum stub and sputter-coated with gold/ (60/40). The specimens were observed and photographed.

In vitro protein digestibility

The *in vitro* protein digestibility of freeze-dried samples of soymilk and tofu was determined by the multienzyme (tryspin, chymotrypsin and peptidase, Sigma Chemical Co., St. Louis, MO) procedure as described by Hsu et al. (1977). The digestibility of sodium caseinate (Sigma Chemical Co.) was determined for comparison.

Available lysine

The available lysine in freeze-dried samples of soymilk and tofu was determined by a procedure described by Carpenter (1960) using modifications suggested by Booth (1971). This procedure is based on the formation of a yellow complex, dinitrophenyllysine (λmax 435 nm) when 1-fluoro-2,4-dinitrobenzene reacts with ε-amino groups of lysine.

Polyacrylamide disc gel electrophoresis

The electrophoretic procedures and the techniques for staining and destaining of gels for protein were performed according to the method described by Maurer (1971). Freeze-dried soymilk (10 mg) and tofu (10 mg) were added to the spacer gel solution (0.5 mL), and 100 μL of this solution was subjected to electrophoresis (4 mA/gel, 1 hr). Triplicate gels were prepared for each sample. One gel was stained for protein using Coommassie blue staining solution (1%). The second gel was subjected to glycoprotein staining using fuchsin-sulfite staining solution (0.5%) according to the method described by Zacharius et al. (1969). The third gel was stained for lipoprotein using an alcoholic solution of Sudan Black B (0.1% in 60% ethanol) according to the method described by Swahn (1952).

Statistical analysis

For all experiments, triplicate samples of soymilk and tofu were prepared and analyzed. Duncan's New Multiple Range Test (Steel and Torrie, 1980) was used to compare means of values from the triplicate samples.

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION was determined on the soymilk and tofu prepared from unprocessed and micronized soybeans (Table 1). The protein content of soymilk prepared from the micronized beans was higher ($p<0.05$) than that of soymilk prepared from the unprocessed beans indicating an increase in water dispersibility of the soybean protein as a result of micronization. The protein content of tofu from the micronized beans was lower ($p<0.05$) than that from the unprocessed beans, suggesting that micronization also affected the coagulation properties of the proteins. The higher ($p<0.05$) percent transmission of the whey (liquid remaining after coagulation of protein) from the unprocessed beans (% whey transmission = 46.8 ± 2.6) was further evidence of the greater protein coagulation when compared with that of micronized beans (% whey transmission = 19.9 ± 0.1). Tofu from the micronized beans showed lower firmness and hardness ($p<0.05$) when compared to tofu from unprocessed beans (Table 1). This suggested that, in the case of the micronized beans, the pro-

Table 2—Effect of coagulation temperature on composition and textural properties of tofu

Whey[d]	Tofu[a]				
Transmission (%)	Yield (g)	Protein (%)	Solid (%)	Firmness (N/mm)	Hardness (N)
19.1 ± 0.1[b]	179.7 ± 6.8[b]	6.58 ± 0.5[b]	17.9 ± 0.5[c]	0.06 ± 0.02[bc]	0.29 ± 0.10[b]
12.2 ± 0.1[c]	180.5 ± 2.9[b]	6.86 ± 0.3[b]	14.1 ± 0.8[a]	0.04 ± 0.01[c]	0.21 ± 0.02[b]
10.4 ± 0.2[c]	219.1 ± 2.3[ab]	8.72 ± 0.6[a]	15.6 ± 0.6[b]	0.07 ± 0.01[b]	0.35 ± 0.03[b]
46.8 ± 2.6[a]	253.6 ± 3.8[a]	9.56 ± 0.8[a]	17.4 ± 1.1[c]	0.11 ± 0.01[a]	0.67 ± 0.08[a]

triplicates. Means in same column with same superscripts are not significantly different ($p<0.05$).
cted from the pressed tofu.
with $CaSO_4.2H_2O$ at 70°C.
e of coagulation (°C).
bean.
ed bean.

Table 3 – Effect of coagulant concentration on composition and textural properties of tofu prepared from micronized soybeans

Concentration of coagulant (M)	Tofu[d] Yield (g)	Protein (%)	Solid (%)	Firmness (N/mm)	Hardness (N)
0.02	170.1 ± 4.5[a]	7.63 ± 1.1[a]	14.8 ± 2.3[a]	0.05 ± 0.01[b]	0.29 ± 0.01[b]
0.03	172.7 ± 7.4[a]	8.94 ± 0.8[a]	16.0 ± 1.0[a]	0.06 ± 0.01[b]	0.32 ± 0.07[b]
0.04	162.8 ± 5.0[a]	7.96 ± 0.1[a]	14.7 ± 1.9[a]	0.09 ± 0.02[a]	0.47 ± 0.04[a]

[a,b] Means of triplicates. Means in same column with same superscripts are not significantly different ($p < 0.05$).
[d] Coagulated with $CaSO_4.2H_2O$ at 70°C.

teins which coagulated had reduced intermolecular binding properties when compared with the coagulated proteins of unprocessed beans. This could explain the relatively inferior textural properties of the tofu from the micronized beans. Kilara and Sharkasi (1986) reported that protein-protein interactions involving intermolecular disulfide bonds of both the 7S and 11S proteins of soybeans could affect the solubility and viscosity properties of the proteins. Previously Roberts and Briggs (1965) reported fewer sulfhydryl groups in soybean 7S protein when compared to the 11S fraction. This difference was cited to explain the greater heat sensitivity of the 7S fraction. Possibly the heat of micronization could have affected the sulfhydryl-disulfide interchange reactions of the 7S and 11S fractions (Hashizume et al., 1975) and this could explain the effect of micronization on textural properties of the tofu.

Micronized bean tofu obtained by coagulation at 90°C gave maximum yield and protein content (Table 2) when compared with coagulation at 70° and 80°C. That same showed maximum firmness and hardness. However, the values were lower than those of unprocessed-bean tofu coagulated at 70°C (Table 1). Wang and Hesseltine (1982) reported decreased yield and increased percent solids of curd as the coagulation temperature for tofu (from unprocessed beans) increased from 60° to 80°C. This was different from results obtained in our study (with micronization) which showed an increase in yield of tofucake and a decrease in percent solids as coagulation temperature for tofu increased from 70° to 90°C. This difference could have been because in our work measurements were made on the processed tofu cake after pressing (Lim et al., 1990), while in the report of Wang and Hesseltine (1982) the measurements were made on the curd before pressing into tofu cake. The coagulation temperature over the range of 70° to 90°C had little effect on textural properties of tofu obtained from the micronized bean. There were no significant differences ($p < 0.05$) between the firmness and hardness of the tofu (from micronized beans) obtained at coagulation temperature of 70° to 90°C. Wang and Hesseltine (1982) reported increased hardness and elasticity as coagulation temperature increased, and little influence of temperature on cohesiveness.

Coagulant concentration affected composition and textural properties of tofu from micronized beans (Table 3). The results indicated that use of calcium sulfate at a concentration of 0.03M gave improved yield and higher protein content of tofu. Use of calcium sulfate at a concentration of 0.04M resulted in improved firmness and hardness. Saio (1979) reported increased hardness of tofu prepared with use of high concentrations of coagulant. The effects of three coagulating agents were compared on composition and textural properties of micronized bean tofu (Table 4). The results indicated that use of citric acid (0.01M) and a mixture of citric acid (0.01M) and calcium sulfate (0.03M) as coagulating agents decreased yield and had little effect on protein content and textural properties of tofu. Somewhat similar results were obtained by Lu et al. (1980) who investigated calcium chloride, calcium acetate, acetic acid, calcium lactate, calcium sulfate and glucono-δ-lactone (GDL) as coagulating agents. They showed that soybean curds prepared with calcium salts had a higher weight yield than those with non-calcium compounds.

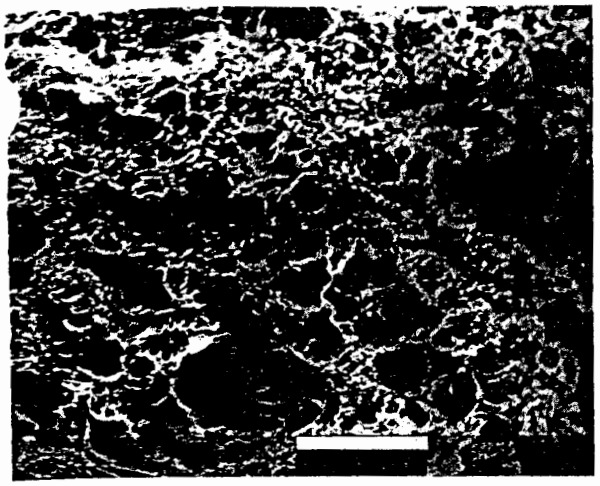
Scanning electron photomicrographs (× 500) of tofu prepared from unprocessed beans and micronized beans were compared (Fig. 1 to 4). The structure of tofu coagulated with calcium sulfate showed a typical 3-dimensional honeycomb-like network with some degree of regularity or uniformity (Fig. 1). The fine structure of tofu obtained from micronized beans (Fig. 2) lacked the typical 3-dimensional honecomb-like network demonstrated by tofu from the unprocessed beans. This structure also showed that the structural network was somewhat collapsed. Likely the heat treatment of micronization affected the tofu microstructure by its effect on protein structure; Lee and Rha (1978) reported that heat treatment which unfolds polypeptide chains affected elasticity and this could be related to stereo-structure and intermolecular interactions. The structure of tofu from the micronized beans showed a lack of regularity associated with that of unprocessed beans. Fig. 3 shows the SEM micrographs of tofu prepared from micronized beans using citric acid instead of calcium sulfate as coagulating agent. The structure was somewhat similar to that of tofu prepared from unprocessed beans. This suggested that use of citric acid instead of calcium sulfate as coagulating agent reduced some of the effects of micronization on the fine structure of tofu. The mechanism by which the citric acid produced this effect was not investigated; however, other researchers (Lu et al., 1980) have shown that organic acids or calcium salts of organic acids affected the coagulation of curd from soymilk. The use of a mixture of calcium sulfate-citric acid as coagulating agent did not have a marked effect on the fine structure (Fig. 4) of tofu from micronized soybeans when compared to use of citrate only (Fig. 2).

The effect of micronization on the *in vitro* protein digestibilities and the available lysine of soymilk and tofu was studied (Table 5). The soymilk from micronized beans showed higher ($p < 0.05$) digestibility than soymilk from unprocessed beans. The temperature (110–115°C) of the micronization process could explain the higher digestibility of the soymilk from micronized beans. The destruction by heat of heat-labile trypsin inhibitors which reduce protein digestibility has been well documented. The digestibilities of the soymilk from unprocessed soybeans was also less ($p < 0.05$) than that of casein (88.2%). However, the digestibilities of tofu obtained from unprocessed (97.2%) and micronized beans (96.7%) were significantly higher ($p < 0.05$) than that of casein. This suggested that certain fac-

Table 4 – Effect of different coagulants on composition and textural properties of tofu prepared from micronized soybeans

Coagulant	Whey[d] pH	Transmission (%)	Tofu[e] Yield (g)	Protein (%)	Solid (%)	Firmness (N/mm)	Hardness (N)
Calcium sulfate[f]	5.90 ± 0.0[a]	14.6 ± 1.4[c]	192.9 ± 2.6[b]	8.94 ± 0.8[a]	16.0 ± 1.0[ab]	0.06 ± 0.01[b]	0.32 ± 0.07[c]
Citric acid[f]	4.21 ± 0.1[d]	16.1 ± 0.2[c]	127.9 ± 9.5[c]	10.44 ± 1.9[a]	16.3 ± 1.3[ab]	0.11 ± 0.03[a]	0.46 ± 0.09[bc]
Citrate sulfate[f]	3.86 ± 0.1[c]	22.9 ± 0.1[b]	130.8 ± 0.2[c]	8.25 ± 0.8[a]	15.3 ± 0.7[a]	0.13 ± 0.02[a]	0.55 ± 0.12[ab]
Calcium sulfate[g]	5.73 ± 0.1[b]	46.8 ± 2.6[a]	253.6 ± 3.9[a]	9.56 ± 0.9[a]	17.4 ± 1.1[b]	0.11 ± 0.01[a]	0.67 ± 0.08[a]

[a,b,c] Means of triplicates. Means in same column with same superscripts are not significantly different ($p < 0.05$).
[d] Whey collected from the pressed tofu.
[e] Coagulated with $CaSO_4.2H_2O$ at 70°C.
[f] Calcium sulfate (0.03 M); citric acid (0.01 M); citrate-sulfate (0.01 M/0.03M)
[g] Unprocessed bean tofu coagulated at 70°C (0.03 M) used as control.



1—SEM photomicrograph of tofu from unprocessed beans
calcium sulfate as coagulating agent, Mag. × 500 (Bar =

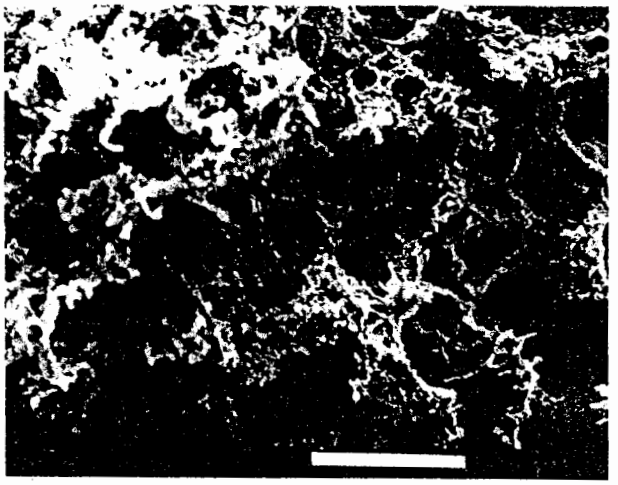
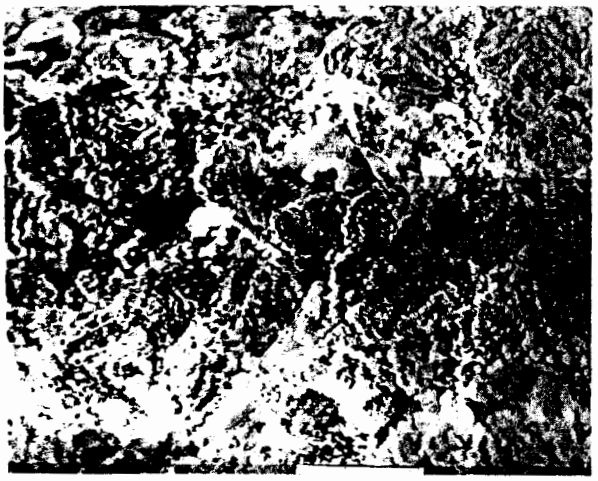


Fig. 3—SEM photomicrograph of tofu from micronized beans using citric acid as coagulating agent, Mag. × 500 (Bar = 40 μ).



2—SEM photomicrograph of tofu from micronized beans
calcium sulfate as coagulating agent, Mag. × 500 (Bar =

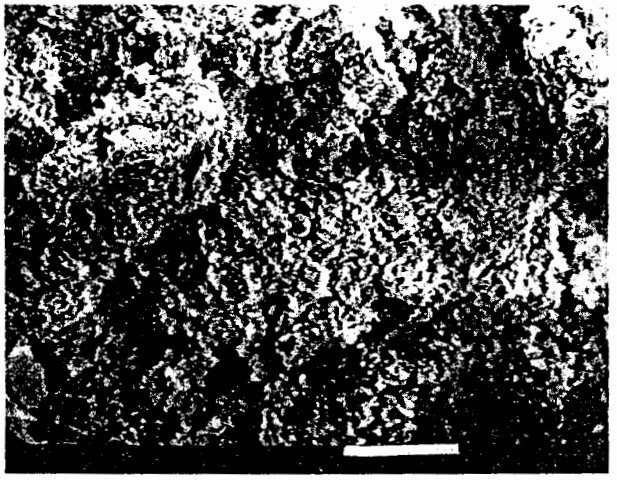


Fig. 4—SEM photomicrograph of tofu from micronized beans using a mixture of citric acid and calcium sulfate as coagulating agent, Mag. × 500 (Bar = 40 μ).

5—In vitro protein digestibility and available lysine content of ca-
soymilk and tofu

	Digestibility (%)		Available lysine (g/16g N)	
ie	Unprocessed	Micronized	Unprocessed	Micronized
ilk	83.2 ± 0.27[a]	86.5 ± 0.84[b]	4.64 ± 0.11[a]	6.14 ± 0.30[b]
	97.2 ± 1.10[c]	96.7 ± 1.21[c]	5.94 ± 0.13[a]	6.60 ± 0.32[b]
n	83.2 ± 0.42[b]	–	–	–

eans of triplicates. Means in same row with same superscripts are not signif-
ntly different ($p < 0.05$).

which reduced protein digestibility remained in the whey
the proteins were coagulated. A reduction of available
e is generally regarded as an indicator of overheating
ızeh-Kanani et al., 1981). In our work, the results showed
the available lysine contents of soymilk (6.14%) and tofu
)%) from micronized beans were higher than those of the
sponding products derived from unprocessed beans (soy-
, 4.64%; tofu, 5.94%). This was somewhat unexpected.
likely explanation is that the effect of the heat of micron-
on resulted in structural changes which led to availability
eviously unavailable ϵ-amino groups.
ne effects of micronization on the protein components and
he interactions between protein-lipid and protein-carbo-
hydrate in tofu and soymilk prepared from the unprocessed and micronized beans were also investigated using polyacrylamide disc gel electrophoresis (PAGE). The interactions could have a significant effect on functional properties such as gelation, water binding capacity, foaming and emulsification (Kinsella, 1979). Electropherograms (PAGE in the absence of dissociation agents) of proteins were obtained from the soymilk prepared from unprocessed (A_1 and A_2) and micronized (B_1 and B_2) (Fig. 5). Results showed some qualitative differences in behavior of proteins of soymilk prepared from unprocessed (A_1) and soymilk of micronized (B_1) soybeans. The bands in the upper portion of the gel from soymilk were not very distinct as a result of what appeared to be diffusion effects. Possibly the non-protein components in the soymilk may have interfered with the proper separation of proteins during electrophoresis under these conditions. The gels which were stained for carbohydrate (A_2 and B_2) demonstrated that one of the bands was associated with carbohydrate material. The gels did not show a positive staining for lipoproteins. Figure 5 also shows some qualitative differences in electrophoretic behavior of protein of the tofu from unprocessed (C_1) and tofu from micronized (D_1) beans. The separation of fractions from tofu was superior to the separation of fractions from soymilk. The results suggested that the major components of tofu were present in the soymilk

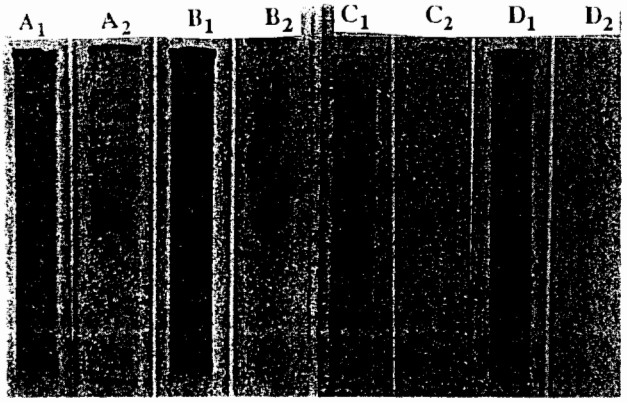


Fig. 5—Electropherograms for polyacrylamide disc gel electrophoresis with tris aminoethane-glycine buffer (pH 8.9) of (a) soymilk from unprocessed (A_1, A_2) and micronized beans (B_1, B_2), gels were stained for protein (A_1, B_1) and for glycoprotein (A_2, B_2), (b) tofu from unprocessed (C_1, C_2) and micronized beans (D_1, D_2), gels of each protein sample were stained for protein (C_1, D_1) and for glycoprotein (C_2, D_2).

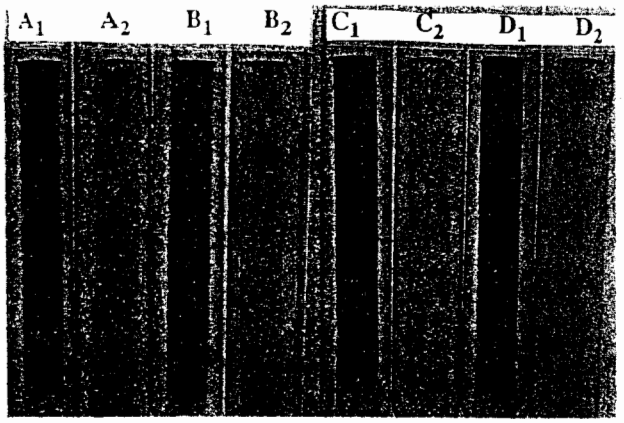


Fig. 6—Electropherograms (SDS electrophoresis) of soymilk (A_1, A_2) and tofu (C_1, C_2) from unprocessed soybean and of soymilk (B_1, B_2) and tofu (D_1, D_2) from micronized soybeans (B_1, B_2); gels A_1, B_1, C_2 and D_2 were stained for glycoprotein.

rom which the tofu was prepared. In both tofu and soymilk, small fraction was associated with carbohydrate material. Micronization apparently have had little effect on protein-carbohydrate interactions.

SDS-electrophoresis of soymilk and tofu from unprocessed and micronized beans (Fig. 6) resulted in similar fractionation patterns. This confirmed the suggestion that micronization did not result in marked changes in the molecular constitution of the proteins and had little effect on protein-carbohydrate and protein-lipid interactions in soymilk and tofu. Note, however, that micronization affected the texture (firmness, hardness) and the fine structure (SEM) of tofu. This apparently was not attributable to any effects on the molecular constitution of the protein.

CONCLUSION

THE INFRARED heating procedure of micronization affected coagulating properties of proteins of soybeans as they relate to preparation of tofu. Changing the appropriate conditions of coagulation, such as coagulating agent and temperature, would not likely improve the coagulating behavior. Consequently, the yield and texture of tofu from micronized soybeans cannot likely be improved. Micronization improved the digestibility of soymilk. In terms of its biochemical properties, the results showed that heat treatment by micronization had only minor effects on electrophoretic behavior of protein components of the soymilk and tofu.

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GYPSUM

(Data in thousand metric tons, unless otherwise noted)

Domestic Production and Use: In 1999, crude gypsum output exceeded 19.4 million tons valued at $134 million. The top producing States were Oklahoma, Iowa, Texas, Michigan, California, Nevada, and Indiana, which together accounted for 72% of total output. Overall, 32 companies produced gypsum at 61 mines in 19 States, and 11 companies calcined gypsum at 65 plants in 27 States. Most of domestic consumption, which totaled about 31.8 million tons, was accounted for by manufacturers of wallboard and plaster products. More than 4 million tons for cement production, almost 2 million tons for agricultural applications, and small amounts of high-purity gypsum for a wide range of industrial processes, such as smelting and glassmaking, accounted for remaining uses. Capacity at operating wallboard plants in the United States was 30 billion square feet per year, while sales were more than 29 billion square feet, representing capacity utilization greater than 98%.

Salient Statistics—United States:	1995	1996	1997	1998	1999e
Production: Crude	16,600	17,500	18,600	19,000	19,400
Byproduct[1]	2,300	2,500	2,700	3,000	3,300
Calcined[2]	16,700	17,000	17,200	19,400	20,600
Wallboard products (million square feet)	24,000	23,700	24,400	26,900	29,100
Imports, crude, including anhydrite	8,160	8,050	8,420	8,680	9,200
Exports, crude, not ground or calcined	79	136	174	166	108
Consumption, apparent[3]	27,000	27,900	29,500	30,500	31,800
Price: Average crude, f.o.b. mine, dollars per ton	7.29	7.10	7.11	7.20	6.92
Average calcined, f.o.b. plant, dollars per ton	17.37	16.88	17.58	18.00	17.02
Stocks, producer, crude, yearend	1,100	1,200	1,200	1,500	1,500
Employment, mine and calcining plant, numbere	6,500	6,300	6,000	6,000	6,000
Net import reliance[4] as a percent of apparent consumption	30	29	28	28	29

Recycling: A relatively small amount of gypsum wallboard is recycled.

Import Sources (1995-98): Canada, 68%; Mexico, 23%; Spain, 8%; and other, 1%.

Tariff: Item	Number	Normal Trade Relations 12/31/99
Gypsum; anhydrite	2520.10.0000	Free.

Depletion Allowance: 15% (Domestic and foreign).

Government Stockpile: None.

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GYPSUM

Events, Trends, and Issues: Construction of new homes, commercial buildings, and office space continued to stimulate wallboard demand and boosted domestic consumption of gypsum. Some forecasts indicate that gypsum demand in North American markets will remain high for the next few years. This demand, however, will depend principally on the strength of the construction industry, particularly in the United States where more than 90% of the gypsum consumed is used for wallboard products, building plasters, and the manufacture of portland cement. Federal funding that was authorized in 1998 for road building and repair through 2003 will continue to spur gypsum consumption in the cement industry. Several large wallboard plants under construction and designed to use only byproduct gypsum will accelerate substitution significantly as they become operational within a few years.

World Mine Production, Reserves, and Reserve Base:

	Mine production		Reserves[5]	Reserve base[5]
	1998	1999e		
United States	19,000	19,400	700,000	Large
Australia	2,100	2,200		
Canada	8,100	8,200	450,000	Large
China	9,000	9,200		
Egypt	2,000	2,000		
France	4,500	4,500		
India	2,400	2,500		
Iran	9,000	9,000		
Italy	2,000	2,000	Reserves and reserve	
Japan	5,300	5,300	base are large in major	
Mexico	7,045	7,100	producing countries, but	
Poland	1,000	1,000	data are not available.	
Spain	7,400	7,400		
Thailand	9,000	9,000		
United Kingdom	2,000	2,000		
Other countries	17,200	17,500		
World total (rounded)	107,000	108,000	Large	Large

World Resources: Domestic resources are adequate, but are unevenly distributed. There are no significant gypsum deposits on the eastern seaboard of the United States, where large imports from Canada augment domestic supplies for wallboard manufacturing in large metropolitan markets. Large deposits occur in the Great Lakes region, midcontinental region, and California. Foreign resources are large and widely distributed; more than 90 countries produce gypsum.

Substitutes: Other construction materials may be substituted for gypsum, especially cement, lime, lumber, masonry, and steel. There is no practical substitute for gypsum in portland cement. Byproduct gypsum generated by various industrial processes is becoming more important as a substitute for mined gypsum in wallboard manufacturing, cement production, and agricultural applications.

eEstimated.

[1]Only byproduct reported as sold or used.

[2]From domestic crude.

[3]Defined as crude + total reported byproduct use + net import reliance.

[4]Defined as imports - exports + adjustments for industry stock changes.

[5]See Appendix C for definitions.

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CALCIUM SULFATE

Calcium sulfate [*7778-18-9*], $CaSO_4$, in mineral form is commonly called gypsum and occurs abundantly in many areas of the world. In natural deposits, the main form is the dihydrate. Some anhydrite is also present in most areas, although to a lesser extent. Mineral composition can be found in Table 1.

Table 1. Gypsum Forms and Composition

Common name	CAS Registry Number	Molecular formula	Composition, wt %		
			CaO	SO_3	Combined H_2O
anhydrite	[*7778-18-9*]	$CaSO_4$	41.2	58.8	
gypsum	[*10101-41-4*]	$CaSO_4 \cdot 2H_2O$	32.6	46.5	20.9
stucco	[*10034-76-1*]	$CaSO_4 \cdot \frac{1}{2}H_2O$	38.6	55.2	6.2

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teenth centur
resistance, led
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Properties

Table 2 lists t

Table 2. Physic

Property
mol wt
transition poin
mp[c], °C
specific gravit
solubility at 2
hardness, Moh

[a]Hemihydrate is
[b]Anhydrous ma
[c]Compound dec

Sources

The natural
quarrying a
The dihydra
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provide a c

The hemihydrate (stucco) is normally produced by heat conversion of the dihydrate from which ¾ H_2O is removed as vapor. The resulting powder is also known as plaster of Paris [*26499-65-0*]. Stucco has the greatest commercial significance of these materials. It is the primary constituent used to fabricate products and in formulated plasters used in job- or shop-site applications.

About 23 million metric tons of gypsum are consumed annually. About 80% is processed into the commercially usable hemihydrate. Uses of gypsum are in fabricated and/or formulated building materials (see BUILDING MATERIALS, SURVEY), Portland cement (qv) set regulation, and agricultural soil conditioning.

Gypsum and its dehydrated form have been used by builders and artists in ornamental and structural applications for more than 5000 years as evidenced by artifacts from the ancient Egyptian and Greek cultures. Processing of gypsum to the hemihydrate in the United States began about 1835 using ore imported from the Canadian Maritime Provinces. Methods for control of the set (hydration time) of hemihydrate conversion to dihydrate were developed by the end of the nineteenth century. This factor, along with the desirable natural property of fire resistance, led to the growth of a significant industry that produces boards and plasters as the primary wall cladding materials in modern building construction.

Properties

Table 2 lists the physical properties of calcium sulfate.

Table 2. Physical Properties of Calcium Sulfate

Property	Dihydrate	Hemihydrate	Anhydrite
mol wt	172.17	145.15	136.14
transition point, °C	128[a]	163[b]	
	163[b]		
mp[c], °C	1450	1450	1450
specific gravity	2.32		2.96
solubility at 25°C, g/100 g H_2O	0.24	0.30	0.20
hardness, Mohs'	1.5–2.0		3.0–3.5

[a]Hemihydrate is formed.
[b]Anhydrous material is formed.
[c]Compound decomposes.

Sources

The natural, or mineral, form of gypsum is most widely extracted by mining or quarrying and used commercially. Natural gypsum is rarely found in a pure form. The dihydrate and anhydrous forms are commonly found together. Impurities in gypsum deposits typically include calcium and magnesium carbonates, oxide(s) of silicon, clays, and small amounts of various soluble salts. The last two items generally have the most undesirable effect on commercial processing and production of prefabricated products. In some cases, the crude ore is beneficiated to provide a commercial feedstock in which the percentage of functional dihydrate

has been increased. Most gypsum commercially used has a purity level of 80% or higher.

The natural ore is quarried or mined in many areas of North America and Europe. Leading North American regions include Canada, Mexico, and in the United States, California, Texas, Nevada, Iowa, Kansas, Ohio, Indiana, and Michigan. In Europe, France, Spain, Italy, the United Kingdom, and Russia have significant deposits of natural gypsum, as does Germany.

Gypsum is found in a variety of natural mineral forms. Most notable is the massive, or rocklike form commonly used in commercial manufacturing operations. However, there are other unique forms of special interest. Among these are alabaster, a fine-grained, relatively soft, rather pure gypsum used almost exclusively by sculptors. Colorado is the principal commercial source in the United States, although alabaster is occasionally found in other deposits. Satin spar is also a pure form of crystalline gypsum that is fibrous in nature. It is translucent in its dense form. Another unique form of gypsum is selenite, high in purity and monoclinic in form. It frequently occurs as an intrusion in more rocklike deposits, but large sheets, up to several meters, of selenite have been found. Sometimes selenite is mistaken for mica because of its platey structure and transparency.

Anhydrite, the anhydrous form of calcium sulfate, occurs frequently in natural mineral deposits. It is naturally dense, and because of its massive state and typical dark grey color, it can usually be distinguished visually from the dihydrate. In addition to occurring naturally, $CaSO_4$ can be obtained by high temperature dehydration of gypsum dihydrate, or by precipitation, although these are not significant factors in the gypsum industry.

Gypsum is also obtained as a by-product of various chemical processes. The main sources are from processes involving scrubbing gases evolved in burning fuels that contain sulfur (see SULFUR REMOVAL AND RECOVERY), such as coal (qv) used in electrical power generating plants (see also COAL CONVERSION PROCESSES), and the chemical synthesis of chemicals, such as sulfuric acid, phosphoric acid, titanium dioxide, citric acid, and organic polymers. In general, the added capital investment and processing costs associated with rendering by-product gypsums suitable as feedstocks for the gypsum board and plaster industry have tended to deter their use where good quality and relatively low cost natural gypsums are readily available. However, high gypsum purity makes by-product sources attractive, especially in regions where natural gypsum is scarce. A notable example of this has been Japan wherein large tonnages of by-product gypsum from its phosphoric acid industry have been used (see PHOSPHORIC ACID AND THE PHOSPHATES). In North America, little phosphogypsum has been used because of objectionable impurities and/or properties. In all areas of the world where gypsum is used, more focus has been given to other sources, ie, chiefly from stack gas scrubbing processes or flue gas desulfurization (FGD).

Decomposition Thermodynamics

The thermodynamic properties of gypsum decomposition, which involve two distinct steps,

$$CaSO_4 \cdot 2H_2O \xrightarrow{\Delta} CaSO_4 \cdot \tfrac{1}{2}H_2O + 1\tfrac{1}{2}\ H_2O$$

$$CaSO_4 \cdot \tfrac{1}{2}H_2O \xrightarrow{\Delta} CaSO_4 + \tfrac{1}{2}\ H_2O$$

have been the subject of much theoretical and practical study. Two forms of the hemihydrate, α and β, have been identified (1). The β-form is obtained when the dihydrate is partly dehydrated in a vacuum at 100°C or under conditions lacking a nearly saturated steam atmosphere. The α-form is prepared by dehydration of gypsum in water at temperatures above 97°C and by dissociation in an atmosphere of saturated steam.

The terms α and β are often used to differentiate two generally accepted, yet controversial forms of hemihydrate. The β-hemihydrate has a higher energy content and a higher solubility than the α-hemihydrate. The α-form is distinguishable from the β-form in that α-form particles disintegrate very little when mixed with water and far less mixing water is required to form a workable slurry. Consequently, the α-form has the ability to produce denser and higher compressive-strength casts and less excess water has to be removed after hydration is complete.

Anhydrite also has several common classifications. Anhydrite I designates the natural rock form. Anhydrite II identifies a relatively insoluble form of $CaSO_4$ prepared by high temperature thermal decomposition of the dihydrate. It has an orthorhombic lattice. Anhydrite III, a relatively soluble form made by lower temperature decomposition of dihydrate, is quite unstable converting to hemihydrate easily upon exposure to water or free moisture, and has the same crystal lattice as the hemihydrate phase. Soluble anhydrite is readily made from gypsum by dehydration at temperatures of 140–200°C. Insoluble anhydrite can be made by heating the dihydrate, hemihydrate, or soluble anhydrite for about 1 h at 900°C. Conversion can also be achieved at lower temperatures; however, longer times are necessary.

Manufacture

Natural Gypsum. Gypsum rock from the mine or quarry is crushed and sized to meet the requirements of future processing or removed for direct marketing of the dihydrate as a cement retarder. Once subjected to a secondary crusher, calcining, and drying, the product is fine-ground. Fine-ground dihydrate is commonly called land plaster, regardless of its intended use. The degree of fine grinding is dictated by the ultimate use. The majority of fine-ground dihydrate is used as feed to calcination processes for conversion to hemihydrate.

***β*-Hemihydrate.** The dehydration of gypsum, commonly referred to as calcination in the gypsum industry, is used to prepare hemihydrate, or anhydrite. Hemihydrate is generally called stucco in North America and plaster in many other continents. In North America, plaster is differentiated from hemihydrate or stucco by the inclusion of additives to control intended use properties, eg, rehydration time, density, coverage, strength, and viscosity.

Kettle calcination continues to be the most commonly used method of producing β-hemihydrate. The kettle can be operated on either a batch or continuous basis. Its construction is shown in Figure 1. The kettle is a cylindrical steel vessel enclosed in a refractory shell with a plenum between. The steel vessel is suspended above a fire box from which heated air flows up and into the plenum surrounding the steel vessel and through multiple horizontal flues that completely penetrate the vessel. The plenum and flues provide heat to the kettle contents before the heated air is exhausted. An agitator with horizontal arms

penetrates the depth of the kettle and is driven from above. Land plaster, usually ground 85–95% through 100 mesh (149 μm) is fed from the top. In batch operation, using an 18.1 metric ton capacity kettle, filling takes 20–30 min. Another 90–120 min are usually required to convert the dihydrate to hemihydrate. The steam released from the dehydration reaction is vented from the kettle top. When conversion to hemihydrate is complete (usually determined by temperature measurement of the kettle contents), the stucco is discharged by gravity through the quick-opening gate located at the periphery and bottom of the steel vessel. A typical temperature pattern for the kettle contents is shown in Figure 2. Approximately 1 GJ/t (950,000 Btu/t) of hemihydrate is required in a well-designed kettle.

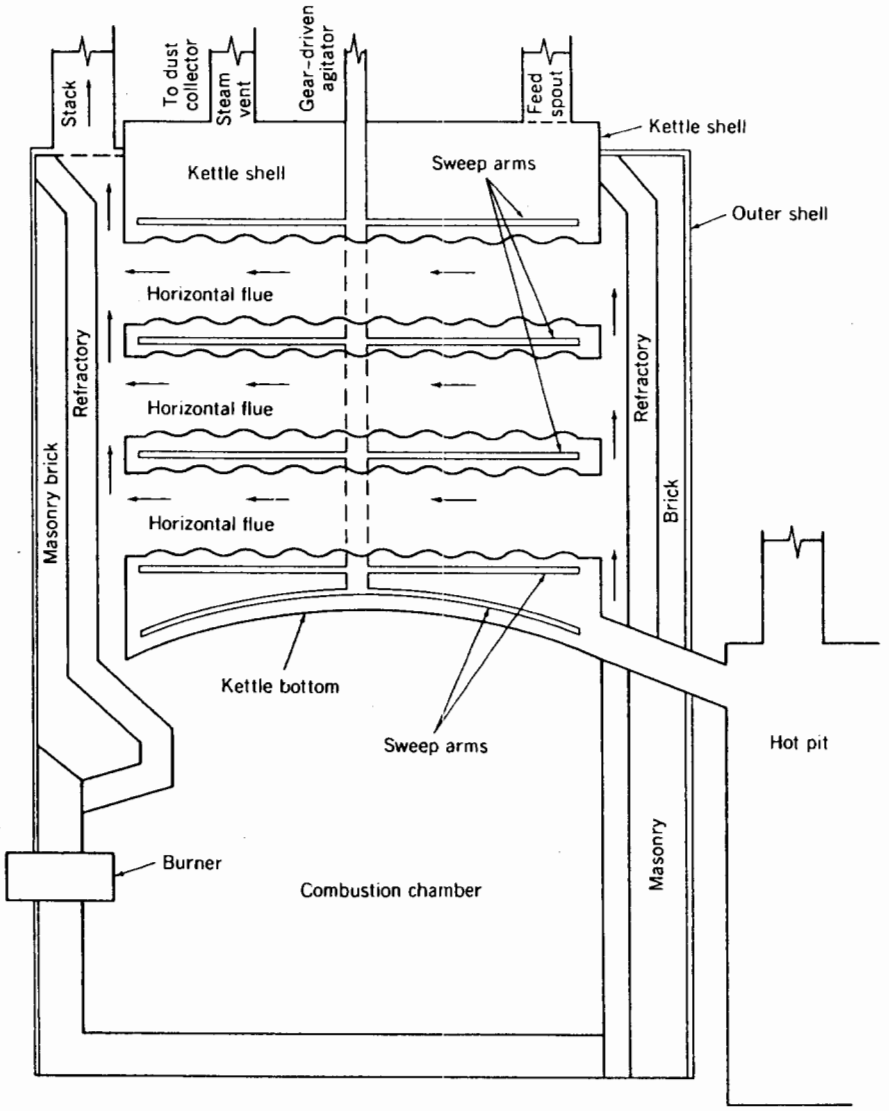

Fig. 1. Generalized vertical cross-section of a calcining kettle.

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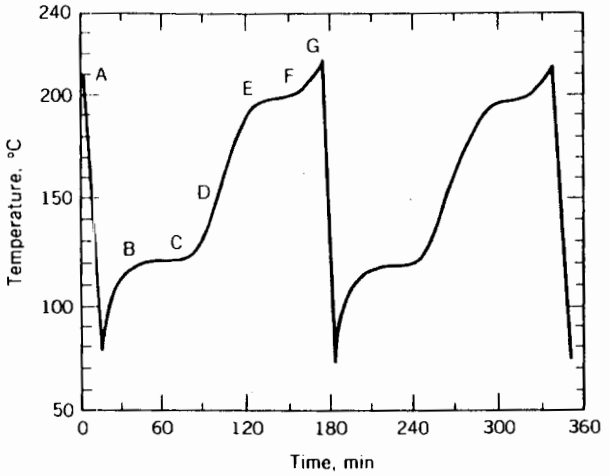

Fig. 2. Time–temperature profile for kettle calcination. Points A–B represent the fill period; B–C, the boil or drag; C–D, falling rate or cook-off; D, discharge for hemihydrate. Points D–E show firing rate to second boil; E–F, second boil; F–G, second cook-off; G, second-settle discharge.

During the fill portion of a kettle cycle, firing rate is usually controlled to maintain the kettle contents at a temperature of approximately 104°C. When the fill is complete, the firing rate is increased to a level dictated by the desired stucco properties. The mass boils at a temperature of 115–120°C. The boil or drag continues for about 1 h, then subsides. Heating continues for a short time period to allow moisture release and the mass temperature increases to approximately 150–155°C if the hemihydrate form is desired, after which firing is reduced and the contents dumped. In practice, owing to the inability to heat all particles of gypsum adequately, the discharged mass often contains small percentages of dihydrate, soluble anhydrite, and at times insoluble anhydrite.

If soluble anhydrite is desired, firing is maintained until a second boil occurs accompanied by a second temperature plateau at about 190°C. Virtually all the water of crystallization has been removed at 215°C. Soluble salts are impurities that increase the vapor pressure within the kettle. Aridized stucco refers to kettle-calcined hemihydrate that has been made with the intentional addition of 0.55–1.1 kilograms of NaCl or $CaCl_2$ per metric ton of land plaster. The stucco characteristic of lower water demand permits higher density and higher strength casts. The hygroscopic nature of the chlorides prevents the use of aridized stucco for some applications.

In another process water is introduced into the hot calcined gypsum mass in a kettle to reduce the temperature of a portion of the mass to below the boiling point of water. The mass is then reheated (2). Stabilized setting and water demand properties are claimed as are water demand levels below those attainable through aridizing.

There is also a technique that permits continuous calcination using kettles (3). A continuous perforated grate charged with a single layer or multiple layers of sized rock has been designed (4) where the bed passes through a machine

wherein hot gases are drawn through the bed. The material is cooled by air at a selected point to control the degree of dehydration.

An air-suspension calcination process for the commercial production of hemihydrate (stucco) from dihydrate gypsum has been patented (5). A schematic representation of this continuous process is shown in Figure 3. Gypsum particles are in intimate contact with heated process air so that only a few seconds of residence time in the process chamber is required to effect dehydration. The β-type material produced is characterized by a high activity level having a naturally rapid time for rehydration to dihydrate, ie, setting time. This process is particularly favorable for commercial fabrication of board. These calcining units are in routine use throughout North America and, in the 1980s, use was extended to Europe and Australia.

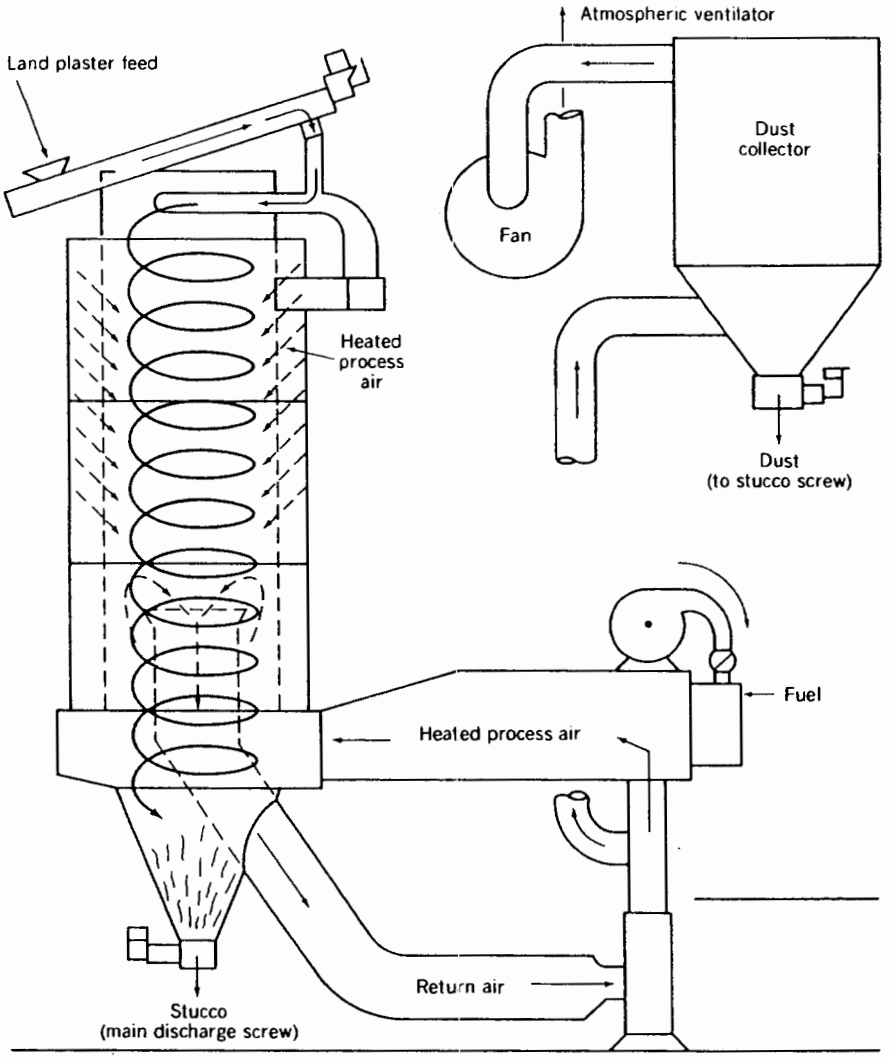

Fig. 3. Generalized vertical cross-section of a calcidyne calciner (5).

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α-Hemihydrate. Three processing methods are used for the production of α-hemihydrate. One, developed in the 1930s, involves charging lump gypsum rock 1.3–5 cm in size into a vertical retort, sealing it, and applying steam at a pressure of 117 kPa (17 psi) and a temperature of about 123°C (6). After calcination under these conditions for 5–7 h the hot moist rock is quickly dried and pulverized.

Another method (7), first reported in the 1950s, has lower water demand. The dihydrate is heated in a water solution containing a metallic salt, such as $CaCl_2$, at pressures not exceeding atmospheric. A third method (8), developed in 1967, prepares very low water-demand α-hemihydrate by autoclaving powdered gypsum in a slurry. A crystal-modifying substance such as succinic acid or malic acid is added to the slurry in the autoclave to produce large squat crystals.

Anhydrite. In addition to kettle calcination (Fig. 1), soluble anhydrite is commercially manufactured in a variety of forms, from fine powders to granules 4.76 mm (4 mesh) in size, by low temperature dehydration of gypsum.

Insoluble anhydrite is manufactured commercially by several methods. Where large rock gypsum is the starting material, beehive kilns are used and 24-h processing times are not unusual. Rotary calciners or traveling grates are often used for small rock feed. Fine-ground gypsum is calcined to the insoluble form in flash calciners. Temperature control is somewhat critical in all methods; low temperatures result in soluble anhydrite being present and too high temperatures dissociate the $CaSO_4$ into CaO and oxides of sulfur.

By-Product Calcium Sulfate. There are many industrial chemical processes that produce by-product calcium sulfate in one of its forms. Whereas the most common is the neutralization of spent sulfuric acid, many of those processes do not produce a commercially useful by-product because of contaminants, particle size, or volume produced. There are, however, six chemical processes that do produce sufficient volume to have potential commercial value. Each is named after its chemical process.

Desulphogypsum or FGD-gypsum are the two names commonly given to the by-product gypsum produced by scrubbing sulfur dioxide out of flue gases (see SULFUR REMOVAL AND RECOVERY). There are three general types of scrubbing processes that produce by-product gypsum: limestone, lime, and dual or double alkali.

The process for limestone scrubbing can be generally described by

Absorption

$$SO_2 + H_2O \longrightarrow H_2SO_3$$

Crystallization

$$H_2SO_3 + CaCO_3 \longrightarrow CaSO_3 + CO_2 + H_2O$$

Oxidation

$$2\,CaSO_3 + O_2 + 4\,H_2O \longrightarrow 2\,CaSO_4{\cdot}2H_2O$$

There are several lime-scrubbing processes being marketed. The generalized

process is described by

Absorption

$$2\ Ca(OH)_2 + 2\ SO_2 \longrightarrow 2\ CaSO_3{\cdot}\tfrac{1}{2}H_2O + H_2O$$

Oxidation/crystallization

$$2\ CaSO_3{\cdot}\tfrac{1}{2}H_2O + O_2 + 3\ H_2O \longrightarrow 2\ CaSO_4{\cdot}2H_2O(s)$$

In the dual or double alkali process, an alkali salt that is considerably more soluble in water than limestone is used. The alkali salt is then regenerated using a second alkali, $CaCO_3$. There are several alkalies used in the absorber; the most common are magnesium sulfite, sodium sulfite, and ammonium sulfite. A typical process using magnesium sulfite is

Absorption

$$MgSO_3 + H_2O + SO_2 \longrightarrow Mg^{+2} + 2\ HSO_3^-$$

Oxidation/crystallization .

$$2\ HSO_3^- + 2\ CaCO_3 \longrightarrow 2\ CaSO_3{\cdot}\tfrac{1}{2}H_2O + 2\ CO_2 + \tfrac{1}{2}\ O_2$$

$$2\ CaSO_3{\cdot}\tfrac{1}{2}H_2O + O_2 + 3\ H_2O \longrightarrow 2\ CaSO_4{\cdot}2H_2O(s)$$

Of all the by-product gypsums from chemical processes, desulphogypsum from coal-fired electric power utility plants has the greatest commercial potential because electric power plants are numerous and many are located near large population centers where there would be a ready market for by-product gypsum wallboard products (see COAL CONVERSION PROCESSES; POWER GENERATION). Utilization of gypsum is dependent on economically removing deleterious chemicals, namely excess chlorides, water-soluble sodium and magnesium, and unoxidized calcium sulfite.

By-product gypsum made by neutralizing waste sulfuric acid from the sulfate process used to manufacture titanium oxide pigment is called titanogypsum (see TITANIUM COMPOUNDS, INORGANIC). This is commonly a two-industry process in that iron-rich ilmenite ore is first processed to obtain iron and the resulting slag is sold to the TiO_2 producers. There are a few locations where titanogypsum is produced in large enough quantities to be considered for commercial use. Limitations are the iron compound contaminants and their average particle size. Titanogypsum has become the second most important source of commercial by-product gypsum after desulphogypsum in the United States.

Phosphogypsum [*13397-24-5*] is the name given to the by-product gypsum residue when phosphate ore is acidulated to extract phosphoric acid. There are several processes commercially used. All of them digest or acidulate tri-calcium phosphate

$$Ca_3(PO_4)_2 + 3\ H_2SO_4 + 6\ H_2O \longrightarrow 2\ H_3PO_4 + 3\ CaSO_4{\cdot}2H_2O$$

Processing techniques vary by means of precipitation. In the United States, environmental considerations render by-product gypsum from all of the processes inappropriate for the building materials industry. Radon and daughter radionuclides are retained in the by-product residue after acidulation as is the heavy metal cadmium (see HELIUM-GROUP GASES; CADMIUM AND CADMIUM ALLOYS). Phosphogypsum's commercial use in the gypsum wallboard industry in Europe and Japan has diminished as desulphogypsum has become more available.

Fluorogypsum is the name ascribed to by-product gypsum from fluorspar acidulation to produce hydrofluoric acid. The chemical reaction

$$CaF_2 + H_2SO_4 \xrightarrow{\Delta} CaSO_4 + 2\,HF(g)$$

produces anhydrite. Over a period of time, the anhydrite converts to gypsum. Contaminants in fluorogypsum, especially the heavy metal beryllium, render fluorogypsum a better road metal, ie, roadbed material, for which it is used, than a building materials product.

Citrogypsum and borogypsum are named after the respective processes and produce sizeable quantities of by-product gypsum in certain locations. However, contaminants preclude commercial use in the gypsum wallboard industry.

Uses

Uncalcined Gypsum and Anhydrite. Calcium sulfate, generally in the form of gypsum, is added to Portland cement (qv) clinker to stop the rapid reaction of calcium aluminates (flash set) (see ALUMINUM COMPOUNDS, ALUMINA). Also, gypsum accelerates strength development. For this reason, gypsum is more properly termed a set regulator, rather than a retarder, for Portland cement. Used in proper amounts it also minimizes volume change. Normal gypsum addition to clinker is 5–6 wt %. Another notable use of uncalcined gypsum is in agricultural soil treatment wherein it is commonly called land plaster. For this use it is finely ground.

Calcined Anhydrite. Soluble anhydrite, or second-settle stucco, has physical properties similar to those of gypsum plaster. It hydrates to the dihydrate rapidly in water. Its outstanding property is its extreme affinity for any moisture, which makes it a very efficient drying agent (see DESICCANTS). In ambient moisture-laden air, it readily hydrates to hemihydrate. Soluble anhydrite, under the trade name Drierite, is widely used as a desiccant in the laboratory and in industry. A small amount is also used as an insecticide carrier. Small amounts of soluble anhydrite are unintentionally produced in most commercial calciners during hemihydrate production.

Keenes Cement is produced from calcined anhydrite (dead-burned), finely ground and intermixed with special accelerator(s). Although the volume of its use has declined greatly since the 1960s, it is available for job-site mixing with hydrated lime as a composite, hand-finished plaster applied generally over an aggregated, gypsum-base (conventional) plaster.

Hemihydrate. The ability of plaster of Paris to readily revert to the dihydrate form and harden when mixed with water is the basis for its many uses. Of

equal significance is the ability to control the time of rehydration in the range of two minutes to over eight hours through additions of retarders, accelerators, and/or stabilizers. Other favorable properties include its fire resistance, excellent thermal and hydrometric dimensional stability, good compressive strength, and neutral pH.

Upon setting, gypsum expands slightly and this property can be used to reproduce the finest detail, down to ca 1 μm, as is done in certain dental and jewelry castings employing the lost wax process. Normal linear expansion upon setting of gypsum plaster is 0.2–0.3%, but by using additives expansion may be controlled for special uses in the range of from 0.03 to 1.2%.

The calcination procedures and processing techniques produce a family of base stuccos best described by the amount of water, in wt %, of the plaster, which must be added when mixing to obtain standard fluidity. The range of fluidity permits casting neat plaster in the dry range of specific gravity of about 0.85–1.8 and consequent dry compressive strength of about 3.5–70 MPa (35–700 atm). Frequently these stuccos are formulated with set and expansion control additives as well as many other materials to meet the needs of a particular application. Properties that limit gypsum plaster usage include plastic flow underload, which is increased under humid conditions, strength loss in a humid atmosphere, and dissolution and erosion in water. Thus gypsum is not normally used for permanent performance structurally or in exposed, exterior locations. To prevent long-term calcination, gypsum products should not be used where temperatures exceed 45°C.

The largest single use of calcined gypsum in North America is in the production of gypsum board. Gypsum wallboard replaced plaster in the United States during the 1960s as the main wall cladding material. During that same time period, new veneer plaster systems were developed as an alternative to gypsum board (drywall) and the classic plastering systems, all of which are specified in building construction (see BUILDING MATERIALS, SURVEY). The veneer plasters are highly proprietary and specially formulated composites that provide good wear-resistant interior wall and ceiling surfaces. They are applied on the construction site either by a one- or two-coat procedure at thicknesses of about 0.19–0.32 cm.

Molding plasters have been used for centuries to form cornices, columns, decorative moldings, and other building interior features. Molding plaster is a good utility plaster where expansion control, high hardness, and strength are not needed. Its miscellaneous uses are numerous. Art plasters are essentially molding plasters modified to increase surface hardness, chip resistance, and to reduce paint absorption of casts made from this material. Orthopedic plasters are used by hospitals and clinics for all types of orthopedic cast work.

A moderate amount of plaster is used in making impressions and casting molds for bridges, etc, by dental laboratories. Both α- and β-plasters are used by the dental trade (see DENTAL MATERIALS). The α-plasters are also tailored to meet the needs of modern industrial tooling, where they are used for master patterns, models, mock-ups, working patterns, match plates, etc. They are the accepted material because their use results in great time and labor savings, as well as excellent accuracy and stability of cast dimensions. Also the material is adaptable to intricate, irregular shapes, complex intersections, and quick modification.

Shipping and Specifications

Gypsum and gypsum products are bulky and relatively low in cost. In North America, factors of varying regional supply and demand for building products not withstanding, the normal economic overland shipping range is about 500 km. For overland shipments there has been a steady shift, starting in the 1950s, from rail to motor transport. In some cases, truck shipments are made from plants directly to building construction sites. For continental coastal and lake region markets, crude gypsum is most often transported in specially designed, rapid unloading ships that deliver from quarries to plant sites where the gypsum is then processed into finished products. During the 1980s, there were reports of increased intercontinental trade in both crude gypsum ore and manufactured goods.

Formulated plasters utilizing specially processed calcined gypsum are packaged in multi-ply paper bags having moisture vapor-resistant liners. This type of packaging protects the contents from airborne moisture keeping the plaster more stable with respect to setting time and mixing water demand over longer periods

Table 3. ASTM Gypsum and Gypsum Product Specifications

ASTM method	Materials
	Gypsum and gypsum plasters
C22-91	Gypsum
C28-91	Gypsum Plasters
C35-89a	Inorganic Aggregates For Use In Gypsum Plaster
C59-91	Gypsum Casting and Molding Plaster
C61-91	Gypsum Keene's Cement
C317-91	Gypsum Concrete
C587-91	Gypsum Veneer Plaster
	Test methods
C265-91	Calcium Sulfate in Hydrated Portland Cement
C471-91	Chemical Analysis of Gypsum and Gypsum Products
C472-90a	Physical Testing of Gypsum Plasters, etc
	Gypsum board products
C36-91	Wallboard (general)
C37-91	Lath (base for plaster)
C79-91	Sheathing
C442-91	Backing Board and Coreboard
C588-91	Base for Veneer Plasters
C630-91	Water-Resistant Backing Board
C931-91	Exterior Soffit Board
C960-91	Predecorated Board
	Test method
C473-87a	Physical Testing of Gypsum Board Products

of warehousing. Manufactured board products are most often bundled, two pieces face to face, stacked in units for transport to dealers' yards, and reshipped to individual job sites as construction schedules dictate. Specialized, labor saving, power driven handling equipment has been developed for stocking boards on construction sites. The ASTM specifications for gypsum and gypsum products are given in Table 3.

Table 4. World Production of Gypsum, 10^3 t[a]

Country	Year 1986	1990[b]
Argentina	462	399
Australia	1,672	1,797
Austria[c]	703	635
Brazil (direct sales plus beneficiated)	629	654
Bulgaria	395	495
Canada (shipments)[c]	8,809	8,207
China	6,536	7,989
Czechoslovakia	743	794
Egypt	906	1,307
France[c]	5,263	5,628
Germany		
eastern states	340	300
western states (marketable)[c]	1,897	1,797
Greece	499	454
India	1,641	1,598
Iran	8,118	7,989
Italy	1,246	1,253
Japan	6,355	6,355
Mexico	4,236	6,005
Morocco	454	454
Pakistan	373	472
Poland[c]	1,108	1,108
Romania	1,598	1,498
Russia	4,603	4,721
Saudi Arabia	373	375
South Africa, Republic of	405	385
Spain	5,066	4,993
Thailand	1,667	5,757
United Kingdom[c]	3,418	3,994
United States[d]	13,983	14,893
Yugoslavia	596	554
other countries	4,195	4,884
Total	*88,289*	*97,744*

[a]Ref. 9.
[b]Estimated.
[c]Includes anhydrite.
[d]Excludes by-product gypsum.

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Table 5. United S

Year	Consum
1976	16,4
1980	17,8
1984	21,0
1988	23,6
1989[g]	22,4
1990	22,6

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Production and Trade

Crude gypsum is the principal form of calcium sulfate shipped in international trade, although the 1980s saw an increase in the volume of fabricated products moved across international borders. Tables 4 and 5 summarize production and trade of gypsum materials for the United States, Canada, Mexico, and other regions of the world. The United States remains both a leading producer and importer of gypsum. In 1989, over 8,400,000 metric tons of crude gypsum were imported into the United States; 67.5% from Canada, 25.3% from Mexico, and the remainder, in order of decreasing quantity from Spain, Australia, China, Morocco, The Dominican Republic, Germany (FDR), and Japan.

Table 5. United States' Crude Gypsum, 10^3 t[a,b]

Year	Consumption[c]	Production	Imports[d]	Exports[d]	Inventories[e]	Representative price $/t[f]
1976	16,499	10,866	5,652	19	1,375	5.51
1980	17,899	11,225	6,680	6	1,492	9.18
1984	21,059	12,988	8,076	5	1,755	8.75
1988	23,673	14,875	8,780	5	1,973	7.34
1989[g]	22,404	14,059	8,439	98	1,867	6.62
1990	22,698	14,893	7,921	117	1,843	5.51

[a]Ref. 10.
[b]Excludes by-product gypsum.
[c]Consumption includes sold or used, minus exports. Data on quantities sold or used is from the quarterly gypsum canvasses of the nongypsum board producers, annual canvasses of all producers, and from data furnished by the Gypsum Association.
[d]Imports and exports are from the Bureau of Census.
[e]Inventories estimated from consumption.
[f]The representative price is the company-reported value per metric ton, fob mine or plant.
[g]Preliminary data.

In 1989, 4,689,000 metric tons of uncalcined gypsum was sold or used; 3,229,000 metric tons for use in Portland cement and the remainder for agriculture and miscellaneous uses. About 17,778,000 metric tons of calcined material was used to produce 1.9 million square meters of board products. Over one million square meters of this material was regular board and about 560,000 m^2 was Type X board.

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CALCIUM MAGNESIUM ACETATE. See ACETIC ACID AND DERIVATIVES, ACETIC ACID; CALCIUM COMPOUNDS, SURVEY.

CALIFORNIUM. See ACTINIDES AND TRANSACTINIDES.

CALKING AND SEALING COMPOSITIONS. See SEALANTS.

CALORIMETRY. See THERMAL, GRAVIMETRIC, AND VOLUMETRIC ANALYSIS.

CALORIZING. See METALLIC COATINGS.

CAMPHOR. See TERPENOIDS.

CANCER CHEMOTHERAPY. See CHEMOTHERAPEUTICS, ANTICANCER.

CANDLES. See WAXES.

CAPACITOR FLUIDS. See HEAT EXCHANGE TECHNOLOGY.

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A Research Note

NUTRITIONAL STUDIES ON SOYBEAN CURD PRODUCED BY CALCIUM SULFATE PRECIPITATION OF SOYBEAN MILK

INTRODUCTION

SOYBEAN PROTEIN is unique among plant proteins by virtue of its relatively high biological value. As a result this oil-rich legume has been the subject of extensive investigation as a source of protein for the human diet. To this end many processes for utilization of soybeans have been developed. A feature common to these processes is some form of heat treatment. The heat treatment is necessary to destroy the antidigestive and growth depressant factors that occur naturally in soybeans (Klose et al. 1948). The heat treatment may also be an important determinant of the flavor of the product (Wilkens et al. 1967). Further processing of the soybeans or soymilk may include extraction, separation and precipitation. All of these treatments may affect the nutritional value of the finished product. With the increasing value of soybean products on the market it is important that we have a complete picture of the implications of processing as they affect human nutrition. Hackler recognized the significance of this problem and the data on processing of soybean milk are quite extensive (Hackler et al. 1965; Hackler and Stillings, 1967). They concluded that heat processing of soybean milk could markedly affect its nutritional value. The nutritive value of many other soybean products, including soybean curd, is covered in an extensive review by Lerner (1972). In view of the considerable variation in nutritive value that can result from processing an evaluation was made of the soybean curd produced by calcium sulfate precipitation of heated soybean milk (Schroder and Jackson, 1972).

MATERIALS & METHODS

Male weanling Sprague-Dawley rats of the University of Alberta strain, equalized with respect to sex and randomized with respect to litter origin, were allocated to two dietary groups at an average age of 21 days and an average weight of 50.2g. The control diet contained casein as the sole source of protein while the test diet contained soybean curd as the sole source of protein. The soybean curd was prepared according to Schroder and Jackson (1972), freeze dried in a RePP, 42-FFD-WS Sublimator (The Virtis Co., Inc., Gardiner, N.Y.) and ground to a fine powder. The rats were housed individually in stainless steel cages, 7 in. wide, 10 in. deep, 7 in. high. The cages were in banks in an air conditioned room maintained at 23°C and a relative humidity of 45–50%. The diets were fed ad libitum and water was available at all times.

The formulation and composition of the experimental diets are given in Table 1. The diets were formulated to meet or exceed the minimal nutrient requirements for growth of the weanling rat as set down by the National Academy of Sciences-National Research Council (1962). The diets were further formulated to be isonitrogenous and isocaloric. Formulation was based on analysis of the major ingredients. Nitrogen was determined by the Kjeldahl method (AOAC, 1965) and gross energy determined with the aid of a Parr oxygen bomb calorimeter (Parr Instrument Co., Moline, Ill.). The crude fat was determined according to the official method for crude fat determination in soy flour (AACC, 1962). Amino acid analysis on the isolated proteins was carried out in the Beckman Spinco model 120-B amino acid analyzer (Beckman Instruments Inc., Fullerton, Calif.) according to Spackman et al. (1958). Hydrolysis of the protein was effected in 6N HCl at 110°C for 24 hr and corrections were made for hydrolytic losses by extrapolation of values back to zero hydrolysis time. All results are reported as percentage of total hydrolysate; however, no measurement of tryptophane was conducted.

The rats were fed the experimental diets for a period of 28 days during which time they were weighed at weekly intervals and their feed consumption recorded. At the time of weekly weighings, wasted feed was collected and recorded, feed consumption being corrected accordingly. Average daily gain, average daily feed, feed conversion (g feed/g gain) and the protein efficiency ratio (PER) were calculated. The data were analyzed statistically by the analysis of variance as described by Steele and Torrie (1962).

Table 1—Formulation and composition of diets used to evaluate the nutritional value of soybean curd

Ingredients	Control %	Test %
Sucrose	48.88	43.38
Casein[a]	25.00	
Soybean curd		42.50
Cellulose	5.00	5.00
Corn oil	15.00	3.00
Mineral mix[b]	5.00	5.00
Fat soluble vitamin mix[c]	0.50	0.50
Water soluble vitamin mix[d]	0.50	0.50
Choline Chloride	0.12	0.12
Composition (by analysis)		
Crude protein (N × 6.25)%	22.94	22.31
Gross energy Kcals/g	4.91	4.67

[a] Vitamin free
[b] Composition, g/100g mix: $CaCO_3$, 30.0; KH_2PO_4, 34·1; NaCl (iodized), 25.0; $MgSO_4 \cdot 7H_2O$, 10.0; $FeCl_2 \cdot 4H_2O$, 0.60; $CuSO_4 \cdot 5H_2O$, 0.157; $MnSO_4 \cdot H_2O$, 0.12; $ZnCl_2$, 0.02; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.003.
[c] Composition, g/100g mix: Vitamin A (10,000 IU/g), 20.0; Vitamin D_3 (35,000 IU/g), 4.0; Myvamix (20,000 IU Vitamin E/lb), 20.0; Corn starch, 56.0.
[d] Composition, g/100g mix: Thiamine HCl, 0.20; Riboflavin, 0.20; Niacin, 1.00; Calcium pantothenate, 0.80; Pyridoxine HCl, 0.10; Vitamin B_{12}, 0.001; Biotin, 0.004; Vitamin K (menadione), 0.02; Sucrose, 97.675.

RESULTS & DISCUSSION

THE DATA for the individual rats were averaged and are presented in Table 2. With either diet the males consumed more feed, gained weight faster and were more efficient than the females. All differences attributable to sex were significant ($P < 0.05$). A significant ($P \leq .05$) sex × diet interaction noted for average daily consumption, average daily gain and protein efficiency ratio shows there is an interaction between the diet and the sex of the rat. Recognizing the mentioned differences, the rats fed the diet containing casein as the sole source of protein consumed more feed, had higher gains, were more efficient both in terms of feed conversion and protein efficiency ratios than were the rats fed the test diet containing soybean curd as the sole source of protein. All differences were significant ($P \leq 0.5$). The feed wastage of soybean diet may reflect a palatability factor in the test diet. The low values of PER of both casein and soybean curd diets are no

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Table 2—Feed intake, gains, feed conversion and PER of rats fed diets containing casein or soybean curd

	Control			Test		
	M	F	M & F	M	F	M & F
Average daily feed g	13.75	11.38	12.57	10.76	10.70	10.73
Average daily gain g	6.88	4.84	5.87	4.37	3.88	4.13
Feed conversion g	2.00	2.35	2.18	2.46	2.76	2.60
Total feed wastage g	13.00	30.00	21.50	322.00	97.00	209.50
PER $\left(\frac{\text{g gained}}{\text{g protein consumed}}\right)$	2.19	1.86	2.02	1.80	1.62	1.71
PER[a]				2.06	2.18	2.12
Protein quality $\left(\frac{\text{Test PER}}{\text{Casein PER}} \times 100\right)$				82.2	87.1	84.6

[a] Corrected to PER for casein of 2.50

Table 3—Amino acid composition (% of diet)

Amino acid	Control (casein) %	Test soybean curd %	NRC minimum requirement %
L-tryptophan			0.15
L-histidine	0.72	0.53	0.30
L-lysine	2.03	1.38	0.90
L-leucine	2.50	1.79	0.80
L-isoleucine	1.66	1.10	0.50
L-phenylalanine	1.34	1.19	0.90
L-methionine	0.78	0.22	0.60
L-threonine	1.10	0.80	0.50
L-valine	1.98	1.18	0.70

doubt partly due to the high protein content (approximately 22%) of the diet.

Hackler et al. (1963) fed acid precipitated soybean curd to rats at levels of approximately 10, 20 and 30% dietary protein. The resulting PER values were 2.20, 1.94 and 1.64 respectively. In the present experiment the level of dietary protein of the soy curd diet was 22.31% and the PER 1.71. The average protein quality (PER soybean curd/PER casein x 100) in this study was 84.6. This is similar to the protein quality of soybean curd calculated from the results of Hackler et al. (1963). The protein quality in their study was 76.9, 80.5 and 87.7 when fed at dietary protein levels of approximately 10%, 20% and 30% respectively. The PER for the soybean curd in this study, 1.71, is also comparable to a PER of ca 1.67 for soybean milk heated at 93°C for 30 min (Hackler and Stillings, 1967) and a PER of ca 1.70 for soybean milk heated at 121°C for 5 min (Hackler and Stillings, 1967). The heat treatment of the soybean curd used in the present work was equivalent to 100°C for 30 min.

The poorer performance of the rats fed the diet containing the soybean curd may have been attributable to an unfavorable balance of certain essential amino acids. Using the figures from the amino acid analysis of the soybean curd and the amino acid composition of casein taken from Crampton and Harris (1969), the amino acid composition of the diets was calculated (Table 3) and compared with the amino acid requirements of the growing rat as set down by the National Academy of Sciences-National Research Council (1962). The test diet meets the amino acid requirements of the rat for all amino acids except methionine. The deficiency of an essential amino acid will result in reduced food intake and concomitantly reduced growth of the rat. The deficiency will also result in an increased feed conversion ratio and a decreased PER, resulting from less efficient utilization of dietary proteins. However the cost of supplementation of the soybean precipitate with methionine would be very small and the resulting product would be a good source of dietary protein.

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TOFU STANDARDS

Recommended by the Standards Committee
and approved by the Board of Directors
and members of the
Soyfoods Association of America

October 28. 1986

III. STANDARD TOFU

A. Ingredients. The basic ingredients in Standard Tofu are whole soybeans, one or more food-grade coagulants (typically a salt, such as magnesium chloride or calcium sulfate, or an acid or acid-forming compound, such as glucono delta-lactone), and water.

Additional technical ingredients (except for spices, flavorings, sweeteners, seasonings, or supplemental protein) may be used, e.g., defoamers, preservatives, or various quality improvers, provided that the ingredient is not a food additive or color additive as defined in section 201 (s) or (t) of the Federal Food, Drug, and Cosmetic Act or is a food additive or color additive as so defined and is used in conformity with regulations established pursuant to section 409 or 706 of the Act.

B. Manufacturing Process.

1) Whole soybeans are ground with or without water, and then cooked with water.
2) The resultant soy slurry goes through an optional filtration process to remove all or part of the soy pulp or fiber.
3) The resulting product, now referred to as Soymilk, is then coagulated to form curds and whey.
4) Whey is then removed before and/or while the curds are pressed.
5) The finished pressed curds may now be referred to as Tofu.

C. Designation According to Consistency and Protein Content*. Standard tofu is divided into four consistencies: soft, regular, firm, and extra firm. These consistencies are classified by the protein content of the tofu immediately after pressing (7). The AOAC method for determining protein content should be used, unless another method is required by the state in which the tofu is made or sold.

1) "Soft Tofu" generally contains from 5.0 to 6.4% protein.

2) "Regular Tofu" generally contains 6.5 to 9.4% protein.

3) "Firm Tofu" generally contains 9.5 to 13.9% protein.

4) "Extra Firm Tofu" generally contains 14% or more protein.

* The Association recognizes that the designations here are guidelines and some manufacturers' products may vary slightly.

Table 1. Representative Composition of Major Tofu Varieties

Variety of Tofu	Food Energy Kcal/100 gm	Moisture %	Protein %	Fat %	Carbos. (w/fibr)	Ratio Prot/Fat
Soft Tofu	63	88.0	6.0	3.5	1.9	1.7
Tofu	79	84.9	7.8	4.3	2.3	1.9
Firm Tofu	102	79.3	10.6	5.3	2.9	2.0
Extra Firm Silken Tofu*	115	79.3	14.0	5.3	2.9	

* Since "silken" refers primarily to a process for making tofu (see section 4B), all major tofu varieties may be manufactured using that process (soft, firm, etc.), and in each case should meet the representative compositions described in this table for that particular variety.

Sources: 3,7,8 (Calories by computation)

D. Terms Associated with Making Tofu.

1) Tofu is typically sold in "cakes," not "blocks."

2) A "coagulant" (the correct technical term) or "curding agent" (a good popular term) is used to coagulate the proteins in soymilk. It is preferable not to use terms such as "solidifier" or "to precipitate."

3) The major commercial tofu coagulants are nigari, magnesium choloride, calcium sulfate, calcium chloride, and glucono delta-lactone. Nigari (a Japanese term, whose English equivalent is "bittern") is the major traditional tofu coagulant in Japan and coastal China. Derived from sea salt or sea water, it is composed primarily of magnesium chloride, but in its unrefined form contains many of the other constituents of sea water. Magnesium chloride is refined nigari. Calcium sulfate (the dihydrate form, also correctly called gypsum) has been used in China since ancient times. It produces tofu containing more than 3.5 times as much dietary calcium as that made with nigari. Calcium chloride yields a tofu that closely resembles that made with nigari but contains much more calcium. Glucono delta-lactone, first used in tofu in Japan during the 1960s, is useful primarily in making varieties of silken tofu. A fine, white crystalline powder prepared by fermenting corn starch, it is widely used by the food industry as an acidulant in baking powders and as a coagulant and pH-lowering agent in dairy puddings and cottage cheeses. Lemon juice and vinegar are sometimes used to make tofu at home.

IV. SILKEN TOFU

A. Ingredients. The basic ingredients in Silken Tofu are whole soybeans, one or more food-grade coagulants (typically a salt, such as magnesium chloride or calcium sulfate, or an acid or acid-forming compound, such as glucono delta-lactone), and water.

Additional technical ingredients (except for spices, flavorings, sweeteners, seasonings, or supplemental protein) may be used, e.g., defoamers, preservatives, or various quality improvers, provided that the ingredient is not a food additive or color additive as defined in section 201 (s) or (t) or the Federal Food, Drug, and Cosmetic Act or is a food additive or color additive as so defined and is used in conformity with regulations established pursuant to section 409 or 706 of the Act.

B. Manufacturing Process.

1) Whole soybeans are ground with or without water, then cooked with water.
2) The resultant soy slurry goes through an optional filtration process to remove all or part of the soy pulp or fiber.
3) The resulting product, now referred to as soymilk, is then coagulated to form curds. Whey may or may not be present.
4) The process of making silken tofu generally includes forming the product in a container (which may be the same container in which it is sold).

C. Designation According to Consistency and Protein Content
Refer to Table 1 on page 6.

VI. COMBINATION TOFU

A. Ingredients. The basic ingredients in Combination Tofu are whole soybeans, a protein-rich soybean derivative, one or more food-grade coagulants (typically a salt, such as magnesium chloride or calcium sulfate, or an acid or acid-forming compound, such as glucono delta lactone), and water.

Additional technical ingredients (except for spices, flavoring, and seasonings) may be used, e.g. defoamers, preservatives or various quality-improvers, provided that the ingredient is not a food additive or color additive as defined in section 201 (s) or (t) of the Federal Food, Drug, and Cosmetic Act or is a food additive or color additive as so defined and is used in conformity with regulations established pursuant to section 409 or 706 of the Act.

B. Manufacturing Process. Combination Tofu is manufactured using any of the processes discussed in IIIB, IVB, and/or VB.

C. Designation According to Consistency and Protein Content*. Combination Tofu may exist in any of four consistencies: soft, regular, firm, and extra firm in accordance with III C. The AOAC method for determining protein content should be used, unless another method is required by the state in which the tofu is made or sold.

1) "Soft" generally contains from 5.0% to 6.4% protein.

2) "Regular" generally contains 6.5 to 9.9% protein.

3) "Firm" generally contains 10.0 to 13.9% protein.

4) "Extra Firm" generally contains 14% or more protein.

*** The Association recognizes that the designations here are guidelines and some manufacturers' products may vary slightly.**

D. Equivalence. The product must be organoleptically equivalent to Standard or Silken Tofu. In accordance with FDA regulation 21 CFR 101.3 (e), the product either must be nutritionally and compositionally equivalent to the standard or silken tofu, or it must be labeled as an imitation of standard or silken tofu.

W. Shurtleff & A. Aoyagi 1975 The Book of Tofu

Two special pieces of equipment, both easy to assemble, will make the work even easier:

*Make a "pressing sack" of the coarsely-woven cotton dishtowel mentioned above, or use a piece of sturdy linen cloth with about the same coarseness of weave as cheesecloth.. Fold the towel or cloth end to end and sew up the sides to form a sack about 15 inches wide and 15 inches deep. Or use a small flour sack with a fairly coarse weave.

*The flat-bottomed colander listed above is for use as a "settling container" which gives its shape to the finished tofu. If a 1-quart strainer or small, round-bottomed colander is used in its place, the tofu will naturally be rounded.

The three settling containers shown in figure 33 can easily be made at home. Container (a) is prepared from a 1½-quart wooden, tupperware, or plastic box with an open top and non-removable bottom. In containers (b) and (c) the bottom is removable, allowing for easy removal of the tofu without immersing the container in water. Good dimensions for the container are 4½ inches square by 4½ inches deep, or 6½ by 3½ by 4½ inches deep. Use a drill or heated icepick to bore 3/8-inch-diameter holes about 1½ inches apart in the bottom and sides of the container. Fashion a flat wooden or plastic pressing lid (with or without holes) to fit down inside the rim of the box. Good woods to use are (lightly oiled) vertical-grain Douglas fir, pine, maple, cedar or cherry. Handmade wooden settling containers (kitchen or community size) can be ordered from Ganesha (p. 316); they are also available at some natural food stores, sold in a kit including pressing sack, settling-box cloths, and nigari.

Ingredients

You will need only the following readily available ingredients:

Soybeans

The soybeans now sold at almost all natural- and health food stores, most co-op stores, and many supermarkets will make good tofu. However, to obtain the highest yield, try buying soybeans directly from a tofu shop in your area (p. 313), for they have been carefully chosen by the tofu maker.

Solidifier

The solidifiers most readily available in the West are Epsom salts, lemon or lime juice, and vinegar. All make delicious tofu, although they are not used in Japanese tofu shops. Japanese-style solidifiers are available from many natural food stores, local tofu shops, Japanese food markets, chemical supply houses (check your phone directory), or your local school chemistry lab. Usable seawater can be retrieved from clean stretches of ocean. Natural *nigari* is available at some salt refineries (p. 315) and can be ordered from Japanese natural food distributors (p. 315); or it can be prepared at home using natural salt (p. 283). We recommend the use of refined nigari unless the natural nigari is certified to have come from a clean source of sea water. While we believe the nigari-type solidifiers are the easiest to use and result in the best tasting tofu, Epsom salts and calcium sulfate seem to give somewhat higher bulk yields and a softer end product by incorporating more water into the tofu. The yield of tofu solids or nutrients is about the same regardless of the type of solidifier used, except that lemon juice and vinegar give rather small yields. (Note: Calcium sulfate, a fine white powder, is sometimes mislabeled in the West and sold as nigari. The latter usually has a coarse, granular or crystaline texture; natural nigari is beige and refined nigari is white.)

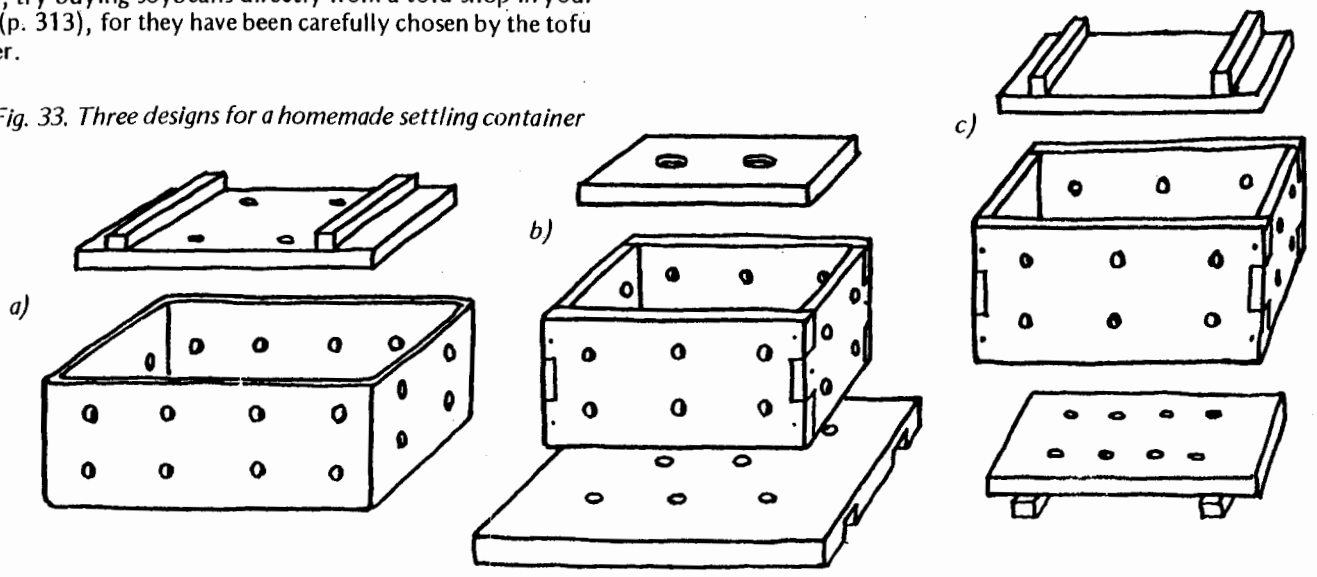
The recipe below calls only for "solidifier." Your choice of solidifier depends upon the type of tofu you want.

For subtly sweet, nigari tofu use: 2 teaspoons magnesium chloride or calcium chloride (refined nigari); or 1½ to 2¼ teaspoons granular or powdered natural nigari; or 1½ to 2½ teaspoons homemade liquid nigari; or 2 to 4½ teaspoons commercially prepared liquid nigari; or 1½ cups seawater (freshly collected; p. 282).

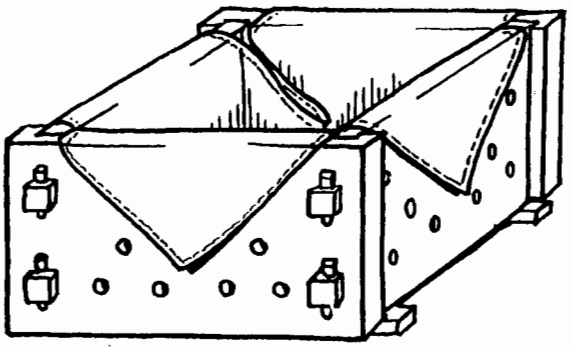
For mild, soft tofu use: 2 teaspoons Epsom salts (magnesium sulfate) or calcium sulfate; or 1½ tablespoons each calcium lactate and lemon juice (the latter being stirred into the soymilk just after the last of the calcium lactate has been added).

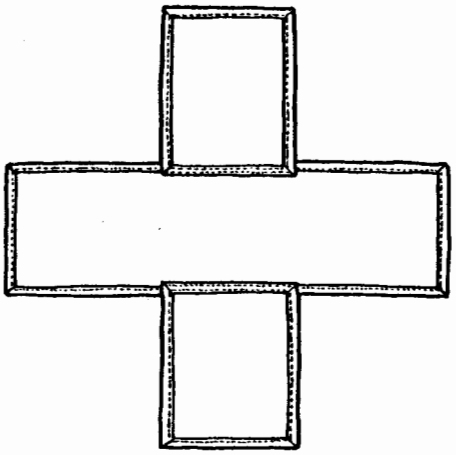
For subtly tart or sour tofu use: 4 tablespoons lemon or lime juice (freshly squeezed); or 3 tablespoons (apple cider) vinegar.

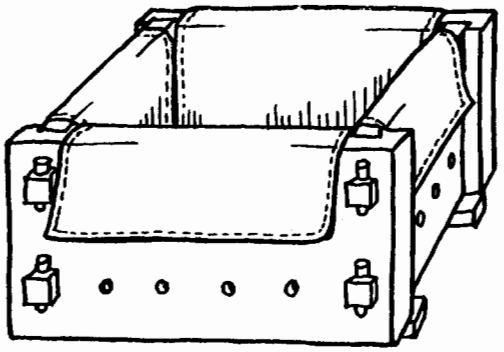
Fig. 33. Three designs for a homemade settling container



either of the two designs shown in the inset. A box of this size will hold the curds made from 8 cups of dry soybeans. A large flat or round-bottomed colander may also be used as the settling container.







Settling Container Cloths: The simplest design is a piece of cheesecloth or a light cotton dishtowel about 20 to 24 inches square which can be arranged diagonally into a square (or round) settling container. A design that allows for slightly better drainage and gives the tofu a smoother surface is made by sewing the cloths to form a square and arranging them in the settling box as illustrated. Sew the cloth to fit your box and seam the edges to prevent fray-

The Care of Your Tools

Wooden boxes and barrels should be dried in the sun after use and stored in a clean dry place. Cloths and sacks should be thoroughly scrubbed in hot whey or hot water as soon as possible after use to prevent soymilk from gradually clogging the mesh; rinse well, dry in the shade, and store in a dry, well-ventilated place to prevent molding.

Solidifiers

Farmhouse tofu makers in Japan have traditionally used either seawater or natural nigari to solidify their tofu. At present, the refined forms of nigari (magnesium chloride or calcium chloride) are also widely used and give virtually the same fine flavor. Although we, like most traditional craftsmen, prefer to use and recommend simple, natural ingredients, the level of contamination of seawater in most parts of the world makes it necessary for us to advise caution in the use of natural seawater or nigari and suggest the use of their refined forms. Nevertheless, for people who still have access to clean seawater, and in the hope that man's large-scale pollution of the environment will soon stop, we would like to give detailed information concerning the composition, preparation, and use of the traditional farmhouse solidifiers. In the process, we will try to explain why the refined forms of nigari make good substitutes.

Seawater: Clean seawater is highly recommended as a solidifier because it is easy to use, makes delicious tofu, requires no further processing or preparation and, if taken directly from the ocean, is available at no cost. Whenever possible, collect the seawater shortly before it is to be used and store it in a clean bottle or other non-corrosible container; it gradually loses its potency as a solidifier the longer it is stored. Do not collect the seawater from near the mouth of a stream or river since it will be relatively dilute and impotent. Do not use water which is cloudy or unclear.

The composition of seawater varies somewhat from place to place throughout the world. A typical sample has the following composition by weight. The remainder is water (H_2O):

	Percent
Sodium chloride (common salt)	2.72
Magnesium chloride	0.38
Magnesium sulfate	0.17
Calcium sulfate	0.13
Potassium chloride	0.09
Magnesium bromide	0.01

Seawater also contains over 60 trace elements, all of which are of nutritional value. In approximate order of abundance are strontium, boron, silicon, nitrogen, aluminum, rubidium, lithium, phosphorous, barium, and iodine, among others.

It is interesting to note that magnesium chloride, magnesium sulfate (Epsom salts), and calcium sulfate are each effective tofu solidifiers used (separately) in modern tofu shops. By using seawater to solidify tofu, their combined action seems to bring out a wide range of complementary flavors and ensure more complete solidification of the different types of soy protein.

Since all seawater contains approximately the same concentration of nigari, it is easy to specify exactly how much seawater will be necessary to solidify tofu made from a given quantity of soybeans. By remarkable coincidence, the required volume of seawater is just equal to the volume of dry soybeans used. Since the concentration of liquid nigari, on the other hand, varies widely and is difficult to measure, it is not easy to specify exactly how much to use in a given recipe.

Tofu prepared with seawater does not taste "salty" because all the salt dissolves in the whey as the latter separates from the curds. However, since a very small amount of whey is inevitably contained in the tofu, even after thorough pressing, the salt contained therein serves as a seasoning, further enhancing the tofu's flavor. Thus, if you have a supply of clean seawater, by all means use it to solidify your tofu. Only if you wished to make your own natural salt would it make sense to first extract salt from seawater, then extract nigari from the salt.

Natural Nigari: Called "bittern" or "bitterns" in the West, *nigari* is the mineral-rich mother liquor that remains after salt is extracted from seawater. All natural sea salt contains some nigari, which gives the salt its hygroscopic propensity to absorb and retain water from the air and imparts to it a subtle bitterness, a slightly gray color, and a concentrated flavor that makes natural salt taste "saltier" and more potent than refined salt. In fact, the refining process is basically the removal of nigari from natural salt to create a pure-white product that is about 99 percent sodium chloride. Generally containing magnesium carbonate additive, refined salt bears about as much resemblance to natural salt as do white bread, white rice, or white sugar to their whole, natural counterparts. The Japanese call natural salt *nami-no-hana*, "the flowers of the waves." It has been regarded as a symbol of purity and is used in a wide array of sacred ceremonies and rituals.

Anyone can make natural salt: to do so, take a large, wide-mouth pot to a clean stretch of ocean, use seawater to fill the pot two-thirds full, then simmer its contents over a driftwood fire until all of the water has evaporated and only moist solids remain. Transfer the solids to a glass jar and cover. (This is a beautiful and fruitful way to spend a day at the ocean!) One gallon of seawater (weighing 8¼ pounds) yields about ¼ pound natural sea salt, almost one-fourth of which consists of minerals other than sodium chloride. Containing all of the trace elements found in seawater, its composition on a moisture-free basis is:

	Percent
Sodium chloride	77.8
Magnesium chloride	9.5
Magnesium sulfate	6.6
Calcium sulfate	3.4
Potassium chloride	2.1
Magnesium bromide	0.2

Using your moist salt (or natural sea salt available at most natural foods stores) you can now prepare your own nigari. For small-scale production, place the salt into a fine-mesh bamboo (or plastic) colander set over the mouth of a non-corrosible (earthenware, glass, wood, or plastic) container (fig. 117). If the salt is dry, sprinkle it lightly with water,

Fig. 117. Making nigari with bamboo colander

then place the salt and container in a cool, damp place. (Or, for faster results, place a large bowl filled with water next to the container and cover the salt, bowl, and container with a large plastic bag or box to form a simple humidifier.) As the salt absorbs moisture from the air, the nigari, a slightly reddish, concentrated liquid, will begin to drip into the empty

container. After several days, depending on the amount of salt and the humidity, there should be enough nigari to solidify the tofu made from 8 cups soybeans.

For larger-scale production, obtain at least 10 pounds (or as much as 50 to 100 pounds) of sea salt. Place the salt in a moistened sack (such as a flour or gunney sack) or on a piece of cotton or linen cloth which is then gathered at the corners to form a sack. Suspend the sack above a large container or, if the container is sturdy and has a wide mouth, place the sack on top of several boards resting across the container's mouth. Set in a cool damp place and allow the nigari to factor out.

In traditional Japanese farmhouses, about 100 pounds of unrefined sea salt were placed in a sack 3 feet long and 2½ feet wide made of woven rice straw. This was either set directly on top of a specially-made wooden container called a "salt boat" (fig. 118) or suspended from the farmhouse rafters over an empty wooden barrel. These sacks could be seen

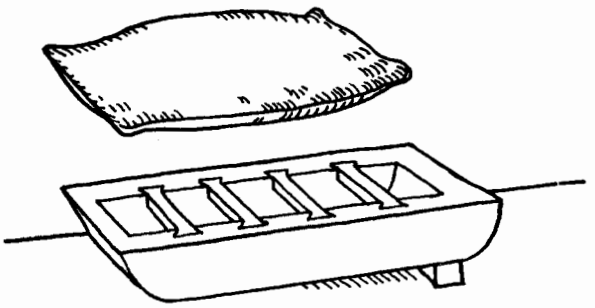


Fig. 118. A "salt boat"

working throughout the year as farmers "refined" their own salt while simultaneously collecting the valuable nigari: thus, full use was made of the natural salt purchased from oceanside salt fields,nothing was wasted, and no energy was consumed in the refining process. The nigari was used for solidifying homemade tofu. The well-drained topmost portions of the salt were used in the preparation of farmhouse miso and shoyu, and for seasoning foods at the dinner table. The lowermost portions, which still contained a small amount of nigari, were used for pickling, since farmers found that the nigari gave vegetables (such as *daikon*) and fruits (such as the *ume*) a crisper texture, firmer skin, and better flavor. Over a period of several weeks during the warm, humid months when nigari could be collected most rapidly, 100 pounds of unrefined (grade 5) natural salt yielded about 10 to 20 quarts of the liquid usually with the following composition (not including its water ɔntent):

	Percent
Magnesium chloride	31
Magnesium sulfate	2
Potassium chloride	2
Sodium chloride	1

It will be seen why refined magnesium chloride and natural nigari produce almost identical flavors in tofu.

Natural nigari is also available from salt refiners (such as Leslie Salt in the U.S.) using natural salt fields. Enormous quantities of bittern are produced as a byproduct of the salt refining process. Although this bittern has not been approved for use in foods by the Food and Drug Administration, it is purified by natural processes; algae eat any organic matter in the salt fields, brine shrimp are introduced to eat the algae, and the shrimp are carefully removed before the brine is made into salt. Most of this nigari is sold for commercial use in tank-truck quantities to produce magnesium chloride, magnesium metal, Epsom salts, potash, or bromine, or to remove the ice from frozen road surfaces. Weighing 10.7 pounds per gallon (specific gravity 1.28 at 60°F), a typical sample of the solids in this nigari shows the following composition by weight:

	Percent
Magnesium chloride	11.8
Sodium chloride (common salt)	6.9
Magnesium sulfate	6.7
Potassium chloride	1.8
Potassium bromide	0.2

For over 1,000 years, up until the beginning of World War II, almost all the tofu made in Japanese farmhouses and tofu shops, and much of the tofu made near the seacoast in China, was solidified with natural nigari. The word "nigari" is composed of the two characters meaning "bitter" and "liquid." Unlike the word "bittern" in the West, "nigari" is well known and widely used in Japan due to its long-standing association with natural sea salt and salt-pickled vegetables as well as with tofu. Magnesium chloride, the main active ingredient in nigari, coagulates the soy protein in soymilk to form curds; in chemical terms, the double-bonded, positive magnesium ion (Mg^{++})combines with a double-bonded negative ion in the protein to form a fruitful and happy marriage.

Until the postwar period, most of Japan's nigari and natural salt were produced by the solar evaporation of seawater in small salt farms located on the seashore in areas with low rainfall, plenty of sunshine, and a high average temperature. Using the an-

cient "raised beach" method, the salt maker carried sea water from the ocean in large wooden buckets suspended from the ends of a shoulder pole. On hot, sunny days, he scattered this water over the surface of a small, level field consisting of clean sand spread several inches thick over a base of hard clay. (In the early part of the twentieth century, the salt fields came to be built at sea level, and the water was run in by a type of irrigation system.) The salt water in the wet sand rose to the surface of the field by capillary action. Here it evaporated and salt crystals formed. These were raked up and placed in a double-level draining vat. The latter consisted of a shallow wooden tub on top of which was mounted a slightly deeper wooden vat with a slatted bamboo bottom covered with a matting of woven rice straw. The sand (in which was deposited the crystallized salt) was placed in the upper vat. Sea water was then poured over it so that the salt dissolved and drained into the lower tub as a concentrated brine. The well-drained sand was scattered back over the field and raked smooth. The brine was then transferred to a large cauldron in which it was further evaporated (simmered) over a wood fire. As the concentration of the brine increased, sodium chloride (common salt) reached its saturation point and crystallized on the bottom of the pot. The crystals were scooped up with a shallow strainer and placed in a tightly-woven bamboo basket. The basket was set on a draining board attached to the edge of the cauldron so that the liquid (nigari) in the salt drained back into the cauldron. The well-drained salt was then put into a 6-foot-deep double-level draining vat—similar in design to but larger than the one used to wash the sand—and was allowed to drain for one week. The liquid remaining in the cauldron, "fresh nigari," was set aside and conserved. The well-drained salt was then sorted into 5 grades. That at the top of the vat, which drained best and contained the least nigari, was considered top-grade and sold at the highest price. That at the bottom of the barrel, grade 5, was relatively moist and bitter; it was sold at the lowest price and was widely used in farmhouses where it was further refined, as described above.

The nigari that remained in the cauldron after all the salt had been removed was cooled, placed into well-seasoned cedar shoyu vats (fig. 9, p. 32), and shipped to tofu shops throughout the country. In some cases it was condensed (by simmering) to twice its concentration or even until it became a solid. Because of its light weight, concentrated (or solid) nigari was easier to transport and was therefore often sold to tofu makers at a lower price.

As the simple salt farms throughout the country were gradually replaced by large scale, industrial salt factories, the lower grades of salt gradually disappeared. By 1931, grades 4 and 5 were no longer available, and by the end of World War II all salt made in Japan was either grade 1 or 2. This meant that farmers could no longer produce their own nigari. Although some farmhouse tofu makers began to order nigari from commercial sources, country tofu gradually started to disappear from the culture.

Food-grade natural nigari is now available at low prices from natural food distributors in both the United States and Japan (see p. 315). It is usually sold in its solid form, which has a coarse, granular texture resembling sea salt, is tan to reddish gray in color, and will dissolve in cold water in less than 1 minute. The solid form is preferable to liquid nigari primarily because the amount necessary to solidify a given quantity of tofu can be specified exactly, whereas with liquid nigari the amount required depends on the concentration. One pound of solid nigari will solidify about the same amount of tofu as 6½ cups of typical liquid nigari or 114 pounds (14 gallons) of seawater. It is obvious from these figures why nigari rather than seawater has been used in most farmhouses and tofu shops in Japan.

Refined Nigari: Because of the present level of contamination in the oceans and the difficulty of obtaining food-grade natural nigari, most Japanese farmhouse craftsmen and many tofu shops throughout East Asia and the United States now use refined nigari—either magnesium chloride or calcium chloride. Although both evoke much the same delicate natural sweetness and bouquet as natural nigari, magnesium chloride gives tofu that is slightly closer in flavor to the traditional, natural product, while calcium chloride is valued for yielding tofu that is rich in calcium. On a farmhouse scale, both solidify the tofu more quickly and are easier to use than the two solidifiers listed below. Both are sold in the form of a granular or crystalline white solid.

Calcium Sulfate and Magnesium Sulfate: When used with soymilk cooked in a metal pot over a wood fire, both of these solidifiers yield delicious, though rather mild-flavored, soft tofu. Natural calcium sulfate (gypsum) has been used to solidify farmhouse and commercial tofu in China for about 2,000 years. Although it has never been used by Japanese farmhouse craftsmen, it is now the most widely used solidifier in tofu shops throughout the world. Its recent popularity is due primarily to its ability to give a slightly larger bulk yield (by incorporating more water into the tofu) and to the ease and speed with which it can be used on a commercial scale. (Keep in

mind, however, that nigari gives tofu with the same yield of solids and nutrients as calcium or magnesium sulfate.) Although present-day natural gypsum is about 97 percent pure, the remaining portions may contain lead or other impurities. To be safe, therefore, use only natural gypsum certified as a food ingredient.

Regardless of the variety of solidifier, use the *minimum amount necessary* to curdle the soymilk. If too much is added, the bulk yield (but not the yield of solids or nutrients) will drop, and the end product will be relatively hard, coarse, and crumbly. Its surface will be less smooth and glossy and contain tiny holes or air pockets. It may also have a slightly bitter taste (which can be alleviated by soaking in cold water as soon as the tofu is removed from the settling container). If the curds forming in the cooking pot separate from the pot's walls and the space in between fills with yellow whey, you have probably added too much solidifier.

Country Farmhouse Tofu

MAKES 15 TO 20 SERVINGS

8 cups soybeans
5 gallons water, approximately
Solidifier:

For subtly sweet nigari tofu use: 3 tablespoons solid granular magnesium chloride or calcium chloride; or 2½ to 4 tablespoons granular or powdered natural nigari; or 2½ to 5 tablespoons homemade liquid nigari, or 3 to 8 tablespoons commercially prepared liquid nigari; or 8 cups clean seawater (freshly collected)

For mild soft tofu use: 3 tablespoons Epsom salts (magnesium sulfate) or calcium sulfate

For subtly tart or slightly sour tofu use: 1¼ cups lemon or lime juice (freshly squeezed), or 1 cup (apple cider) vinegar

Prepare in advance:

On the evening of the previous day: Place beans in pressing pot and rinse with water, stirring vigorously with paddle or hands. Drain in colander, rinse again, and re-drain. Combine beans and 1½ gallons water in pressing pot and soak for 8 to 10 hours (or in very cold weather for as long as 15 to 20 hours).[1]

If using a wood fire, gather firewood, preferably oak or other fragrant hardwood. Prepare cooking site and lay (but do not light) a small fire.

Rinse out pressing pot, sack, and rack. Place sack and rack in pressing pot and set pot 6 to 8 feet away from fire.

Moisten settling-box cloths and use to line bottom and sides of settling container. See that cloths fit closely against all inside edges and corners of container and are free of large wrinkles. Set container aside for later use.

Pour soaked beans into colander, rinse well under running water, and allow to drain.

After making the above preparations, proceed as follows:

1) Light fire and begin to heat 4¾ gallons water in covered cooking pot. While water is heating, combine about 2½ cups soaked beans with 2 2/3 cups water in blender and purée at high speed for 2 to 3 minutes or until smooth. Transfer purée (gô) to purée container and repeat until all beans are used. (If using a foodmill or meat grinder, grind beans without adding water, and add 5½ quarts more water to cooking pot. If using grinding stones, see Note 2.)

2) When water in cooking pot comes to a boil, transfer 1 gallon to hot water pot. Add soybean purée to water in cooking pot, rinsing out purée container and blender with a little water from hot water pot to retreive any remaining purée. Taking care that pot does not boil over, heat over high heat, stirring bottom of pot frequently with wooden paddle to prevent sticking. When foam suddenly rises in pot, quickly lift pot off fire (or use tongs or a shovel to remove fire from under pot) or turn off heat.

3) Place pressing pot next to cooking pot. While a second person holds sack down inside pressing pot with mouth of sack open, ladle and then pour hot purée into sack. Rinse out cooking pot with a little water and pour into sack. Lift sack out of barrel, quickly place pressing rack across barrel's mouth, and set sack on top of rack. Twist hot sack closed and fold neck across top of sack. Balance a heavy pressing weight directly on top of neck in center of sack and press for 2 to 3 minutes. (Or press sack with lever press.) Adding your full body weight, press for about 1 minute more to expel as much soymilk as possible.

4) Remove weight and bounce sack on rack to loosen pressed okara. Open sack on rack and pour in 1 gallon hot water from hot water pot, dampening entire surface of okara. Stir okara briefly with paddle or ladle, then twist sack closed and re-press for 2 to 3 minutes. Open sack, shake okara into one corner of sack, twist closed again, and press for 1 to 2 minutes more. Adding your full body weight, press for several minutes more, or until soymilk no longer drips into pressing pot. Bounce sack on rack to loosen okara, then empty okara into purée container and set aside. Dry hot water container thoroughly, measure in solidifier, and set aside.

5) Scrub out and rinse cooking pot, return it to the fire and pour in soymilk. Stoke fire, increasing heat to medium-high. Bring soymilk to a boil, stirring bottom of pot frequently to prevent sticking. Reduce heat to medium and cook for 5 minutes, then turn off heat. (Or place lid on pot, then rake or shovel all burning wood and coals out from under pot.)

6) Add 1½ quarts water to solidifier in hot water container. (Do not add additional water to seawater.) Using paddle or ladle, stir soymilk clockwise to form a swift whirlpool, stop paddle abruptly near side of cooking pot with blade

Calcium Sulfate Concentration Influence on Yield and Quality of Tofu From Five Soybean Varieties

NONG SUN and WILLIAM M. BREENE

ABSTRACT

A mini-process is described for making tofu from 50-g quantities of soybeans. It was used to compare yield and quality among 5 Minnesota grown varieties (Vinton, Corsoy, Hardin, Stine 2510, Stine 2810) and to determine optimum concentration of the coagulant, $CaSO_4$. Product yield (wet basis), solids recovery, protein recovery and textural quality (Texture Profile Analysis hardness and fracturability) were optimal at 0.02 N $CaSO_4$ for all 5 varieties. Negative correlations were found between $CaSO_4$ concentration and both yield ($r = -0.90$ to 1.00) and protein recovery ($r = -0.96$ to 1.00) for all varieties.

Key Words: tofu-quality, soybean, coagulants, tofu-yield, calcium sulfate

INTRODUCTION

SOYBEAN VARIETY has been shown to influence yield and quality of tofu (Skurray et al., 1980; Kamel and De Man, 1982; Wang et al., 1983; Lim et al., 1990) due to variations in composition, e.g., protein, oil, ash and phosphorus content, among others. However, in order to make valid comparisons among varieties, it is important to produce tofu by a standardized procedure because manufacturing variables can profoundly influence yield and quality. These variables include time and temperature of presoaking soybeans prior to making soymilk (Watanabe et al., 1964; Shurtleff and Aoyagi, 1979; Hsu, 1983; Hsu et al., 1983), the water: soybean ratio in the soymilk (Shurtleff and Aoyagi, 1979; Beddows and Wong, 1987a), time and temperature of heating the soymilk (Watanabe et al., 1964; Saio, 1979; Shurtleff and Aoyagi, 1979) temperature and extent of stirring during coagulation (Wolf and Tamura, 1969; Wang and Hesseltine, 1982; Beddows and Wong, 1987b) and type and concentration of coagulant (Wantanabe et al., 1964; Appurao and Narasinga Rao, 1975; Saio, 1979; Shurtleff and Aoyagi, 1979; Skurray et al., 1980; Tsai et al., 1981; Wang and Hesseltine, 1982; Wang, 1984; Yasuda and Hokama, 1984; De Man et al., 1986; Beddows and Wong, 1987c).

The coagulant of choice among most tofu makers is calcium sulfate; Tsai et al. (1981) and Wang and Hesseltine (1982) found it superior to other calcium salts. Nigari, a by-product of the maufacture of sea salt, consists mainly of magnesium sulfate, but can vary in composition and has not been approved for use in the U.S. (Shurtleff and Aoyagi, 1979). Glucono delta lactone (GDL) is used to make soft (silken) tofu, but is not suitable for making firm (Chinese style) tofu if used alone (Tsai et al., 1981).

The objective of our study was to determine how yield and quality might be influenced by the concentration of coagulant, calcium sulfate, in tofu made from five different Minnesota grown soybean varieties using a small-scale procedure to mimic industrial practices.

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MATERIALS & METHODS

Materials

Five Minnesota-grown, light-hilum soybean varieties from the 1987 harvest were obtained from North Country Seed, Inc., Trimont, MN. They were selected to include the popular choice of many tofu makers (Vinton), two varieties very popular among growers (Corsoy and Hardin) and two newer introductions (Stine 2510 and Stine 2810).

All chemicals used in the analyses were reagent grade and were obtained from Sigma Chemical Co., St. Louis, MO.

Hydration rate

Fourteen 50-g samples of soybeans of each variety were immersed in distilled 22°C water in separate beakers. After soaking at 22°C for 2, 4, 6, 8, 10, 12 or 14 hr, duplicate samples were drained on a screen for 30 sec and weighed to determine the amount (g) of water absorbed.

Soymilk and solids content

Washed soybeans (50 g) were soaked in 500 mL beakers at 22°C for 12 hr in about 400 mL tap water, drained, rinsed once with 400 mL tap water, combined with 500 mL tap water and ground for 2 min in an Osterizer seven-speed blender at "liquefy" speed. Five drops of Antifoam A emulsion (Dow Corning Corp., Midland, MI) were added and the slurry was boiled in an aluminum saucepan for 15 min and immediately filtered through a muslin cloth (one thickness) to remove the okara.

Soymilk solids content was determined using an Abbe refractometer (American Optical Model 10450) and a standard curve in which refractive index (Y axis) was plotted vs oven dry solids content (X axis) of 1-mL soymilk samples in the solids range 4 to 11%. Refractive index was determined at room temperature and correlated highly with oven dry moisture results. This quick method was recommended for industry use by Johnson and Wilson (1984).

Preparation of tofu

A 325-mL portion of hot soymilk was poured into 40 mL of Food Grade calcium sulfate ($CaSO_4 \cdot 2H_2O$, Van Waters & Rogers, Inc. Seattle, WA) solution of the appropriate concentration in a 500 mL beaker. For final concentrations in soymilk of 0.01, 0.02, 0.03, 0.04 and 0.06N $CaSO_4$, this required 0.63, 1.26, 1.89, 3.15, and 3.78 $CaSO_4$/40mL, respectively. The 365 mL of soymilk/coagulant was held at 70°C for 10 min in a water bath while coagulation occurred. The soybean curd was then transferred, taking care to avoid breakage, to a specially designed 7.5 × 7.5 × 7.5 cm cheesecloth-lined polymethyl methacrylate form and pressed for 2 hr by placing a 5 kg weight on the 56.25 cm^2 plate covering the curd. The six-sided form could be easily assembled and disassembled for cleaning.

Tofu yield, protein and solids recovery, and proximate analysis

Yield is expressed as wet weight of tofu obtained from 50g soybeans. Protein recovery is expressed as the amount of protein in the tofu dry matter divided by the amount of protein in the soybean dry matter times 100. Solids (dry matter of tofu) recovery was determined by the oven dry method.

About 1 kg of soybeans of each variety was ground in a Thomas Wiley Mill to pass a 1-mm diameter mesh sieve using liquid nitrogen to prevent moisture loss and protein denaturation and to keep them in the solid state.

Samples were analyzed for moisture (Williams and Baker, 1982; AOAC 14.081, 14.004); oil (Link, 1973, AOCS AC 3-44); ash

milk with the coagulant. After keeping at 70°C in a water bath for 10 minutes, the tubes were centrifuged for 10 minutes at 1,000 x g. The curds were collected and weighed. Their moisture contents and total solids were determined by drying at 105°C for 24 hours. The nitrogen content of the solids was also determined.

Texture evaluation

Curds used for texture evaluation were made as previously described except that 400 ml of soybean milk was used and the curds were pressed in a square wooden box by placing weight (10 g/cm²) on the top for 1 hour.

The texture profiles were determined by compressing a 2.5 x 2.5 x 2 cm tofu cube to 0.5 cm thickness (75% deformation) with an Instron universal testing machine. The load cell scale was 0-50 kg, cross-head speed 2 cm/min., and the chart speed 20 cm/min. The compression test was repeated twice, and the time lag between the two was 2 minutes. At least three cubes were tested from each sample.

Effect of heat treatment

In making soybean milk, soybean slurry is first boiled. This heat treatment is essential not only for protein denaturation[24] to obtain proper curd formation but also for improving nutritional value and reducing beany flavor. The effects of boiling (98°C) on *in vitro* digestibility of soybean milk are shown in Fig. 1. As boiling time increased, the digestibility of soybean milk increased and reached a maximum of 97.3% after 15 minutes. These results agree with the theory that proper heat treatment increases the digestibility of soybean proteins owing to

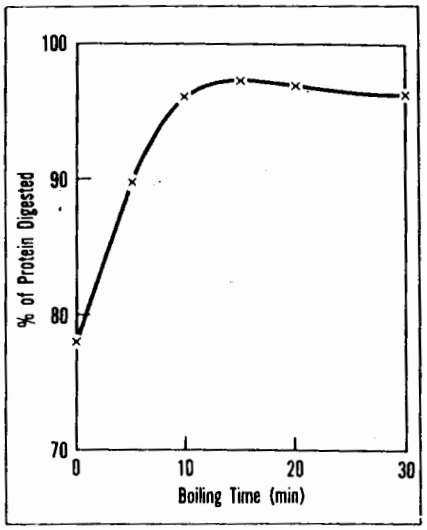

Fig. 1. In vitro digestibility of soybean milk as affected by duration of boiling.

destruction of heat-labile trypsin inhibitors and possibly other biologically active components, but an excessive amount of heat may adversely affect the nutritive value[25]. Hackler *et al*[26] reported that the protein efficiency ratio (PER) of heat-processed milk is dependent upon both temperature and time. They found that heating soybean milk at 93°C did not give maximum PER until 60 minutes, whereas at 121°C maximum PER was obtained in 5 minutes. However, none of the soybean milk samples had a PER value equal to that of soybean meal.

Data in Table 1 indicate the destruction of cystine and methionine after extended boiling, amounting to 30% destruction after boiling for 30 minutes. The destruction of cystine in overheated soybean product is not unexpected; Evans *et al*[27] have thoroughly documented the sensitivity of cystine in soybeans to heat treatment. Hackler and Stillings[28] also examined the effects of heating on amino acid composition of soybean milk. They found no significant changes in amino acid composition at 93°C; however, at 121°C, cystine and tryptophan decreased as the cooking time was increased from 0 to 120 minutes.

Watanabe *et al*[20] reported that boiling soybean slurry for more than 20 minutes not only reduces the total solids recovery and tofu yield but also affects the tofu texture. Thus, they suggested boiling the slurry for 10 minutes. The authors' own data on *in vitro* digestibility and amino acid composition also indicate that maximum nutritive value of soybean milk can be ensured by boiling for 10-15 minutes.

Tofu often is consumed without further cooking; therefore, sufficient heat treatment is necessary to destroy the antinutritional factors and to attain the maximum nutritional value of the soybean milk.

Table 1. Effect of boiling time on the essential amino acid composition of soybean milk

Amino acids	Boiling time (minutes) 0	5	10	15	20	30
Threonine	5.01	5.02	5.05	5.01	5.13	4.79
Valine	6.04	6.03	6.25	6.06	5.93	5.67
Cystine	1.22	1.28	1.25	1.11	1.08	0.86
Methionine	1.47	1.40	1.28	1.11	1.12	1.06
Isoleucine	5.88	5.88	6.11	6.11	5.89	5.63
Leucine	9.81	9.71	10.07	10.08	9.90	9.67
Tyrosine	4.82	4.82	4.70	4.80	4.46	4.87
Phenylalanine	6.52	6.55	6.67	6.45	6.42	6.22
Lysine	8.17	7.95	8.23	8.14	8.09	7.89

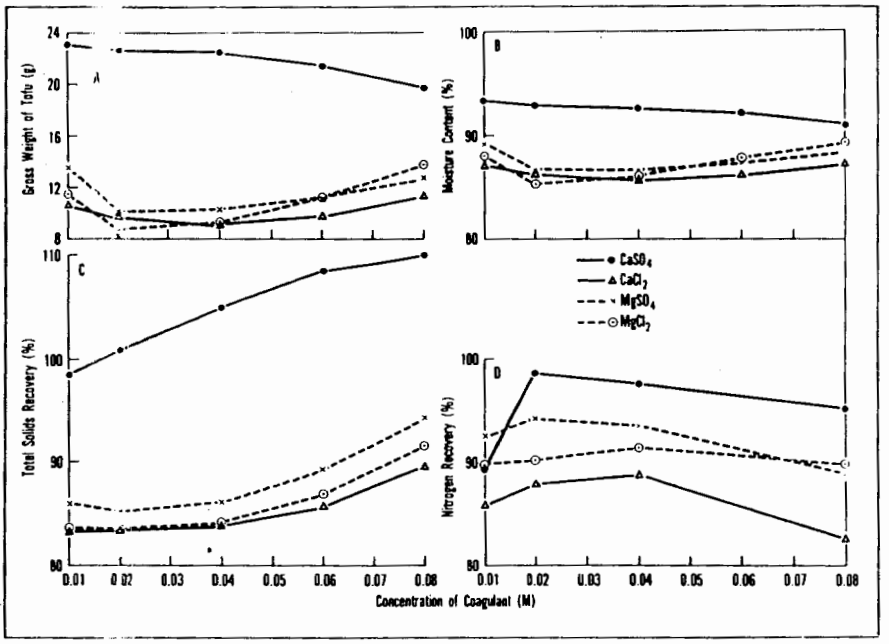

Fig. 2. Relationship of concentration and type of coagulants to the yield of tofu.

Effects of coagulants

Data in Fig. 2 show that both the ionic concentrations and the coagulants used affect the gross weight and the moisture content of the final product, as well as the total solids and nitrogen recoveries. When the final concentrations of any one of these salts in soybean milk were between 0.01 to 0.10M, good curd formation was noted. No curd was observed when the final concentrations were lower than 0.008M or higher than 0.1M, although in some cases thickening of soybean milk occurred.

Except when calcium sulphate was used, gross weight, moisture content of tofu, and total solids recovery decreased as the concentration of salt increased from 0.01 to 0.02M, remained about he same up to 0.04M, and then steadily increased at the higher concentrations. The percentage of nitrogen recovery, on the other hand, increased as the concentration of salt increased from 0.01 to 0.02M, remained the same at 0.02-0.04M, and then decreased at higher concentrations. In studying the bind-

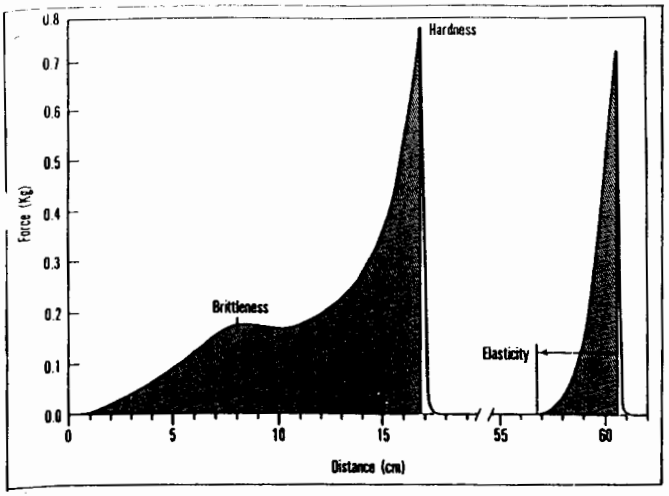

Fig. 3. Force-distance curve of tofu obtained from Instron universal testing machine.

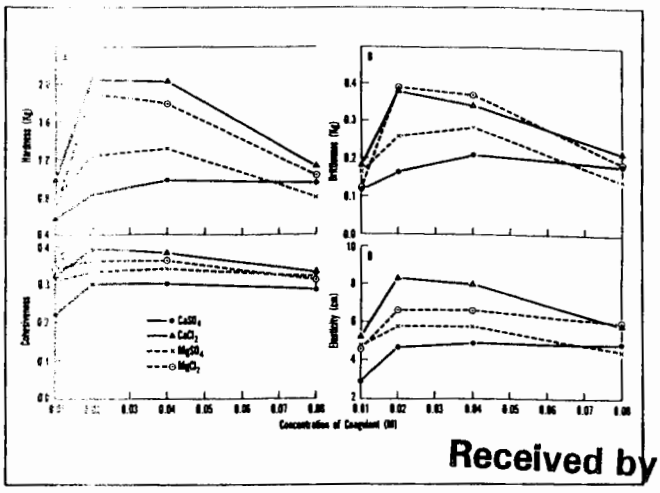

Fig. 4. Relationship of concentration and type of coagulants to the texture characteristics of tofu.

ing of unfractionated soybean proteins with calcium ion, Appu Rao and Rao[12] observed that at higher concentrations of calcium ion the extent of precipitation decreased and the protein was soluble again.

Because of the limited solubility of calcium sulphate, the actual ionic concentration at each level is uncertain and the concentration gradient is less than that indicated. Therefore, the limited solubility of calcium sulphate can partly account for the smaller variation noted in tofu made with the coagulant, as compared to that with the other three salts. The insolubility of calcium sulphate probably also contributes to the more than 100% solids recovery observed in tofu made with this salt. Among the four salts studied, calcium sulphate resulted in tofu with the greatest weight. The weight differences are due not only to the higher moisture content of the curd made with calcium sulfate than of curd made with the other three salts under the same experimental conditions but also could be due to the high solids recovery, or a combination of both.

The present study shows that salt concentrations between 0.02-0.04M had the least effect on the four quantities investigated and also resulted in the highest nitrogen recovery. Therefore, use of salt at a level between 0.02 to 0.04M is more likely to yield reproducible product with high nitrogen recovery. For the same reason, calcium sulfate seems to be preferred.

Effects of coagulant conditions

The texture parameters were evaluated according to Bourne's definitions[29]. Brittleness is the force of first major peak, hardness the force required for a 75% deformation, cohesiveness the ratio of the two shaded areas as shown in Fig. 3, and elasticity the recovered height after the first compression measured by the distance. Fig. 3 is a mirror image of a force-distance curve of tofu made with calcium sulphate. Similar curves were obtained from tofu made with the other three salts. In some samples, the breaking point or brittleness is not clearly defined. However, no explanation can be found for this.

When the concentration of the coagulant was increased from 0.01 to 0.02M, significant increases in hardness, brittleness, cohesiveness, and elasticity of all curd samples were noted (Fig. 4). No significant effect was observed at concentrations between 0.02 to 0.04M, but above that range these measurements of the curds decreased steadily, except when calcium sulphate was used as coagulant.

The hardness and brittleness of the curds also were influenced by the types of salts used. Calcium chloride and magnesium chloride resulted in curds with much greater hardness and brittleness than did calcium sulfate and magnesium sulfate, suggesting that anions have a greater effect than cations on these two parameters. The difference appears to be due to some type of interactions. In a study by Aoki[30] on the effect of salts on the gelation of soybean proteins, anions were found to have a stronger effect on water-holding capacity than did cations.

There was no significant difference in cohesiveness of the curds made with the four salts (Fig. 4C), indicating that cohesiveness may be an inherent texture characteristic of a given protein. The present study also showed that the elasticity of curd was not greatly affected by the type of coagulants used. Lee and Rha[31] found heat treatment that unfolds the polypeptide chains[24] has a dominant effect on the elasticity of the curd, and they speculated that elasticity may depend mainly on stereo structure and intermolecular reaction.

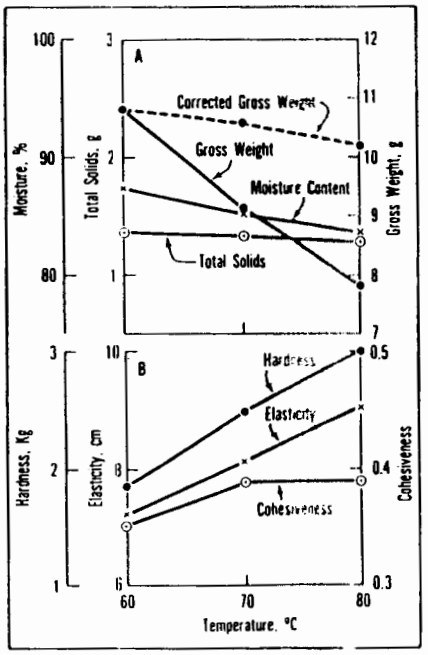

Fig. 5. Effect of coagulation temperature on tofu.

Effect of coagulation temperature

The effect of milk temperature at the time of adding coagulants on the yield and texture of the resulting curd is exemplified by the curd prepared with 0.02M calcium chloride at 60-80°C. As shown in Fig. 5A, the gross weight and the moisture content of the curds decreased as the temperature increased, whereas the total solids remained about the same. The difference in the gross weight appears to be derived mainly from the difference in the moisture content of the curds, as indicated by the dotted line in Fig. 5A, which is the gross weight of the curds after equalizing the moisture content.

Increasing hardness and elasticity of the curds was noted as the temperature increased (Fig. 5B). However, coagulation temperature appears to exert little influence on the cohesiveness of the curds.

Effect of mixing on coagulation

Traditionally, smooth and firm curd is made by pouring coagulants into the soybean milk without further mixing, because stronger mixing would result not only in hard curd as illustrated in Table 2 but also in curd with air pockets. Extra stirring significantly increases the hardness and reduces the gross weight and moisture content of the curds. The relationship between stirring speed and volume of tofu was investigated by Watanabe *et al*[20], who found that increasing stirring speed decreased tofu volume.

Conclusion

Although making tofu is a simple process, the phenomenon of protein precipitation

Table 2. Effect of mixing on coagulation temperature in making tofu

Coagulants	Gross Weight		Moisture Content		Hardness	
	A	B	A	B	A	B
	g		%		kg	
$CaSO_4$	170	153	87.1	85.6	0.80	0.97
$CaCl_2$	120	105	81.1	78.8	3.12	4.21

A. Coagulant and soybean milk were mixed by pouring one solution into the other.

B. After adding the coagulant, extra stirring was applied.

and polymerization is a rather complicated one. As shown in this study, many factors comes into play during the process and each exerts an effect reflected in the final product.

It has been suggested that the calcium coagulation is due mainly to the cross-linking between protein molecules by the ion[32,12]. However, the site of cross-linking in the protein molecules is still under debate. Also, the relationships between the ion binding to the soybean protein and the precipitation phenomenon of the protein are not completely understood. It is, therefore, difficult to speculate on the mechanisms of these effects.

This study, nevertheless, suggests that the quality and quantity in processing tofu can be manipulated by the selection of coagulation conditions, such as type and concentration of coagulants, temperature, and the mode of mixing, as well as the pressure applied to remove the whey. Uniform products can then be obtained from the same lot of soybeans or even from the same variety of soybeans by observing a selected set of conditions. Based on the authors' results, calcium sulphate ($CaSO_4 \cdot 2H_2O$) is the most suitable as coagulant for making tofu of high bulk weight, high nitrogen recovery and firm but not hard texture. To attain reproducible products with smooth texture, the coagulation is accomplished by pouring an appropriate amount of calcium sulphate suspension (10% volume of soybean milk) into 70°C soybean milk so that the final concentration of the salt in the milk is 0.02M.

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MODIFICATION OF CERTAIN FOODSTUFFS

continued from page 6

nected with one another: Formation of hydrogen peroxide, Formation of gluconic acid, Removal of oxygen, Removal of glucose. The removal of glucose and oxygen from a foodstuff proceeds as follows:

In the manufacture of egg powder an important part is played by the inhibition of the Maillard reaction, a reaction of compounds containing aldehyde and amino groups (as a rule glucose and protein), which is undesirable in this connection. The addition of glucose oxidase lowers the glucose content to 0.1%, which is sufficient to prevent the undesired browning reaction during the product's drying and storage.

Fig. 10 shows a processing line developed by Scott in the US for the manufacture of Salmonella-free egg-powder with a low content of microorganisms.

Finally, let us consider the deliberate removal of oxygen for the protection of water-oil emulsions, e.g. mayonnaise. The removal of the unwanted oxygen with a glucose oxidase-catalyse preparation prevents the development of an oxidative rancidity in mayonnaise for at least 6 months, which is combined with a retention of the original depth of colour. Some data are given in Table 2.

Conclusion and summary

The use of enzymes is generally allowed in the German Federal Republic provided that it is not restricted or wholly forbidden by specific decrees. In the production of these materials the manufacturer takes care to achieve a satisfactory quality according to standards drawn up internationally.

The examples given indicate that enzyme products used at the right place for the right purpose can improve the quality and the nutrient and organoleptic properties of foodstuffs and can also improve production processes. An additional benefit is the almost invariable reduction of costs.

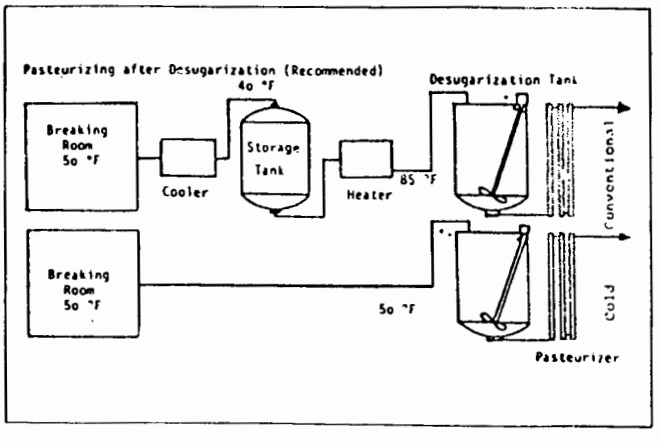


Fig. 10 Manufacture of egg-powder using Fermco de sugarization with GOD (Scott and Kliss, 1962)

Table 2. Influence of treatment with GOD on stability of mayonnaise

Effect of Glucose Oxidase-Catalase Treatment of Mayonnaise on its Stability

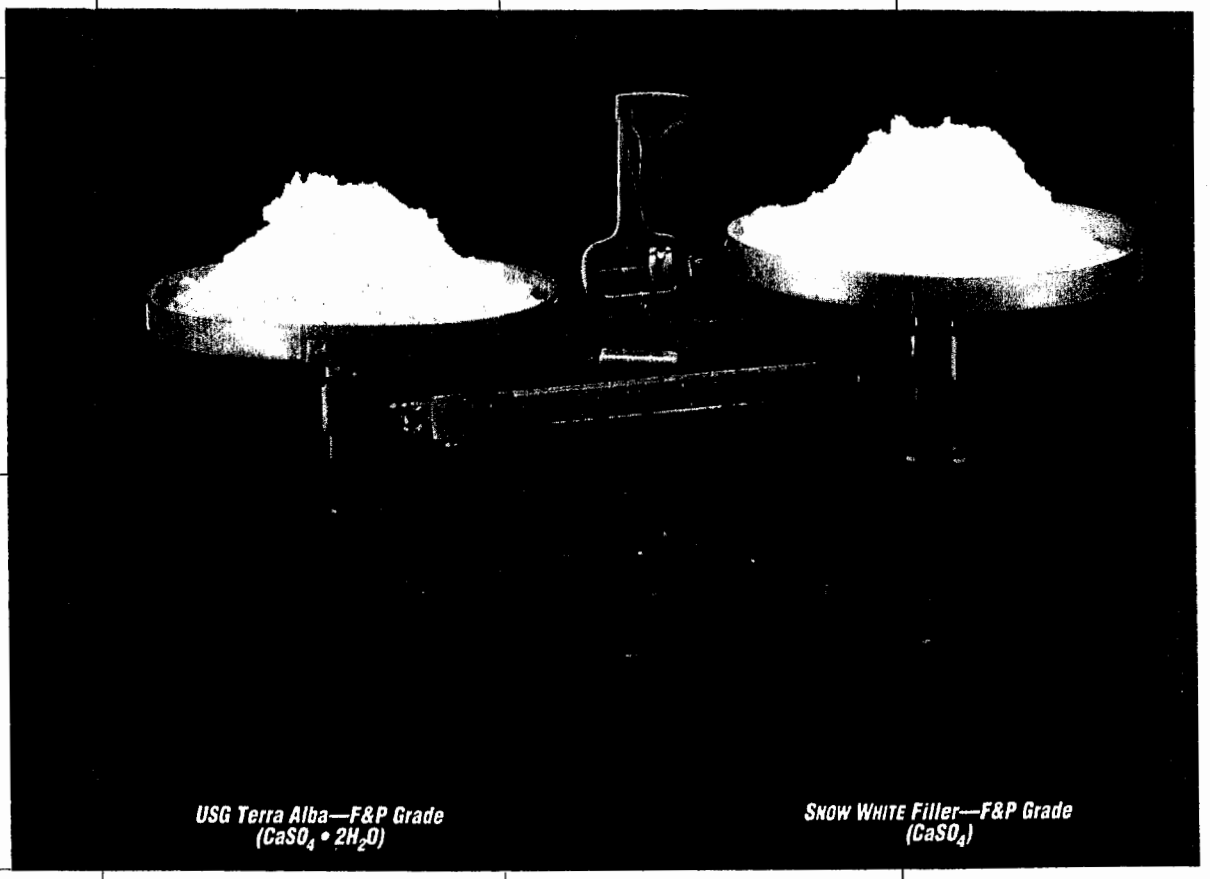
Age (months)	Organoleptic[2]		Colour[3]		Peroxide value (mM/kg averages)	
	Control	Enzyme treated	Control	Enzyme treated	Control	Enzyme treated
0	+	+	+	+	0.2	0.2
2	+	+	+	+		
3	–	+	+	+	6.2	0.2
4	–	+	–	+		
5	–	+	–	+		
6	0	+	0	+	14.6	0.8

[1] From Bloom *et al.* (1956).
[2] For organoleptic evaluation +, fresh; –, slightly rancid; 0, rancid.
[3] For colour evaluation: +, deep yellow; –, pale yellow, 0, v pale yellow.

Calcium Sulfate for Food and Pharmaceutical Uses

USG® Terra Alba—F&P Grade
SNOW WHITE® Filler—F&P Grade

High-purity additives for high-quality products



USG Terra Alba—F&P Grade ($CaSO_4 \cdot 2H_2O$)

SNOW WHITE Filler—F&P Grade ($CaSO_4$)

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Susan Dichter
Market Sales Manager

USG

USG

United States Gypsum Company
Industrial Gypsum Division

USG Terra Alba and SNOW WHITE Filler—Food and Pharmaceutical Grades—meet a diversity of product requirements

USG Terra Alba-F&P Grade ($CaSO_4 \cdot 2H_2O$), the *dihydrate* form of calcium sulfate, is made by fine-grinding and air-separating a select, high-purity white gypsum containing about 20% water of crystallization.

SNOW WHITE Filler-F&P Grade ($CaSO_4$), the *anhydrous* form of calcium sulfate, is made by high-temperature calcining of select, high-purity gypsum which is then ground and air-separated into a white powder.

In increasing volume, U.S. Gypsum Calcium Sulfate products serve the food, beverage, and pharmaceutical industries as an economical source of supplemental calcium. They are used in enriched flour and breads, cereals, baking powder, yeast foods, bread conditioners, canned vegetables and artificially sweetened jellies and preserves.

In beer manufacturing, the calcium ion, together with the needed buffering action as provided by proper water correction, promotes proper gelatinization of the starch in the cooker mash, as well as protein degradation and starch conversion. Thus, yield in the main mash is increased. In addition, the color of the wort is improved, and better precipitation and floeculation of undesirable protein complexes are achieved. The result is a paler, smoother-tasting beer with improved stability and shelf life.

For pharmaceutical applications, calcium sulfate is extensively used as a diluent serving as an excellent inert extender while it supplies dietary calcium.

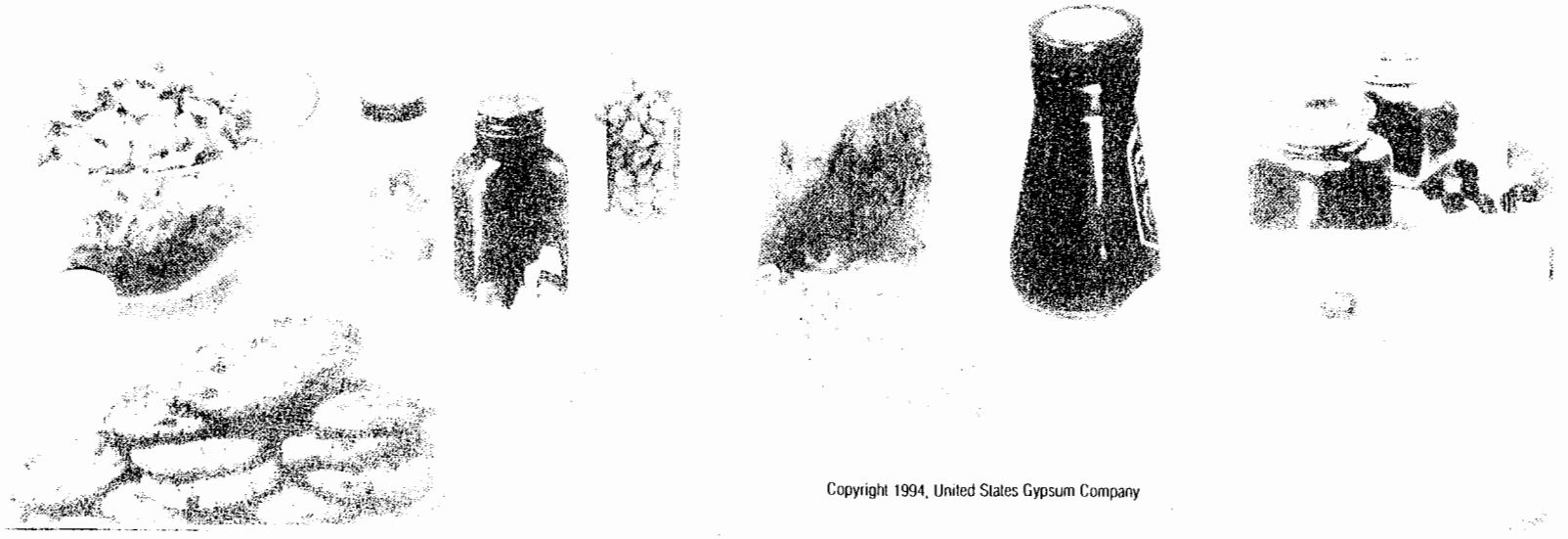
Typical Analyses

	USG Terra Alba-F&P Grade	SNOW WHITE Filler-F&P Grade
Total calcium[1]	23.1%	29.2%
CaO	32.31%	40.92%
SO_3	45.22%	57.46%
$CaSO_4$	0.39%	97.68%
$CaSO_4 \cdot 2H_2O$	97.1%	—
$CaCO_3 \cdot MgCO_3$	1.52%	0.77%
SiO_2 and Insolubles	0.24%	0.13%
$Fe_2O_3 \cdot Al_2O_3$	0.12%	0.12%
Water loss 250 °C	20.31%	0.33%
Brightness index–min.[2]	84.4	97.1
Oil absorption[3]	23.5	26.5
Specific gravity	2.32	2.96
Bulk density–pcf		
Loose	42.0	44.0
Compacted	70.0	80.0
Bulking values		
Lbs. per solid gal.	19.38	24.43
Solid gals. per lb.	0.0518	0.0406
Solubility (70 °F) per 100 cc of H_2O	0.26 grams	0.26 grams
pH-10% slurry	7.3	10.4
Refractive Index	1.52	1.56
Through 100 mesh—min.	100%	100%
Through 325 mesh—min.	93%	97%
Avg. particle size—microns	12-15	7-9

[1] Conversion of calcium content: Milligrams of calcium per lb. of USG Terra Alba-F&P Grade computed as follows: 1 lb. = 454 grams = 454,000 mg 23% x 454,000 mg = 104,420 • 1 lb. of Terra Alba-F&P Grade = 104,420 mg of calcium. Milligrams of calcium per lb. of SNOW WHITE Filler-F&P Grade computed as follows: 1 lb. = 454,000 mg 29% of 454,000 mg = 131,660 mg • 1 lb. of SNOW WHITE Filler-F&P Grade = 131,660 mg of calcium.

[2] Brightness index was determined on a Beckman DU Spectropholometer using magnesium oxide as the standard.

[3] Oil absorption is the amount of linseed oil in cubic centimeters required to wet 100 grams of filler.



SNOW WHITE Filler-F&P Grade, because of its whiteness, is used successfully to extend TiO_2 in white cake icings at a much lower cost. This filler also functions as a stabilizer and supplies enrichment calcium.

USG Terra Alba-F&P Grade and SNOW WHITE Filler-F&P Grade are manufactured only at U.S. Gypsum's Southard, Okla. plant. Careful quality-control tests are conducted on a regular basis in a modern well-equipped laboratory. These products are *guaranteed* to meet the specifications of Food Chemicals Codex and National Formulary as listed below:

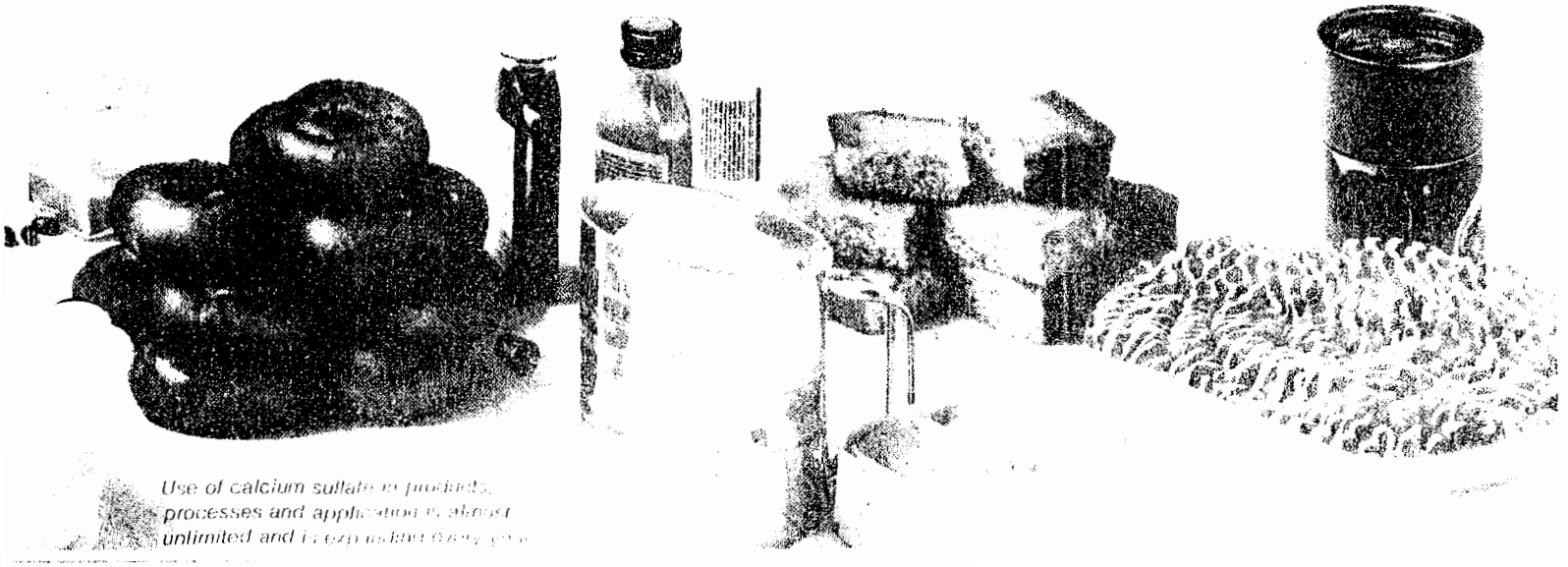
	Food Chemicals Codex	National Formulary
Arsenic	3.0 ppm max.	3.0 ppm max.
Selenium	30.0 ppm max.	30.0 ppm max.
Fluorine	30.0 ppm max.	30.0 ppm max.
Heavy metals	10.0 ppm max.	10.0 ppm max.
Iron	—	100.0 ppm max.
Lead	10.0 ppm max.	—
Calcium assay	98.0% min.	98.0% min.

Upon request, U.S. Gypsum will supply a *continuing guarantee* to customers using USG Terra Alba or SNOW WHITE Filler-F&P Grade. Each shipment is batch-coded to show day, month and year of manufacture. Representative samples for reference are maintained at Southard for five years from date of shipment.

Calcium sulfate is an approved additive on the Food and Drug Administration GRAS (Generally Recognized As Safe) list of food additives. Approvals for the use of calcium sulfate in specific food products for nutritional and functional uses are listed in FDA Regulations, Title 21, Food and Drugs, Parts 1 to 199, Revised April 1, 1985 as follows:

Sections	Uses
133.111 (c) 2	With benzoyl peroxide in Caciocavallo siciliano cheese
133.141 (c) 2	With benzoyl peroxide in Gorgonzola cheese
133.165 (c) 2	With benzoyl peroxide in Parmesan and reggiano cheese
133.181 (c) 3	With benzoyl peroxide in Provolone and pasta filata cheese
133.183 (c) 2	With benzoyl peroxide in Romano cheese
133.195 (c) 1	With benzoyl peroxide in Swiss and emmentaler cheese
136.115 (a) 2	Enriched bread, rolls and buns
137.105 (a) 5	Flour
137.165 (b)	Enriched flour
137.185 (b)	Enriched self-rising flour
137.235 (a) 3	Enriched corn grits
137.260 (a) 3	Enriched corn meals
137.305 (a) 3	Enriched farina
139.115 (a) 3	Enriched macaroni products
139.117 (b) 2	Enriched macaroni products with fortified protein
139.155 (a) 3	Enriched noodle products
150.141 (a) 5	Artificially sweetened fruit jelly
150.161 (a) 5	Artificially sweetened fruit preserves and jams
155.170 (a) 2 xi	Firming agent in canned peas
155.190 (a) 2 i	Firming agent in canned tomatoes
155.200 (c) 6	Firming agent in canned potatoes
155.200 (c) 6	Firming agent in canned green sweet peppers, red sweet peppers, or lima beans
155.200 (c) 6	Firming agent in canned carrots
175.300 (xxvi)	Resinous & polymeric coatings - Pigments and colorants
178.3297	Colorants for polymers
182.90	Substances migrating to food from paper and paperboard products
184.1	GRAS
184.1230	Nutrient and/or Dietary Supplement (a) Product: Calcium Sulfate (b) Meets specifications Food Chemicals Codex (c) Anticaking, coloring, adjunct, dough strengthener, drying, firming, leavening, formulation aid, nutrient supplement, pH control, processing aid, stabilizer and thickener, synergist, and texturizer. (d) Conditions of Use: This substance is generally recognized as safe when used in accordance with good manufacturing practices. (e) Waiver—prior sanctions

Calcium sulfate is also considered safe as a migrating substance in food contact surfaces. It is acceptable for mixture with pigments and colorants used in food containers.



Use of calcium sulfate in products, processes and applications is almost unlimited and is constantly growing.

Shipping and Ordering

Fillers are shipped in bags or in bulk. Only carload and truckload orders are accepted; however, LTL quantities are available in many areas through U.S. Gypsum distributors.

Commodity descriptions for freight-rate classification: USG Terra Alba–"Ground Gypsum"; SNOW WHITE Filler–"Calcined Gypsum."

For further information contact:

United States Gypsum Company
Industrial Gypsum Division
125 South Franklin Street
Chicago, Illinois 60606-4678
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מועצת הרבנים דשיקגו

RABBI GEDALIA DOV SCHWARTZ
/ *h Din*
MOSHE KUSHNER
Administrator
RABBI DOVID JENKINS
Kashruth Administrator

8 Tevet 5761
3 January 2001

בס"ד

TO WHOM IT MAY CONCERN:

After investigation of the raw material and processing methods employed by UNITED STATES GYPSUM COMPANY, we find that the following products are acceptable for use in kosher food products:

TERRA ALBA F&P and SNOW WHITE® F & P FILLER

These materials are made from high purity deposits of calcium sulfate dihydrate which are pulverized and contain no additives whatsoever.

These products are acceptable for kosher use for year-round including Passover.

We will review the above annually.

Dovid Jenkins

Rabbi Dovid Jenkins
Kashruth Administrator

DJ:rp

ENCYCLOPÆDIA BRITANNICA

ale

fermented malt beverage, full-bodied and somewhat bitter, with strong flavour of hops. Popular in England, where the term is now synonymous with beer, ale was until the late 17th century an unhopped brew of yeast, water, and malt, beer being the same brew with hops added. Modern ale, usually brewed with water rich in calcium sulfate, is made with top-fermenting yeast and processed at higher temperatures than the lager beers popular in the United States. Pale ale has up to 5 percent alcohol content; the darker strong ale contains up to 6.5 percent.

Information about this topic in other articles:

ale

beer production
Types of beer
from beer

beergeeks.com

Water

As we have seen the primary ingredients used in making beer can have a wic on the over all flavor composition of beer. Component-wise beer consists, oı average, of about 4-5.5 % alcohol and 2-3% other flavor components. So, fr is safe to assume that the over whelming majority of beer is made of water a fact, is roughly 92-93 %. This, in turn, means that the overwhelming majorit components of beer are from the water.

Beer historians, experts, and many advertisers will go to great lengths to exp great the "mountain springs" a particular beer uses and will argue that it is b water that various styles of beer can be seen emerging in different parts of th at the same time. That is to say it is because of the water that is in one area tl appearance of pale ales occurs while in others we see the emergence of dopp and alt beers. It is suggested that local water sources can be held responsible as most brewers would have used the community water supply to make their far as those commercials advertising the benefits of mountain springs are co consider what wild life can do to a mountain spring.

As the English beer makers are quick to say it is because of the water that th tastes so special. Many of the breweries in this country get their water supply very deep mineral rich wells and so, consequently their beers have a distinct "minerally" character. Bass, a commercial brewery, maintains this "water In concept and it is indeed a major contributor to the taste of their beer. The tov which Bass originates is Burton-on-Trent and their water is high in calcium which renders Bass' special taste.

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Water Preparation

As we have seen, water, also known as brewing liqueur, is the largest ingred beer. It is what differentiates beer from bread and contributes many flavor characteristics. As a general rule of thumb, for home brewing, it is important that if the water is fit for human consumption then it is fit for the production However with the increase of environmental contaminants and with attempts sources of algae and other harmful bacteria it is necessary for many municip supplies to treat water with healthy dosages of chlorine, and other chemicals additives can, and often do, contribute undesirably to the flavor of the beer.

For the extract brewer water is less of a concern because when the ingredien mash at the factory all of the essential minerals, metals and ionic compounds been added. For the all grain brewer a little attention to water may be needed an important factor when considering pH, which will play an important role alpha-almayse activity.

It is important that the water used for making beer is not distilled as distillin removes many of the minerals that are important in the making of beer and c out these, result in unstable fermentation. However, for the homebrewer who to do complex water adjustments, starting with mineral free or deionized wa be the best place to start. It is usually easier to add minerals than it is to take away.

As a rule of thumb any water, if fit for human consumption, is fit for making That means if your tap water is consumable, with out first having to treat it, can be used for making beer. However, one will frequently notice a bleach o "swimming pool" like aroma emanating from a freshly poured glass of tap w This is because most tap water has been treated mildly with chlorine so as to harmful bacteria and algae. As a simple test pour a glass of ordinary tap wat take note of the smell. Set it a side and come back to it in an hour. You'll not if not all, of the chlorine smell has gone. This is because chlorine rapidly evaporates... So, what does this mean for you and your beer? If not taken ca these chlorine aromas and tastes, where there's aroma there's taste, will carry finished beer. To rectify this problem simply measure out the water you'll ne brew day the night before. Remembering to account for evaporation let the v over night and you'll have plenty of chlorine free water for your batch of bee

A simpler, but pricier method is to buy a decent charcoal based filter. A goo

that can attach to your sink's spigot will usually start at around $40 but, in th run, will cost you about 29 cents per gallon. Not only will it improve your bi water but also it will greatly improve your drinking water. When water is lef over night you have the benefit of maintaining the natural "hard" parts of the Similarly, a filter should effectively remove the chlorine and any other odd characteristics present in your water but not strip it of its natural salts, miner metals. For healthy fermentation and good tasting beer it is necessary to hav a variety of minerals salts and metals because, if removed, all kinds of weird will happen.

If your water is really funky (murky, rusty, smelly or just generally unpleasa may consider alternatives such as bottled water or reverse osmosis. If you us water find one that gives you lots of information about the contents (i.e. min content, methods of treatment etc...). Reverse osmosis will take care of pret any problem you have with your water but if this is the best solution for you water adjustment will be required. If you are uncertain about your water ther a specialist and your local home brew shop.

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Designing the Water

As I said before it is no real surprise that certain beer styles have appeared ai developed in different areas of the globe. Brewers have always developed be recipes with the ingredients most readily available. Composing 95-88% of th all finished beer water has always played an extensive role in beer styles hen contributing significantly to the overall taste of a beer. Therefore, if a brewe to emulate certain styles of beer, from the water up, it is necessary to have sc understanding of the chemistry of water and how it will affect the brews you

The first thing a home brewer who wants to do serious water adjustment for achieving specific styles of beer needs to do is acquire an analysis of the bre water. For municipal water sources all one needs do is call the local water de and ask for an analysis of the water for that area. Most are more than willing provide this information on request and, by law, are required to.

The most important aspects of water composition for the purpose of brewing calcium, sulfate, magnesium, chloride, sodium, carbonate and bicarbonate. S secondary ions in water include iron, copper, silicate, zinc and manganese. T that may be found at a home brew supply shop to add to your brewing liquei calcium sulfate, calcium chloride, magnesium sulfate, sodium chloride and c carbonate.

> Calcium is perhaps the single most important ion. It reacts with phosp are naturally present in malt and which provide acidity to the mash.
>
> Sulfate does not contribute significantly to the chemical reactions in th brewing process but can and does make stylistic contributions to the c of the beer. It will, however, allow for extra hop utilization. In excess 450-550 parts per million sulfates will promote off or undesirable flav
>
> Magnesium ions promote reactions similar to calcium but less signific is present in malt and should not exceed 30 parts per million. More tha will contribute sour-bitter-salty sensations.
>
> Chloride, like sulfate, contributes stylistically to a beer and does not c importantly to reactions in the brewing process. It will increase malty which will become apparent in portions of about 200-250 parts per mi

Sodium becomes a concern when using a water softener. Softeners wi the hard mineral in water but add sodium. Sodium, in combination wit chloride, will help with stylistic alterations but in excess of 150 parts p million salty undesirable characteristics will be derived.

Carbonate will inhibit crucial chemical reactions in the beer making p neutralizes acids and inhibits the benefits of calcium. It will lower ma and can lend to darker run-offs, which is undesirable in lighter beers. I of about 50 parts per million carbonates will begin to create undesirab characteristics in beer. However, carbonates are inhibited by darker m heavily roasted malts because of their acidity

Iron should not exceed .5 parts per million as amounts more than this render a blood like character.

Copper for healthy yeast activity trace amounts of copper are needed b amount more than .1 parts per million. Above that level copper proves yeast.

Silicate is of no real significance to home brewers.

Zinc and Manganese should not exceed .2 parts per million to avoid m flavor.

Brewing Water Analysis

	Pilsen	Pittsburgh	Munich	Vienna	Burton-on-Trent
Calcium (Ca++)	7	32	75	200	270
Magnesium (Mg++)	2	6	18	60	60
Sodium (Na+)	2	20	2	8	30
Carbonate (HCO3--)	15	45	150	125	200
Sulfate (SO4-)	5	72	10	120	640
Cloride (Cl-)	5	31	2	12	40
Total Dissolved Solids	35	179	275	850	1200

All numbers are parts per million.
Pittsburgh data from 1992 City of Pittsburgh Water Analysis
Pilsen, Munich, Vienna data from "Brewing Lager Beer" by Greg Noonan
Burton-on-Trent data from "Pale Ale" by Terry Foster

Description

Calcium: Calcium is the principal mineral of hardness, having come from the water's passage over limestone, dolomite, gypsum or calcified gypsiferous shale. Calcium increases mash acidity and inverts malt phosphate.

In appropriate amounts, calcium is advantageous to the brew. It stimulates enzyme activity and improves protein digestion, stablizes the alpha amylase, helps gelatinize starch and improves lauter runoff. Calcium also extracts fine bittering principles of the hop and reduces wort color. A calcium precipitate formed with potassium phosphate improves hot-break flocculation. It is also an essential part of yeast-cell composition; small amounts of calcium neutralize substances toxic to yeast such as peptone and lecithin. During aging, it improves clarification, stability, and flavor of the finished beer.

In excess, however, calcium precipitation with organic phosphates interferes with runoff filtering and robs the wort of phosphate, a necessary yeast nutrient. Calcium levels are usually 5 to 200 ppm; its solubility is greatly affected by the anions in solution with it.

Magnesium: Magnesium is the secondary mineral of hardness. It is essential as a co-factor for some enzymes and as a yeast nutrient. In small concentrations of 10 to 30 ppm, it accentuates the beer's flavor, but it imparts an astringent bitterness when it is present in excess. Over 125 ppm it is cathartic and diuretic. Usually found at levels of 2 to 50 ppm, its solubility is less affected by carbonate anions in solution than is calcium.

Sodium: The sour, salty taste of sodium accentuates beer's flavor when it is found in reasonable concentrations. It is poisonous to yeast and harsh tasting when it is in excess. Usually found at levels of 2 to 100 ppm, it is very soluble.

Carbonate: Carbonate is a strongly alkaline buffer formed by the reaction of atmospheric carbon dioxide with hydroxides of alkaline-earth and alkali metals. Carbonates go into solution as hydrogen carbonates (HCO3-, "bicarbonates"), which are strong buffers. Bicarbonates form by the reaction of a carbonate ion

with a molecule each of carbon dioxide and water.

Carbonates resist increases in mash acidity by neutralizing acids as they are formed. It also hinders gelantinization of starch by alpha amylase, impedes trub flocculation during the cold break, and increases risk of contamination in the ferment. It contributes a harsh, bitter flavor overwhelming in delicate lagers, and carbonate in excess of 200 ppm is tolerable only when a dark-roasted malt is used to buffer its excessive acidity. Preferably, carbonate should be less than 50 ppm when pale malt or infusion mashing is used.

Sulfate: Sulfate is weakly basic, and its alkalinity is overcome by most acids. It is fairly soluble. It gives beer a dry, fuller flavor, although the taste is somewhat sharp. With sodium and magnesium it is cathartic. Above 500 ppm it is strongly bitter and levels are best kept less than 150 ppm.

Chloride: Chloride is very weakly basic and readily neutralized. It accentuates bitterness, increases stability of any solution, and improves clarity. The "salt" taste of chloride generally enhances beer flavor and palate fullness, but the salt flavor can be reduced with calcium and magnesium. Usually found at levels of 1 to 100 ppm, chloride levels should never by more than 100 ppm for light beers and 350 ppm for beers above 12 degrees Balling (SG of 1.049).

Treatment

Adding 1 gram to 1 gallon of water increases the mineral content as follows:

	Calcium	Magnesium	Sodium	Carbonate	Sulfate	Chloride
chalk	105			158		
gypsum	60				140	
epsom salt		25			100	
table salt			110			170

All numbers are parts per million.

from "Brewing Lager Beer" by Gregory J. Noonan and Zymurgy All-Grain Special Issue 1995.

Carnegie Mellon University / School of Computer Science / wsawdon@cs.cmu.edu

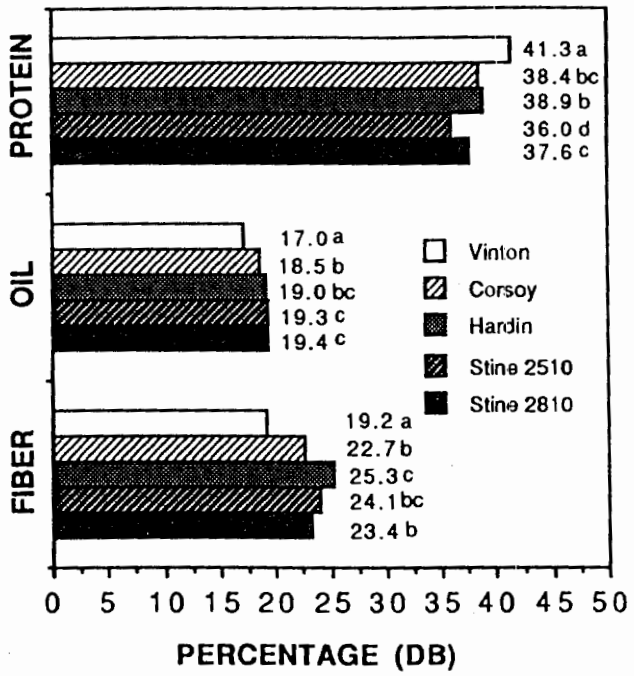

Fig. 1—Dry basis (DB) protein, oil and neutral detergent fiber contents of five soybean varieties. Means of duplicates; different letters adjacent to values denote differences (P<0.05).

liams and Baker, 1984, AOAC 14.082, 7.009) and crude protein (Williams and Baker, 1984, AOAC 2.057, modified Kjeldahl method).

Neutral detergent fiber (NDF)

Neutral detergent fiber (NDF) was determined by the modified method of Van Soest and Wine (1967). Duplicate 1.00±0.10g samples of soybeans or tofu were weighed in tared 30 mL Pyrex glass filtering crucibles; 100 mL of neutral detergent solution was added. The crucibles were placed in a Tecator Fibertec System M 1020 Hot Extractor and samples were boiled for 1 h. The digested samples were rinsed well several times with hot tap water. Residues were rinsed again twice with acetone and dried overnight in a 100–110°C oven.

Mineral content

Ground soybean samples containing about 1g of dry matter were accurately (±0.001g) weighed into 20-mL high-form silica crucibles and dry-ashed in a muffle furnace at 485°C for 10–12 hr. Crucibles were covered during ashing. Ash samples were each equilibrated with 10 mL 2N HCl at room temperature for 3 hr followed by transfer of the supernatant to a 7 mL plastic disposable tube for simultaneous direct analysis of Ca, Mg, Na, K, P, Fe, Mn, Al, Cu, Zn, Cd, Cr, Ni, Pb and B by Inductively Coupled Plasma (ICP) Atomic Emission Spectrometry.

Textural properties and color

The Texture Profile Analysis (Szczesniak, 1975) parameters of hardness and fracturability were determined on 2.0 cm diameter by 2.0 cm high cylindrical samples on a Model 1122 Instron Universal Testing Machine (0-2 kg load cell capacity). Samples were taken such that compression force was exerted on the same plane as the compression force during tofu pressing. The samples were compressed from 2 to 0.5 cm (75% deformation). Crosshead and chart speeds were 20 and 50 mm/min, respectively. Nine replicate samples were tested for each 50-g soybean batch of tofu.

A Minolta Chroma Meter CR-200 was used for color reflectance determination. One determination was made for each variety of soybeans or each batch (50 g soybeans) of tofu.

Statistical analysis

Data were analyzed by ANOVA using the Statistical Analysis Systems program (SAS, 1985). Means comparisons were made by Least Significance Differences (LSD) procedure ($p<0.05$).

RESULTS & DISCUSSION

Analyses of soybeans

Vinton had the highest protein and the lowest oil content while Stine 2510 had the lowest protein content but a higher oil content that was the same as those of Stine 2810 and Hardin (Fig. 1). Vinton had the lowest and Hardin the highest NDF content. Negative relationships existed between protein and fiber ($r = -0.71$) and protein and oil content ($r = -0.87$). This was in agreement with Caviness (1973) and Wang et al. (1983) who found that protein and oil contents of soybeans correlated negatively.

Moisture, size and color data are listed in Table 1. Size was expressed in terms of the weight (g) of 100 soybeans. Seeds of Vinton were the largest and Corsoy the smallest. L values showed Vinton, Corsoy and Hardin to be lighter-colored than Stine 2510 and 2810. Redness (a) ranged from 2.7 in Hardin to 5.1 in Stine 2510; yellowness (b) ranged from 25.8 to Vinton to 33.6 in Hardin. All five varieties had yellow hila. Soybeans with light hila are preferred for tofu making (Wang et al., 1983).

The total ash contents of the five varieties were essentially the same (Table 1). The main differences among individual minerals that might relate to tofu yield and quality were higher contents of Ca in Vintron and lower Mg in Stine 2510. A strong linear relationship existed between protein and Ca contents. P content varied somewhat and K content was similar among varieties. The contents of trace minerals were also similar (data not shown). Their contribution to tofu yield would be minor and their main contribution to tofu quality would be nutritional.

Hydration of soybeans

To make tofu, soybeans are presoaked to soften their cellular structure, reduce the amount of energy required to grind them, and increase the subsequent rate of nutrient extraction. The hydration rate depends on the temperature of the soaking water and the variety and age of the soybeans; the colder the water, the slower the hydration (Shurtleff and Aoyagi, 1979; Hsu et al., 1983). In our studies, hydration occurred rapidly and similarly in all five soybean varieties up to 4 hr after which the rates leveled off. Complete hydration required about 12 hr, which was similar to results reported by Hsu et al. (1983). All varieties had similar hydration rates which appeared to be normal. Therefore, a 12-hr soak was employed in our studies. Typical soaking times at ambient temperature can vary from

Table 1—Moisture, size, color (Hunter L) and proximate analyses of five soybean varieties[a]

Variety	Moisture %	g/100 beans	L	% Ash	Ca	Mg	P	K
					mg/100 g			
Vinton	10.4	23.0	65.6	5.3	269	307	644	1976
Corsoy	11.8	16.2	66.0	5.2	250	252	493	1953
Hardin	11.6	18.2	65.8	5.3	254	259	530	2010
Stine 2510	10.5	18.0	59.5	5.3	237	256	543	2039
Stine 2810	10.5	17.1	57.8	5.3	256	292	571	2018

[a] Means of duplicates.

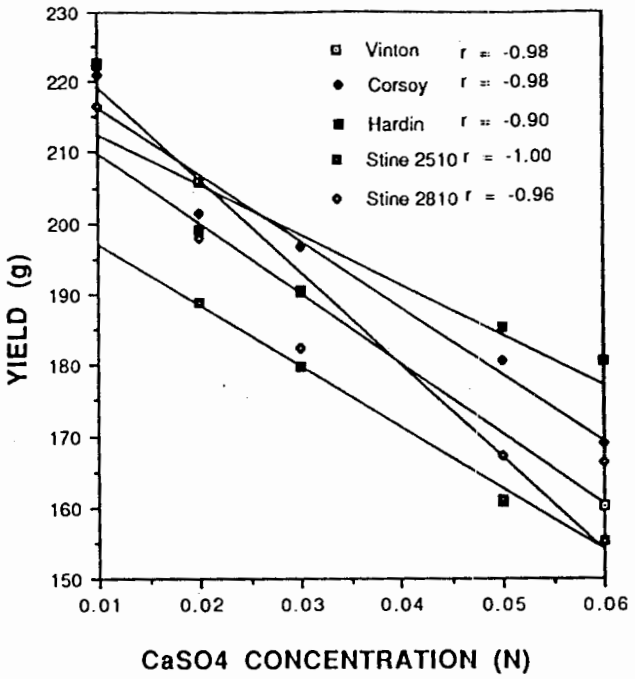

Fig. 2—Effect of soybean variety and $CaSO_4$ concentration in soymilk on yield of fresh tofu from 50g of soybeans.

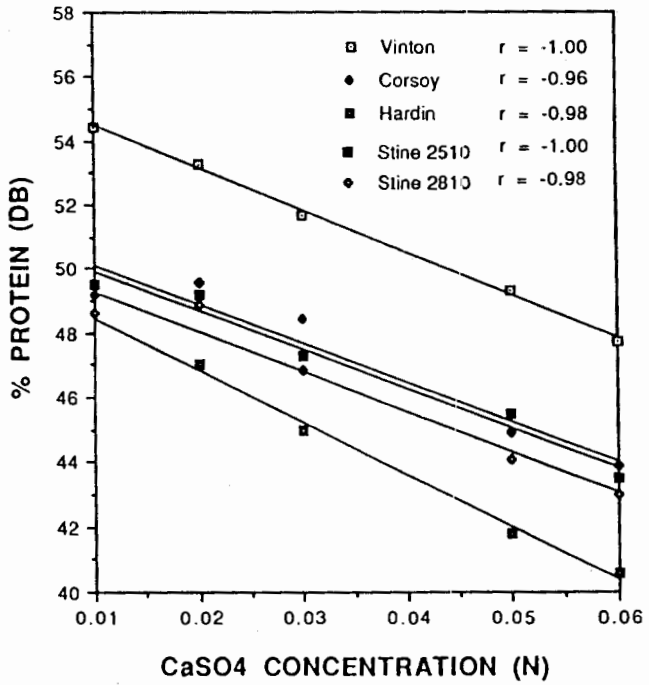

Fig. 3—Effect of soybean variety and $CaSO_4$ concentration in soymilk on protein content (dry basis) of tofu from 50g of soybeans.

8 to 10 hr in summer to 10 to 20 hr in winter in Japan; hydration rates differ among varieties (Watanabe et al., 1964). Soybeans that are small and hard, older than 6 mo and/or high in oil may have impaired hydration. Too much or too little moisture uptake by beans prior to soymilk extraction can affect quality and yield of tofu (Shurtleff and Aoyagi, 1979).

$CaSO_4$ concentration effects on tofu yield and quality.

Yield and quality of tofu can be affected by concentration of the coagulant, $CaSO_4$. The effects of soybean variety and

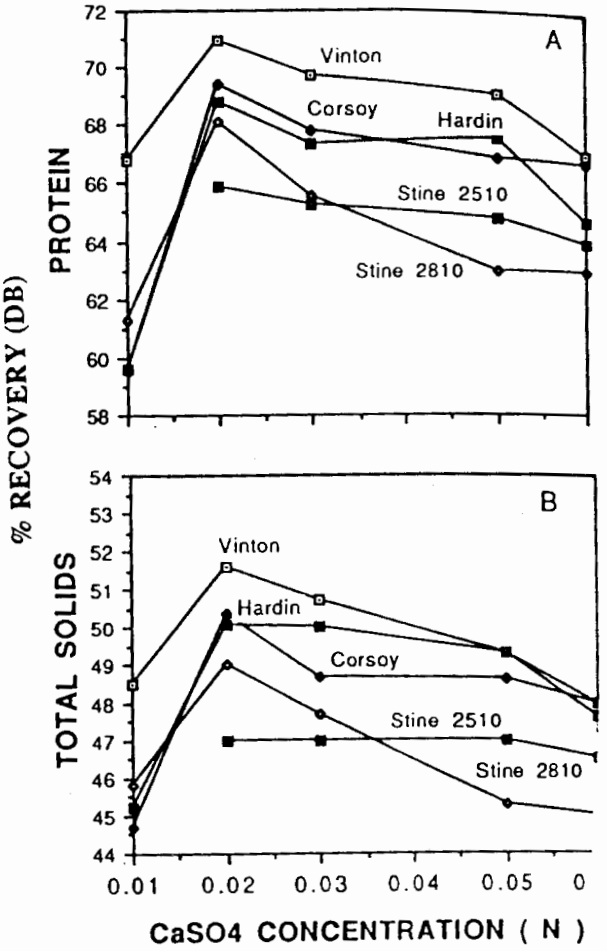

Fig. 4—Effect of soybean variety and $CaSO_4$ concentration soymilk on percentage recovery of protein (A) and total so (B) in tofu.

$CaSO_4$ concentration on yield, percent protein in tofu, t solids recovery, protein recovery, fracturability and hard of tofu are shown in Fig. 2 through 5. Negative linear reg sion relationships were found between $CaSO_4$ concentra and both yield (Fig 2) and percent protein (Fig 3) in tofu. decrease in yield with increasing $CaSO_4$ (calcium ion) con tration could be due to increasing syneresis and loss of v from the curd as more bonding occurred thus making the tein matrix more dense and compacted. The regression were roughly parallel for tofu yield from Corsoy, Stine and Stine 2510 compared to a lesser decrease in yield increasing $CaSO_4$ concentration for Hardin and a greater c sponding decrease for Vinton. Thus, Vinton was the mos sitive to changes in $CaSO_4$ concentration.

The percent protein in tofu (DB) showed parallel decr with increased coagulant concentration for all five var (Fig 3) indicating that increased syneresis resulted in lo soluble protein (whey protein) along with water. The dat reflect overall differences in protein content of the five so varieties, decreasing in the order Vinton, Corsoy, Hardin, 2810, Stine 2510. Although increasing $CaSO_4$ concent produced the most dramatic decrease in yield for Vin corresponding decrease in protein content was not obse Therefore, yield reduction must have been due prima loss of water through syneresis.

Figure 4 shows maximum recovery of both protein an solids in tofu at 0.02N $CaSO_4$, with recoveries much lo 0.01N $CaSO_4$ and a gradual decrease from 0.02N to $CaSO_4$. At the lower concentration, bridging of protei ecules by calcium ion is not sufficient to form a firm g

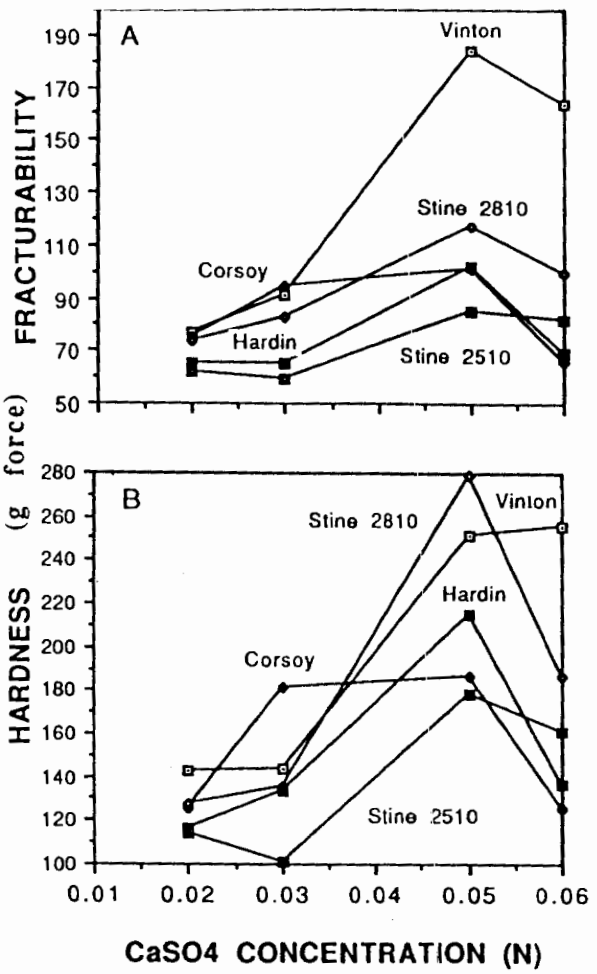

Fig. 5—Effect of soybean variety and $CaSO_4$ concentration in soymilk on fracturability (A) and hardness (B) of tofu.

higher concentrations, increased bridging and the resulting compaction of the protein matrix cause increased syneresis and loss of water, whey protein and other solubles. Although the highest tofu yields were obtained at 0.01N $CaSO_4$ (Fig. 2), this was due mainly to a high moisture content in the pressed product. These tofu products did not assume a distinct cubical form and did not retain their shape after cutting. At 0.02N $CaSO_4$, the tofu had an even texture and good retention of cut shape.

Figure 5 depicts the effects of $CaSO_4$ concentration on TPA fracturability and hardness. Tofu made with 0.01N $CaSO_4$ was too soft to enable sampling for the TPA procedure. As coagulant concentrations increased above 0.02N $CaSO_4$, the trend was toward an increase in fracturability and hardness up to 0.05N $CaSO_4$ and then a decrease at 0.06N $CaSO_4$. Vinton tofu showed the greatest increase in fracturability with increasing $CaSO_4$ concentration and considerably higher fracturability than the others at the 0.05N and 0.06N levels (Fig. 5A). Vinton tofu recovered the highest percentage of total solids and protein at 0.05N $CaSO_4$, whereas Stine 2810 tofu recovered the least (Fig. 4). They were similar in hardness at that coagulant level and both were harder than the other 3 (Fig. 5B). These tofus were all sorfter than those described by Wilson et al. (1983) who used a different coagulation temperature. Increasing the $CaSO_4$ concentration to 0.06N produced little, if any, change in hardness of Vinton tofu (Figure 5B), but a dramatic decrease in that of Stine 2810 tofu. This suggested that soybean solids other than protein may play a role in tofu textural quality. This could be through interactions between carbohydrates and proteins as hypothesized by Lin et al. (1990).

No formal sensory texture evaluations were made. Or thor (NS) is Chinese and a lifelong consumer of tofu, that tofu texture was best and quite acceptable at 0.02N C Also, as $CaSO_4$ concentration was increased above 0.02N tofu products became harder, coarse and rubbery and ha appearance of precipitates rather than gels. Miura and K yasu (1981) found sensory hardness correlated highly wit strumental hardness, so it appears that instrumental har is a valid and useful parameter for quality evaluation of These observations that addition of $CaSO_4$ to soymilk at 0 produced optimum yields and textural quality of tofu we agreement with previous reports (Saio, 1979; Yasuda and ama, 1984).

CONCLUSIONS

A LAB SCALE mini-procedure could differentiate betv soybean varieties for relative potential for tofu making. H ever, additional work is needed to verify that this method c predict the behavior of different soybean varieties and proc ing variables under large scale tofu processing conditions. concentration of the coagulant $CaSO_4$ in the soymilk aff the yield, protein and solids recoveries and textural quality tofu from a given quantity of soybeans. These were optim at a $CaSO_4$ concentration of 0.02N in soymilk from 5 diffe varieties of Minnesota-grown soybeans. For screening v eties, we recommend the mini-procedure described here ar $CaSO_4$ concentration of 0.02N in the soymilk.

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MATERIAL SAFETY DATA SHEET
USG® Terra Alba F & P

United States Gypsum Company
125 South Franklin Street
Chicago, Illinois 60606-4678

Product Safety: 1 (800) 507-8899
Version Date: October 1, 1999
Version 3

SECTION I
PRODUCT IDENTIFICATION

PRODUCT: USG® Terra Alba F & P
CHEMICAL FAMILY: Gypsum (Calcium Sulfate Dihydrate, $CaSO_4 \cdot 2H_2O$)

SECTION II
INGREDIENTS

MATERIAL	%	TLV (mg/m^3)	PEL(mg/m^3)	CAS NUMBER
Gypsum	90-98	10	15(T)/5(R)	13397-24-5
Limestone	0-10	10	15(T)/5(R)	1317-65-3
Crystalline Silica	<5	0.1(R)	0.1(R)	14808-60-7

(T) = Total (R) = Respirable

All ingredients of this product are included in the U.S. Environmental Protection Agency's Toxic Substances Control Act Chemical Substance Inventory. All components of this product are included in the Canadian Domestic Substances List (DSL) or the Canadian Non-Domestic Substances List (NDSL).

INFORMATION FOR HANDLING AND IDENTIFICATION OF CHEMICAL HAZARDS

NFPA Ratings:	Health: 0	Fire: 0	Reactivity: 0	Other: N/A
HMIS Ratings:	Health: 0	Fire: 0	Reactivity: 0	

Personal Protection: Use eye and skin protection. Use NIOSH/MSHA-approved respiratory protection when necessary.

0 = Minimal Hazard 1 = Slight Hazard 2 = Moderate Hazard 3 = Serious Hazard 4 = Severe Hazard

SECTION III
PHYSICAL DATA

Appearance and Odor: Off white to white powder; low odor
Melting Point: 1450°C - decomposes
Specific Gravity: 2.32 – 2.96
Solubility in Water: 0.26%

SECTION IV
FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used): Not combustible
Extinguishing Media: Use extinguishing media appropriate for surrounding fire.
Special Fire Fighting Procedures: None
Unusual Fire and Explosion Hazards: None

SECTION V
HEALTH HAZARD DATA

This product can release nuisance dust in handling or during use. Eye, skin, nose, throat and upper respiratory irritation can occur with dust exposure.

EFFECTS OF OVEREXPOSURE:

ACUTE:

EYES: Direct contact can cause mechanical (particulate) irritation of eyes. If burning, redness, itching, pain or other symptoms persist or develop, consult physician.

SKIN: No toxic effects from powdered gypsum are noticed where air contains contaminate to excess. This material exhibits some affinity for moisture, and frequent exposures may have a drying effect on the skin. Possible itching and irritation may be experienced. This may lead to dermatitis.

INHALATION: Inhalation of dusts from this product may irritate the nose, throat, lungs, and upper respiratory tract. Persons subjected to large amounts of this dust will be forced to leave area because of nuisance conditions such as coughing, sneezing and nasal irritation. If respiratory symptoms persist, consult physician.

INGESTION: This product is gypsum. Gypsum is non-toxic, however, ingestion of a sufficient quantity could lead to mechanical obstruction of the gut, especially the pyloric region.

CHRONIC:

INHALATION: None known for gypsum. Prolonged and repeated exposure to respirable crystalline silica can result in lung disease (i.e., silicosis) and/or lung cancer.

EMERGENCY AND FIRST AID PROCEDURES:

EYES: Flush thoroughly with water for 15 minutes to remove particles. If irritation persists, consult physician.

SKIN: Wash with mild soap and water. A commercially available hand lotion may be used to treat dry skin areas. If skin has become cracked, take appropriate action to prevent infection and promote healing.

INHALATION: Leave the area of dust exposure and remain away until coughing and other symptoms subside. Other measures are usually not necessary, however, if conditions warrant, call physician.

INGESTION: Ingestion of sufficient quantity may result in mechanical obstruction of, the gut. If there is any discomfort, consult physician.

TARGET ORGANS: Eyes, skin, lungs, and respiratory system.

MEDICAL CONDITIONS WHICH MAY BE AGGRAVATED: Pre-existing upper respiratory and lung disease such as, but not limited to, bronchitis, emphysema and asthma.

PRIMARY ROUTES OF ENTRY: Inhalation, eyes, and skin contact.

CARCINOGENICITY OF INGREDIENTS:

MATERIAL	IARC	NTP
Crystalline Silica	Group 1	Anticipated

In June, 1997, the International Agency for Research on Cancer (IARC) classified crystalline silica (quartz and cristobalite) as a human carcinogen. In making the overall evaluation, the IARC Working Group noted that carcinogenicity in humans was not detected in all industrial circumstances studied. Carcinogenicity may be dependent on inherent characteristics of the crystalline silica or on external factors affecting its biological activity or distribution of its polymorphs.

IARC states that crystalline silica inhaled in the form of quartz or cristobalite from occupational sources is carcinogenic to humans (Group 1).

SECTION VI
REACTIVITY DATA

STABILITY:	Stable
INCOMPATIBILITY:	Acids
HAZARDOUS POLYMERIZATION:	Will not occur.
HAZARDOUS DECOMPOSITION:	Above 1450°C could produce SO_2 & CaO.

SECTION VII
SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED:

Avoid creating excessive dust. Wear appropriate protective equipment. Scoop up material from spillage into a waste container for disposal, or if not contaminated by foreign material it may be reclaimed for processing.

WASTE DISPOSAL METHOD:

Dispose of in accordance with local, state and federal regulations.

SECTION VIII
SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION:

Not typically necessary under normal conditions of use. Avoid inhalation of dust. Dust created from mixing or handling may cause eye, nose, throat or upper respiratory irritation. Wear a NIOSH/MSHA-approved dust respirator if TLV is exceeded and/or when dusty conditions exist. Provide general ventilation and/or local exhaust ventilation to meet TLV requirements.

PROTECTIVE EQUIPMENT:

Gloves or protective clothing are usually not necessary but may be desirable in specific work situations. Wear adequate clothing to minimize drying of skin. Wear safety glasses or goggles for eye protection to avoid particulate irritation of the eye.

SECTION IX
SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE:

Store in a dry place. Minimize exposures in accordance with good industrial hygiene practice. During handling wear the appropriate respiratory, eye and skin protection if warranted per environmental conditions.

ΔCAUTION!

Dust may cause eye, skin, nose, throat or upper respiratory irritation. Avoid inhalation of dust and eye contact. Provide good general ventilation and/or local exhaust to reduce dust exposure. If dusty conditions exist, use NIOSH/MSHA-approved respiratory protection. Wear eye protection to avoid particulate irritation of eye. If eye contact occurs, flush thoroughly with water for 15 minutes. If irritation persists, call physician. Product safety information (800) 507-8899.

KEEP OUT OF REACH OF CHILDREN

END

Physicochemical Properties and Tofu Quality of Soybean Cultivar Proto†

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The objective of this study was to investigate the physicochemical properties and tofu quality of Proto soybean grown at two different lots. The bean size, proximate chemical composition, weight of seed coat, total carbohydrate and nonstarchy polysaccharides (NSP), 11S/7S protein ratio, total and free cysteine, and amino acid patterns of the beans were determined. Tofu was made according to a laboratory method that employed calcium sulfate as a protein coagulant. The yield, texture profile, color, and NSP content of tofu were determined. Proto soybean produced tofu with excellent yields and good tofu characteristics. The two samples differed in size but did not differ in the physicochemical properties and in the yield and firmness of tofu. The sulfur-containing amino acids retained in tofu were only 40–50% of that in the raw soybean.

Keywords: *Proto soybean; physicochemical properties; tofu quality*

INTRODUCTION

Tofu is a soybean protein gel-like product and has been widely consumed in the Orient for more than 2000 years. For most Americans, tofu is receiving attention because it is low in calories and is cholesterol-free. A typical soft tofu is characterized by a bland taste and fine texture with an 84–90% moisture content (Kohyama et al., 1993).

Tofu manufacturers desire soybeans that are uniform in size and that provide a high yield and quality product. Yield and quality are affected by several factors, such as variety or cultivar (Skurray et al., 1980; Wang et al., 1983), soybean growth environment (Wang et al., 1983), and tofu-processing methods (Wang et al., 1983; Beddows and Wong, 1987a–c; Shen et al., 1991). Soybean varieties differ in chemical components, including proteins, lipids, and minerals, that may influence yield and quality of tofu (Skurray et al., 1980; Wang et al., 1983; Lim et al., 1990; Sun and Breene, 1991; Shen et al., 1991; Schaefer and Love, 1992).

Processing factors, which affect the quality of tofu, include soaking time and temperature, grinding temperature, soy milk heating rate, stirring speed, type and concentration of coagulant, method of adding coagulant to soy milk, and the weight and time of press (Lu et al., 1980; Tsai et al., 1981; Wang and Hesseltine, 1982; Beddows and Wong, 1987a–c; Sun and Breene, 1991). Beddows and Wong (1987a) reported the water/bean ratio in the range of 11–12:1 had maximum yield, but the ratio of 10:1 gave the best quality of tofu. Beddows and Wong also (1987b) noted that a faster heating rate produced better tofu quality due to more protein being retained in the tofu. Saio (1979) reported a higher stirring speed during soy milk heating produced tofu with a firmer texture. The scanning electron microscopic (SEM) and transmission electron microscopic (TEM) images of tofu showed a stronger stirring produced a denser network of tofu structure (Saio, 1979).

The textural characteristics are the important determinants for consumption. Protein content in soybean and soy milk affects tofu texture significantly. Wang et al. (1983) and Shen et al. (1991) reported that varieties higher in protein content produced firmer and more springy tofu texture. Soybean storage proteins, the ratios of 7S and 11S, differed among varieties. Tofu made from 11S protein was significantly harder than that from 7S protein (Saio et al., 1969). The 11S tofu also had greater cohesiveness and elasticity than the 7S tofu (Saio, 1979). The larger protein molecule (11S) had more sulfhydryl–disulfide interchange reaction during heat aggregation, resulting in a larger protein network, which showed a harder texture (Saio, 1979). The ratio of 11S/7S soybean protein affects the textural properties of tofu (Saio et al., 1969, 1974, 1979; Saio and Watanabe, 1978; Skurray et al., 1980).

Phytic acid can bind both protein and calcium to produce a colloidal precipitate, which results in a softer tofu during processing (Saio, 1979; Prattley and Stanley, 1982). However, Lim et al. (1990) worked on nine varieties of soybeans and found no correlations between phosphorus content and hardness of tofu, although they found that smaller size soybeans had higher phytic acid content. Schaefer and Love (1992) found that phytic acid and copper ion contents were significantly correlated with tofu texture. Moreover, coagulant type and concentration play a significant role in the textural characteristics of tofu (Lu et al., 1980; Tsai et al., 1981). The texture of tofu is affected by various protein coagulants. Calcium sulfate, magnesium sulfate, calcium chloride, and glucono-δ-lactone are widely used for manufacturing different types of tofu (Tsai et al., 1981; deMan et al., 1986; Shen et al., 1991; Sun and Breene, 1991; Skurray et al., 1980).

In industrial practice, large size soybeans are preferred for tofu making. The total amount of the seed coat in the small soybeans is higher than in the large

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soybeans. The higher amount of the seed coat in the small soybeans is considered by the tofu industry to have a negative effect on the yield and the quality of tofu (personal communications with the tofu industries). However, the influence of the size of soybean on the yield and quality of tofu has not been reported in the scientific literature. Proto soybean is a new food soybean cultivar grown in the upper northern plains of the United States and has not been studied with respect to physicochemcial properties and tofu quality.

The objectives of this study were to investigate the physicochemical properties and tofu quality of small and large size Proto soybean and to investigate the effect of the added seed coat to the large Proto soybean on tofu quality.

MATERIALS AND METHODS

Samples. Large (LS) and small seeds (SS) of the Proto cultivar soybean grown in two lots in 1992 were obtained from the Sinner Brothers and Bresnahan Co. (Casselton, ND). The pedigrees of Proto cultivar included Chippewa 64, Provar, Pridesoy II, and experimental lines developed at the University of Minnesota—St. Paul (Orf et al., 1991). After harvest, beans were stored at 5 °C until tofu processing. Antifoaming agent (containing 89.5% glycerol fatty acid ester, 8% lecithin, 2% $MgCO_3$, and 0.5% silicon resin) was obtained from Kaoh Co. (Wakayama, Japan). Food grade coagulant (a mixture of $CaSO_4 \cdot 2H_2O$ and natural nigari) was obtained from Taiwan Salt Workers (Tainan, Taiwan).

Characteristics of Soybean Size and Seed Coat. The number of soybean seeds per 100 g was counted. Two hundred grams of large and small soybeans was soaked in tap water for 8 h. The seed coat was peeled by hand, freeze-dried over 24 h, and vacuum-dried (<25 mmHg) for 5 h at 100 °C to obtain a moisture-free sample. The percentage of seed coat in the large and small seeds was calculated on a dry weight basis.

Proximate Analysis of Soybean and Tofu. Tofu was freeze-dried. The soybean and freeze-dried tofu were ground to pass a 60-mesh screen. Moisture, proteins, lipids, and ash were determined according to AOAC Methods (AOAC, 1990) 925.10, 955.04, 945.16, and 942.05, respectively.

Phytic Acid Determination. Phytic acid in soybean was extracted according to the method of Wang et al. (1988). Phosphorus in the extract (total) and soluble fraction was determined according to the method of Fiske and Subbarow (1925). The difference between total and soluble phosphorus was insoluble phosphorus (Bartlett, 1959) from phytic acid.

Soy Milk Preparation and Solids Content Measurement. Three different types of soybean samples, i.e., large size soybean, small size soybean, and large size soybean plus 0.5% seed coat (LSP), were used for tofu study. The large size soybean containing 0.5% seed coat, which was peeled from small soybeans, was used to determine the effect of seed coat on tofu yield and quality. The reason for selecting 0.5% was that the seed coat of the small soybean was 0.5% (w/w) greater than that of the large seeds. Five batches of soybeans (600 g each batch) for each bean type were washed and soaked in tap water at 15–18 °C for 8 h. Hydrated soybeans were drained and ground with 5.4 L of tap water in a high-speed grinder (Chan Shen Machinery Co., Taoyuan, Taiwan), which was equipped with an automatic centrifugal filter to separate the residue and soy milk.

To increase the solid extraction from the soybean, the residue from each grinding was stirred (washed) in 5.4 L of water for 5 min and filtered to obtain filtrate. The filtrate was used to replace water for the grinding of the second batch of soybean. In this study, the soy milk (approximately 5 L) from the first batch was not used for tofu making. The reason for not using the first batch was that soybeans are usually ground with the washed water in the tofu industry to increase yield. The total volume and degree Brix of soy milk were measured. The degree Brix of the soy milk was determined using a refractometer (AutoAbbe, Model 10500, Buffalo, NY) at 20 °C. The solid content of soy milk was estimated from a standard curve, which was constructed previously in our laboratory using solid content of various concentrations of soy milk determined by oven-drying versus degree Brix (Johnson and Wilson, 1984).

In a similar manner, the residues from the second, third, and fourth batches were washed, and the filtrates were used to grind the third, fourth, and fifth batches, respectively. The soy milk from the second to the fifth batches was used to prepare tofu. Therefore, tofu making was replicated four times for each soybean type.

Tofu Making. Four and a half liters of soy milk was used for one mold (268 × 268 × 70 mm) of tofu preparation. After adding antifoaming agent (0.07% of the raw soybean weight) was added, soy milk was cooked to boiling with gentle stirring and kept for 5 min at approximately 95 °C. After cooling to 88 °C, the soy milk was poured into a container containing 50 mL of suspended coagulant (3% of the raw bean weight). The coagulated soy milk was transferred into the wooden mold immediately and pressed with a 12-lb steel plate for 40 min. After pressing, the cloth was removed, and the tofu was allowed to stand for 5 min before the weight was measured. The yield of tofu was expressed as the weight of tofu on the basis of weight of soybean contributing to 4.5 L of soy milk.

$$\text{yield of tofu} = \text{wt of tofu/540 g of soybean}$$

$$\text{540 g of soybean} = 600\ \text{g}\left(\frac{\text{4.5 L, soy milk taken for tofu processing}}{\text{5.0 L, total volume of soy milk from 600 g}}\right)$$

Textural Analysis. The texture profile of tofu was measured using an Instron Universal Testing Machine (Model 1000, Instron Co., Canton, MA) equipped with a 500-kg weight beam. A 5-kg load cell was used with a cross-head control at 20 mm/min. A cylindrical plunger 5 cm in diameter was used to compress the tofu. The chart paper speed was 20 mm/min. A piece of tofu (1.5 cm height with 5 cm diameter) was cut for texture profile analysis. Six tofu cakes from each batch were measured by compressing twice to 25% of the original height of each cake. Hardness, fracturability, elasticity, and cohesiveness were calculated using the Instron Texture Profile analysis curve as described by Wang and Hesseltine (1982) and Bourne (1968).

Color Analysis. The Gardner Lab Model XL-23 colorimeter (Gardner Lab Inc., Bethesda, MD) was used to measure the surface color of tofu. The instrument was standardized using a standard white tile ($L = 91.94$, $a = -1.03$, $b = 1.14$).

Total Nonstarchy Polysaccharide (NSP) and Total Carbohydrate (CHO). A 200-mg sample of defatted soybean flour or defatted dried tofu was analyzed for total NSP (Englyst and Cummings, 1988). The sample was dispersed and hydrolyzed by α-amylase and pullulanase. The residue from enzymic digestion was followed by acid hydrolysis. The sample for total CHO determination was prepared without enzymic digestion. The neutral sugars in the hydrolyzed solution were reduced and derivatized to alditol acetates, which were quantitated using a Hewlett-Packard (Model 5890 Series II, Avondale, PA) gas chromatograph. Standard sugars, L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose, and D-glucose, were used. D-Allose was used as an internal standard. The uronic acid was determined according to the procedure of Scott (1979).

Total Cystine/Cysteine and Free Cysteine. The methods for determining for total cystine/cysteine and free cysteine (−SH group) were those of Felker and Waines (1978) and Chang et al. (1982). A 150–200-mg sample was finely ground with double-distilled water and diluted to 10 mL. Total cystine/cysteine in the sample was analyzed in 9 M urea after reduction with $NaBH_4$. Free cysteine was analyzed without reduction. Samples with or without reduction were reacted with dithionitrobenzoic acid (DTNB). Absorbance at 412 nm was taken approximately 20 min after DTNB was added. A standard curve was constructed using glutathione.

Table 1. Characteristics of Raw Soybeans of the Proto Cultivar[a]

seed size	no. of seeds per 100 g of beans	dimensions: length, mm	width, mm	thickness, mm	% seed coat in soybeans
large	501a	7.9a	7.0a	6.0a	5.9a
small	622b	7.0b	6.4b	5.3b	6.4b

[a] Means with different letters in the same column differ significantly ($p < 0.05$).

Extraction and Determination of 7S and 11S Proteins. The method for extraction and determination of 7S and 11S was modified from those of Nagano et al. (1992) and Arrese et al. (1991). Soybean flour and freeze-dried tofu were defatted and extracted in water at pH 7.5. The slurry was extracted for 1 h and centrifuged at 9000*g* for 30 min. The protein content of the extracts was determined by using the biuret method. The extract was diluted to contain 2 mg/mL. A phosphate buffer containing sodium dodecyl sulfate (SDS) was added to the protein extracts, and the mixture was heated for 2 min in boiling water. A 50-μL portion of the protein extract was used for polyacrylamide gel electrophoresis (PAGE), using the procedure of Laemmli (1970). For 7S and 11S protein quantification, gel was scanned with a densitometric scanner (Model GS300, Hoefer Scientific Instruments Co., San Francisco, CA). The ratio of 7S to 11S protein was calculated from the sum of the area of their subunits.

Amino Acid Composition. Amino acids were determined using acid hydrolysis, followed by precolumn phenyl isothiocyanate (PITC) derivatization and reversed-phase high-performance liquid chromatography (RP-HPLC) (Bidlingmeyer et al., 1984; Chang et al., 1989).

Statistical Analysis. One-way analysis of variance of the data was performed using a Statistical Analysis System package (SAS, 1990). When differences were observed, the least significant difference (lsd) test was used to analyze the significance of the treatment ($p < 0.05$).

RESULTS AND DISCUSSION

Characteristics of Soybean. The number of seeds in 100 g of soybean and percentage (w/w) seed coat of the soybean are listed in Table 1. The large size soybean ranged from 490 to 512 seeds/100 g, and the small size soybean ranged from 609 to 630 seeds/100 g. The length, width, and thickness of the large size Proto soybean were greater than those of the small size Proto soybean. The percentage of seed coat of the small soybean was 0.5% higher ($p < 0.05$) than the large size soybean (Table 1). Because the seed coat weights differed significantly between the two soybean samples, seed coats separated from the small seeds were added to the large bean for tofu testing to determine its effect on tofu properties.

Proximate Analysis. The protein content (Table 2) of the large and small size soybeans was lower than the protein content of 45.6% reported in the original cultivar registration document (Orf et al., 1991). The protein content of Proto soybean was greater than that of tofu soybean cultivars Vinton, Amsoy 71, Corsoy, and Hardin (Lim et al., 1990; Sun and Breene, 1991; Schaefer and Love, 1992). No significant differences ($p > 0.05$) were found in lipid, ash, and phytic acid contents for both sizes of soybeans. In the chemical composition of tofu, there were no differences ($p > 0.05$) in protein, lipid, and ash contents between the two sizes of soybean.

Solid Content and Yield. The solid content of soy milk and yield of tofu are shown in Figure 1. No significant difference ($p > 0.05$) in solid content of soy milk was observed among large size soybean, small size soybean, and LSP soy milk. In the tofu industry, the residues from the first grinding are normally washed with water two to three times to extract all of the proteins from the soybeans to increase yield. In this study, the degree Brix of the filtrate was approximately 2. The degree Brix of the soy milk increased from approximately 7.0 to 7.2 to 8.6 to 8.8 when the filtrate was used to replace water for grinding the soybeans. Using a refractometer to measure the concentration of solid is rapid and is good for quality control in tofu processing.

In the industrial manufacturing of tofu, large size soybeans are preferred for making tofu. However, our study showed no significant difference in the yield of tofu between the large and small size soybeans of the same cultivar (Figure 1). Tofu produced from the large size soybeans containing peeled seed coat did not differ significantly ($p > 0.05$) from the large and small soybean. Shen et al. (1991) reported that solid content of the soy milk was related to the yield of tofu. The solid contents of the soy milk derived from the three soybean samples were similar (approximately 7.3%).

The tofu yield of Proto was greater than the values reported for other soybeans that were processed using calcium sulfate as a coagulant. The fresh tofu yield of Proto (approximately 5.5–5.6 g/g of bean) was greater than the 3.5–4.6 g/g yield range of five soybean cultivars reported by Sun and Breene (1991), the 4.4–5.3 g/g yield of nine soybean cultivars reported by Lim et al. (1990), and the 4.4–4.8 g/g yield of six Japanese/Chinese cultivars (Taira, 1990).

Table 2. Proximate Composition of Proto Soybean and Tofu[a,b]

size of soybean	protein (%)	lipid (%)	ash (%)	CHO[c] (%)	phytic acid (mg/g)
		Raw Soybean			
large	43.60a	18.06a	5.43a	33.91	12.73a
small	42.77b	18.61a	5.52a	33.10	12.05a
		Tofu			
large	5.56a	2.46a	0.43a	2.29	ND[e]
small	5.21a	2.78a	0.51a	2.26	ND
LSP[d]	5.27a	2.65a	0.53a	1.99	ND

[a] Means with different letters in the same column differ significantly ($p < 0.05$). [b] The proximate composition of soybean was calculated on the basis of dry matter. [c] CHO% = 100% − (protein + oil + ash)%. [d] LSP, large size soybean plus 0.5% seed coat. [e] ND, not determined.

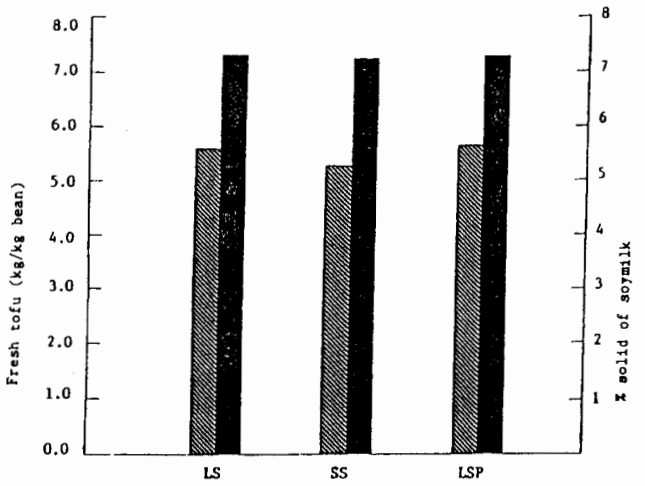

Figure 1. Percent solids of soy milk and fresh tofu yield from large (LS) and small size soybean (SS) and the large size soybean plus 0.5% seed coat (LSP): (slashed bar) yield; (black bar) % solid.

Table 3. Texture Characteristics of Tofu Made from Small and Large Size Soybeans and the Large Size Soybean plus 0.5% Seed Coat[a]

size of soybean	hardness (g)	fracturability (g)	elasticity (cm)	cohesiveness
large	2551a	511a	0.85a	0.335a
small	2509a	455b	0.68b	0.328a
LSP[b]	2549a	494a	0.78ab	0.346a

[a] Means with different letters in the same column differ significantly ($p < 0.05$). [b] LSP, large size soybean plus 0.5% seed coat.

Table 4. Color Characteristics and Subjective Test of Tofu from Small and Large Size Soybeans and the Large Size Soybean plus 0.5% Seed Coat[a]

size of soybean	Hunter *Lab*			subjective test	
	L	*a*	*b*	color	texture
large	86.41a	0.42a	15.79a	creamy white	soft and smooth
small	85.93a	0.36a	16.25a	creamy white	soft and smooth
LSP[b]	86.25a	0.45a	16.80a	creamy white	soft and smooth

[a] Means with different letters in the same column differ significantly ($p < 0.05$). [b] LSP, large size soybean plus 0.5% seed coat.

The high yield of Proto soybean may be partly related to its high protein content. Proto soybean had a higher protein content than the five soybean cultivars (protein ranging from 36 to 41%) reported by Sun and Breene (1991). Lim et al. (1990) and Shen et al. (1991) reported positive correlations of protein content of nine varieties of soybeans with fresh yield of tofu made with GDL or calcium sulfate. However, protein was not the single factor to affect yield. Calcium, ash, phytic acid, and lipid contents also are related to tofu yield (Lim et al., 1990; Shen et al., 1991). It is difficult to compare the yield of various soybean cultivars reported in the literature since various processing methods were used.

Texture Characteristics. Tofu made from Proto soybean had a cohesive and smooth texture, which is one of the desirable characteristics. The small size soybean had less ($p > 0.05$) fracturability than the large size soybean and LSP tofu (Table 3). Small size soybean tofu had less elasticity than large size soybean tofu. However, there were no significant differences in hardness and cohesiveness of tofu between the two sizes of Proto soybean (Table 3). The hardness of tofu was affected by the types of coagulants and protein content of soy milk (Wang and Hesseltine, 1982; deMan et al., 1986; Shen et al., 1991). The hardness of Proto tofu produced in this study approached that of a commercial regular-style tofu and was greater than a Kinugoshi-style tofu produced by the House Foods and Yamauchi Inc. (Los Angeles, CA).

Color Characteristics. White or creamy white color is a desirable tofu characteristics. All of the tofu produced from this research showed a creamy white color. The *L*, *a*, and *b* values among large and small size soybeans and LSP tofu did not differ (Table 4). The *L* value ranged from 85.93 to 86.41 (Table 4), indicating that the whiteness of tofu was close to the standard white tile ($L = 91.94$).

Total NSP and CHO. The NSP is composed of cell wall polysaccharides (the main ingredient of dietary fiber), including cellulose, hemicellulose, and pectic substances, which may cross-link with calcium to affect the texture of tofu (Shen et al., 1991). Total CHO includes total NSP, sugars, and starches in the soybean. It is known that soybean has very little starch. The major carbohydrates in soybean are NSP and sugars. No literature has reported the relationship between NSP and texture profiles. The results (Table 5) showed that a slightly higher ($p < 0.05$) content of NSP was found in small size Proto soybean than in large size Proto soybean. However, the total NSP and total CHO of tofu did not differ between these two sizes of soybean. The NSP in tofu was the polysaccharide fraction that was soluble in water and was extracted into soy milk during grinding. The NSP might have interacted with protein molecules or been trapped in the protein network in the final tofu product.

Total and Free Available Cysteine and 11S/7S Ratio. Lee and Rha (1978) and Utsumi and Kinsella (1985) reported that the soybean curd strength was related to soybean intermolecular binding force, including hydrogen bond, disulfide bond, and hydrophobic interaction. The small size Proto soybean had higher total available cysteine than large size Proto soybean (Table 6). However, free cysteine contents were similar. Taira and Taira (1972) studied the bean protein components in 30 Japanese cultivars of food soybeans grown in three locations and found the 11S/7S protein ratio ranged from 0.7 to 1.4, with most beans in the range of 0.8–1.2. The 11S/7S ratio of large size Proto soybean was higher than that of the Japanese cultivars.

Saio (1979) reported the 11S/7S ratio in soy milk strongly affected the textural properties of tofu. He found 11S protein Ca tofu was harder than 7S Ca tofu because the free sulfhydryl group in 11S tofu was higher than that in 7S tofu. Taira (1990) reported the protein to lipid ratio affected the hardness of tofu, but the 11S/7S ratio did not correlate with the yield and hardness of tofu. Figure 2 shows a typical SDS–PAGE electro-

Table 5. Total Nonstarchy Polysaccharide (NSP) and Total Carbohydrate (CHO) in Proto Soybean and Tofu[a]

size of soybean	neutral sugars[b]						uronic acid	total NSP
	Rha	Ara	Xyl	Man	Gal	Glu		
	Nonstarchy Polysaccharides							
raw								
large	0.40	2.86	1.50	2.92	5.17	4.88	2.24	19.48a
small	0.48	3.08	1.67	3.08	4.96	4.98	2.40	20.63b
tofu								
large	0.07	0.39	0.09	0.52	1.38	2.36	0.26	5.07a
small	0.09	0.29	0.07	0.52	1.11	2.53	0.27	4.88a
	Total Carbohydrate							
tofu								
large	0.11	0.89	0.36	1.41	1.96	2.66	0.26	7.65a
small	0.10	0.84	0.36	1.55	2.02	2.88	0.27	8.01a

[a] Data are g/100 g sample on a dry weight basis. Means with different letters in the same column differ significantly ($p < 0.05$). [b] Rha, rhamnose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose.

Table 6. Total Cystine/Cysteine, Free Cysteine, and 11S/7S Ratio of Proto Soybean[a]

seed size	total cystine/cysteine (g/100 g of protein)	free cysteine (g/100 g of protein)	11S (%)	7S (%)	11S/7S (%)
large	1.93a	0.18a	50.59a	33.28a	1.52a
small	2.39b	0.19a	49.11a	35.33a	1.39a

[a] Means with different letters in the same column differ significantly ($p < 0.05$).

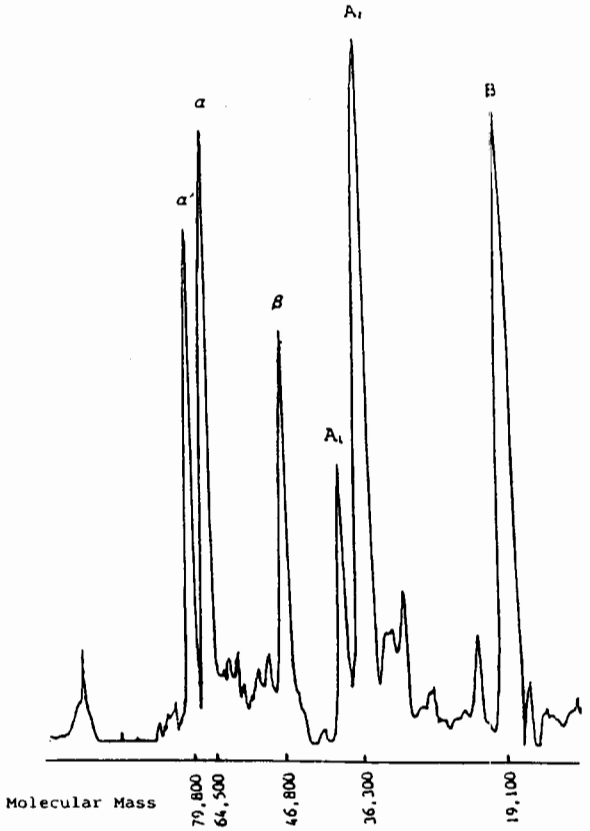

Figure 2. Densitometric scanning of 7S and 11S globulin protein subunits of the large size Proto soybean. 7S subunits include α, α′, and β subunits. 11S subunits include A and B subunits.

phoregram of Proto soybean proteins. There were no differences in 11S/7S ratio and free cysteine content between the large and small size soybean (Table 6).

Amino Acid Analysis. Amino acid composition analysis (Table 7) showed no difference ($p > 0.05$) between the large and small size Proto soybean in both raw bean and tofu. However, aspartic acid, serine, phenylalanine, methionine, and total available cysteine and cystine decreased, and glutamic acid increased after tofu processing. The cystine/cysteine content might have been reduced because the tofu gels did not include the cystine-rich Bowman–Birk trypsin inhibitors (small 2S proteins) in soybean (Odani and Ikenaka, 1973). The sulfur amino acids, methionine and cystine/cysteine, are the limiting essential amino acids of soybean proteins. Therefore, tofu processing might decrease the protein quality slightly due to the decrease in the sulfur amino acids. This is the first study to report the effect of tofu making on the amino acid composition of soybeans.

Conclusion. This study showed that tofu with excellent yield and quality can be made from Proto soybean. No difference existed between two sizes of Proto in yield, color, appearance, and amino acid composition. This study suggested that the size of the same soybean cultivar does not affect the quality and yield of tofu.

Table 7. Amino Acid Patterns of Soybean and Tofu[a]

amino acid	amino acids (g/100 g of protein)			
	soybean		tofu	
	large seeds	small seeds	large seeds	small seeds
Ala	4.5	4.3	5.7	5.5
Arg	7.0	7.2	7.2	7.6
Asp	10.2	11.0	4.7	4.5
Cys	1.9	2.4	0.6	0.7
Glu	18.6	19.2	23.5	22.4
Gly	4.1	4.3	5.0	5.4
His	2.4	2.6	2.8	2.6
Ile	4.3	4.5	5.1	5.0
Leu	7.2	7.9	8.7	8.4
Lys	6.3	6.5	6.9	7.6
Met	1.5	1.6	1.0	0.8
Phe	5.0	5.1	3.6	3.5
Pro	5.4	5.6	6.2	6.6
Ser	5.1	5.4	3.5	3.7
Thr	4.2	3.8	4.0	3.9
Tyr	2.6	2.8	2.5	2.1
Val	6.0	6.2	5.4	6.2
total	96.3	100.2	96.4	96.5

[a] All amino acids were determined according to the HPLC procedure of Bidlingmeyer et al. (1984) except total cystine/cysteine (Cys), which was determined according to the colorimetric method of Felker and Waines (1978).

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TEXTURE AND MICROSTRUCTURE OF SOYBEAN CURD (TOFU) AS AFFECTED BY DIFFERENT COAGULANTS

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Abstract

The coagulating properties of five coagulants and the nature of the curd obtained from soymilk was investigated. Viscosity changes during coagulation were studied using a Nametre Vibrating Sphere Viscometer and texture measurements were made by compression and computer assisted analysis. pH and amount of solids in the whey were determined. The microstructure of the tofu was examined by scanning electron microscopy. It was observed that $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ coagulated the milk instantly while $CaSO_4.1/2H_2O$, glucono delta lactone (GDL) and $MgSO_4.7H_2O$ acted comparatively slowly. The texture of the curd was greatly influenced by type and concentration of coagulant. Curd obtained with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ was coarse, granular and hard, whereas $CaSO_4.1/2H_2O$ and GDL (fresh solution) gave a very smooth, soft and uniform curd. Among the five coagulants studied, 0.75% $CaSO_4$ and 0.4% GDL (fresh solution) appeared to be most suitable for making tofu of high bulk weight and smooth texture.

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Introduction

Tofu, a soybean derived curd, is a low-cost, high protein product which has been widely used in the Orient. In a study by Muto et al. (1963), tofu was judged to be nutritionally equivalent to the protein derived from a mixture of eggs, fish and liver. Depending on the kind and concentration of coagulant used, as well as stirring during coagulation and pressure applied to the curd, tofu ranges in hardness from soft to firm with a moisture content of 70 to 90% and protein content of 5 to 16%.

Making tofu is a relatively simple process but due to its bland nature, its textural properties play a big role in influencing quality and consumer acceptability. Shurtleff and Aoyagi (1984) presented a good review on the manufacturing of tofu. The variety of soybeans used may affect the quality of the tofu (Kamel and deMan, 1982; Skurray et al., 1980) and this is considered to be due to differences in protein content of the soybeans and the ratio of 7S and 11S proteins. Saio (1979) reported that higher solids in soymilk correlated with harder tofu and increasing coagulating temperature with increased hardness of tofu. Recently, Wang and Hesseltine (1982) investigated some of the coagulating conditions in tofu processing and reported that to obtain a good curd, the concentration required for commonly used salts was in the range of 0.01 to 0.1M.

One of the most important factors in determining the texture of tofu is the selection and addition of a coagulant at the proper concentration. This study was conducted to get more detailed information on the coagulating properties of different coagulants and the nature of the curd obtained under different conditions. The curds prepared in the laboratory were compared with some commercial tofu samples.

Materials and Methods

Preparation of tofu

The yellow hilum Ontario soybeans used in this study contained 10% moisture, 16.8% fat and 36.6% protein. The soybeans were made into milk by the following procedure: 300 g of beans were soaked overnight at 20°C. The soaked beans were drained, rinsed and blended for 4 min at high speed in a Waring blender with 750 ml of water. The resultant slurry was mixed with 800 ml of boiling water and strained through a filter cloth. The soymilk contained 10% total solids with 4.7% protein and 2.5% fat. Fresh soymilk was used to make tofu.

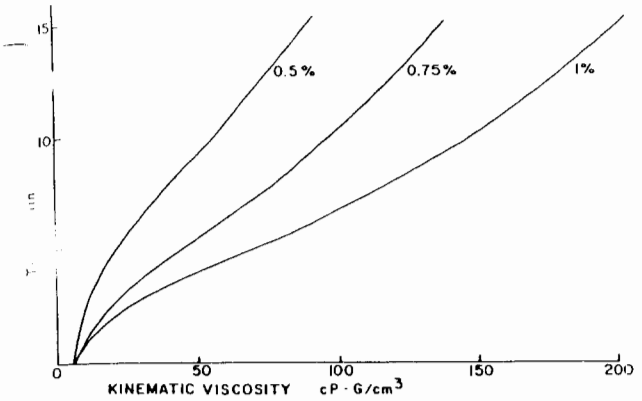

Fig. 1. Development of kinematic viscosity with time of soymilk with 0.5%, 0.75% and 1.0% added $CaSO_4.1/2H_2O$.

Table 1. Effect of coagulant type and concentration on viscosity of soymilk.

Coagulant	Concentration		Kinematic viscosity (cP.g/cm³) at time (min)			
	%	M	0	5	10	15
$CaSO_4.1/2H_2O$	0.50	0.034	7.3	29.8	72.8	106.3
	0.75	0.052	8.5	45.9	98.3	135.3
	1.00	0.069	9.3	70.5	153.0	213.5
$CaCl_2.2H_2O$	0.15	0.010	20.9	19.6	24.4	29.8
	0.20	0.014	23.5	22.0	25.1	30.1
$MgSO_4.7H_2O$	0.30	0.012	27.8	41.8	55.0	68.4
	0.40	0.016	45.0	83.5	105.0	128.0
$MgCl_2.6H_2O$	0.20	0.010	12.2	24.5	39.0	52.1
	0.30	0.015	31.2	27.8	35.8	44.6
	0.50	0.025	40.3	33.7	42.2	51.8
GDL (heated)	0.30	0.017	12.0	28.3	52.0	75.0
	0.40	0.022	13.0	53.5	91.0	117.5
GDL (fresh solution)	0.40	0.022	3.9	81.7	172.0	244.0

(Table 3) and even with the same pH of whey, the coagulants behaved differently. For example, the curd obtained with 0.3% $MgCl_2.6H_2O$ was three times harder than the curd obtained with 0.5% $CaSO_4.1/2H_2O$ although the pH of whey was 6.03 in both cases.

Moisture content of tofu and solids in whey

Results in Table 3 show that the coagulant used affects the amount of whey liberated and, therefore, the weight and moisture content of the final product. With increase in coagulant concentration, there was a decrease in moisture content of the tofu. With increase in coagulant concentration, the structure of tofu became more porous separating more whey and leaving less moisture in the tofu.

There was a general trend towards decrease in solids in the whey with increase in coagulant concentration. However, the difference was not significant. The solids content of the whey increased dramatically when the coagulant concentration used was lower than the minimum concentration listed in Table 3.

Scanning electron microscopy

Figures 2a and 2b are micrographs of the critical-point dried

Table 2. Effect of coagulant type and concentration on texture of curd.

Coagulant	Conc. %	Peak force (N)	Force at 25% compression (N)	Firmness (N/mm)
$CaSO_4.1/2H_2O$	0.50	–*	0.47	0.11
	0.75	–	0.54	0.14
	1.00	–	0.87	0.21
$CaCl_2.2H_2O$	0.15	1.09	0.54	0.14
	0.20	2.66	1.34	0.30
$MgSO_4.7H_2O$	0.30	0.84	0.44	0.11
	0.40	1.97	0.96	0.25
$MgCl_2.6H_2O$	0.20	0.80	0.44	0.09
	0.30	2.88	1.43	0.36
	0.50	3.25	2.37	0.62
GDL (heated)	0.30	1.13	0.58	0.15
	0.40	2.52	1.26	0.32
GDL (fresh solution)	0.40	1.24	0.76	0.19

*Samples disintegrated before 50% compression was reached.

Table 3. Effect of coagulant type and concentration on pH of whey, solids in whey, amount of whey and % moisture of tofu.

Coagulant	Conc. %	pH of whey	Solids in whey %	Whey %	Moisture of tofu %
$CaSO_4.1/2H_2O$	0.50	6.04	3.1	16.17	91.0
	0.75	5.97	3.2	17.50	89.4
	1.00	5.94	3.0	18.93	89.1
$CaCl_2.2H_2O$	0.15	5.98	3.3	36.57	87.4
	0.20	5.94	3.3	45.33	86.4
$MgSO_4.7H_2O$	0.30	6.09	3.2	33.11	88.2
	0.40	6.07	3.2	47.83	86.4
$MgCl_2.6H_2O$	0.20	6.25	3.2	40.00	88.4
	0.30	6.03	3.1	56.23	85.0
	0.50	5.89	2.7	59.67	82.8
GDL (heated)	0.30	5.52	3.5	42.10	89.2
	0.40	5.27	3.5	49.33	84.8
GDL (fresh solution)	0.40	5.41	3.3	19.43	88.5

(CPD) and freeze-dried (FD) tofu coagulated with 0.4% fresh GDL. The network structure appeared to be similar in the two pictures, although the CPD sample seemed to have shrunken considerably. CPD has been shown to cause shrinkage (Cohen, 1977). Freeze drying appeared to be more appropriate for observing tofu structure. In Figures 3 and 4, SEM micrographs of tofu coagulated with different coagulants show clearly different fine structures. The microstructures as indicated in these pictures can be easily related to the visually observed texture. Tofu obtained with GDL (fresh solution) was judged best in texture on the basis of smoothness, and the micrograph showed a fine and uniform honeycomblike structure (Fig. 3a). The structure was very uniform with smaller holes than those prepared

The following coagulants were used: $CaSO_4.1/2H_2O$ or plaster of Paris; $CaCl_2.2H_2O$; $MgCl_2.6H_2O$; $MgSO_4.7H_2O$ and glucono-δ-lactone (GDL). To make tofu, 300 ml of fresh soymilk was heated to near boiling and the required amount of coagulant dissolved or suspended in 7.5 ml of water. The hot soymilk and coagulant were poured simultaneously into a glass container ensuring good mixing without stirring. The curd was left to set for 15 min and then transferred to a perforated plastic container with a diameter of 9 cm and lined with a filter cloth. The curd was pressed by applying weights (31.4 g/cm^2) for 15 min. After pressing, the curd was left in running water for 1 h and then stored in a refrigerator.

Viscosity

A Nametre vibrating sphere viscometer (Nametre Co., Edison, N.J.) was used to follow changes in viscosity. The hot soymilk and coagulant were poured simultaneously into a glass container ensuring good mixing, and the vibrating sphere was immediately immersed to the mark. The kinematic viscosity was measured as a function of time. This provides a non-destructive method of measuring changes in viscosity.

Texture

For texture evaluation, the mechanical part of an Instron Universal Testing Machine was used. The original load sensing mechanism was replaced with a Daytronic load cell (cap. 12 kg) and a Daytronic 9000 strain gage amplifier-indicator (Daytronic Corp., Miamisburg, OH). The signal voltage was fed to an A-D converter (AI13 Interactive Structures, Bala Cynwyd, PA) and from there to an Apple IIe computer. The instrument output was stored on floppy disks and analyzed using a program developed by the Statistical and Engineering Research Institute, Agriculture Canada, Ottawa, Ont. (Buckley et al., 1984). The information obtained by this system included: peak force (N), time to peak (s), deformation to peak (mm), firmness (N/mm), and force at different points (N). Cylindrical samples were prepared from the curd with a boring tube and wire cutter, sample dimensions were 20 mm diameter and 20 mm height. Samples were compressed by a flat plate to 50% deformation using a crosshead speed of 10 mm/min. Peak force at 50% compression was measured as well as force at 25% deformation.

Moisture

For moisture determination about 100g of tofu was homogenized in a blender and 3–5g dried on a steam bath for 15 min followed by forced air oven drying at 98°–100°C overnight. Total solids in the whey was determined by drying for 15 min on a steam bath and 3 h in the oven at 98°–100°C.

pH

pH of the whey was measured using a Fisher Accumet pH meter model 825 MP.

Scanning Electron Microscope Observations

A scanning electron microscope (ETEC Autoscan) was used to examine the fine structure of tofu coagulated with different coagulants. The procedure used for sample preparation was that of Saio (1981) with some modifications. Small pieces of (< 2 mm cube) were fixed at room temperature with 5% glutaraldehyde in 0.1M phosphate buffer (pH 6.7) for 90 min. After five washes in 0.1M phosphate buffer (pH 6.7) at 10 min intervals, they were postfixed in 1% osmium tetroxide in the same buffer for 90 min at room temperature. The fixed samples were rinsed five times with phosphate buffer at 10 min intervals. Dehydration was done using a 10% incremental ethanol series, leaving samples at each concentration for 15 min followed by three rinses with 100% ethanol. The samples were then rinsed three times with chloroform. Critical point drying (CPD) was conducted using CO_2.

For freeze drying, the samples were dehydrated using the ethanol series, frozen in liquid nitrogen and transferred to a Polaron E5300 freeze drier and dried for 24 h.

All of the samples were mounted on stubs and sputter coated with 20–30 nm of gold palladium (60:40) using a Technics Hummer V Sputter Coater. The observations were made at 10 kV.

Making of commercial tofu

Tofu was made in a commercial tofu plant (Victor Food Products Ltd., Toronto, Ont.) by a semiautomatic process using the optimum concentration of coagulants based on laboratory experience.

Results and Discussion

Results are reported only for those concentrations of coagulants which gave curds with clear or nearly clear whey. The minimum coagulant concentration required was 0.5% $CaSO_4.1/2H_2O$, 0.15% $CaCl_2.2H_2O$, 0.3% $MgSO_4.7H_2O$, 0.2% $MgCl_2.6H_2O$ or 0.3% GDL.

Viscosity

The coagulation rates as measured with the Nametre vibrating sphere viscometer are shown in Table 1. The coagulation rate was very rapid with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ and visible whey separation occurred at an early stage. A more gradual increase in viscometer readings was obtained with $CaSO_4.1/2H_2O$, $MgSO_4.7H_2O$ and GDL. With these coagulants up to 10 min were required for curd formation and no whey separation was observed. The recordings of viscosity when using different concentrations of $CaSO_4$ are presented in Fig. 1.

Saio (1979) reported that GDL only coagulates soymilk when heated. GDL activity is influenced by temperature and time of preparation. Curd obtained by addition of fresh cold GDL solution to hot soymilk (near boiling) was very smooth and similar to curd obtained with $CaSO_4$. When the GDL solution was left at room temperature for 30 min, a very hard curd was obtained and even harder curd resulted with a hot (90–95°C) GDL solution. In the latter case, the curd was grainy, less cohesive and similar to curd made with $MgSO_4$. The active coagulant in GDL is gluconic acid and when a freshly prepared GDL solution is aged more gluconic acid is formed. The pH of a 0.4% GDL solution dropped from 3.0 after 10 min to 1.7 after 4 h. GDL has been reported to recover more protein in the tofu (Shurtleff and Aoyagi, 1984) and is used for making silken tofu.

Texture

Results of textural evaluation are presented in Table 2. Reported values are means of six replicates. Peak force values for $CaSO_4.1/2H_2O$ produced curd are not reported because the samples fell apart before reaching 50% compression. The hardest curd was obtained with 0.5% $MgCl_2.6H_2O$. It appears from these data that curd firmness can be affected by using various coagulants at different concentrations.

pH

According to Lu et al. (1980) pH, not the calcium ion concentration, is by far the most important factor in the precipitation of soy protein. These authors reported that protein starts to coagulate when the pH drops to about 6.0, therefore, according to Lu et al. (1980), the addition of salt should be stopped when the pH approaches 6.0. In this study, with all the coagulants except GDL, the pH of whey was in the range of 5.89 to 6.

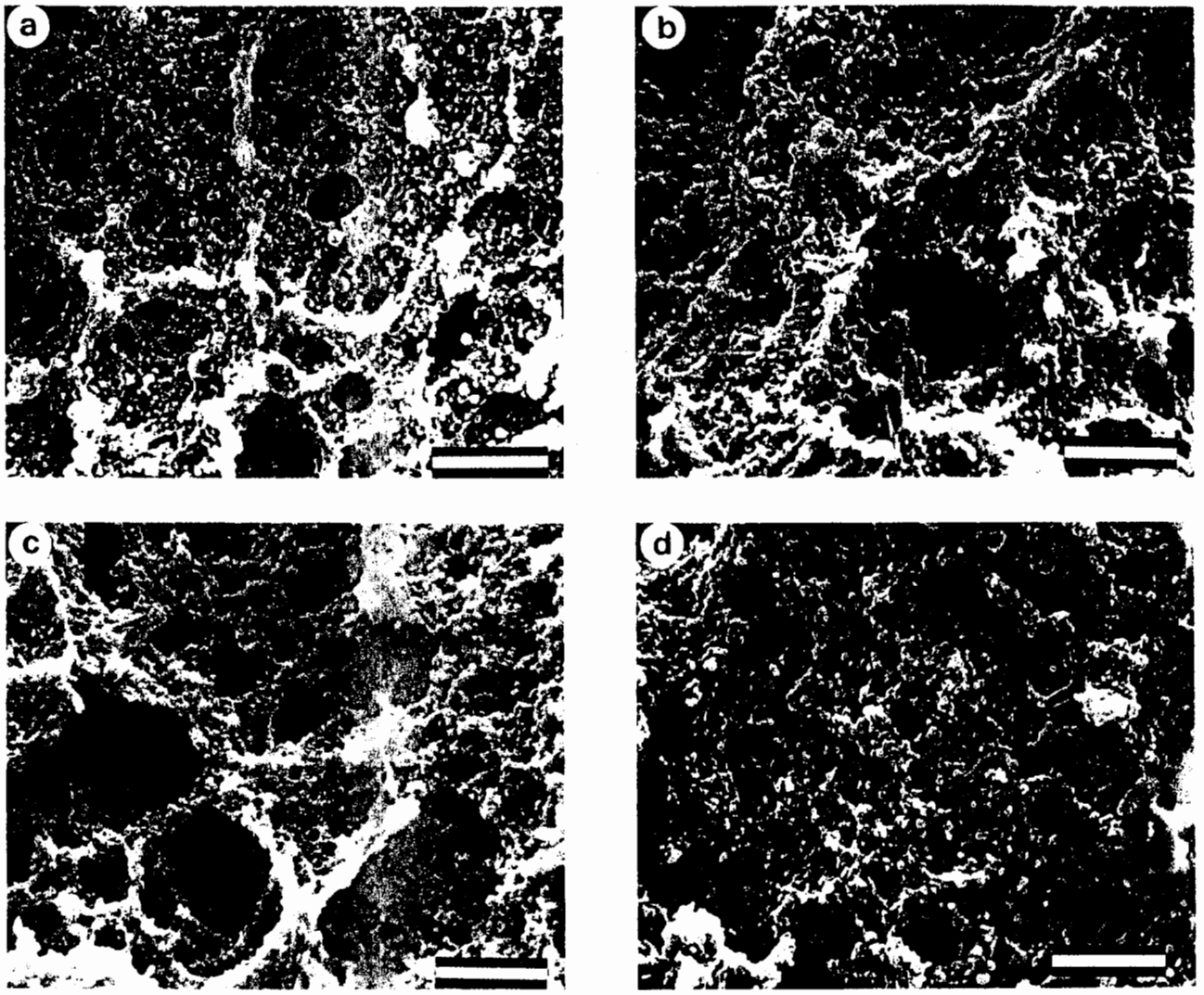

Fig. 4. SEM-images of freeze dried tofu prepared with different coagulants. (Bar = 5 μm).

a) – 0.75% $CaSO_4.1/2H_2O$
b) – 0.30% $MgCl_2.6H_2O$
c) – 0.15% $CaCl_2.2H_2O$
d) – 0.30% $MgSO_4.7H_2O$

Texture profile analysis with curve smoothing using a personal computer system. J. Texture Studies. **15**. 247–261.

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Discussion with Reviewers

W.J. Wolf: Is pH a factor in the coagulation of tofu with GDL? The pH values in Table 3 for GDL are all significantly lo[illegible]

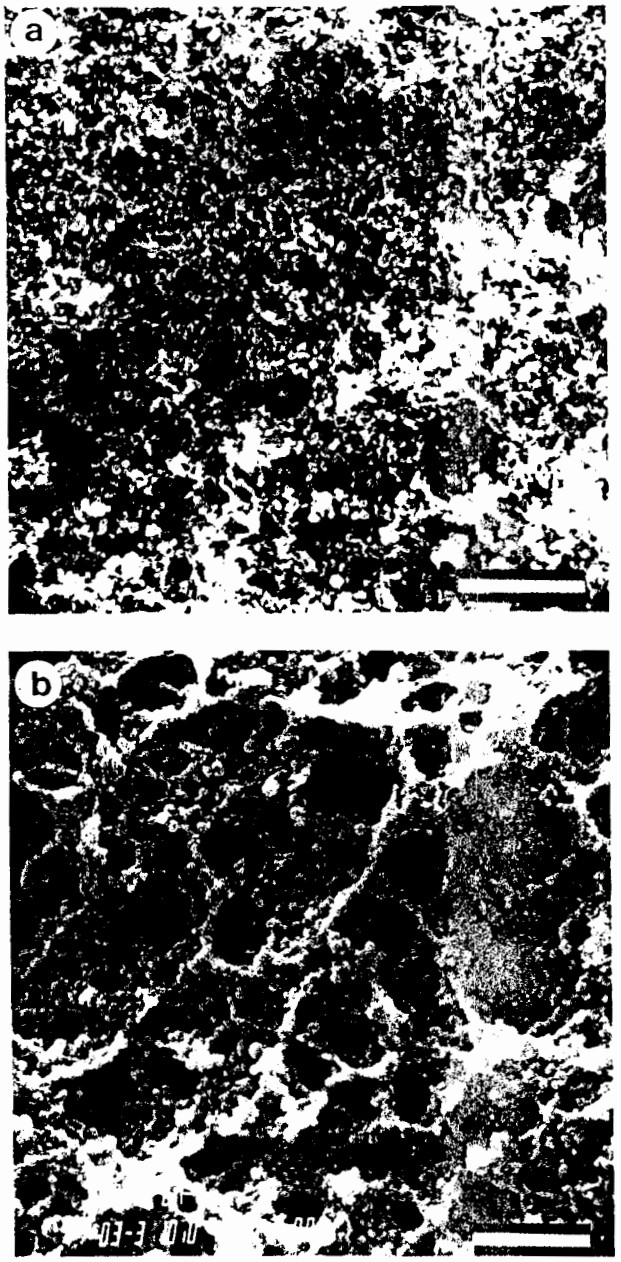

Fig. 2. Comparison of critical point drying (a) and freeze drying (b) of tofu coagulated with 0.40% GDL (fresh solution.) (Bar = 5 μm).

with $CaCl_2.2H_2O$, $MgCl_2.6H_2O$ and $MgSO_4.7H_2O$. Coagulation with $CaSO_4.1/2H_2O$ gave a structure similar to that obtained with GDL but less uniform. The SEM pictures taken at higher magnification (Fig. 4) of curd made with $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$ appear to show similar structures. The networks of these samples were not as fine and continuous as those obtained with GDL and $CaSO_4.1/2H_2O$. $MgSO_4.7H_2O$ gave a more continuous and uniform structure than curd prepared with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$.

Commercial tofu

The process used in the plant was basically the same as used in the laboratory. However, in the plant the curd is broken up during the transfer to the press. Due to this difference, the moisture content of tofu made in the plant was slightly lower than tofu made in the laboratory and turned out to be harder (Table 2 and Table 4).

Table 4. Texture and moisture content of some commercial tofu samples.

Sample	Coagulant	Peak force (N)	Force at 20% compression (N)	Firmness (N/mm)	Moisture %
A	$CaSO_4$	1.47	0.93	0.22	87.57
B	$MgSO_4$	3.08	1.76	0.45	83.41
C	$CaSO_4$	1.12	0.82	0.17	88.68
D	$MgSO_4$	3.68	2.06	0.45	83.60
E	$CaCl_2$–$CaSO_4$	11.19	6.23	1.63	70.05
F	$CaCl_2$–$CaSO_4$	10.63	5.44	1.64	73.79

For good curd production and clear whey formation, the concentration of coagulants on a molarity basis ranged from 0.0 to 0.02M except for $CaSO_4.1/2H_2O$ which required a minimum of 0.03M. It is not feasible to decide on a common optimum concentration for all of the coagulants as has been pointed out in some other studies (Wang and Hesseltine, 1982; Tsai et al., 1981). Tsai et al. (1981) noticed a dramatic change in the texture of tofu when increasing the concentration of coagulants above 0.03N (0.015M). The present study reemphasizes the different behaviour of various coagulants.

Table 4 lists results of texture and moisture analyses of some commercial tofu samples. Texture and moisture content of samples A, B, C and D fall in the range obtained with the laboratory made tofu.

It has been suggested that the coagulation of soymilk is due to the crosslinking between protein molecules by divalent cations (Saio et al., 1967). However, the site of crosslinking is still under debate. Saio et al. (1967) suggested that the free carboxyl group of soybean protein is the major site of calcium binding and phytic acid also acts as a binding site. According to Appurao and Narasinga Rao (1975) a probable binding site on the protein molecules is the imidazole group. In addition to uncertainties about the binding sites of the soy proteins, there is a lack of understanding of the mechanism of coagulation with GDL.

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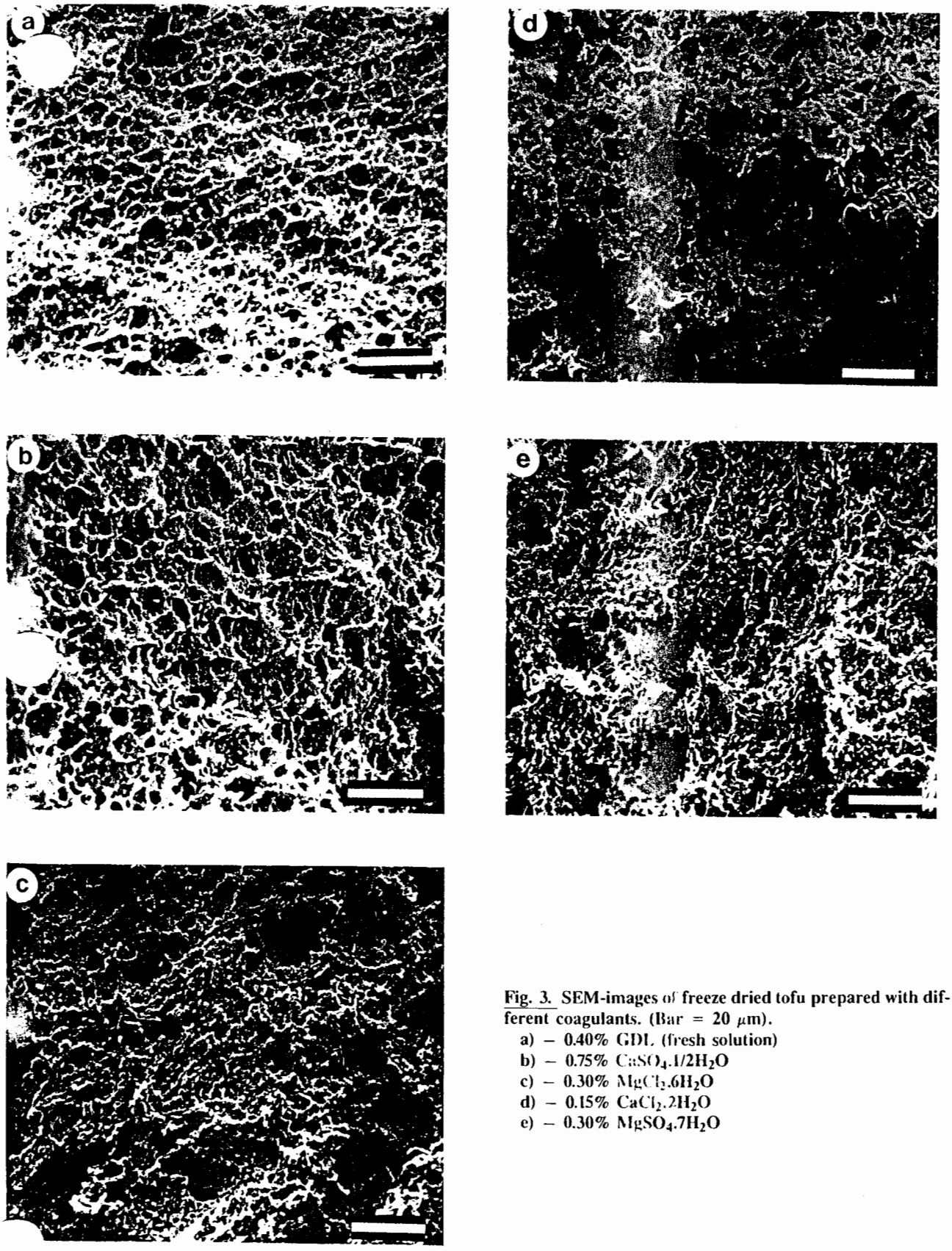

Fig. 3. SEM-images of freeze dried tofu prepared with different coagulants. (Bar = 20 μm).

a) – 0.40% GDL (fresh solution)
b) – 0.75% $CaSO_4.1/2H_2O$
c) – 0.30% $MgCl_2.6H_2O$
d) – 0.15% $CaCl_2.2H_2O$
e) – 0.30% $MgSO_4.7H_2O$

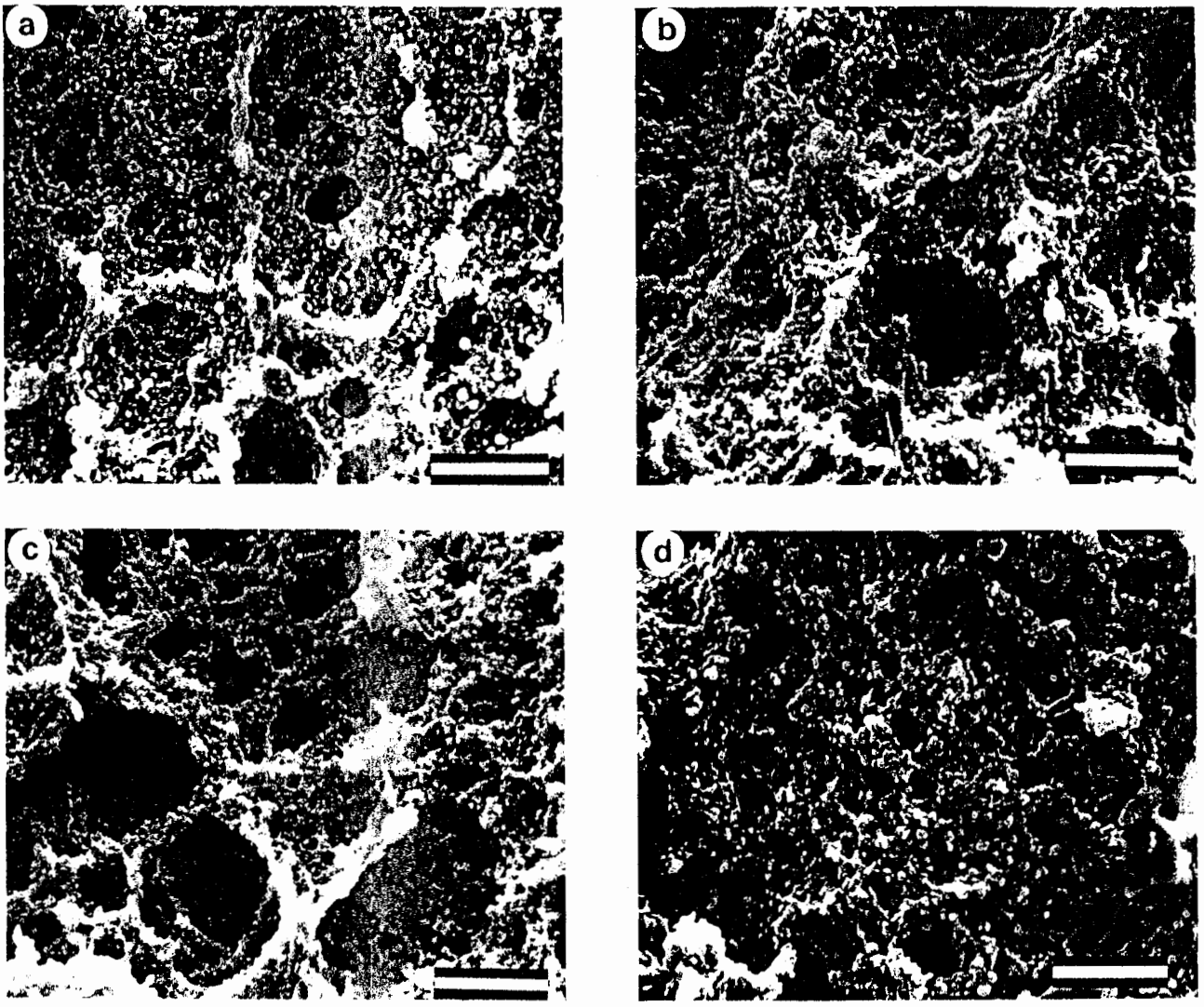

Fig. 4. SEM-images of freeze dried tofu prepared with different coagulants. (Bar = 5 μm).

a) – 0.75% $CaSO_4.1/2H_2O$
b) – 0.30% $MgCl_2.6H_2O$
c) – 0.15% $CaCl_2.2H_2O$
d) – 0.30% $MgSO_4.7H_2O$

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Discussion with Reviewers

W.J. Wolf: Is pH a factor in the coagulation of tofu with GDL? The pH values in Table 3 for GDL are all significantly l

or any of the other coagulants. Moreover, pH values of obtained by GDL coagulation are all below 6 and are approaching the isoelectric range for the proteins.

ors: acts by opening of the lactone ring to form ic acid. This occurs when GDL is dissolved in water even m temperature as is demonstrated in the paper by monitore pH of a GDL solution at room temperature. Saio (1979) ed that GDL-solution should be added to cold soymilk en reheated. In industry this means cooling of the soynd then reheating with GDL solution. It is better to disthe required amount of GDL in water just before addition atch of hot soymilk coming from the production line as e with the other coagulants. The release of gluconic acid temperature results in a very uniform curd as shown in M photograph. When the GDL solution is left at room rature for a longer time the reaction with hot soymilk is cid precipitation and produces a coarse curd.

wer III: How do you define texture and what proof is there eak force," "force at 25% compression" and "firmness" re texture?

ors: Texture can be defined as "The way in which the s constituents and structural elements are arranged and ined into a micro- and macrostructure and the external estations of this structure in terms of flow and deforma- Instrumental analysis of texture involves measurement of nical properties such as resistance to deformation, in this eak force and force at 25% compression and also the /strain ratio which is defined as firmness.

K. Saio: As shown in Fig. 4 the hardness of tofu is influenced by the concentration and kinds of coagulants. Japanese like tofu because of its texture and bland flavor. Different coagulants are used for various tofu types, e.g., $CaCl_2$ for kori tofu, $CaSO_4$ or $MgSO_4$ or $Mg\ Cl_2$ (with phosphoric or citric acid) for hard tofu and GDL and $CaSO_4$ (alone or with GDL) for silken tofu. It was mentioned in the paper that tofu coagulated with GDL had the best smooth, soft and uniform texture but do you think North Americans prefer such silken tofu to the hard kind?
Authors: From our experience it appears that North Americans prefer the firmer styles of tofu. These seem to be more suitable for western style cooking and are preferred in salads.

K. Saio: Is it possible to distinguish the differences of coagulation state among soybean varieties with a Nametre vibrating sphere viscometer?
Authors: We have tested this on 17 different varieties of soybeans grown in Ontario, and found no significant differences between them. However, these soybeans were harvested at the same time and stored under identical conditions. We suspect that storage conditions have a greater effect than varietal differences and this is now being investigated.

Coagulation Conditions in Tofu Processing

Hwa L Wang and C W Hesseltine*

Tofu is a highly gelatinous food product derived from soybean milk. The authors examine the method of preparation. Amino acid analysis, protein digestibility measurement, and nitrogen determination are discussed. The effect of heat treatment and coagulants on the final product are also examined.

Introduction

Over the past decade, the eating habits of Western consumers have slowly changed. A growing interest in foods of plant origin has become evident, especially among younger Americans and those living in large cities. These consumers may have medical, philosophical, or religious reasons for choosing more vegetable foods. One food product that is gaining general acceptance and attention is soybean curd or tofu.

Tofu, a highly hydrated gelatinous product, has been the most important source of protein in the Orient since Liu An (179 B.C. –122 B.C.) of the Han dynasty invented the method of preparation[1]. Today, tofu is prepared essentially the same way that it was made in 2,000 years ago. Soybeans are washed, soaked, and ground with water. After the bean slurry has been boiled and filtered through cheesecloth, it yields a milk-like product known as soybean milk. When bittern or nigari (the bitter mother liquor that remains after salt is crystallized from seawater), gypsum, or vinegar is added to hot soybean milk, curd forms; this curd is pressed to form a soft whitish cake or tofu.

Several investigations[1-4] have described procedures for making tofu based on the traditional method and modified for laboratory equipment and small-scale preparation. Although gel formation of isolated soybean proteins[5-10] and their bindings with Ca(II) and Mg(II) ions[11-14] have been investigated by many workers, various factors involved in making tofu from whole soybeans that may affect quality and quantity of tofu in a quantitative manner have not yet been studied.

Schroder and Jackson[15] reported that calcium sulphate precipitation of soybean milk resulted in a bland soybean curd and that the pressure applied can affect the curd texture. Lu and his coworkers[16] found calcium acetate and calcium chloride to be good coagulants. Tseng et al[17] showed that tofu prepared by calcium salt precipitation has a higher calcium content and also a higher Ca/P ratio than that prepared by a coagulant such as glucono-delta-lactone. Thus, they suggested that tofu can help to correct the imbalanced Ca/P ratio in American diets.

In the Orient, a fine-textured tofu is necessary for acceptance. Recently, Saio[18] reviewed various factors affecting hardness of tofu and found that heating temperature of soybean milk, type and concentration of coagulants, as well as temperature and stirring during the addition of the coagulant, all influence the product's hardness.

With the rapid development of markets outside the Orient, the need for quantitative information on making tofu has become more apparent, so that uniform, good-quality products can be produced. It is with this subject that this study is concerned. Chemical constituents of soybeans[18] and also varieties of soybeans[3] have been reported to affect tofu preparation, which will be investigated in a separate study.

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Preparation of soybean milk

Corsoy soybeans of the 1980 U.S. crop were used throughout this study. Soybeans were washed and soaked in water at room temperature (20-22°C) for 16 hours to reach complete hydration (135% hydration)[19]. The soaked beans were drained, rinsed, and homogenized for 2 minutes in a Brinkmann homogenizer with the addition of enough water to give a water-to-dry beans (prior to soaking) ratio of 10:1 on a weight basis[20]. The slurry was brought to a boil and kept at boiling temperature for 15 minutes or other lengths of time as indicated. It was then filtered through double-layered cheesecloth.

Amino acid analysis

Freeze-dried soybean milk samples containing 100 mg protein were hydrolyzed for 24 hours by refluxing in 6N hydrochloric acid evaporated to dryness followed by evaporation with three small volumes of water, and dissolved in citrate buffer at pH=2.2. A portion of the hydrolyzate was analyzed with a Glenco Model MM-100 amino acid analyzer, and data were computed automatically using the method of Cavins and Friedman[21].

In vitro measurement of protein digestibility

Pepsin and pancreatin digestion was carried out by the method of Akeson and Stahmann[22]. Freeze-dried soybean milk samples containing 100 mg protein were incubated with 1.5 mg pepsin in 15 ml of 0.1N hydrochloric acid at 37°C for 3 hours. The digestion mixtures were neutralized with 0.2N sodium hydroxide and then incubated for an additional 24 hours after the addition of 4 mg pancreatin in 7.5 ml of pH 8.0 phosphate buffer in the presence of 50 ppm merthiolate. The undigested protein and larger peptides were removed by picric acid. Supernatants were then passed through a column containing anion exchange resin to remove picric acid. The eluents were analyzed for nitrogen, and the percentage of protein digested was expressed as the ratio of nitrogen recovered from the eluent to the nitrogen in freeze-dried soybean milk used for digestion. Nitrogen was determined by micro-Kjeldahl analysis[23].

Evaluation of coagulation conditions

For this evaluation, tofu-making was carried out in 50 ml centrifuge tubes. Reagent grade calcium chloride $CaCl_2 \cdot 2H_2O$, magnesium sulphate $MgSO_4$ and magnesium chloride $MgCl_2 \cdot 6H_2O$ obtained from Fisher Scientific Co. and food-grade calcium sulphate (Terra Alba) $CaSO_4 \cdot 2H_2$) from United States Gypsum were used for coagulants.

Appropriate amounts of calcium sulphate were placed in each tube and then suspended in 2 ml boiling water. For the other three coagulants, various amounts of concentrated solutions were pipetted into each tube and then diluted to 2 ml. The tubes were kept at 70°C in a water bath, and 23 ml of soybean milk at 70°C was delivered into each tube by a Brewer automatic pipetting machine. The action of the delivery served the purpose of mixing the

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