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THERMOSET PROTEIN-STARCH GELS AS COMPOSITE FOODS

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EXECUTIVE SUMMARY

Gels are unique foods for their textural properties vary from a liquid-like, viscous appereance to a solid-like behavior. In these sense most processed foods can be regarded as gels. This research studied fundamental bases, characterization and applications of mixed gels based on proteins and polysaccharides.

Mixed gels are important because they better simulate highly demanded foods, may be tailored to give different textures and nutritional properties, and can be fabricated from a wide variety of local ingredients. Gels under study were of a thermosetting nature (became solid-like after heating the solutions) and contained cassava starch (CS), κ-carragenan (κ-C), a fish paste (FP) and a whey protein isolate (WPI). Products developed used local, raw materials from Bolivia and Chile (except for WPI) and simulated popular and nutritious foods such as yoghurt, soft cheeses and seafood analogues.

Fabricated mixed gels were characterized by their mechanical and rheological properties (e.g., related to texture), thermal phase transitions and microstructural characteristics (light and electron microscopy). End point determination studies considered the effect of relevant variables such as total solids in the gel (TS), phase composition ($1>\vartheta_S>0$), pH and temperature. Kinetic studies followed the development of viscoelastic properties as a function of time at a fixed temperature or during heating at a fixed rate.

Unique results of this research at the scientific level can be summarized as follows:

a) Quantitative data on the rheological, mechanical and structural properties of WPI gels is now available.

b) Unusual reinforcing properties of CS on WPI gels have been found and a structural hypothesis advanced.This may lead to important product developments.

c) Kinetic aspects for the gelation of mixed CS/WPI systems have been studied in detail and a kinetic model has been developed.

d) A simple method to follow the kinetics of gelling of WPI proteins based on the water retention capacity has been implemented.Mechanical and rheological properties are strongly dependent on phase composition.

e) Infrastructure and know-how derived from the project assisted in the study of caking phenomena in foods.

f) The concept of a microstructural approach to study food processing has been developed, advanced and accepted by the international scientific community.

From the international cooperation viewpoint achievements of this project were:

a) Strong links were developed between food technology groups from the P. Universidad Católica de Chile (PUC) and U.A. Gabriel Rene Moreno of Santa Cruz, Bolivia (UAGRM) through training, equipment purchases, courses, local visits, and personal contacts. Four graduate students (two from UAGRM) and two undergraduates were supported by the project. The two trained Bolivian food scientists teach now at UAGRM.

b) The PI was elected to lead the Ibero American Network on Physical Properties of Foods (RIPFADI) which includes over 200 researchers from 11 countries and in 1993 was awarded the International Award of the Institute of Food Technologists (US)

Advances in institutional buildup were significant:

a) The project was instrumental in consolidating at PUC what is probably the strongest laboratory in Food Materials Science in Latin America, as assessed by scientific productivity, modern equipment, and institutional links to U.S. and European universities and research centers.

b) It helped to consolidate the work of a Center of Food Technology at UAGRM through new trained personel, equipment, courses to industry and new research lines.

c) Other sources of funding have become now available from local grants, the PUC and private companies to continue work.

RESEARCH OBJECTIVES

Nowhere in the world is the need for foods based on new ingredients perceived more dramatically than in less developed countries (LDCs). Previous efforts in this direction have failed in that new foods have remained as nutritious powders, overlooking the capacity of poor urban and rural populations to adopt new eating habits. As an example, the consumption of yoghurt - a structured protein gel- has had an explosive growth in Chile and neighboring countries and is now a popular low cost, nutritious food.

This research attempts to study the fundamental bases of mixed gel fabrication to produce desirable textures using mainly a whey protein isolate (WPI) as a source of protein, in combination with cassava starch (CS). Also studied was a mixture of fish paste and κ-carrageenan. The specific objectives of this research were:

1. To assess the feasibility of food fabrication methods based on gel precursors (mainly protein/starch) by heating.
2. To study biochemical phase transformations, rheological properties and microstructures of fabricated composite gels and the bases for controlling their development.
3. To correlate results at the biochemical, rheological and microstructural levels to develop a basic understanding of their relationship in structure formation.
4. To develop institutional linkages and train professionals from the Universidad Autónoma Gabriel Rene Moreno, Santa Cruz, Bolivia, as well as building scientific capabilities in the area of food materials at the P. Universidad Catolica..

METHODS AND RESULTS

I. Gelation of whey proteins

I.1. Introduction

Whey is the soluble fraction of milk that is separated from the casein curd during cheese manufacturing. The main proteins in whey are: β-lactoglobulin (β-Lg), α-lactalbumin (α-La), proteose-peptone, immunoglobulins and serum albumin (BSA). Protein products derived from whey proteins such as concentrates and isolates (aprox. 70 and 90% protein, respectively) have important commercial applications in foods, a major one being their tendency to form irreversible heat-induced gels. β-Lg and BSA are the major gelling proteins in whey, but since the former is 10-20 times more abundant in whey products it is considered the primary gelling protein.

Most examples to be illustrated in this report correspond to gelation of a commercial whey protein isolate (WPI) containing 95% protein and prepared by ion exchange, obtained from Le Sueur Isolates (Le Sueur, MN) under the trade name BIPRO. BIPRO consists of 73% β-Lg, either monomer (61%) or dimer (13%), 16% α-La and 6% BSA, thus it may be considered a concentrated form of β-Lg. All three fractions occur naturally in whey as globular proteins folded into compact three-dimensional structures in which the reactive hydrophobic amino acids side chains are buried in the interior of the protein leaving most of the polar side chains to the exterior. The structure is maintained by weak noncovalent bonds, and in the case of β-Lg, by a few disulfide bonds (Kinsella and Whitehead, 1980).

The subject of gelation of whey protein products and of constituent proteins has been reviewed many times in the past, denoting that it is a topic of enormous relevance both scientifically and commercially (Paulsson et al., 1986; Mulvihill and Kinsella, 1987; Katsuta, 1990; Ziegler and Fogeding, 1990). Heat-induced gelation of these compounds depend on many factors such as composition, extent of denaturation, pH, temperature, concentration, heating rate, ionic strength and presence of specific ions, among others.

I.2. Rheological Characterization of the Gelation Process: "Curing" Experiments.

It was necessary first to fully characterize the gelation properties of the whey isolate to be used. Heat-induced gelation of whey proteins starts with a translucent solution that

increases in viscosity due to formation of protein aggregates and finally sets into a gel spanning over the vessel. The gel can support its own weight under gravity (within a given observation time) and can transmit mechanical signals from one side of the sample to the other. Because of the gradual development of viscoelasticity, gels and the gelation process are best characterized by small amplitude dynamic testing, normally involving the application of a sinusoidal oscillatory stress or strain under constant frequency. The measured response is usually resolved into an in-phase elastic component (G') and a 90° out-of-phase viscous component (G"), the storage and loss moduli, respectively. Due to the fragile nature of the network that sets in, deformation should be kept small and within the elastic range. The subject of rheological characterization of dairy products has been reviewed recently by Shoemaker et al. (1992).

In a "curing" experiment (a term borrowed from polymer science) a characteristic curve of log G', log G" (or tan δ = G"/ G') versus time is obtained for a given set of conditions as heating proceeds at constant temperature (Figure I.1). Curing runs provide the kinetics of gelation and may be used to derive fundamental information on the gelling mechanism as well as the effect of process variables on the gel properties. As expected for a liquid, initially G" is greater than G', indicating that the starting solution is relatively more viscous than it is elastic. Rapidly, G' starts to increase faster than G" and the system develops a greater elasticity or "solid-like" behavior. Eventually both moduli appear to reach a pseudo-plateau value larger for G' than for G", although an equilibrium value is seldom measured within practical times. Sometimes G" passes through a maximum before decreasing as the network is consolidated; absence of a maximum in the G" curve may be interpreted as if the gel is formed by a stiff network (Ross-Murphy, 1992). Cooling further increases the magnitude of the limiting storage modulus. This rheological transition is reversible since upon subsequent re-heating to the curing temperature the gel exhibits a similar pseudoequilibrium modulus, corroborating that thermotropic gelation is irreversible (Figure I.1). Changes in G' during heating ($\Delta G'_{heat}$) relative to those during cooling ($\Delta G'_{cool}$) are considered as the relative contributions of hydrophobic interactions and hydrogen bonding to the final value of G' (Beveridge et al, 1984). When curing experiments are carried out at increasing concentrations but under the same heating and cooling conditions, the pseudo-plateau values increase dramatically (Figure I.2). Plots of log G' at the pseudo-plateau after cooling versus log concentration shown in Figure I.3 predict that at high concentrations an almost constant power law region exists with an exponent close to 3. Paulsson et al. (1990) demonstrated that the power exponent for the dependence of the storage modulus of β-Lg gels with concentration in the pH range 4 to 5.5 was close to 2.5,

suggesting that the gelation characteristics of WPI are dominated by the presence of this protein fraction The power law diverges to higher values of the exponent at the minimum concentration for gelation. According to Clark (1992) values of the power exponent vary for common gelling food components such as several proteins and polysaccharides from 2 for strong, concentrated gels to 5 for weaker systems.

The development of the elastic (E) or Young´s modulus (determined by axial compression at small deformations) during curing present a similar behavior as for G'. The mechanical modulus of selfstanding gels after cooling to room temperature is commonly assumed to be related to the textural properties (Langley and Green, 1989; Aguilera and Kinsella, 1991). Interestingly, a good correlation (R^2= 0.98) exists between the limiting values of log E and log G' for WPI gels, emphasizing that the predominant properties of the food gel are due to physicochemical interactions developed during heating.

I.3. Transitions during Gel Formation

Globular proteins taking a single conformation in their native state (N) are transformed to the unfolded or denatured state under certain conditions (e.g., heating). Achievement of the gel state (G) in globular proteins appears to involve transitions from the native to the unfolded state and into an aggregated state . This sequence of events give rise to the following reactions proposed by Schmidt (1981) for formation of thermally-induced protein gels, based on Ferry's (1948) two-step mechanism:

Step I:	$N \rightarrow D\ (U)$
Step II:	$D\ (U) \rightarrow A$
Step III:	$A \rightarrow S$
Step IV:	$S \rightarrow G$

Each of these fundamental steps postulated to be involved in gelation are discussed in further detail in the accompaning paper by Aguilera (1995).

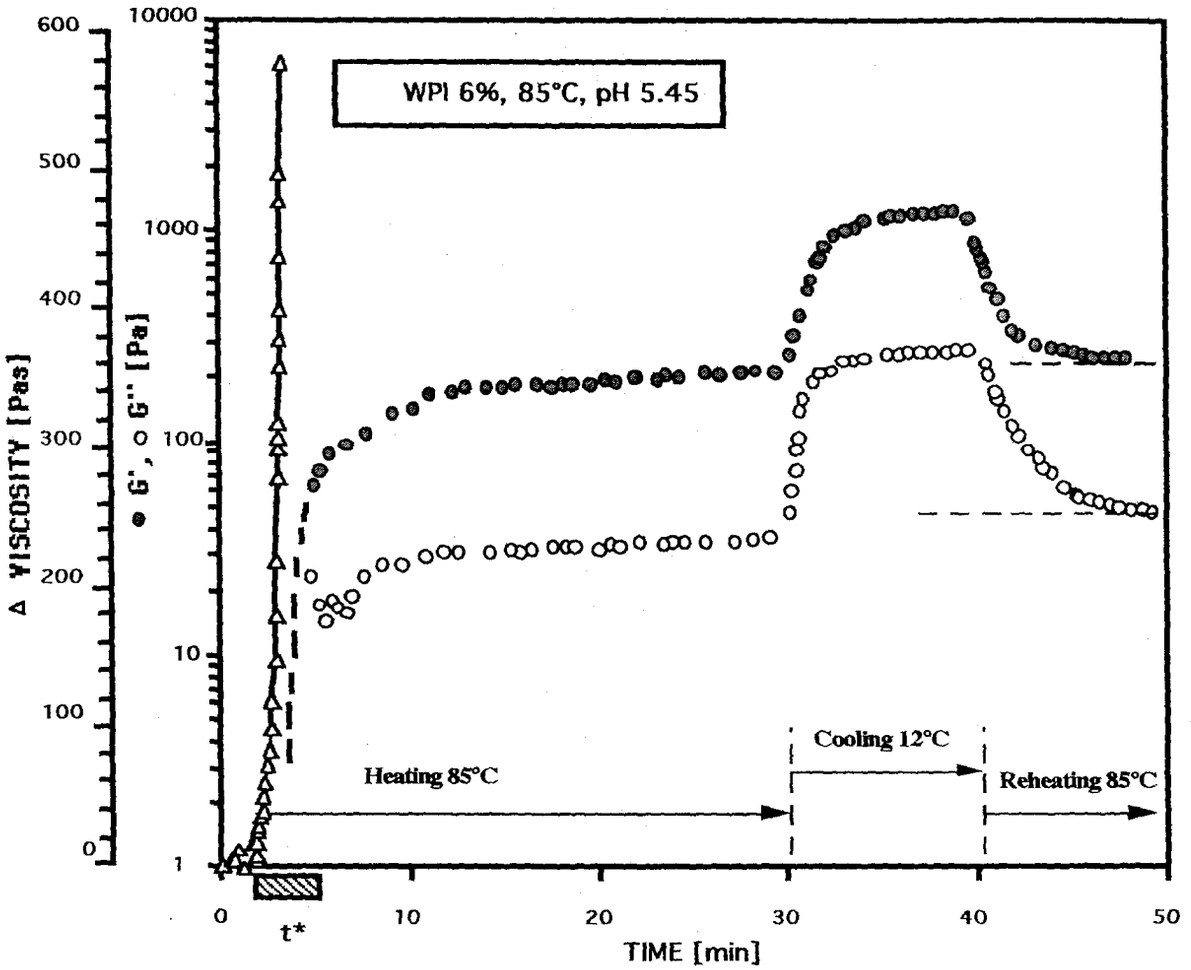


Fig.I.1- **The curing experiment**. Changes in shear viscosity, storage (G') and loss (G'') moduli during gelation of a whey protein isolate (pH: 5.45; T curing: 85°C; total solids: 6%) under cycling temperature conditions.

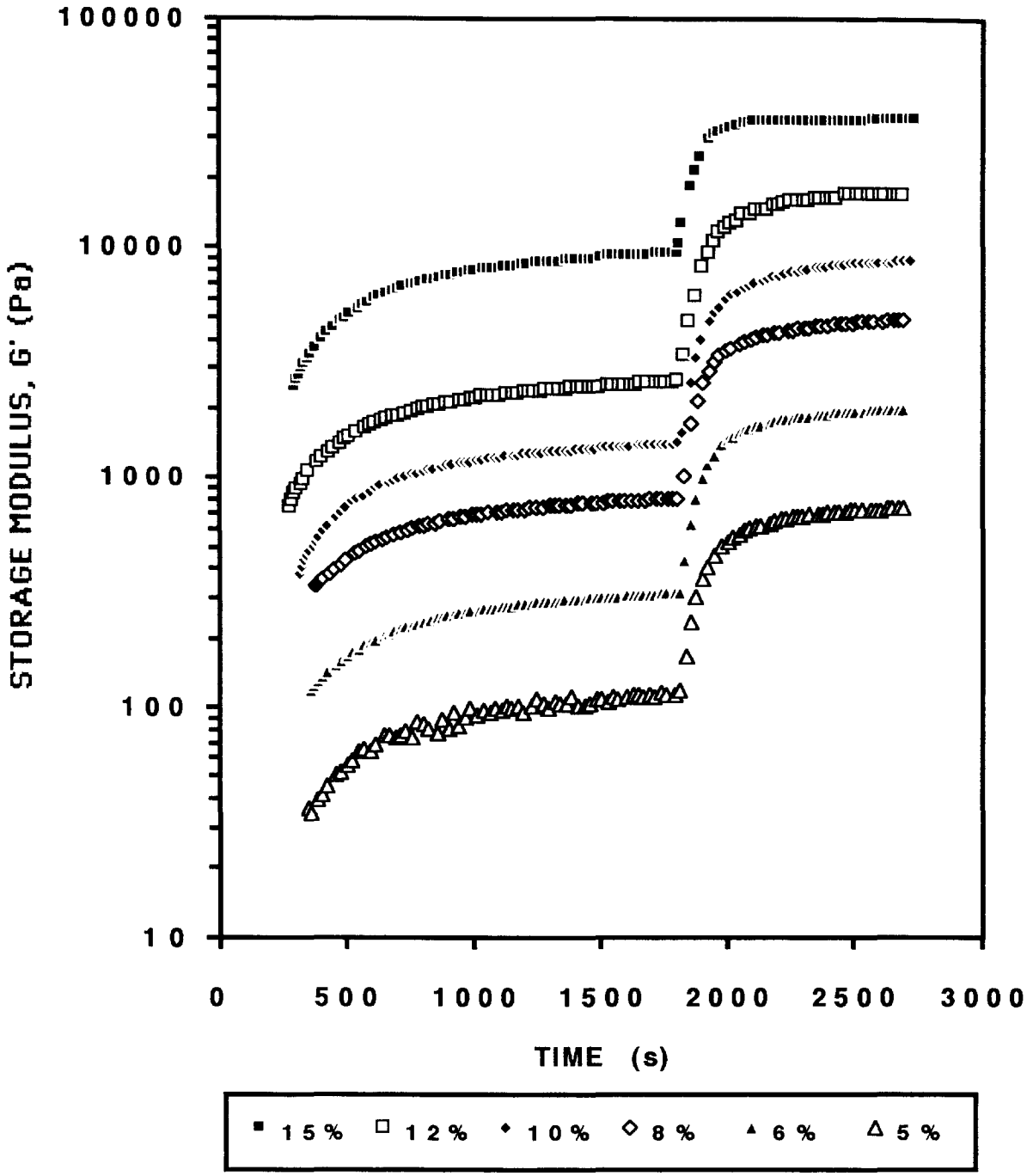


Fig. I.2- **Development of the Storage Modulus G'** of whey protein gels at different concentrations during heating for 30 min at 80°C, followed by cooling at 12°C (pH 5.45).

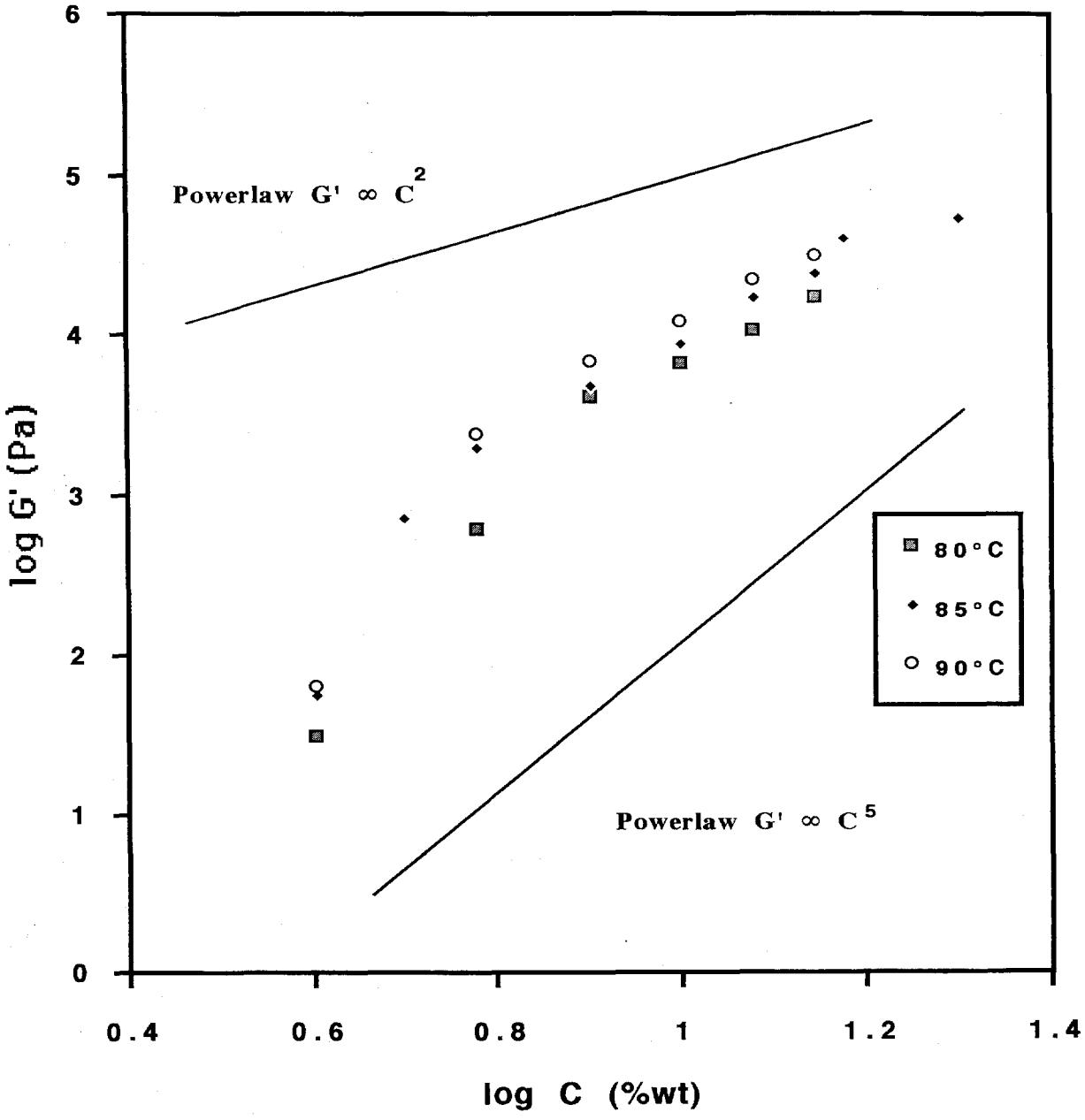


Fig. I.3- **Log G'** (of pseudo plateau after cooling at 12°C) vs log C data for whey protein isolate gels cured at different temperatures.

II. Rheological, thermal and microstructural properties of whey protein-cassava starch mixed gels

II.1. Introduction

Starch and proteins are responsible for most of the desirable characteristics of food products derived from cereals and other grains. An excellent example are the many textures of bakery products and their relation to the interactions of two biopolymers, wheat starch and gluten, in the presence of water. When a rubber-like polymeric system contains a substantial amount of water and is viscoelastic in nature, it is termed a gel and in many processed foods, protein and polysaccharides are found as mixed gels (Morris, 1991; Blanshard, 1988: Brownsey and Morris, 1988).

Starches are known to lose their crystallinity when heated in water at temperatures above 60-70°C and "gelatinize". During gelatinization, starch granules in suspension absorb water, swell and amylose leaches out into solution. Cooling converts the mixture into an opaque and viscoelastic "mixed" gel composed of swollen and "porous" starch granules embedded in an amylose gel (Morris, 1991; Biliaderis, 1992). The gel network appears to develop on cooling by the formation of ordered junction zones based on amylose double helices (Clark and Ross-Murphy, 1987). Whey protein solutions (concentration > 5-7% protein) form irreversible particle gels when heated to a temperature >75°C. The mechanism of thermal gelation of whey proteins involves an initial denaturation/unfolding step followed by aggregation into protein particles sticking together as a network (Mulvihill and Kinsella, 1987). Apparently, exposure of non-polar amino acid side chains on the surface of aggregates stabilize the network through hydrophobic interactions (Mitchell et al., 1990).

With the advent of novel separation techniques, purified and more functional sources of protein are now available to participate in complex gel systems. Cassava is and abundant source of carbohydrate used as food in many countries of South America and Africa but its study in mixed systems is largely unreported. In particular, knowledge of the rheological properties of a mixed starch/whey protein gel is important in understanding and predicting its flow properties during processing as well as the final texture of products (Biliaderis, 1992). Mixed gels of protein and non-starchy polysaccharides have been studied by Clark et al. (1983) and Braudo et al. (1986), among others. Starch is an ubiquitous source of calories in foods and a common filler in some comminuted meat and fish products. For example, addition of different starches to fish protein gels showed no apparent detrimental rheological

effects (Wu et al., 1985). However, specific interactions and synergistic effects are highly desirable to create new textures and accomplish ingredient substitution.

The objective of the present work was to characterize cassava starch/whey protein gels of thermotropic nature by mechanical and dynamic oscillatory rheometry and to interpret the results by studying their thermal transitions and microstructure.

II.2. Materials and methods

Materials

Whey protein isolate, WPI (BIPRO, Le Sueur Isolates, MN), 92.7% protein; commercial food-grade cassava starch, CS (FRABOL, Santa Cruz, Bolivia) with 0.1% protein and 0.3% lipids; distilled water. Corn starch (Corn Products, Santiago,Chile) was used as a reference in some experiments. NaCl and sucrose were of technical grade.

Preparation of gels

Blends of 10% total solids (TS) were prepared from stock solutions of WPI, CS and corn starch in distilled water, and adjusted to pH 5.75 with a 1M HCl solution. Sucrose and salt were added when required. Mixed solutions were kept agitated to avoid sedimentation of starch granules and transferred to glass tubes (100x12.3 mm diameter), sealed with rubber stoppers and held vertically in a thermoregulated water bath at 85±1°C (T_{gel}) for 30 min. Heating rate, measured at the axis of the tube with a fine thermocouple, was approximately 10-15°C/min. The tubes were cooled in an ice/water bath for 15 min and stored overnight at 5°C. Gels were removed from the glass tubes, equilibrated at 20°C for 1 hr and cut into cylindrical sections 11 mm long for mechanical testing using a template and parallel piano wires. All gelation experiments were replicated once.

Mechanical and rheological tests

Cylindrical sections of gels (at least four) were axially compressed to 5% deformation (within the elastic range) in a mechanical testing machine model TA.TX2 (Texture Technol. Corp., Scarsdale, NJ) at a deformation speed of 0.2 mm/s. An axial compression stress was calculated by dividing the force at 5% deformation by the initial cross section of the gels, since area changes during testing were negligible. Small amplitude oscillatory testing of solutions as a function of time at a constant temperature of 85°C was performed in a Physica rheometer model Rheolab MC 120 (Physica Messtechnik GmbH, Stuttgart, Germany) using a coaxial cylinder system geometry and a thermostated water bath. The storage and loss

modulus (G' and G", respectively) were determined at a frequency of 1 Hz and under a maximum deformation of 0.5% (within the linear viscoelastic regime) to assure preservation of the gel structure.

Calorimetry studies

To study the effect of heating on thermal transitions of pure and mixed solutions of CS and WPI a Perkin Elmer DSC-7 Series 1020 (Perkin Elmer Corp., Norwalk, CT) was used. Portions (15-20 mg) of well mixed solutions at 10% TS were transferred into aluminum DSC pans and immediately sealed. Duplicate samples were scanned at a heating rate of 10°C/min in the temperature range 40-95°C using an empty pan as reference. The heating rate was similar to that during gelation of solutions in the glass tubes.

Microscopy

Several gel samples in the range $0<\vartheta_S<1$ were examined under the microscope (only four are reported). Duplicate samples for microscopy were cut with a razor blade from the center of a gel and studied in a JEOL JSM 25 SII scanning electron microscope at 25 kV after fixation in 2.5% glutaraldehyde in 0.2M cacodilate buffer (pH 7.3), dewatered in a graded series of acetone solutions and dehydrated by critical point drying in a Sorvall unit. Dehydrated specimens were fractured, placed in metal stubs and the exposed surface was coated with gold.

II.3. Results and Discussion

Important variables in gelation of globular proteins are the concentration and pH of the sol, temperature and time. It is known that mechanical properties of WPI gels improve as the holding temperature increases above 75°C and that curing times of 15 min are sufficient to promote adequate gelation (Aguilera and Kessler, 1989; Aguilera and Kinsella, 1991). Results from axial compression tests for pure gels are presented in Figure II.1. The ratio of the standard deviation to the mean value of E varied between 3.8 and 7.2% for all determinations reported in this paper. Since a deformation of 5% falls within the elastic range, these values correspond to the elastic modulus, E. In the pH range 4-8, solutions (10% TS) of WPI formed strong gels below pH 5.8 and did not gel above pH 6.5. The CS solution did not form self-standing gels at any pH while corn starch formed weaker gels than WPI, almost independent of pH (Figure II.1, inset). Similarly, while WPI gels at pH 5.75 became stronger at increasing concentration of total solids, CS solutions did not form self-standing gels even at 15% TS. Corn starch showed an almost linear relation between E and concentration, for solutions > 5% TS (Figure II.1). Cassava starch had a low lipid content

(0.3%) that suggested no interference of amylose-lipid complexes in the mechanical properties. Conditions selected for the study of mixed gels were: 10% TS, pH 5.75, T_{gel} = 85°C and 30 min curing time.

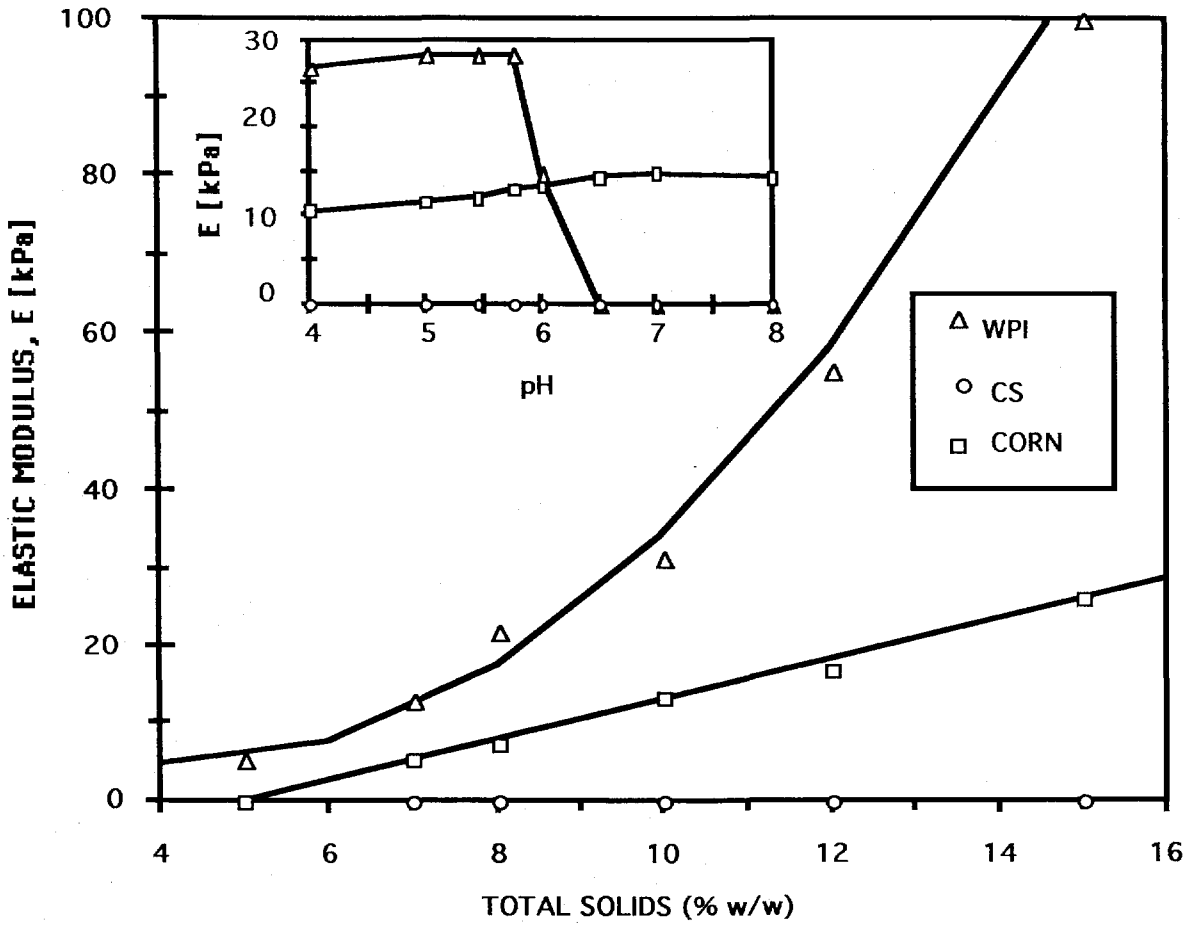


Fig. II.1- **Effect of total solids (TS) at pH 5.75 and pH at 10% TS (inset)** on the elastic modulus (E) of pure gels of WPI, cassava and corn starch, formed by heating the solutions to 85°C for 30 min and cooling.

Figure II.2 shows the effect of the starch fraction ϑ_S [starch/(WPI + starch), w/w] on the elastic modulus (E) of mixed gels at 10% TS and pH 5.75. Care has to be taken in comparing these values with others reported in the literature under larger deformation, as the force increases with strain (i.e., Aguilera and Kinsella, 1991). Substitution of WPI by CS in the range $0<\vartheta_S<0.4$ had the effect of increasing E over the value of the pure WPI gel (ϑ_S = 0). This synergistic effect had a maximum E at ϑ_S = 0.2-0.3 which was 1.6 times higher than

that for the WPI gel. Further replacement of WPI by CS in the range $0.3<\vartheta_S<0.7$ resulted in decreasing moduli and for $\vartheta_S > 0.7$ no self-standing gels were formed.

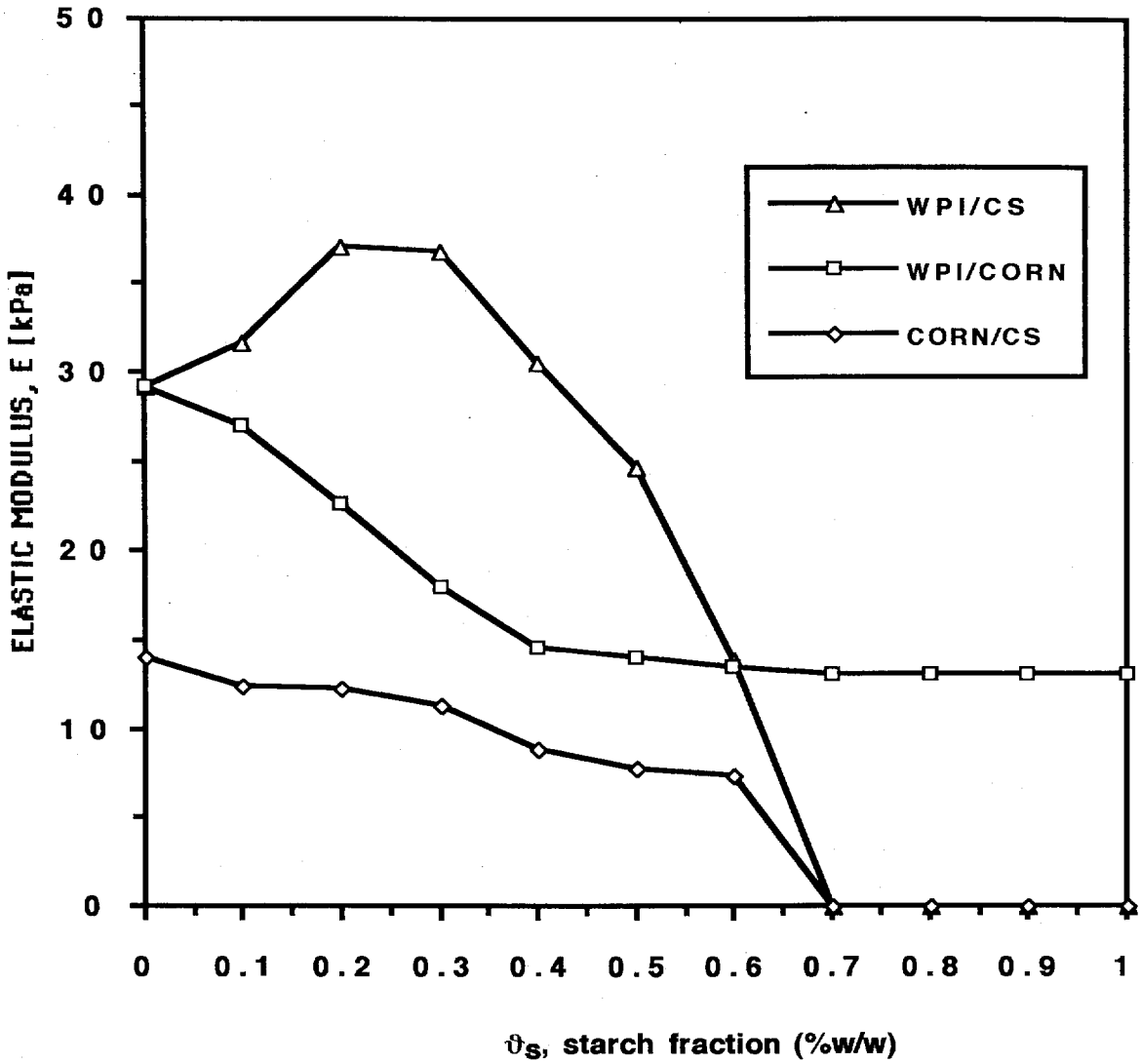


Fig. II.2- **Effect of starch fraction** ϑ_S [starch/(WPI+starch] on the elastic modulus (E) of mixed gels (10% TS, pH 5.75, T= 85°C).

The effect of ϑ_S on E for WPI/corn starch gels was different (Figure II.2). Increasing the starch fraction decreased E almost linearly from the value for pure WPI ($\vartheta_S = 0$) to one similar to that of pure corn starch at $\vartheta_S = 0.4$. Elastic modulus of mixed gels in the range

$0.4<\vartheta_S<1$ were similar to that of the pure corn starch gel, hence, WPI played the role of an inert, contributing to the TS effect. A weight fraction of WPI > 0.6 ($\vartheta_S = 0.4$) was required to develop associations that followed a mixing rule (e.g., linear dependence with concentration) but not to synergistic effects. Corn/CS blends yield gels weaker than those of pure corn starch and did not form gels for $\vartheta_S > 0.7$ (in this case ϑ_S refers specifically to the fraction of CS). It can be concluded that for $\vartheta_S > 0.7$ the non-gelling effect of CS predominates when mixed either with WPI or corn starch.

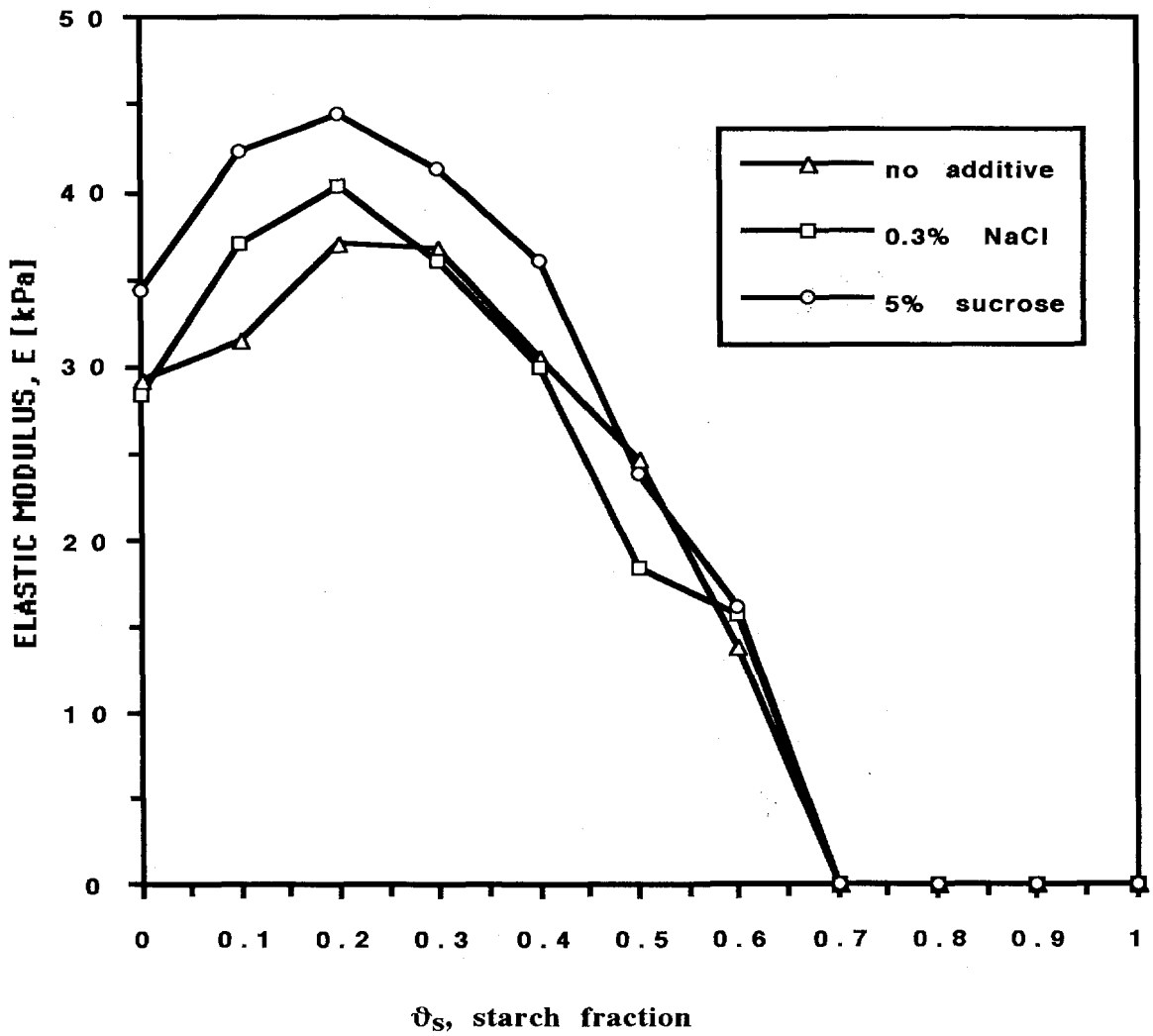


Fig. II.3- **Effect of starch fraction (ϑ_S) and addition of sucrose and salt** on the elastic modulus (E) of mixed WPI/CS gels. (10% TS, pH 5.75, T= 85°C).

It was of interest to observe the effect of incorporation of salt and sugar to mixed WPI/CS gels since food gels usually have these solutes added (Figure II.3). Addition of sucrose (5%) and salt (2%) did not alter the dependence of E with ϑ_S observed in Figure II.2 and led to higher E values. This further reinforcing effect can be ascribed to a reduced availability of water by the presence of these solutes and a concomitant increase in the effective concentration of both biopolymers (Labuza, 1975). Further work needs to be done on the effect of these two solutes in the mechanical and rheological properties of these mixed gels.

Dynamic experiments

Gelation kinetics were studied by small-amplitude oscillatory rheometry experiments at constant temperature (85°C) and monitoring the increase in the storage modulus (G'), the elastic (solid-like) component of the viscoelastic properties (Figure II.4). Data showed a rapid initial increase in modulus representative of the transition from sol to gel. At longer times the rate of increase of G' was slower, reaching an apparent steady value (pseudo-plateau) in the semi log plot. Values of G' were almost one order of magnitude higher than those of G", the loss module (not shown), typical of true gels. G' values reached at the pseudo-plateau region followed the same behavior with ϑ_S as E values of the axial compression tests, e.g., the highest G' corresponded to ϑ_S=0.2, and values for ϑ_S>0.5 were very low. These data suggest that structural features of mixed WPI/CS gels responsible for mechanical and rheological properties are already established at the moment of gelation and are not related to cooling or aging (retrogradation) phenomena.

Muhrbeck and Elliason (1991) measured values of the storage moduli of cassava starch/ bovine serum albumin (BSA) gels as a function of protein fraction (1 - ϑ_S) at 10% TS, heated to 90°C and cooled to 25°C. As reported here, gels with ϑ_S > 0.6 showed low G' (< 0.1 kPa) and when the protein fraction increased (ϑ_S decreased) the modulus increased sharply. Unfortunately, no values were reported by these authors in the range $0<\vartheta_S<0.25$.

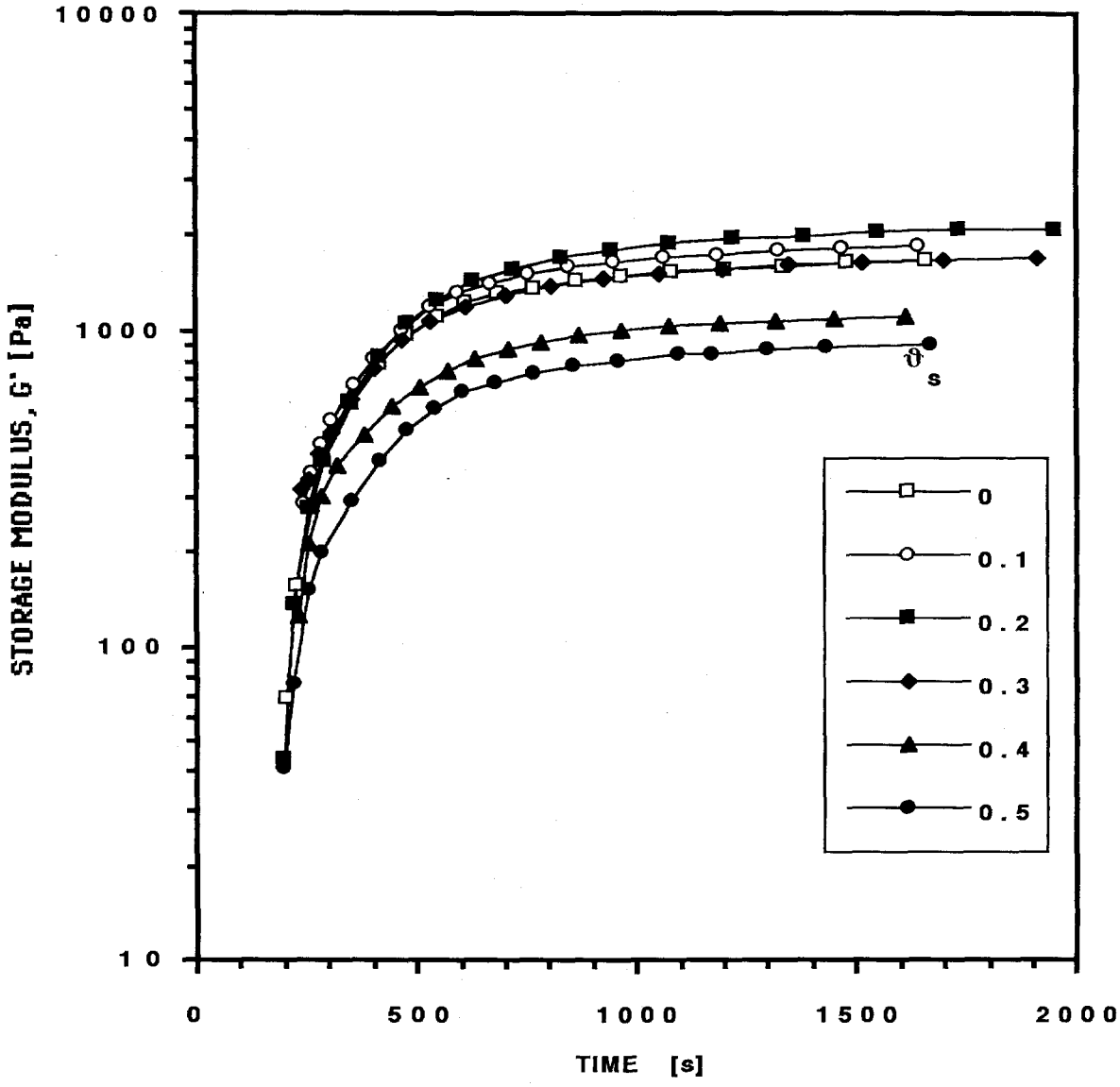


Fig. II.4- **Effect of starch fraction (ϑ_s) on the storage modulus (G')** of mixed WPI/CS gels. (10% TS, pH 5.75, T= 85°C).

Thermal properties of gels

DSC scans were performed to find out the effect on gelation of the presence of one biopolymer on the thermal transitions of the other. Preliminary thermograms (not reported) of

a WPI solution (10% TS) showed that the peak of the denaturation endotherm shifted from 82.2 to 74.3°C as pH increased form 4 to 6. At pH 6.5 no denaturation peak was found, coincidental with absence of gelation reported in Fig. II.1.

Thermograms of WPI/CS solutions of 10% TS and pH 5.75 are presented in Figure II.5. The pure CS solution (ϑ_s = 1.0) showed a single endothermic peak at 66.0°C, coinciding with early DSC work of starch-water mixtures in excess water where only one endotherm represented the starch "gelatinization" process (Lelievre, 1992). This value is the same as the midpoint gelatinization temperature (T_g) reported by Zobel (1988) for tapioca (cassava). A broad denaturation peak between 66 and 79°C was evident for the pure WPI solution (ϑ_s = 0.0) corresponding to a "denaturation" temperature (T_d) for WPI of 74.5°C, close to the value of T_d = 78.8°C (extrapolated at 0°C/min heating rate) reported by Bernal and Jelen (1985) for a whey protein concentrate at pH 5.5. As shown by these same authors, T_d decreases by increasing pH or reducing the heating rate and is dependent on the relative proportions of β-lactoglobulin (T_d = 75.9 - 81.2°C), α-lactalbumin (T_d = 61.0 - 61.5°C) and serum albumin (T_d = 71.9-74.0).

Only the denaturation peak near T_d of pure WPI predominated for ϑ_s=0.1 and single gelatinization peaks close to T_g of pure CS were notorious for solutions of $\vartheta_s \geq 0.8$. Hence, the behavior of the thermal property of these blends may be regarded as typical of pure WPI or pure CS solutions, respectively. Instead, solutions of $0.1<\vartheta_s<0.8$ showed two peaks: a low-temperature peak around 64.8-65.3°C and a high-temperature peak between 73.8-74.3°C, as if both, gelatinization of starch and denaturation of proteins, occurred unaffected by the presence of the other component. A special feature of blends with ϑ_s = 0.2 and 0.3 was a well developed T_d peak and a smaller but noticeable T_g peak. In solutions with a higher proportion of CS (e.g., $0.3<\vartheta_s<0.7$) the gelatinization peak outweighed the contribution of the denaturation peak, which correlated well with the poorer gelling properties of these mixtures.

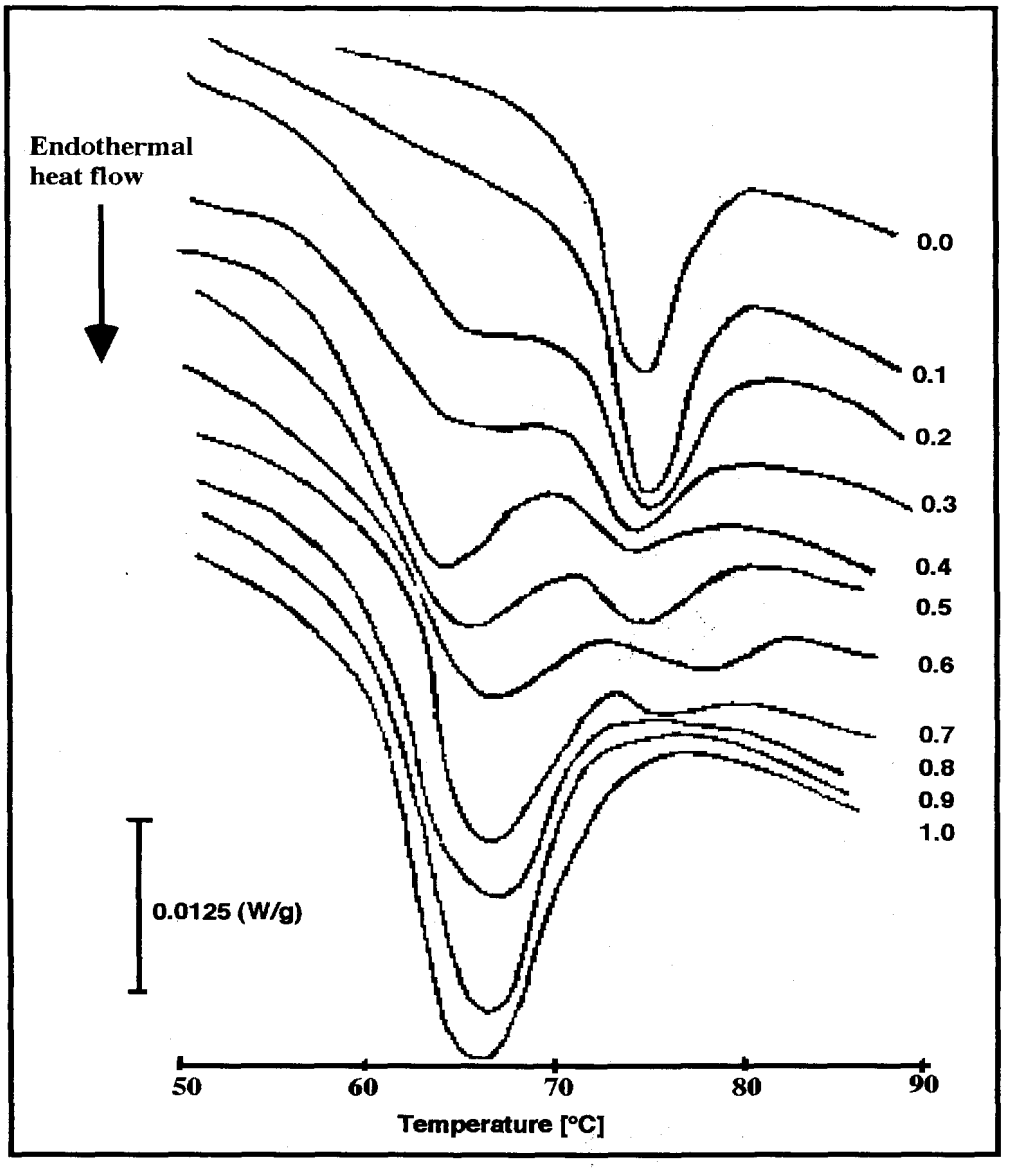


Fig. II.5- **Tracings of DSC thermograms** for WPI/CS blends (10% TS, pH= 5.75) as a function of starch fraction (w/w).

Microstructural aspects

The microstructure of gels as studied by scanning electron microscopy is presented in Figures II.6 and II.7. In the single component WPI gel proteins aggregated into almost spherical units 0.2-0.3 μm in diameter which in turn packed into clusters forming the network (Figure 6A). This basic microstructure is typical of gels from whey protein

(Aguilera and Kinsella, 1991) and β-lactoglobulin (Stading et al., 1993) formed under similar conditions of pH, concentration and heating rate.

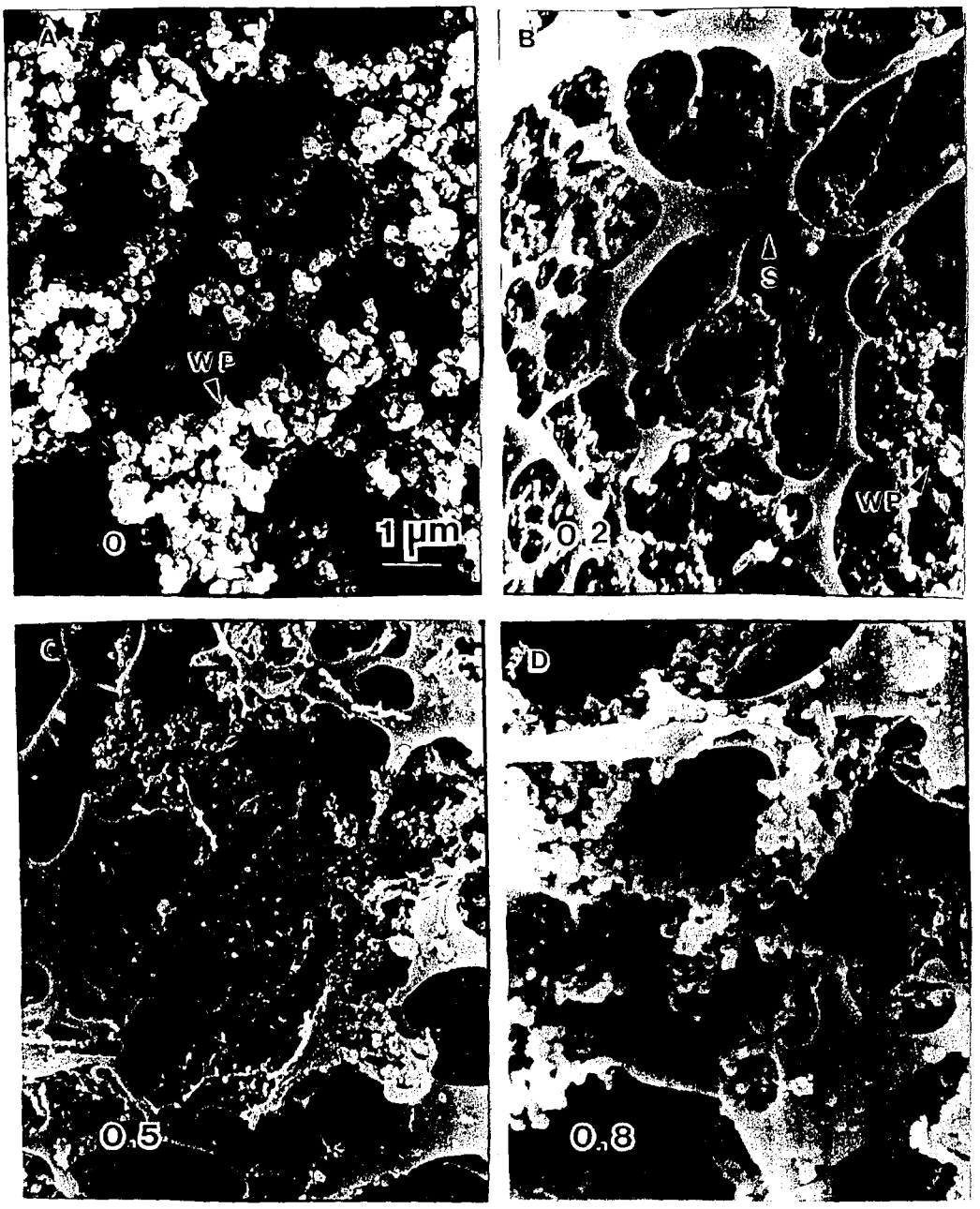


Fig. II.6- **SEM micrographs of gels** with starch fraction (w/w) indicated in the lower left corner. (WP = whey protein isolate network; S = starch network).

The WPI/CS gel at $\vartheta_S = 0.2$ showed that both components formed independent and well-developed networks of an interpenetrating nature. This microstructural feature is in opposition to the suggestion by Muhrbeck and Eliasson (1991) that the predominant network in a mixed gel is that formed first during heating, in this case CS for $T_g < T_d$, and supports the argument that kinetic aspects of gelation as well as phase separation may be determinant in the development of microstructure. The CS mesh appeared as smooth and fine strands independent of the WPI particle-type matrix, and the presence of remnants of starch granules could not be found, as if they disintegrated completely during heating. Mixed gels of ϑ_S = 0.5 and 0.8 (Figure II.6C and II.6D) also exhibited independent WPI and cassava starch networks and no remnants of starch granules. In these gels the extent of the WPI matrix was reduced and frequently disrupted by the CS network, in agreement with the poorer mechanical and rheological properties exhibited. At $\vartheta_S = 0.8$ the microstructure was typical of a phase-separated or composite gel network rather than of an interpenetrating one (Aguilera, 1992) with portions of gelled WPI entrapped in the weaker CS network.

Figure II.7 presents the microstructure of WPI/cassava starch and WPI/corn starch gels at $\vartheta_S = 0.2$ prepared under the same conditions. While interpenetrating WPI and CS networks were clearly observed in the WPI/CS gel (Fig. II.7A), the mixed gel containing corn starch was of the filled-type, with collapsed starch granules acting as embedded particles in a predominantly WPI gel (Fig. II.7B). Also noticeable was the lack of adhesion between the surface of shrunk corn starch granules and the WPI matrix and the absence of an independent amylose network. These two features may account for the poorer mechanical properties of this gel relative to the WPI/CS gel (Fig. II.2). Further work is necessary to extend these results to other gelling conditions.

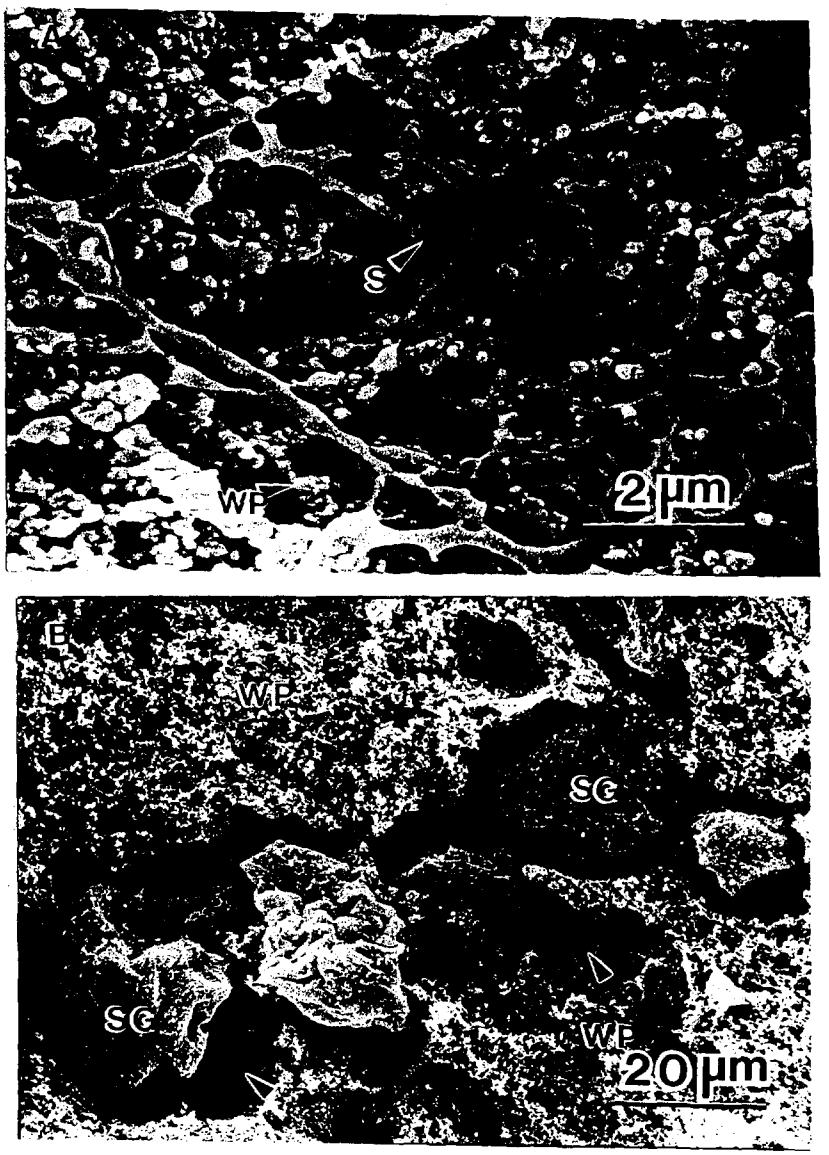


Fig. II.7- **SEM micrographs of mixed gels** (ϑ_S= 0.2). A) WPI/cassava starch gel, and B) WPI/corn starch gel. Arrows point at separation between starch granules and matrix. (WP = whey protein isolate network, S= starch network, SG = starch granules).

Relation of thermal and mechanical data with microstructure

Gelation of mixed biopolymer solutions leading to structure formation is still not fully understood and remains mostly on a conjectural basis. The process appears to be heavily dominated by phase-separation phenomena as it is known that most biopolymer systems are thermodynamically non-compatible. Brownsey and Morris (1988) have classified two-component mixed gel networks as Type I, when a soluble polymer is entrapped inside the network of the other, and Type II, where both biopolymers form a gel network. In this latter case the spatial distribution of the two components will depend on the degree of mixing/demixing during gelation.

It can only be speculated at this point that amylose gelation in CS at low ϑ_S is possible by rapid solubilization from disintegrated cassava starch granules, demixing and phase separation into a polymer rich phase. Partial support to this hypothesis may come from ample evidence by Russian scientists that most protein/polysaccharide systems form phase-separated solutions (Braudo et al., 1986). Specifically, there are suggestions that amylose gelation in particular, occurs via aqueous two-phase separation into polymer-rich and polymer-deficient regions leading to turbid gels (Miles et al., 1985; Clark and Ross-Murphy, 1987). Formation of a continuous network of CS at low and not at higher ϑ_S may be interpreted by the excluded volume effect as proposed by Braudo et al. (1986). As the presence of whey proteins in solution increases, water may become a poorer solvent for starch (which gelatinizes first), increasing its effective concentration in one of the microphases and eventually reaching the concentration threshold for gelation and network formation.

There are a number of physicochemical differences between corn and cassava starches that may help explaining their different gelling behavior (Balagopalan et al., 1988). Cassava starch has a slightly lower gelatinization range than corn (59-70°C versus 62-73°C). CS granules swell on hydration to a diameter almost 3 times larger than corn starch granules and continue to swell after pasting (Wurzburg, 1986; Vasanthan and Hoover, 1992). Paste viscosity of a cassava starch slurry as followed in a Brabender amylograph peaks at around 75-80°C and upon cooling it readily breaks down (Eggleston et al., 1993). Corn starch solutions instead, develop into a low viscosity paste on heating but show a high peak viscosity on cooling (retrogradation). Also, amylose is released faster from cassava starch granules than from corn starch granules (Vasanthan and Hoover, 1992).Sols of corn starch having a relatively high level (ca. 22-28%) of moderately-sized amylose molecules form opaque gels on cooling while cassava starch which has a lower amylose content (ca. 18%)

but bulkier amylose molecules only thicken to a translucent paste on cooling but do not form gels (Wurzburg, 1986).

II.4. Conclusions

As revealed by SEM and thermal analysis, a commercial whey protein isolate and cassava starch mixed in solutions at 10% TS and pH 5.75 appear to form a mixed gel with independent networks when heated at 85°C. Elastic moduli (E) and storage moduli (G') of mixed gels were maximum at $\vartheta_S = 0.2$, where both biopolymers formed continuous, interpenetrating networks and gelatinization of CS and denaturation of WPI occurred to a noticeable extent. Kinetics of development of the storage modulus suggests that physical and structural characteristics of mixed gels develop already during heating and not on cooling. Basic microstructures may be induced in mixed WPI/starch gels by varying the relative proportions of the two components and the nature of the starch fraction. These microstructures are responsible for several mechanical and rheological properties of mixed gels.

III. Gelation kinetics of cassava starch/ whey protein blends as followed by dynamic oscillatory rheometry

III.1. Introduction

Kinetics of gelation for heat-set gels of cassava starch (CS) and a whey protein isolate (WPI) are reported based on small deformation oscillatory shear rheological measurements. Loss moduli (G´) of pure gels were followed with time as a function of concentration (4-14%) and temperature (70-90°C). Gelation of WPI was modeled as a single first order reaction while gelation of CS followed a two step reaction with a reversible step.

Kinetics of formation of mixed gels (10% total solids, pH 5.45) having different proportions of CS ($0<\vartheta_S<1$) were followed by dynamic and mechanical methods. Unusual reinforcing effects were found for $\vartheta_S < 0.3$, particularly since CS does not gel at these low concentrations. Properties of mixed gels depended also on the thermal history of the curing experiment, with gelation of WPI being the critical step. Microstructures of synergetic mixed gels showed interpenetrating individual networks of CS and WPI, suggesting that phase separation occurs during gelation.

IV. Interpretation of mechanical properties of mixed CS/WPI gels

IV.1. Introduction

Data previously reported on the dependence of the elastic modulus (E) on starch fraction (ϑ_S) showed synergistic effects at $\vartheta_S < 0.3$ (see section II). It is known that starch granules initially dispersed in water hydrate and swell as they are heated. Cassava starch has a gelatinization range from 59 to 70°C where granules swell to a diameter 6 to 7 times their original size and continue to expand after pasting (Wurzburg, 1986; Vasanthan and Hoover, 1992). Paste viscosity of a cassava starch slurry as followed in a Brabender amylograph peaks at around 75-80°C and upon cooling it readily breaks down (Eggleston et al., 1993). Also, amylose is rapidly released from cassava starch and only thicken to a translucent paste on cooling but do not form gels (Wurzburg, 1986). All this background suggests that as a mixed WPI/CS solution is heated above 60°C, starch granules swell and imbibe part of the water that becomes unavailable for the solution, increasing the effective concentration of WPI in the remaining aqueous phase. It may be speculated also that some amylose may leach out and interact with the WPI.

IV.2. Materials and Methods

This hypothesis was tested by a hot-stage microscopy study carried out at Unilever Research Laboratories, Colworth House (Bedford). A Leitz Orthlux II microscope equipped with a hot-stage, Linham TMS 91 temperature programming device and JVC video camera (3-CCD) model KY-F30B was used to observe in a video monitor, record and photographed. A drop of solution (or blend) was placed on a 32mm diam. glass cover slip and covered with a 22mm cover slip, press, blotted to remove water and sealed on the edges with nail varnish to avoid dehydration. All experiments started at 40°C at a heating rate of 30°C/min to a final temperature of 85°C. Filters were used to get birefringence or enhanced contrast.

IV.3. Model

The mechanical model considers a two phase system: hydrated/swollen starch granules embedded in a WPI network of effective concentration higher than the nominal concentration in the starting solution. The equation proposed for the composite is:

$$\mathbf{E}_{Comp} = (1-\vartheta_S)\ \alpha\ \mathbf{E}_{WPI} + \mathbf{E}_{CS}$$

where $\mathbf{E}_{Comp}$ = Elastic modulus of the mixed gel (or composite)

ϑ_S = weight fraction of CS in total solids of mixed gel

α = correction factor for changing from nominal to effective concentration of WPI.

$\mathbf{E}_{WPI}$ = Elastic modulus of the WPI gel at nominal concentration

$\mathbf{E}_{CS}$ = Elastic modulus of the starch gel at nominal concentration

IV.4. Results and Discussion

Hot-stage microscopy clearly showed that starch granules imbibe water and swell after heating above approx. 65°C (Figure IV.1). When the temperature reaches 75°C WPI starts to gel around already swollen starch granules and if the relative proportion of WPI is much larger than that of CS (e.g., $\vartheta_S < 0.3$) a composite gel of a WPI network filled with swollen CS granules is formed when the final temperature of 85°C is reached. Hence, the unusually high E value of WPI/CS gels at $\vartheta_S < 0.3$ is, at least partly explained, by an increase in the E of the WPI network due to its higher effective concentration at the gelation point (75 to 85°C) and by the reinforcing effect of embedded hydrated CS granules in the WPI gel. Not reported here is extensive microscopy work performed by SEM, TEM and cryo-SEM that supports the hypothesis that WPI and CS gel as separate phases. Gels high in CS are weak because the strong WPI is dispersed as microgels in a continuous starch phase.

A correction factor α was adjusted from experimental data for total solids concentrations of 10, 12, 15 and 18% and the range $0<\vartheta_S<0.5$. Figure IV.2 shows the relationship between the nominal WPI concentration in solution and the effective WPI concentration as derived from mechanical property data and the dependence of E on WPI concentration. Figure IV.3 depicts the prediction of the model and experimental data for a mixed gel having 10 - 18% total solids.

In conclusion, a structural mechanical model has been developed based of microscopic observation for mixed WPI/CS gels. This model can be used to tailor gels to specific "textures" simulating specific local foods by addition of appropriate colors, flavors and condiments.

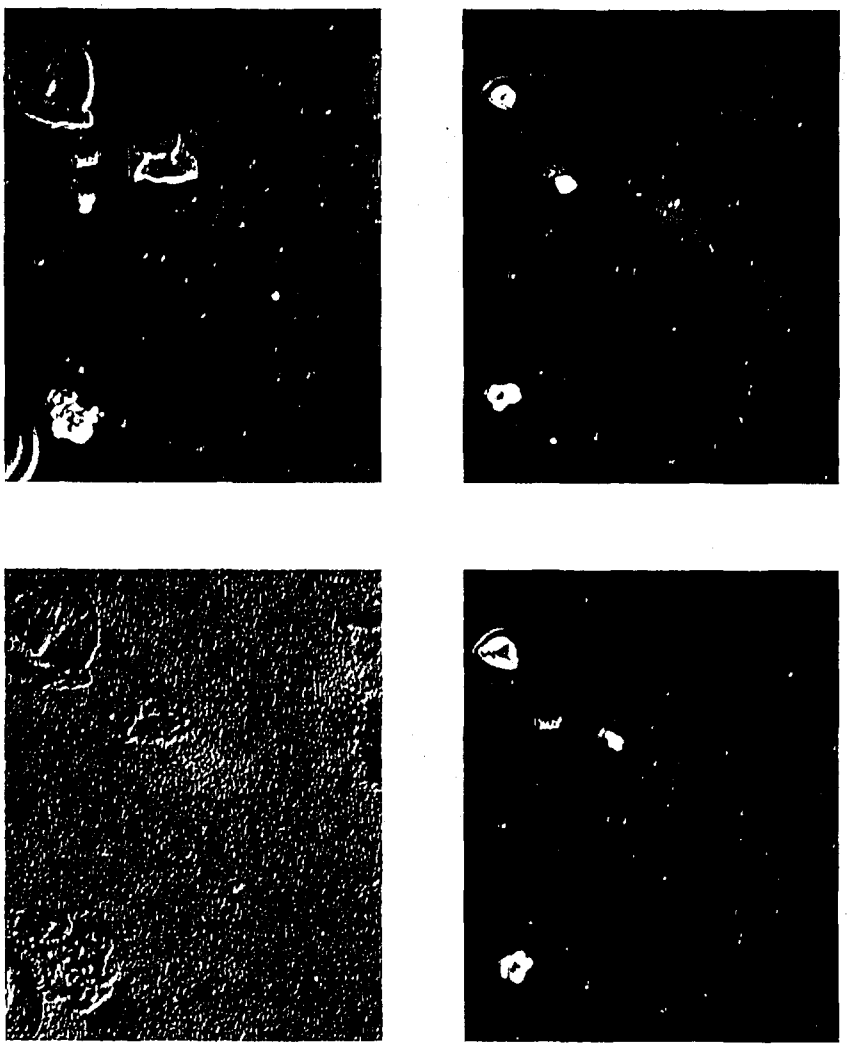


Fig. IV.1. **Hot-stage microscopy** of a WPI-CS blend heated at 85°C.

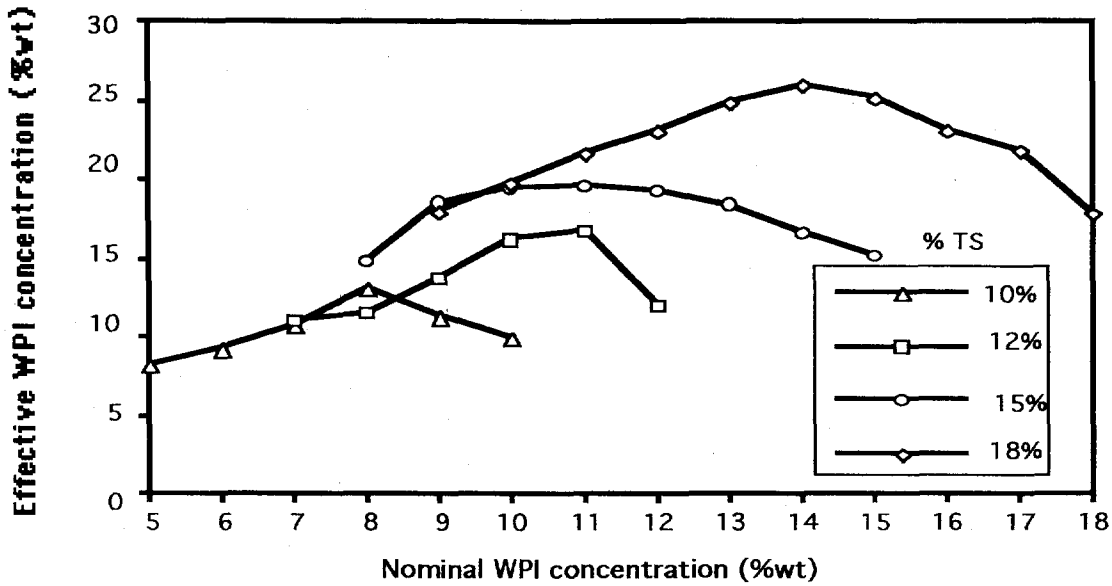
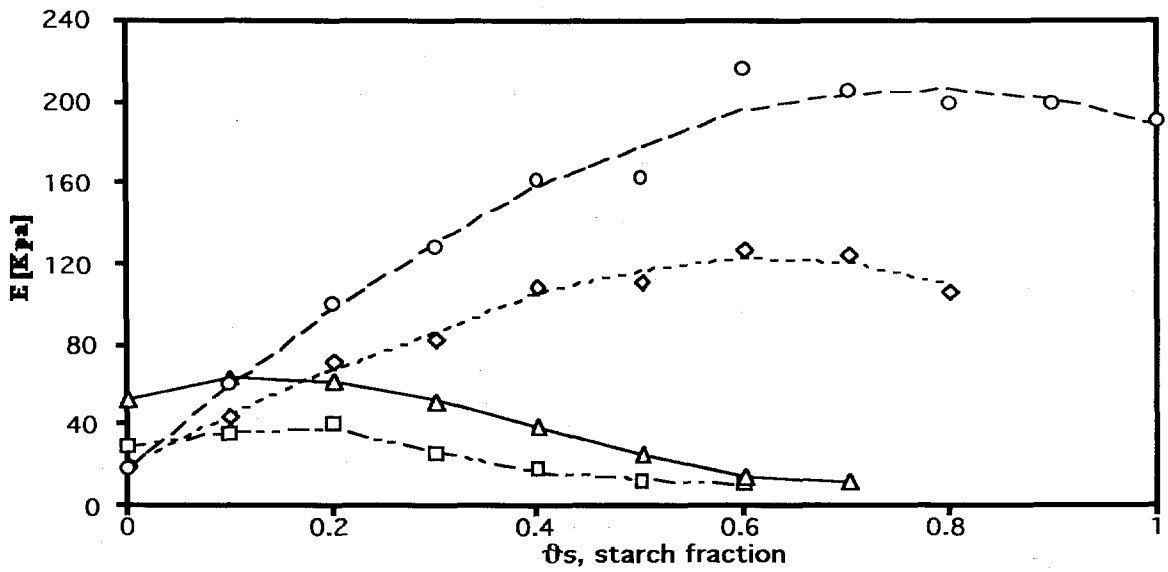


Fig. IV.2- **Relationship between nominal and effective WPI concentration** as derived from mechanical property data



TS%(wt)	10%	12%	15%	18%
experimental data	□	△	◇	○
model prediction	— · — · —	————	- - - - - - -	— — — —

Fig. IV.3- **Comparison of the E values obtained by the model prediction and experimental data** for mixed gels having 10-18% total solids. (pH 5.75)

V. Gelation of surimi and κ-carrageenan blends

V.1. Introduction

Surimi is the Japanese term for a high-protein paste made from fish muscle that forms a gel called *kamaboko* when heated to 80°C (Susuki, 1987). This fabricated gel is of economic and nutritional relevance to less-developed countries of the Pacific coast of South America where over 90% of fish catches go to fish meal. Surimi gels produced from the main species (fatty pelagic fish) are of poor textural quality and color

κ-carrageenan (κ-C) is a natural polysaccharide of high molecular weight extracted from red algae and formed from repeated units of sulfated galactose and 3,6 anhydrogalactose. κ-C forms rigid and strong thermoreversible gels of low syneresis and sensitive to K and Ca ions. When heated κ-C goes into solution and upon cooling it gels. Chile is a producer and exporter of κ-C which is being increasingly used as fat binder, water holding agent and in meat products.

The objective of this study was to produce improved fish gels from local ingredients based on the properties of κ-C and the effect of KCl.

V.2. Materials and Methods

κ-C was obtained from FMC (Philadelphia, PA) to have a standardized sample. Surimi was prepared from fresh horse mackerel *(Trachurus murphyi)* by a procedure described in Aguilera et al. (1992). Methods for determination of mechanical and rheological properties, thermal transitions and microstructural aspects were similar to those described previously.

V.3. Results and Discussion

Figure V.1 shows how the storage modulus G' changes as κ-C is added to fresh fish paste during a heating/cooling cycling. Two very interesting effects take place: first, as heating occurs, gelation of myofibrillar protein is paralleled by solubilization of κ-C, which ensures good mixing at the microstructural level and formation of a strong matrix on cooling. Second, after cooling a significant strengthening effect takes place even at low concentrations of κ-C (0.5%) and at 2% the modulus increases to almost 8 times that of the pure kamaboko

gel, reaching a value close to 70 kPa. Part of the reinforcing effect comes also from the water binding effect of the hydrocolloid which leaves less hydration water for protein gelation (Wu et al., 1985).

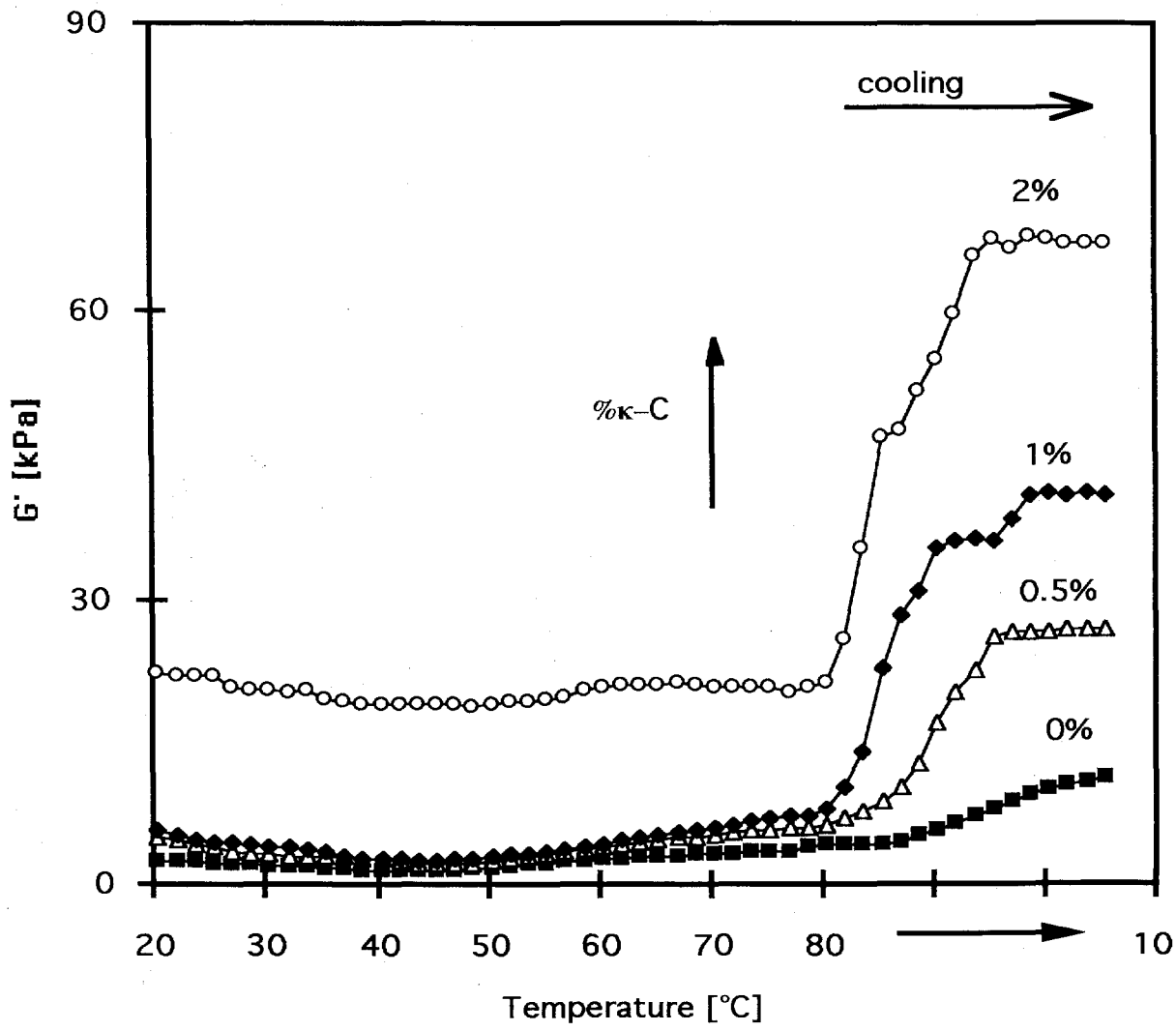


Fig. V.1- **Development of the Storage Modulus G'** of fresh surimi with different κ-C content during heating.

Figure V.2 summarizes the effect of addition of KCl, κ-C and both additives combined on the elastic modulus (E) based of fresh surimi (20% total solids). Addition of KCl increases slightly the E when added up to 2%, while κ-C increases drastically the

strength of the mixed gel after addition in the same proportion. Interestingly, only a combination of κ-C (2%) and KCl (0.5%) further increased the rigidity of the gel.

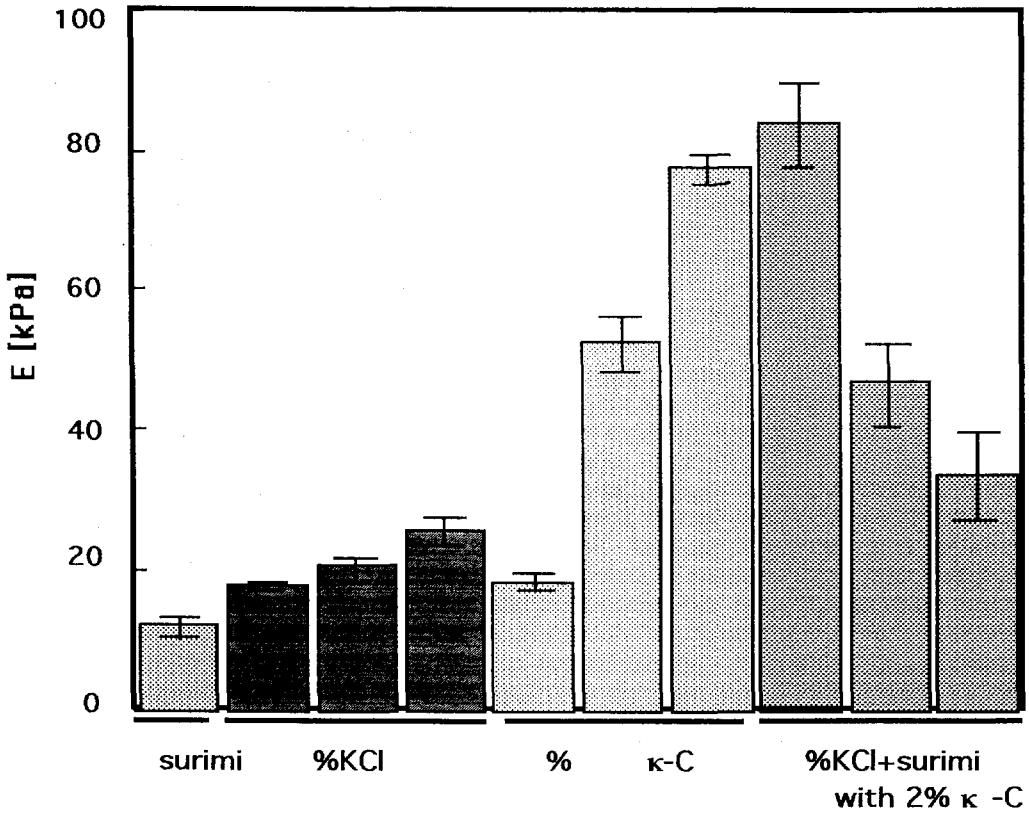


Fig.V.2- **Effect of addition** of KCl, κ-C and both additives combined on the elastic modulus (E).

Figure V.3 is a schematic of all relevant findings of this study, including interpretation of SEM microscopy. It can be concluded that much firmer fish gels can now be manufactured from low-cost fatty fish by addition of κ-C and KCl. These results should be of enormous importance to the fish processor in that restructured fish products of good textural properties can now be manufactured for food uses.

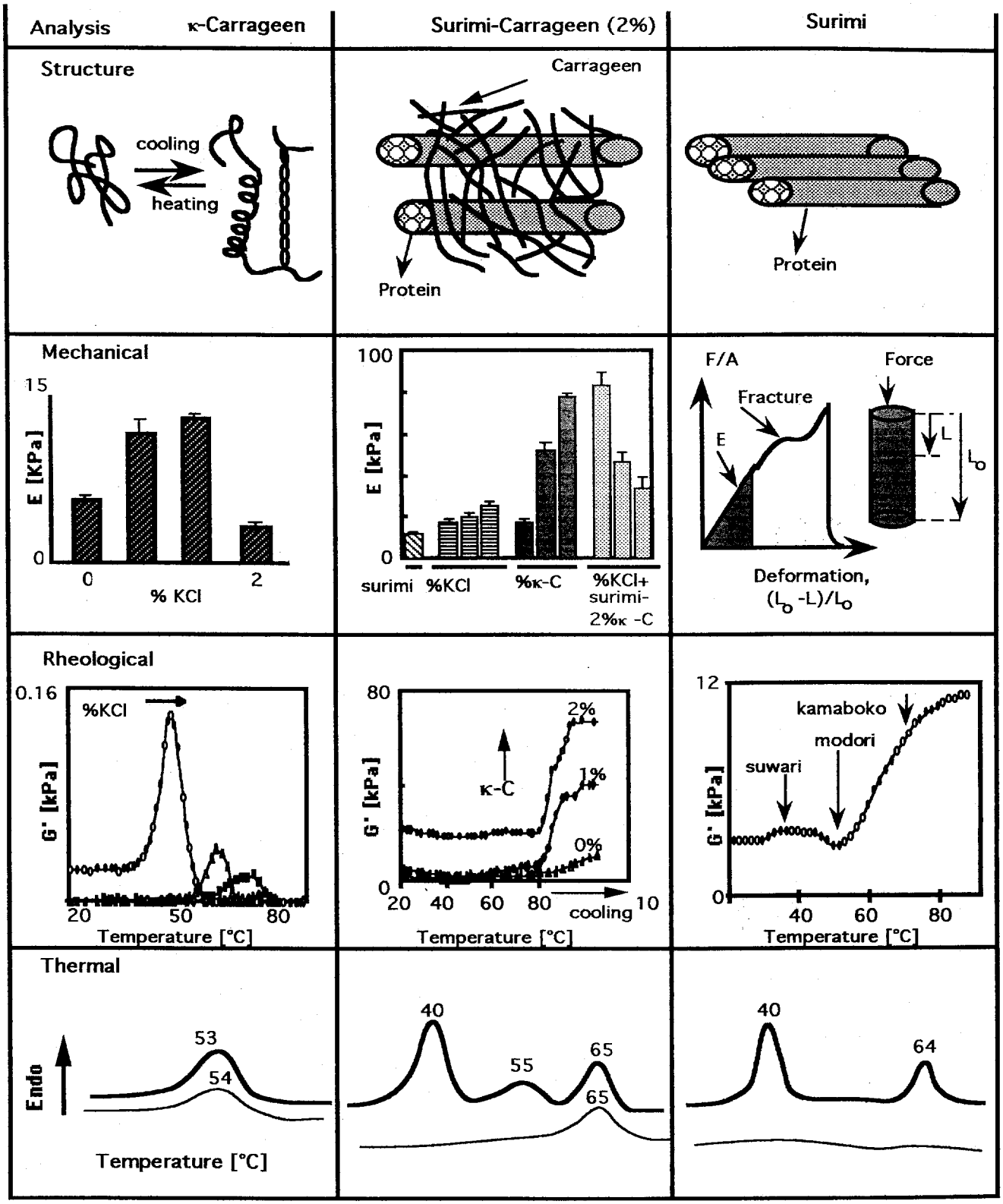


Fig. V.3- Scheme of the relevant findings for the gelation of surimi, κ -carrageenan and their blends.

IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

The project is likely to have an important impact in the development of new structured foods in Latin America. Results are being disseminated at the academic level in national and international congresses and scientific publications in top scientific journals. In the near future a course will be offered to the Chilean and Bolivian food industries on structuring of foods and the use of modern equipment for quality evaluation and control. Technology transfer to other LA scientists has been done through RIPFADI (the Ibero American Network on Physical Properties of Foods) by courses on gels in Brazil, Argentina and Uruguay. In the near future a course will be offered to the Chilean and Bolivian food industries on structuring of foods and the use of modern equipment for quality evaluation and control. Food technology research in Latin America is now being regarded as a source of novel applied concepts in structured foods. The PI has been invited by Nestle Research Center near Lausanne to spend four months working on gels and structured foods with the famous Russian scientist Prof. Tolstoguzov.

During the period of the project the PI received two important distinctions: The 1993 International Award of The Institute of Food Technologists of the US, the first Ibero American to receive this award in 35 years, and the Berger Distinguished Lectureship in International Food Science Development at Cornell University.

A total of 6 students were trained by the project (4 MSc and 2 Engineer degrees). The technician partly funded by AID is now in charge of the Biomaterials Lab on a half-time basis.

Institutional linkages have been developed with UAGRM and other universities in Bolivia through annual visits. As result of this project courses have been offered by professors from the Chemical Engineering Department at universities of Tarija and Oruro with an estimated total of 200 participants. Graduates of the program are now assistant professors in the Department of Food Engineering, UAGRM, participate in industry-university projects and use equipment bought for research and teaching. Interviews to Drs. Salek and Aguilera were telecast through regional TV. The Consulate of Bolivia in Santiago has invited Prof. Aguilera to several cultural and scientific meetings.

The project has been very important to consolidate the Biomaterials Laboratory at the Department of Chemical Engineering. This is probably the most updated food materials

laboratory in Latin America under one single roof. The equipment gathered in the last five years include a Perkin Elmer DSC-7 differential scanning calorimeter, a computerized Physica rheometer with dynamic oscillatory capabilities, a JEOL 5300 scanning electron microscope and sample coating apparatus. The project has been instrumental in a recent grant awarded by the University to the PI (US$ 30,000) to upgrade the SEM facility. Modern image gathering equipment, a color video monitor and VCR, image analysis and photography software will be purchased. All these equipment will continued to be used by other researchers from the University, graduate students from national and Latin American institutions.

PROJECT ACTIVITIES/OUTPUTS

Papers published in international journals

Aguilera, J.M. 1995. Gelation of whey proteins. *Food Technology* 49(10):83-89.

Aguilera, J.M., Rojas, E. 1996. Rheological, thermal and microstructural properties of whey protein- cassava starch mixed gels. *J. Food Sci.* (in press)

Papers in preparation for international journals

Aguilera, J.M. y Rojas G. Gelation kinetics of cassava starch/ whey protein blends as followed by dynamic oscillatory rheometry. In preparation for *Food Hydrocoll.*

Aguilera, J.M. and Ortiz, J. Rheological and thermal behavior of surimi/κ-carrageenan mixed gels. In preparation

Papers in books and Proceedings

Aguilera, J.M., Rojas, E. y Lozano, S. 1994. Rheological, mechanical ad microstructural properties of protein-starch gels. *Proceedings of the Fourth European Rheology Conference*, pp. 209-211.

Invited Conferences

Aguilera, J.M. Reología y microestructura de geles mixtos almidón-proteina. Simposio Internacional sobre Proteinas em Alimentos, Sao Paulo, Brazil 18-20 de Abril, 1995. Invited Conference.

Aguilera, J.M. 1994. Gelation of whey proteins. Invited Symposium paper. 1994 IFT Annual Meeting, Atlanta, GA

International Conferences

Aguilera, J.M., J.Ortiz, G. Rojas, H. Jorquera, P. Baffico. Rheological and thermal behavior of surimi/κ-carrageenan mixed gels. I Congresso Ibero-Americano de Engenharia de Alimentos, Campinas, Brasil 5-9 Noviembre, 1995.

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National Conferences

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Aguilera, J.M. y Ortiz, J. Efecto de la adición de kappa carragenato en el proceso de gelificación de surimi de jurel con distinto contenido de KCl. XI Congreso Nacional de Ciencia y Tecnología de Alimentos, Viña del Mar 15-17 de Noviembre 1995.

Thesis

Undergraduated

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Raffo, Alejandra. 1993. Propiedades mecánicas de geles de surimi-kappa carragenato. Thesis submitted for the Engineer degree, Pontificia Universidad Católica de Chile.

Flores, Andrés. 1993. Estudio de gelificación mediante sinéresis por centrifugación. Thesis submitted for the Engineer degree, Pontificia Universidad Católica de Chile.

Bafico, Pablo. 1995. Modelos mecánicos de geles proteina/almidón. Thesis submitted for the Engineer degree, Pontificia Universidad Católica de Chile.

Magister

Rojas, Erick. 1993. Estudio de geles mixtos de proteina/almidón. Thesis submitted for the Master in Engineering Sciences degree, Pontificia Universidad Católica de Chile.

Gajardo, F. 1993. Estudio acerca de la gelificación de proteinas del suero de leche. Thesis submitted for the Master in Engineering Sciences degree, Pontificia Universidad Católica de Chile.

Rojas, G. 1994. Estudio de la gelificación de proteinas de suero y almidón de yuca mediante pruebas de reología dinamica. Thesis submitted for the Master in Engineering Sciences degree, Pontificia Universidad Católica de Chile.

Ortiz, J. 1995. Effecto de κ-carragenato y KCl en la gelificación de surimi de jurel *(T. murphii)*. Thesis submitted for the Master in Food Sciences degree, Universidad de Chile.

PROJECT PRODUCTIVITY

FUTURE WORK

Future work will continue through the application of the "Microstructural Approach to Food Research and Development", a concept coined by the PI that involves an active integration of microstructural aspects to research. After all food technology is the controlled preservation, modification, creation or destruction of structure in foods. More specifically in the foreseable future work will continue in the areas of extremely low dehydration of foods, frying of potato slices, extraction of natural resources and mixed gels. Funding in excess of US$ 200,000 will become available in the next three years.

The PI is negotiating the new edition of his book "Microstructural Principes of Food Processing and Engineering" with a British editorial house.Also, in collaboration with the publisher Technomics (Lancaster, PA) is editing a book on "Topics of Food Technology" in Spanish aimed at graduate students and practitioners from the food industryin Latin America.

Nestle Research Center in Lausanne (Switzerland) has invited Dr. Aguilera for a 4-month stay to work with their leading scientist in gels and structured foods (Prof. Tolstoguzov) during January-April, 1996 and develop further the microstructural approach.

Trained personnel in Bolivia will continue teaching and research while assisting the local dairy industry in improving their fabrication technologies for cheeses, desserts and yoghurts.

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ENCLOSED MATERIAL

PUBLICATIONS

1. Whey proteins, J.M. Aguilera

2. Caking phenomena in amorphous food powders, J.M Aguilera, J.M. del Valle and M. Karel.

3. Rheological, mechanical and microstructural properties of protein/starch gels, J.M Aguilera, E. Rojas, S. Lozano.

4. Rheological, thermal and microstructural properties of whey protein-cassava starch mixed gels, J.M Aguilera.

5. Microstructural and imaging analyses as related to food engineering, J.M Aguilera.

CONGRESSES AND CONFERENCES

1. 1994 IFT Annual Meeting, Atlanta, June 25-29, 1994.
-Aguilera, J.M. Gelation of whey proteins.
-Aguilera, J.M. y Rojas, E. Structure-property relationships in whey protein/starch mixed gels.

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-Aguilera, J.M. Microestructura de Alimentos.
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3. I Congresso Ibero-Americano de Engenharia de Alimentos, Campinas, Brasil, 5-9 Noviembre, 1995.
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5. 1994 Summer Meeting, American Chemical Society, Washington, DC
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6. Faraday Discussion No 101, Gels, Paris, 6-8 Septiembre 1995.
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7. X Congreso Nacional de Ciencia y Tecnología de Alimentos, Pucón 11-13 Noviembre 1993.
-Raffo, M.A. y Aguilera, J.M.Propiedades mecánicas de geles de surimi-kappa carragenato.

THESIS

Undergraduated

1. Rosales, Cecilia. 1993.
2. Raffo, Alejandra. 1993.
3. Flores, Andrés. 1993.
4. Bafico, Pablo. 1995.

Magister

5. Rojas, Erick. 1993.
6. Gajardo, F. 1993.
7. Rojas, G. 1994.
8. Ortiz, J. 1995.

PUBLICATIONS

Gelation of Whey Proteins

Whey proteins undergo gelation through a series of steps involving denaturation, aggregation, strand formation, and network formation

Jose M. Aguilera

□MACROSCOPICALLY, GELS ARE MULTICOMPONENT PHYSICOchemical systems having a substantial liquid phase but exhibiting solid-like behavior. An updated discussion of the phenomenological definition of a gel can be found in Almdal et al. (1993).

Protein solutions are known to form reversible or irreversible gels upon heating. In particular, solutions of most globular proteins in foods, e.g., egg white, soybean, and whey proteins, form aggregates and heat-set gels as temperature is increased. The gelation of proteins has been reviewed many times since the seminal paper by Ferry (1948), and gelation specifically of food proteins has been reviewed by Clark and Lee-Tuffnell (1986), Clark and Ross-Murphy (1987), Ziegler and Foegeding (1990), and Grinberg et al. 1992).

Gelation of polymers occurs by an enhancement of the interactions between the dissolved polymer macromolecules (or its aggregates) due to a partial decrease in the solubility of the polymer (or some of its functional groups) in the solvent, brought about by one of several external factors (e.g., pH, heat, ionic strength). If a balance is achieved between the attractive and repulsive forces associated with interactions between the polymer and the solvent, a two-phase system may set in: the gel network (of constant composition) and the solution, or syneresis, of the gel (Lipatov, 1988). Gelation involves transition from a sol (polymer solution) to a gel where the polymeric network may be considered to have infinite molecular weight at the gelling point. However, an important difference between polymer gelation and gelation in foods is the high proportion of solvent (water) present in the latter.

Although only thermal gelation will be covered in this article, it is known that other mechanisms may be used to induce gel formation of whey proteins. The term "whey proteins" will be used to represent generic commercial products (concentrates and isolates) as well as individual proteins in the whey of milk.

Whey Proteins and Gelation

Whey is the soluble fraction of milk that is separated from the casein curd during cheese manufacturing. The main proteins in whey are: ß-lactoglobulin (ß-Lg), α-lactalbumin (α-La), proteose-peptone, immunoglobulins, and bovine serum albumin (BSA). Protein products derived from whey proteins, such as concentrates and isolates (approximately 70 and 90% protein, respectively), have important commercial applications in foods, a major one being their tendency to form irreversible heat-induced gels. ß-Lg and BSA are the major gelling proteins in whey, but since the former is 10–20 times more abundant in whey products, it is considered the primary gelling protein.

Most examples to be illustrated here correspond to gelation of a commercial whey protein isolate (WPI) containing 95% protein and prepared by ion exchange, obtained from Le Sueur Isolates (Le Sueur, Minn.) under the trade name Bipro. It consists of 68–73% ß-Lg (as monomer or dimer, approximately 5:1 ratio), 16–19% α-La, and 6% BSA; thus, it may be considered a concentrated form of ß-Lg. All three fractions occur naturally in whey as globular proteins folded into compact three-dimensional structures in which the reactive hydrophobic amino acid side chains are buried in the interior of the protein, leaving most of the polar side chains to the exterior. The structure is maintained by weak non-covalent bonds, and, in the case of ß-Lg, by a few disulfide bonds (Kinsella and Whitehead, 1989). Relevant properties of whey proteins related to thermal gelation (molecular weight, isoelectric pH, and denaturation temperature) are presented in Table 1.

*Table 1—**Molecular Weight**, isoelectric pH (pI), and denaturation temperature of whey proteins*

Protein fraction	MW (kD)	pI[a]	Td[b] (°C)
β-Lactoglobulin	18.6	5.3	76–82
α-Lactalbumin	14.2	4.8	59–62
Bovine serum albumin	66.0	5.1	72–74
Whey protein isolate	—	—	77–88

[a]From Kinsella and Whitehead (1989)
[b]From Bernal and Jelen (1985)

Gelation of whey protein products and constituent proteins has been reviewed many times in the past, denoting that it is a topic of enormous relevance both scientifically and commercially (Paulsson et al., 1986; Mulvihill and Kinsella, 1987; Katsuta, 1990; Mangino, 1992). Heat-induced gelation of these compounds depends on many factors, such as composition, extent of denaturation, pH, temperature, concentration, heating rate, ionic strength, and presence of specific ions, among others.

Rheology of Gelation

Heat-induced gelation of whey proteins starts with a translucent solution that increases in viscosity due to formation of protein aggregates and finally sets into a gel spanning the vessel. The gel can support its own weight under gravity (within a given observation time) and can transmit mechanical signals from one side of the sample to the other.

Because of the gradual development of viscoelasticity, gels and the gelation process are best characterized by small amplitude dynamic testing, normally involving the application of a sinusoidal oscillatory stress or strain under constant frequency. The measured response is usually resolved into an in-phase elastic component G′ and a 90° out-of-phase viscous component G″, the storage and loss moduli, respectively. Due

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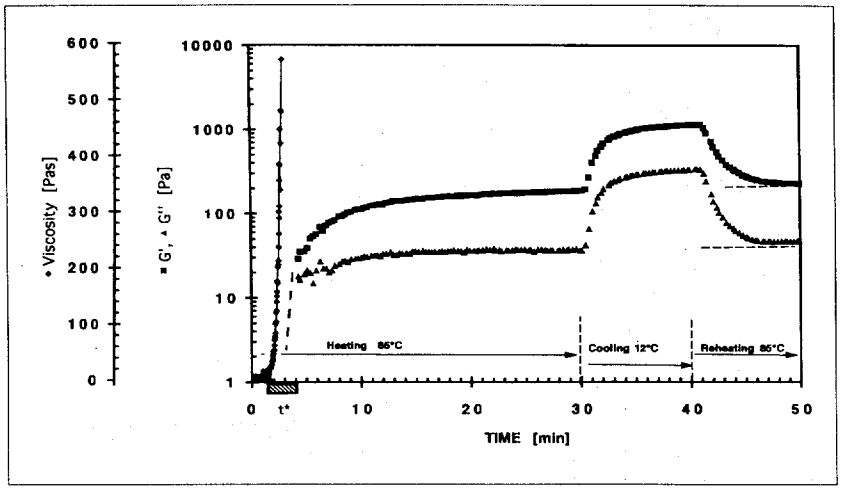

Fig. 1—**The Curing Experiment**. *Changes in shear viscosity and storage (G′) and loss (G″) moduli during gelation of a whey protein isolate (pH=5.45; T curing=85°C; total solids=6%) under cycling temperature conditions*

to the fragile nature of the network that sets in, deformation should be kept small and within the elastic range. The rheological characterization of dairy products has been reviewed by Shoemaker et al. (1992).

In a "curing" experiment (a term borrowed from polymer science), a characteristic curve of log G′, log G″ (or tan δ = G″/G′) vs time is obtained for a given set of conditions as heating proceeds at constant temperature (Fig. 1). Curing runs provide the kinetics of gelation and may be used to derive fundamental information on the gelling mechanism as well as the effect of process variables on the gel properties. As expected for a liquid, G″ initially is greater than G′, indicating that the starting solution is relatively more viscous than it is elastic. Rapidly, G′ starts to increase faster than G″, and the system develops a greater elasticity or "solid-like" behavior. Eventually both moduli appear to reach a pseudo-plateau value larger for G′ than for G″, although an equilibrium value is seldom measured within practical times.

Sometimes G″ passes through a maximum before decreasing as the network is consolidated; absence of a maximum in the G″ curve may be interpreted as if the gel is formed by a stiff network (Ross-Murphy, 1992). Cooling further increases the magnitude of the limiting storage modulus. This step is reversible, since upon subsequent reheating to the curing temperature, the gel exhibits a similar pseudoequilibrium modulus, corroborating that thermotropic gelation is irreversible. Changes in G′ during heating ($\Delta G'_{heat}$) relative to those during cooling ($\Delta G'_{cool}$) are considered as the relative contributions of hydrophobic interactions and hydrogen bonding to the final value of G′ (Beveridge et al., 1984).

When curing experiments are carried out at increasing concentrations but under the same heating and cooling conditions, the pseudo-plateau values increase dramatically (Fig. 2). Plots of log G′ at the psuedo-plateau after cooling vs log concentration (Fig. 3) predict that at high concentrations an almost constant power-law region exists with an exponent close to 3. Paulsson et al. (1990) demonstrated that the power exponent for the dependence of the storage modulus of ß-Lg gels with concentration in the pH range of 4–5.5 was close to 2.5, suggesting that the gelation characteristics of WPI are dominated by the presence of this protein fraction. The power law diverges to higher values of the exponent at the minimum concentration for gelation. According to Clark (1992), values of the power exponent vary for common gelling food components, such as several proteins and polysaccharides, from 2 for strong, concentrated gels to 5 for weaker systems.

The development of the elastic modulus E or Young's modulus (determined by axial compression at small deformations) during curing presents a similar behavior as for G′ (Fig. 4). The mechanical modulus of self-standing gels after cooling to room temperature is commonly assumed to be related to the textural properties (Langley and Green, 1989; Aguilera and Kinsella, 1991). Interestingly, a good correlation (R^2=0.98) exists between the limiting values of log E and log G′ for WPI gels, emphasizing that the predominant properties of the food gel are due to physicochemical interactions developed during heating (Fig. 5).

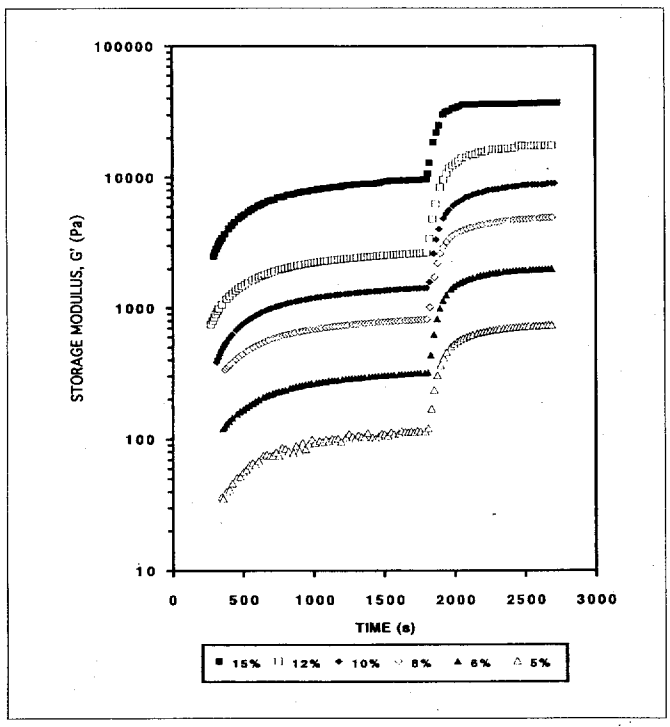

Fig. 2—**Development of the Storage Modulus G′** *of whey protein gels at different concentrations during heating for 30 min at 80°C, followed by cooling at 12°C (pH 5.45)*

The Sol/Gel Transition

Gelation involves a change in the macroscopical state of matter, namely a "transition" from the sol to the gel state. The term transition refers to "a change of state induced by variations in the temperature or pressure" (Sperling, 1992). First-order transitions of low-molecular-weight compounds from their solid (S) or crystalline (C) state to their liquid (L) or vapor (V) state are well known: melting (C or S→L) and boiling (L→V).

From the polymer physics viewpoint, gel formation proceeds via a first-order transition from a disordered molecular state in a solution to a more or less ordered state in a network. The gel point or gel time t* represents the instant when the solution (sol) becomes a viscoelastic solid (gel) or, in the polymerization model of gelation, is the time when an infinite network is formed. As shown in Fig. 1, in the vicinity of t* the steady-

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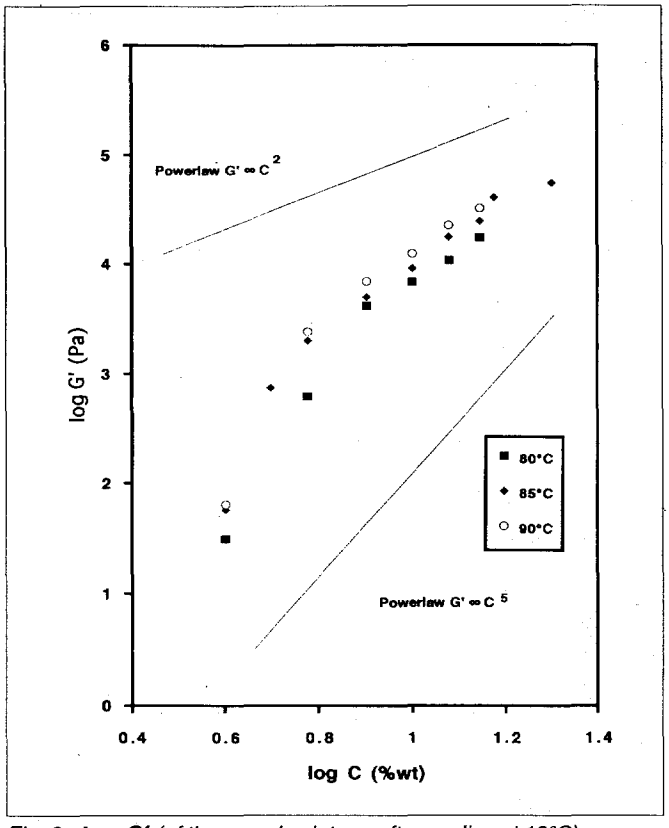

Fig. 3—**Log G'** *(of the pseudo plateau after cooling at 12°C) vs log C data for whey protein isolate gels cured at different temperatures*

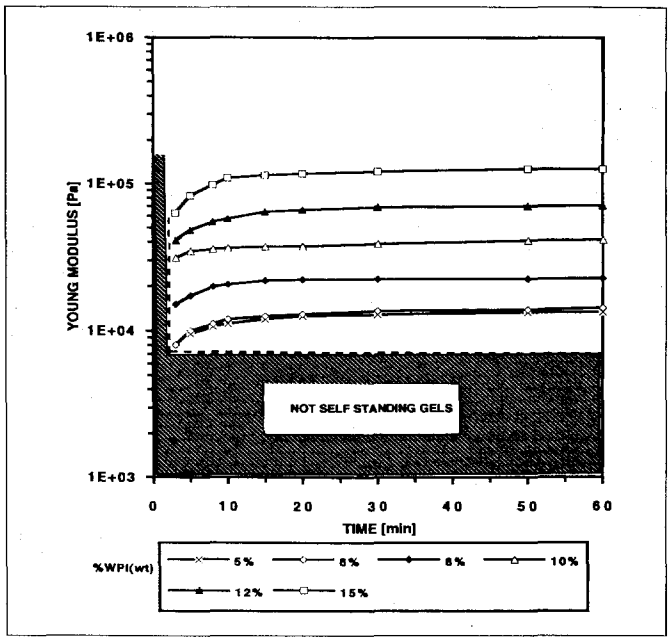

Fig. 4—**Development of the Young's (Elastic) Modulus E** *of whey protein gels at different concentrations during curing for 60 min at 85°C. Testing was performed at 20°C by axial compression of self-standing gels within the elastic range (5% deformation)*

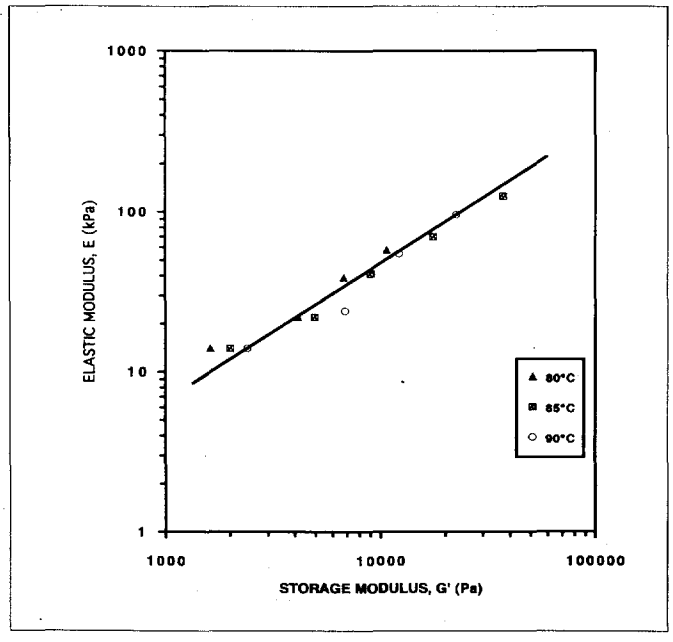

Fig. 5—**Correlation Between the Elastic Modulus E and Storage Modulus G'** *at the cooling pseudoplateaus of whey protein gels at different concentrations (pH 5.45) and curing temperatures*

shear viscosity diverges to infinity (Tang et al., 1993). Its estimation from the curing curve is usually done at the crossover point for G″ and G′ or by extrapolating the rapidly rising G′ to the time axis (Ziegler and Foegeding, 1990).

Similarly, in thermotropic gelation, there is a critical concentration of active molecules C* and a well-defined temperature T* below which gelation does not occur. When critical conditions for gelling are met, many properties show a discontinuity; in particular, elastic behavior is observed at lower and lower frequencies, whereas for viscoelastic solutions this behavior is observed only at high frequencies (Guenet, 1992). The instant of the sol/gel transition is less important in food technology than in polymer science because food gels exhibit higher mechanical moduli which are achieved at times much longer than t* (see Fig. 1).

A number of studies have been undertaken using both chemical shift and spin-spin nuclear magnetic resonance (NMR) relaxation techniques. For a given polymer solution, it is consistently found that T2 (the spin-spin relaxation time) for sols is higher than for gels, both showing a linear dependence with concentration but different slopes. It has been suggested that the gel point be defined as the interception of the two lines, as both sol and gel should approach a common T2 value at this condition (Gajardo, 1993).

Molecular Transitions

Since polymeric systems such as proteins do not attain a totally crystalline form in the solid state or vaporize (they decompose before the boiling point), common first-order transitions found in low-molecular-weight compounds are irrelevant. However, in heat-induced gelation of globular proteins, other "transitions" occur at the molecular level that are intrinsic to the protein: denaturation (D)→unfolding (U)→aggregation (A), as shown in Fig 6. These transitions can also be pressure-induced, and gels thus produced are different in texture, digestibility, and flavor than heat-induced gels (Hoover, 1993).

Globular proteins taking a single conformation in their native state (N) are transformed to the unfolded or denatured

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state under certain conditions (e.g., heating). Achievement of the gel state (G) in globular proteins appears to involve transitions from the native to the unfolded state and into an aggregated state. This sequence of events gives rise to the following reactions proposed by Schmidt (1981) for formation of thermally induced protein gels, based on Ferry's (1948) two-step mechanism:

Step I: N→D(U)
Step II: D(U)→A
Step III: A→S
Step IV: S→G

Step I: Denaturation (Unfolding) of Native Proteins. When temperature is increased, and as a result of opposing temperature dependencies of the entropic and enthalpic interactions stabilizing a protein molecule, unfolding occurs usually in the range of 60–80°C (Myers, 1990). Denaturation of proteins is considered an intramolecular first-order transition in which compact molecules begin to unfold into random coils at the so-called "denaturation" temperature (Grinberg et al., 1992). Thermally induced gels from globular proteins are known to require a certain degree of unfolding of the protein as a first step (Clark and Ross-Murphy, 1987) but not total unfolding, which rarely occurs below 100°C. An interesting observation for interpretation of the gelation process is that as unfolding proceeds, the amount of water bound to the protein increases (Mangino, 1992).

The subject of denaturation of proteins in general and of whey proteins in particular is too complex to be discussed here; it is known to depend on the state of native proteins, pH, ionic strength, heating rate, etc. Ample information on denaturation kinetics (e.g., extent of denaturation as a function of time and temperature) for ß-Lg and α-La in skim milk was presented by Dannenberg and Kessler (1988). Recent studies using reverse-phase high-performance liquid chromatography suggest that unfolding of whey proteins by heat begins at 40°C and proceeds slowly to achieve 10% denaturation at 62°C. After renaturation to almost the initial state at 65°C, % denaturation follows a linear relationship with temperature and is 95% complete at 85°C (Parris and Baginski, 1991). The renaturation step at 65°C may be interpreted as a collapse of the unfolded domains into native-like structures (Jaenicke, 1991). Since opening of the molecular structure exposes a larger amount of reactive sites for intermolecular interactions, gelation of whey proteins is normally observed above 70°C.

It has been postulated recently (Hirose, 1993) that initial steps in gelation may involve a conformational intermediate state in which the secondary structure of the globular protein is either intact or slightly expanded but where intramolecular motions of side chains are released and surface hydrophobicity is increased. Hypothetically, the transformation from the native to this so-called "molten globule" state is analogous to an intramolecular glass transition. The subject of the degree of unfolding and the physical state of the molecules necessary for gelation should be further addressed to gain a more basic understanding of the gelling mechanism.

Step II: Aggregation of Unfolded Molecules. Aggregation of denatured or partially denatured globular proteins in solution has been recognized as an integral part of the heat-induced gelation process (other names for this step are oligomerization and association). Network development in legumin (11S fraction of broad beans) solutions close to the gelation threshold involved formation of "aggregates" with mean Stokes diameters of about 0.2 μm (Grinberg et al., 1992). Only 80% of the total protein underwent this type of association, while the rest remained in solution as lower-molecular-weight units. Matsudomi et al. (1992) showed that while a 0.2% solution of ß-Lg heated for 30 min at 80°C formed soluble aggregates, a similar α-La solution did not. However, when a mixture of both diluted solutions was heated, they interacted to form soluble aggregates through a thiol–disulfide interchange reaction, an interaction that was critical in formation of the gel network and markedly enhanced the hardness of gels. Similarly, addition of α-La to BSA induced formation of soluble aggregates through the same mechanism, to form a finer and more uniform network leading to transparent gels (Matsudomi et al., 1993).

Heating dilute ß-Lg solutions at pH values substantially above or below the isoelectric pH and at low ionic strength did not lead to visible aggregation (Harwalkar and Kalab, 1985b). However, aggregates of ß-Lg of approximately 0.5 μm in diameter were observed by Harwalkar and Kalab (1985a) using transmission electron microscopy when a dilute solution (1%, ionic strength 0.1 and 0.4) was heated at 90°C, pH 2.5 and 4.5. Aggregation of a dilute solution (1.5% w/w, pH 4.8) of ß-Lg as a function of time when heated at 80, 85, and 90°C was followed using a particle size analyzer (Aguilera and Stanley, 1993). Protein units in the original solution exhibited a mean diameter of 7 nm, which coincides with the size reported for the ß-Lg dimers, while soluble aggregates of approximately 1 μm in diameter were formed after 40–60 sec independent of the heating temperature.

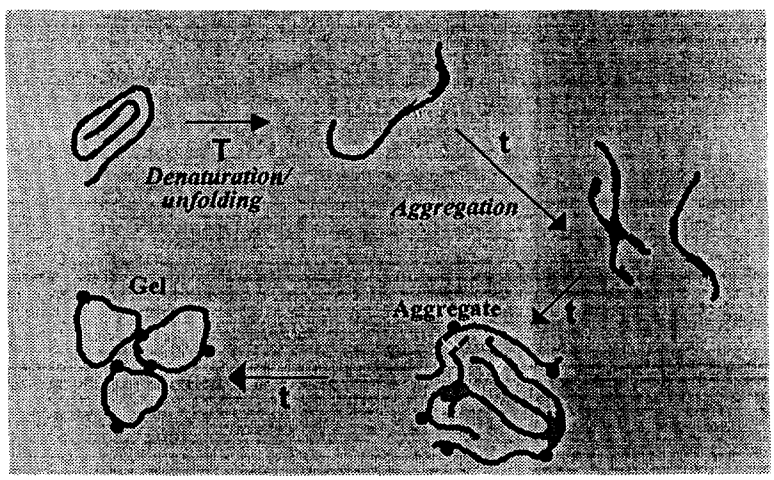

Fig. 6—**Schematic of Gelation of Whey Proteins**

The molecular mechanisms responsible for the formation of aggregates during and after protein unfolding are still obscure. It may be surmised that aggregation proceeds through a sequence of (unimolecular) unfolding reactions and bimolecular association steps to yield higher-order polymeric structures, as is the case of folding of native proteins. Those interested in the subject may find some answers in the review paper by Jaenicke (1991), but it is evident that this topic is of particular relevance to the formation of macromolecular protein assemblies such as gels. It is generally assumed that the driving forces for aggregation are nonspecific interactions between hydrophobic regions of unfolded polypeptide chains, but hydrogen bonding and ionic interactions are likely to participate as well (Clark and Ross-Murphy, 1987). Stabilization of soluble

aggregates may even proceed through disulfide crosslinking between polypeptide chains, as suggested by Hillier et al. (1980) for whey concentrates, and corroborated by experiments in which active sites are blocked. Obviously, this subject is of primary importance, as aggregates are the basic building blocks in the network of aggregate protein gels.

The amount and state of water within and outside these aggregates is another area of needed research, as some authors have postulated that gelation proceeds sometimes through liquid–liquid phase separation into a solvent-rich phase and a polymer-rich phase vitrified at (or below) the glass transition temperature (Callister et al., 1990). Gels of globular proteins may be metastable forms of a glassy network immersed in a solution, a theory in accord with the proposed molten globule state.

Step III: Strand Formation of Aggregates. The most outstanding microstructural feature of aggregate gels made from whey proteins is the presence of a homogeneous network of connected protein particles or aggregates forming a three-dimensional matrix with interstices filled by a liquid (or aqueous solution). An excellent overview and interpretation of the microstructure of food biopolymer gels has been presented by Hermansson (1988).

Tombs (1974) postulated two models for the formation of globular protein gels from aggregates: random clustering and a "string-of-beads" structure. ß-Lg, BSA, and whey protein products are known to form either type of gel, depending on the pH and ionic strength. For example, ß-Lg forms both aggregate and "fine-stranded" thermally induced gels, depending on the pH (Stading and Hermansson, 1991). The network of aggregated or particulate gels is composed of more-or-less spherical particles linked together, forming the strands of the network. ß-Lg particulate gels formed at a fast heating rate (5–12°C/min) showed strands of almost spherical particles of uniform size (about 0.5 µm in diameter) linked as a "string-of-beads" in a chain of single particles. Those formed at slower heating rates resulted in thicker strands of larger particles, with broad particle size distribution (0.5–2 µm in diameter) fused together in many points (Stading et al., 1993).

Several stabilizing forces affecting "gelation" have been suggested at the molecular level: electrostatic forces, covalent bonds, and hydrophobic interactions (Mangino, 1992). Although it is not clear whether they stabilize the aggregates themselves or the strand of aggregates, it appears that the primary overall stabilizing forces are hydrophobic interactions (Clark et al., 1981) and possibly ionic interactions (Kohnhorst and Mangino, 1985).

Step IV: Association of Strands and Network Formation. An ultimate level of association occurs between the network strands themselves. Protein gels are usually divided into physical gels and entangled networks. The former are strong gels, while the latter are formed by the topological entanglement of chains and are gelled networks at frequencies higher than some typical entanglement lifetime or are viscoelastic liquids without an equilibrium modulus (Ross-Murphy, 1992). Whey protein gels appear to be physical gels of infinite molecular weight.

Other Possible Transitions in Gels

A reversible volume transition of chemical gels between a swollen phase→collapsed phase in response to physical and chemical stimuli such as temperature, solvent composition, pH, etc., and their application in foods has been discussed by Tanaka (1992). Since commonly studied heat-set whey protein gels are irreversible, this volume transition has not been reported.

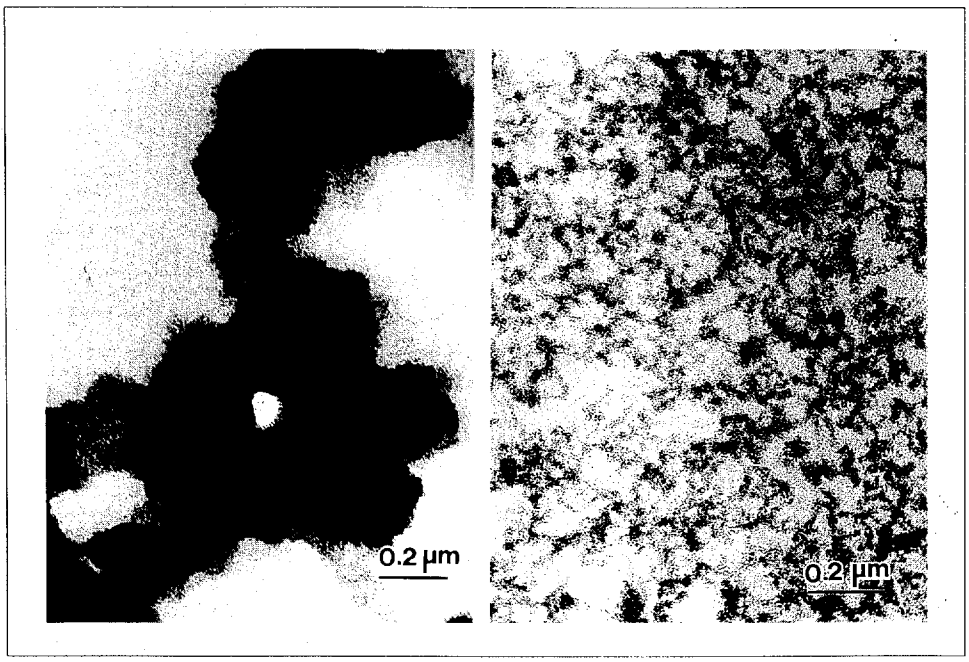

Fig. 7—**Microstructure of WPI Gels**. *Left, transmission electron micrograph of a gel formed at pH 5.7 (opaque); right, gel formed at pH 6.3 (translucent)*

However, it is well known that an opaque→transparent (translucent) transition exists for globular protein gels (Doi, 1993). Transparent gels have a molecularly homogeneous network, whereas nontransparent gels consist of colloidal particles or aggregates (larger than a quarter of the wavelength of light, i.e., 150 nm). Transparent, fine-stranded ß-Lg gels were formed in the pH range of 7–9, while opaque, aggregated structures resulted close to the isoelectric pH (e.g., 4–6). Transition from one gel type to the other can be triggered by a small shift in pH (~0.1 unit) before heating (Hermansson, 1988; Doi, 1993). Whey protein isolates and concentrates form opaque aggregated gels at intermediate pH and at high ionic strength, and the pH range of the transition appears to be broader than for pure ß-Lg gels (Stading et al., 1993). Fig. 7 shows the wide microstructural differences of translucent and opaque WPI gels whose only difference is the pH of the starting sol (6.3 and 5.7, respectively).

Another conceivable transition for globular protein gels is that of single-phase biphasic structures referred to in Aguilera (1992) and demonstrated by microscopy for many gelling systems (Gotlieb et al., 1988; Clark and Ross-Murphy, 1987). Mixing of two polymer solutions most frequently results in a two-phase system. This has been extensively reported for protein–polysaccharides systems as well (Braudo et al., 1986). Even if two polymer solutions do mix, they are generally found to phase-separate at some higher temperature rather than a

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lower temperature (the lower critical solution temperature). Hence, depending on whether gelling occurs before, during, or after the demixing process, one-phase or complex multiphase gel structures may form. This is a subject of enormous importance in gelling of food materials but one extremely difficult to address from a basic viewpoint, for it involves coupling of kinetic and thermodynamic data.

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Caking phenomena in amorphous food powders

José M. Aguilera, José M. del Valle and Marcus Karel

Caking of free-flowing powders during storage is a deleterious phenomenon that is ubiquitous in the feed, fertilizer and pharmaceutical industries, and of economical importance for low-moisture foods. Among other subjects related to caking of amorphous powders, the following aspects are reviewed in this article: (1) physical and morphological changes, and quantitative procedures to assess caking; (2) proposed mechanisms of caking phenomena; (3) examples of caking in food materials; (4) the relationships between storage-induced caking and other phenomena and the glass transition of amorphous powders; (5) methods for estimating the evolution of caking with time from the physical properties of powder samples and environmental conditions; and (6) measures to minimize the occurrence of caking phenomena.

Caking is a deleterious phenomenon by which a low-moisture, free-flowing powder is first transformed into lumps, then into an agglomerated solid and ultimately into a sticky material, resulting in loss of functionality and lowered quality. The problem is ubiquitous in the food, feed, fertilizer, pharmaceutical and related industries[1–3]. This review deals, in the main, with an important form of caking in foods, namely that of amorphous powders by viscous flow. Caking may also occur as a result of recrystallization, either after fat melting or after solubilization at crystal surfaces; surface wetting followed by moisture equilibration or cooling; or electrostatic attraction between particles[4].

A strict definition of caking is difficult to formulate, because changes in a particulate system depend on temperature, moisture and position within the powder, and involve many different stages, including bridging, agglomeration, compaction and liquefaction (Fig. 1). At any given stage, lumps may be few or numerous, of different sizes and of varying degrees of hardness.

In practice, a quantitative measure of caking is highly desirable for following changes in the condition of a powder over time. The 'caking index' (a termed coined by the authors) is defined as the state of the system at any time relative to an initial state. Two morphological indicators of the state of the system are: the ratio of instant system porosity to initial system porosity, $p(t)/p_0$; and the ratio of interparticle bridge diameter to particle diameter, $D_{bridge}/D_{particle}$ (see Fig. 1).

Because of the diversity and inconsistency of the terms used to describe caking, some definitions are proposed based on their most common use in the literature. Bridging, the initial stage in caking, occurs as a result of surface deformation and sticking at contact points between particles, without a measurable decrease in system porosity. At the onset of caking, small interparticle bridges may even disintegrate under mild shaking. Agglomeration, a later stage, involves an irreversible consolidation of bridges but the high porosity of the particulate system is maintained, resulting in particle clumps with structural integrity. The mechanisms of interparticle bonding have been reviewed by Rutland[2] and Schubert[5]. Compaction, an even more advanced stage in caking, is associated with a pronounced loss of system integrity as a result of thickening of interparticle bridges owing to flow, reduction of interparticle spaces, and deformation of particle clumps under pressure. In the final stage of caking, interparticle bridges disappear as a result of sample liquefaction and extensive flow owing to the high moisture content. This stage usually involves the solubilization of low molecular weight fractions and the presence of hygroscopic behavior[6].

Although most publications state only whether caking is observed and under what conditions, quantitative

Stage	$p(t)/p_0$	$D_{bridge}/D_{particle}$	Morphology
Free flowing	1	0	
Bridging	→1	→0	
Agglomeration	<1	>0	
Compaction	→0	→1	
Liquefaction	0	1	

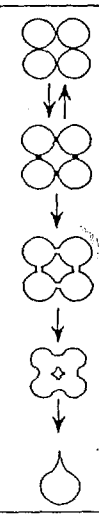

Fig. 1
Stages during a typical caking process, indicating the changes in system porosity [$p(t)/p_0$] and the ratio of interparticle bridge diameter to particle diameter [$D_{bridge}/D_{particle}$] at each stage.

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(T_g^a = 5°C)[11], glucose (T_g^a = 31°C)[11] and sucrose (T_g^a = 62°C)[11], and the T_g of fructose/glucose/sucrose blends equilibrated to 30% RH (w = 0.05 g water/g material), similar in composition to dehydrated fruit juices, can be estimated to be below typical room temperature values[19]. This also explains why it is recommended that the walls of spray dryers used to dehydrate these types of products are cooled to reduce stickiness problems[20,21].

The T_g^a of amorphous lactose is 101°C (Ref. 11) and has a T_g that is below room temperature at ~0.05 g water/g material (Ref. 19), which is usually the maximum moisture content of dried milk products showing adequate chemical stability. As expected, the stability against caking of several dairy-based infant formula powders increased with increasing amounts of high molecular weight carbohydrates (maltodextrins), which raised the T_g of the samples[22,23].

In addition to amorphous sugars, low molecular weight inorganic salts[6] and protein hydrolyzates[17,24] are known to exhibit hygroscopicity and to cause caking problems during storage. A fraction mainly containing nitrogenous compounds (presumably amino acids) was held responsible for the hygroscopicity and caking of spray-dried fermented soy sauce powders[24].

An important related phenomenon is aggregation of proteins in the dried state. There are examples in the literature of aggregation reactions (e.g. thiol–disulfide interchanges, other covalent bonds mediated by lysine residues, and conformational changes) resulting from water sorption during processing, storage and delivery. The aggregation of biomolecules in the 'dry' state has important implications in the modern biotechnology industry, as it may be responsible for a pronounced decrease in biological activity[25,26].

Caking and related phenomena

Collapse, stickiness and caking appear to be related phenomena. When a solid matrix (usually freeze dried) reaches its 'collapse' temperature (T_c), an initial occurrence of interparticle bridging (or surface caking[22]) is manifested as a loss of structure and decrease in sample volume[23]. At the 'sticky point' temperature (T_s), a sharp increase in the force required to stir a powder is observed[27], which is indicative of a more advanced stage of caking[22]. Figure 3 presents the effect of sample moisture content on T_g, T_c and T_s of dehydrated orange juice and an amorphous fruit juice model system, as assessed by several authors using different instruments, together with the predicted T_g of mixtures of water and sucrose, the main component in anhydrous fruit juices. Although some data scattering is evident, T_g values for mixtures of water and the main non-aqueous component of a sample appear to predict the phenomena of collapse and stickness as a function of temperature reasonably well.

Some caution should be exercised in the measurement and interpretation of glass transition temperatures, which vary not only because of instrumental differences and variations in the physical events that constitute the definition of T_g, T_c and T_s, but also because of the broad

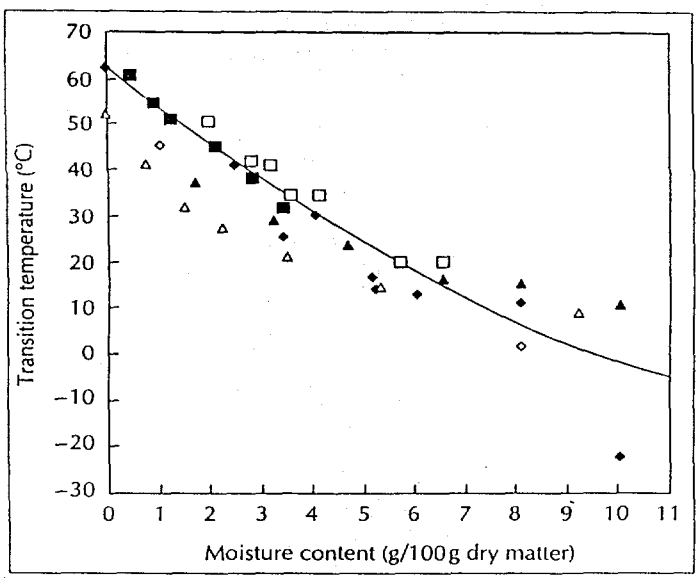

Fig. 3

Effect of sample moisture content (w) on glass transition temperature (T_g), sticky point temperature (T_s) and collapse temperature (T_c) of dehydrated fruit juices and an amorphous fruit juice model system. (◇), midpoint T_g determined using differential scanning calorimetry[19]; (◆), T_g determined using thermal mechanical analysis (V.T. Huang, unpublished); (□), T_s of an amorphous sucrose and fructose (7:1) model system[15]; (■), T_s of spray-dried orange juice with added glucose[20]; and T_c of slow- (△) and fast-frozen (▲) lyophilized orange juice[23]. The curve represents the predicted T_g of amorphous sucrose using the Gordon–Taylor equation[28] and equation parameters estimated by Roos and Karel[11].

temperature range over which the glass transition occurs. The T_s and T_c values measured for several dairy-based infant formula powders by using the techniques of Lazar *et al.*[27] and Tsouroflis *et al.*[23], respectively, were dependent on the scanning rate (i.e. the heating rate), and T_s was found to be higher than T_c which, in turn, was 30–50°C higher than T_g at a constant scanning rate[22]. These differences were attributed to variations in sample size and viscous flow time constants between the methods.

Caking kinetics

An obvious goal for food technologists is to be able to predict the degree of caking of a food powder during storage. When a dry powder is packed, it picks up water from the moist air in the headspace (or through the package material or seal) to a degree that depends on the sorption characteristics of the food (if a pseudo-equilibrium can be assumed). The caking rate of the powder will depend on its instantaneous moisture content (through its effect on T_g) and the ambient temperature.

Modeling the kinetics of caking is a difficult task because many factors are known to affect it, both internal [such as particle size (D_p), charge, hygroscopic behavior, and the physical state of particles] and external

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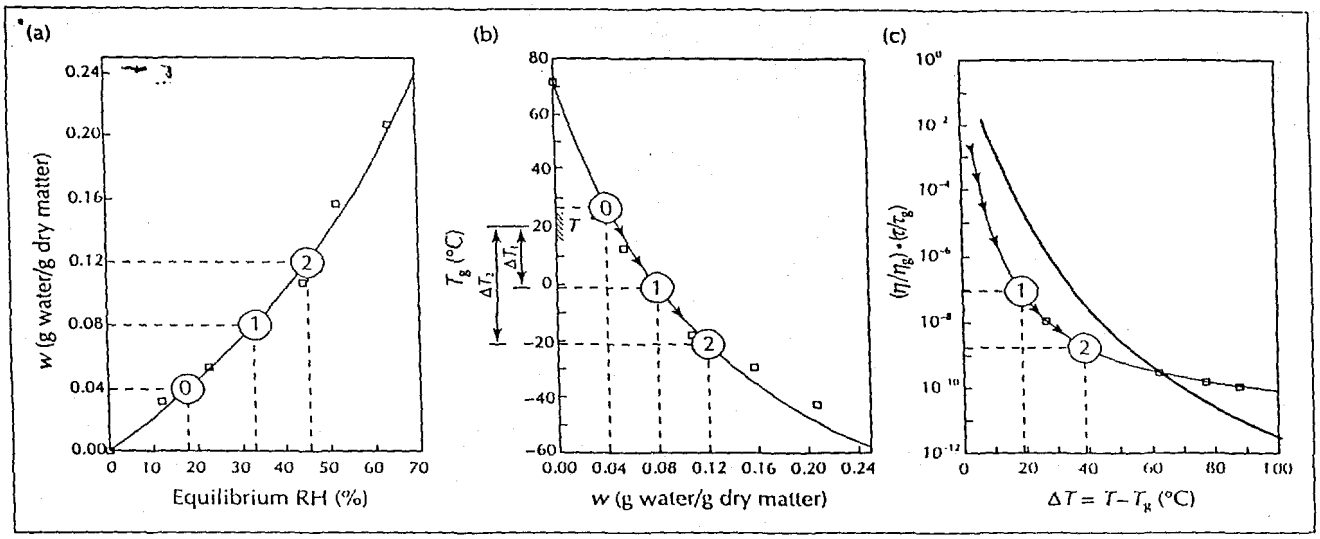

Fig. 5

Data required to predict the advance of caking in fish protein hydrolyzate powder samples. (a), Sorption isotherm: (□), experimental data from Aguilera *et al.*[17]; (—), model using Henderson's equation[33] (Eqn 3). (b), Phase diagram plot: (□), experimental data from Aguilera *et al.*[17]; (—), model using the Gordon–Taylor equation[28] (Eqn 4). (c), Williams–Landel–Ferry (WLF) plot of relaxation times for caking: (□), experimental data from Aguilera and del Valle[29]; (—), universal WLF plot for viscosity of polymers (Eqn 2); (—), model using a WLF-type relationship[10] with adjustable C_1 and C_2 coefficients (Eqn 5). Condition ⓪ indicates the stability of powder samples against caking; condition ① signals the onset of caking phenomena (just perceptible in a few days); and condition ② indicates that powder samples cake fast enough to allow the easy identification of the defect. See text for further details.

In the fish hydrolyzate example, caking started to be apparent as samples reached average moisture contents of 0.04–0.08 g water/g dry matter, corresponding to a delay factor of ~6 h. This reveals nothing about the actual moisture content on the surface of the particles. Hence, factors that should be controlled when establishing actual caking kinetics include water vapor transmission rate and uniform exposure of particles to the vapor phase.

Microstructural aspects of caking

Morphological changes in a powder undergoing caking can be best visualized using microscopical techniques[34]. Thus, it is surprising that microscopical analysis has not been used to any great extent to characterize caking, but it is likely that quantitative information related to caking phenomena and the determination of a caking index (see Fig. 1) will be easily derived by microscopy and the aid of computational image analysis in the future.

Amorphous components in spray-dried powders are in the metastable amorphous state and exposure to high relative humidity results in an irreversible transition to the crystalline state. Amorphous lactose crystallizes and releases water during equilibration at 42–52% RH (at 25°C). Scanning electron microscopy (SEM) was used to follow caking of skim milk powder stored at 20°C during equilibration at 43–94% RH (Ref. 35). After two weeks, changes had occurred only for powder equilibrated at >54% RH, whereas at ≥74% RH lactose crystallization as well as bridging between particles were observed extensively. Similar microstructural observations were made for spray-dried powders of whole milk supplemented with lactose[36].

Microstructural changes in lyophilized samples of fish myofibrillar protein hydrolyzate were also observed using SEM[29]. Samples equilibrated for three weeks to ≤44% RH retained their original highly porous structure with fine walls. At 56% RH, pore walls were observed to thicken, whereas at 67% RH they had fused into thick and continuous masses, reducing considerably the porosity of the system. The sample equilibrated to 75% RH presented evidence of crystalline-like structures.

Image processing by digitization can be used to correct defects in the original microstructural image (e.g. non-informational noise), enhance or suppress data, select important features, extract numerical data (e.g. particle area or characteristic size) and perform statistical analysis. Figure 6 depicts three images from the SEM micrograph of milk powder in an agglomerated state that can be used to characterize stages in caking and caking kinetics by the analysis of geometrical features.

Prevention of caking

As should be evident at this point, the strict control of moisture content and storage at low temperatures, when possible, are key factors in minimizing the effects of caking of powders. However, in many instances, anti-caking agents are added to hygroscopic food powders to improve their flowability and/or to inhibit caking; they achieve this by one of several mechanisms.

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RHEOLOGICAL, MECHANICAL AND MICROSTRUCTURAL PROPERTIES OF PROTEIN-STARCH GELS

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1. INTRODUCTION

Starch and proteins, major biopolymers of cereals and legumes, are responsible for characteristic textural properties of grains, pulses and products from their flours. A typical example are bakery products, where the different textures of breads and pastries around the world are basically derived from the interactions of wheat starch and gluten. With the advent of novel separation techniques, purified sources of protein and starches are becoming available to be used as food in mixtures but their rheological properties are yet to be determined.

Knowledge of the thermomechanical properties of mixed starch/protein gels is important in understanding and predicting the flow properties during processing as well as the final texture of products (Biliaderis, 1992). Few aqueous mixed gels based on polysaccharides and proteins but of the thermoreversible nature have been studied, in particular, those containing agar and gelatin (Clark et al., 1983). The rheological properties of fish protein gels containing various types of starch was explained by the properties of each component (starch and protein) without any specific interaction (Wu et al., 1985).

Whey proteins form irreversible gels by heating above 70°C while starch gels formed above the gelatinization temperature (ca. 65°C) are generally regarded as filled gels in which swollen starch granules are embedded in an amylose gel matrix (Biliaderis, 1992).

The objective of the present work was to characterize starch/protein gels of thermotropic nature from a mechanical and dynamic viewpoints and to interpret the results by studying their microstructure.

2. MATERIALS AND METHODS

2.1 Materials

Whey protein isolate, WPI, (BIPRO, Le Sueur Isolates, MN), 92.7% protein; commercial food-grade cassava starch, CS, (FRABOL, Santa Cruz, Bolivia), with 0.1 and 0.3% protein and lipids, respectively; distilled water. Sucrose and sodium chloride (NaCl) were technical grade.

2.2 Preparation of gels

Mixed solutions of 10% (w/w) total solids (TS) were prepared from stock solutions of WPI and CS in distilled water or in solutions of NaCl and sucrose, and adjusted to pH 5.75 with a 1M HCl solution. Mixed solutions were kept agitated to avoid sedimentation of starch granules and transferred to glass tubes (100x12.3 mm diameter), sealed with rubber stoppers and held vertically in a water bath at 85±1°C for 30 min. The tubes were cooled in an ice/water bath for 15 min and stored overnight at 5°C. Gels were removed from the glass tubes, equilibrated at 20°C for 1 hr and cut into cylindrical sections 11mm long using a template and parallel piano wires.

2.3 Mechanical and rheological tests

Gel samples (at least 4) were compressed to 5% deformation (within the elastic range) in a mechanical testing machine model TA.TX2 (Texture Technol. Corp., Scarsdale, NJ) at a deformation speed of 0.2 mm/s. A compression stress was calculated dividing the maximum force at 5% deformation by the initial cross section of the gels. Changes in the cross sectional area during testing were negligible. Dynamic testing of solutions was performed at 85°C in a Physica rheometer model Rheolab MC 120 (Physica Messtechnik GmbH, Stuttgart, Germany) using a coaxial cylinder system. The storage and loss modulus (G' and G", respectively) were determined at a frequency of 1Hz and under a maximum deformation of 0.005 to assure preservation of the gel structure.

2.4. Microscopy

Samples for microscopy were selected and cut from the center of the gel and studied in a JEOL JSM-25 SII scanning electron microscope at 25 kV after fixation in 2.5% glutaraldehyde in 0.2M cacodilate buffer (pH 7.3), dewatered in a graded series of acetone solutions and dehydrated by critical point drying in a Sorvall unit.

3. RESULTS AND DISCUSSION

Important variables in the gelation of globular proteins are the concentration and pH of the sol, temperature and heating rate. Thus, conditions for the present work (10% TS, pH 5.45, 85°C, and 10°C/min) were chosen based on previous work and are known to produce firm and opaque protein gels (Aguilera and Kessler, 1988; Aguilera and Kinsella, 1991). Cassava starch failed to form a gel by heating to 85°C in the pH range 4 to 8, and total solids concentration 4 to 15 % (w/w) even though it had a low lipid content that assured no formation

of amylose-lipid complexes that are known to affect the rheological properties.

Figure 1 shows the effect of the starch fraction ϕ_S on the elastic modulus (E) of gels. Care has to be taken in comparing these results with those reported previously for mechanical tests under larger deformations (i.e., Aguilera and Kinsella, 1991). Addition of CS to whey protein in the range $0<\phi_S<0.4$ had the surprising effect of reinforcing the structure, as shown by the higher E values. An E maximum, 1.6 times higher than for a pure WPI gel, occurred for $\phi_S=0.2$. Increase in the content of CS led to an almost linear decrease in modulus up to $\phi_S>0.7$; with further addition of CS, no mixed gel was formed.

Addition of sacarose (1-5%) or NaCl (0.1- 0.3%) induced a further increase in E values to the extent that a mixed WPI/CS gel, $\phi_S=0.2$ and having 0.3% salt or 5% sucrose presented a compressive stress almost twice of that of a pure WPI gel (Figure 1). It may be postulated that addition of these solutes bound free water, reducing the effective concentration of solvent. and increasing the effective concentration of the polymers.

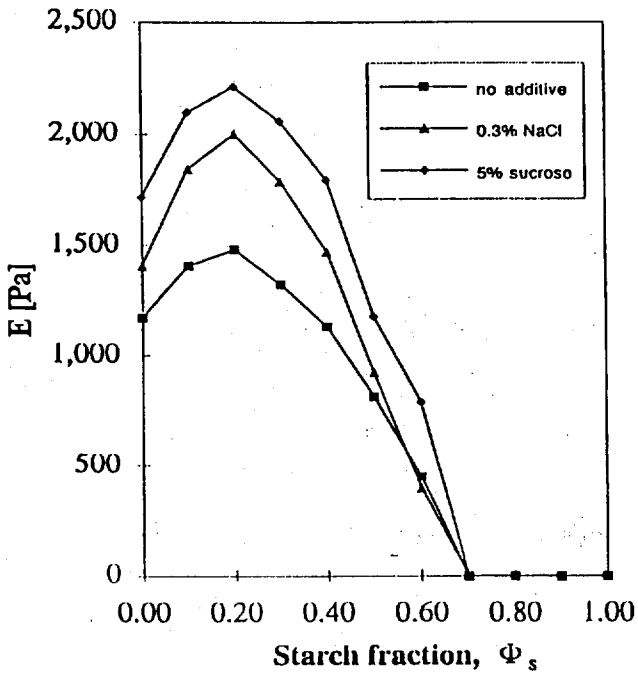


Figure 1. Effect of starch fraction (ϕ_S) and addition of salt and sucrose on the elastic modules (E) of mixed WPI/CS gels. (10% TS, pH 5.75, T=85°C).

From the applications viewpoint, these results show that up to 40% of WPI could be replaced by CS without affecting the elastic modulus of gels. Addition of salt or sugar, common additives in food processing, far from affecting negatively the texture, allow further levels of substitution of WPI.

Kinetics of development of the storage modulus (G') at 85°C of mixtures of WPI and CS are shown in Figure 2. The G' profiles show a rapid initial increase in modulus followed by a slower increase (pseudo plateau) at longer times. G' values were almost one order of magnitude higher than G" (not shown), typical of true gels. G' values in the pseudo plateau region for different levels of substitution followed the same pattern as E values of the compression tests, e.g., the highest storage modulus corresponded to $\phi_S=0.2$, and values for $\phi_S>0.7$ were very low.

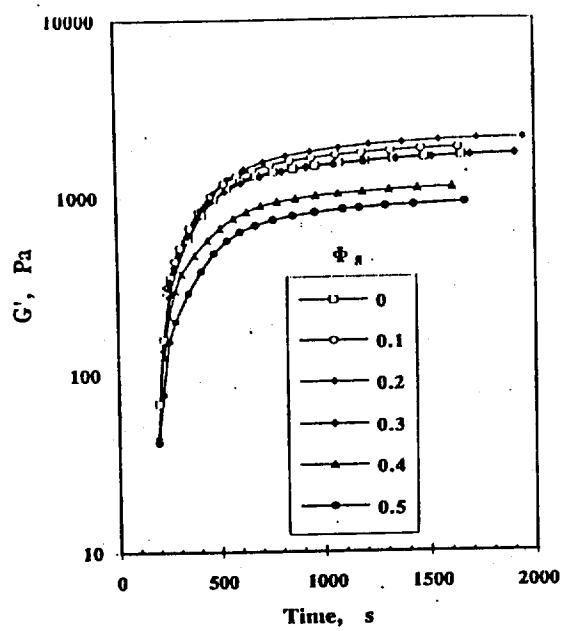
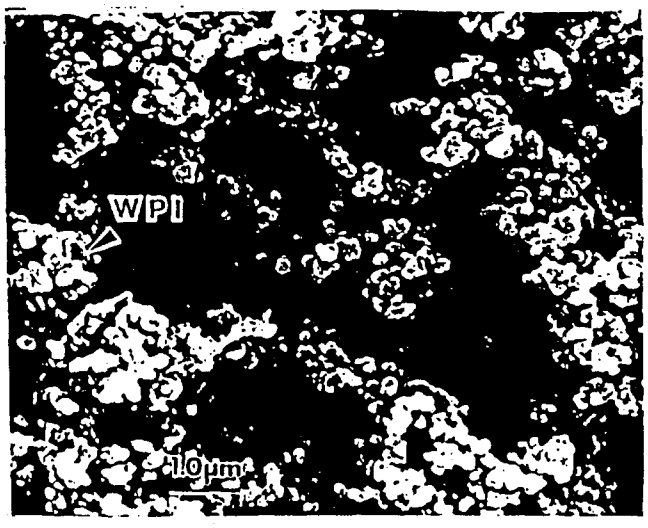


Figure 2. Effect of starch fraction (ϕ_S) on the storage modulus (G') of mixed WPI/CS gels. (10% TS, pH 5.75, T=85°C).

The microstructure of mixed gels as studied by scanning electron microscopy revealed independent networks for WPI and cassava starch fractions (Figure 3). Whey proteins aggregated into almost spherical units 0.2-0.3 µm in diameter densely packed while YS gelled as a fine sponge. WPI/CS gels at $\phi_S= 0.2$ showed well-developed intermeshing networks of WPI and AS. It is interesting to note that each

polymer developed its own network and no copolymer structure could be perceived. Non-gelling WPI/CS solutions ($\phi_S > 0.7$) failed to form a continuous WPI network.



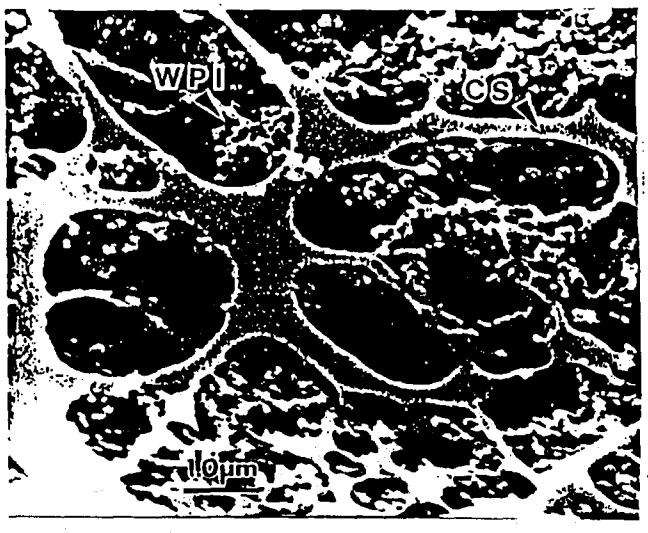


Figure 3. Scanning electron micrographs of pure and mixed gels. Top: Pure WPI gel; Bottom: mixed WPI/CS gel for $\phi_S = 0.2$.

Formation and reinforcement of the gel structure may be explained in part by phase separation. As the effective concentration of a biopolymer increases in one of the microphases, the concentration threshold for gelation may be reached and the polymer network may be formed.

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RHEOLOGICAL, THERMAL AND MICROSTRUCTURAL PROPERTIES OF WHEY PROTEIN- CASSAVA STARCH MIXED GELS

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ABSTRACT

Mixed gels of cassava starch (CS) and a whey protein isolate (WPI), obtained by heating solutions of 10% total solids, pH 5.75 to 85°C, were characterized as a function of the starch fraction, ϑ_S, by axial compression, small-amplitude oscillatory rheometry, differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Gelation did not occur for $\vartheta_S > 0.7$. In the range $0<\vartheta_S<0.4$ mixed gels showed higher mechanical (E, elastic modulus) and rheological (G', storage modulus) properties than pure gels, with maximum values for $\vartheta_S = 0.2$-0.3. Viscoelastic measurements as a function of time showed that gels containing higher levels of WPI developed a larger G'. Blends of both biopolymers showed independent thermal transitions in DSC runs, related to gelatinization and denaturation phenomena. Microstructure of mixed gels showed continuous strands of WPI particle aggregates and a smooth network for CS with an interpenetrating network formed at $\vartheta_S = 0.2$. Several gel microstructures (interpenetrating, composite and filled) were obtained by varing ϑ_S and the source of starch.

KEY WORDS: whey proteins, cassava starch, rheology, microstructure, gels

INTRODUCTION

Starch and proteins are responsible for most of the desirable characteristics of food products derived from cereals and other grains. An excellent example are the many textures of bakery products and their relation to the interactions of two biopolymers, wheat starch and gluten, in the presence of water. When a rubber-like polymeric system contains a substantial amount of water and is viscoelastic in nature, it is termed a gel and in many processed foods, protein and polysaccharides are found as mixed gels (Morris, 1991; Blanshard, 1988: Brownsey and Morris, 1988).

Starches are known to lose their crystallinity when heated in water at temperatures above 60-70°C and "gelatinize". During gelatinization, starch granules in suspension absorb water, swell and amylose leaches out into solution. Cooling converts the mixture into an opaque and viscoelastic "mixed" gel composed of swollen and "porous" starch granules embedded in an amylose gel (Morris, 1991; Biliaderis, 1992). The gel network appears to develop on cooling by the formation of ordered junction zones based on amylose double helices (Clark and Ross-Murphy, 1987). Whey protein solutions (concentration > 5-7% protein) form irreversible particle gels when heated to a temperature >75°C. The mechanism of thermal gelation of whey proteins involves an initial denaturation/unfolding step followed by aggregation into protein particles sticking together as a network (Mulvihill and Kinsella, 1987). Apparently, exposure of non-polar amino acid side chains on the surface of aggregates stabilize the network through hydrophobic interactions (Mitchell et al., 1990).

With the advent of novel separation techniques, purified and more functional sources of protein are now available to participate in complex gel systems. Cassava is and abundant source of carbohydrate used as food in many countries of South America and Africa but its study in mixed systems is largely unreported. In particular, knowledge of the rheological properties of a mixed starch/whey protein gel is important in understanding and predicting its flow properties during processing as well as the final texture of products (Biliaderis, 1992). Mixed gels of protein and non-starchy polysaccharides have been studied by Clark et al. (1983) and Braudo et al. (1986), among others. Starch is an ubiquitous source of calories in foods and a common filler in some comminuted meat and fish products. For example, addition of different starches to fish protein gels showed no apparent detrimental rheological effects (Wu et al., 1985). However, specific interactions and synergistic effects are highly desirable to create new textures and accomplish ingredient substitution.

The objective of the present work was to characterize cassava starch/whey protein gels of thermotropic nature by mechanical and dynamic oscillatory rheometry and to interpret the results by studying their thermal transitions and microstructure.

MATERIALS AND METHODS

Materials

Whey protein isolate, WPI (BIPRO, Le Sueur Isolates, MN), 92.7% protein; commercial food-grade cassava starch, CS (FRABOL, Santa Cruz, Bolivia) with 0.1% protein and 0.3% lipids; distilled water. Corn starch (Corn Products, Santiago,Chile) was used as a reference in some experiments. NaCl and sucrose were of technical grade.

Preparation of gels

Blends of 10% total solids (TS) were prepared from stock solutions of WPI, CS and corn starch in distilled water, and adjusted to pH 5.75 with a 1M HCl solution. Sucrose and salt were added when required. Mixed solutions were kept agitated to avoid sedimentation of starch granules and transferred to glass tubes (100x12.3 mm diameter), sealed with rubber stoppers and held vertically in a thermoregulated water bath at 85±1°C (T_{gel}) for 30 min. Heating rate, measured at the axis of the tube with a fine thermocouple, was approximately 10-15°C/min. The tubes were cooled in an ice/water bath for 15 min and stored overnight at 5°C. Gels were removed from the glass tubes, equilibrated at 20°C for 1 hr and cut into cylindrical sections 11 mm long for mechanical testing using a template and parallel piano wires. All gelation experiments were replicated once.

Mechanical and rheological tests

Cylindrical sections of gels (at least four) were axially compressed to 5% deformation (within the elastic range) in a mechanical testing machine model TA.TX2 (Texture Technol. Corp., Scarsdale, NJ) at a deformation speed of 0.2 mm/s. An axial compression stress was calculated by dividing the force at 5% deformation by the initial cross section of the gels, since area changes during testing were negligible. Small amplitude oscillatory testing of solutions as a function of time at a constant temperature of 85°C was performed in a Physica rheometer model Rheolab MC 120 (Physica Messtechnik GmbH, Stuttgart, Germany) using a coaxial cylinder system geometry and a thermostated water bath. The storage and loss modulus (G' and G", respectively)

were determined at a frequency of 1 Hz and under a maximum deformation of 0.5% (within the linear viscoelastic regime) to assure preservation of the gel structure.

Calorimetry studies

To study the effect of heating on thermal transitions of pure and mixed solutions of CS and WPI a Perkin Elmer DSC-7 Series 1020 (Perkin Elmer Corp., Norwalk, CT) was used. Portions (15-20 mg) of well mixed solutions at 10% TS were transferred into aluminum DSC pans and immediately sealed. Duplicate samples were scanned at a heating rate of 10°C/min in the temperature range 40-95°C using an empty pan as reference. The heating rate was similar to that during gelation of solutions in the glass tubes.

Microscopy

Several gel samples in the range $0<\vartheta_s<1$ were examined under the microscope (only four are reported). Duplicate samples for microscopy were cut with a razor blade from the center of a gel and studied in a JEOL JSM 25 SII scanning electron microscope at 25 kV after fixation in 2.5% glutaraldehyde in 0.2M cacodilate buffer (pH 7.3), dewatered in a graded series of acetone solutions and dehydrated by critical point drying in a Sorvall unit. Dehydrated specimens were fractured, placed in metal stubs and the exposed surface was coated with gold.

RESULTS AND DISCUSSION

Axial compression stress of gels

Important variables in gelation of globular proteins are the concentration and pH of the sol, temperature and time. It is known that mechanical properties of WPI gels improve as the holding temperature increases above 75°C and that curing times of 15 min are sufficient to promote adequate gelation (Aguilera and Kessler, 1989; Aguilera and Kinsella, 1991). Results from axial compression tests for pure gels are presented in Figure 1. The ratio of the standard deviation to the mean value of E varied between 3.8 and 7.2% for all determinations reported in this paper. Since a deformation of 5% falls within the elastic range, these values correspond to the elastic modulus, E. In the pH range 4-8, solutions (10% TS) of WPI formed strong gels below pH 5.8 and did not gel above pH 6.5. The CS solution did not form self-standing gels at any pH while corn starch formed weaker gels than WPI, almost independent of pH (Figure 1, inset). Similarly, while WPI gels at pH 5.75 became stronger at increasing concentration of total solids, CS solutions did not form self-standing gels even at 15% TS. Corn starch showed an almost linear relation between E and concentration, for solutions > 5% TS

(Figure 1). Cassava starch had a low lipid content (0.3%) that suggested no interference of amylose-lipid complexes in the mechanical properties. Conditions selected for the study of mixed gels were: 10% TS, pH 5.75, T_{gel} = 85°C and 30 min curing time.

Figure 2 shows the effect of the starch fraction ϑ_S [starch/(WPI + starch), w/w] on the elastic modulus (E) of mixed gels at 10% TS and pH 5.75. Care has to be taken in comparing these values with others reported in the literature under larger deformation, as the force increases with strain (i.e., Aguilera and Kinsella, 1991). Substituion of WPI by CS in the range $0<\vartheta_S<0.4$ had the effect of increasing E over the value of the pure WPI gel ($\vartheta_S = 0$). This synergistic effect had a maximum E at ϑ_S = 0.2-0.3 which was 1.6 times higher than that for the WPI gel. Further replacement of WPI by CS in the range $0.3<\vartheta_S<0.7$ resulted in decreasing moduli and for $\vartheta_S > 0.7$ no self-standing gels were formed.

The effect of ϑ_S on E for WPI/corn starch gels was different (Figure 2). Increasing the starch fraction decreased E almost linearly from the value for pure WPI ($\vartheta_S = 0$) to one similar to that of pure corn starch at $\vartheta_S = 0.4$. Elastic modulus of mixed gels in the range $0.4<\vartheta_S<1$ were similar to that of the pure corn starch gel, hence, WPI played the role of an inert, contributing to the TS effect. A weight fraction of WPI > 0.6 ($\vartheta_S = 0.4$) was required to develop associations that followed a mixing rule (e.g., linear dependence with concentration) but not to synergistic effects. Corn/CS blends yield gels weaker than those of pure corn starch and did not form gels for $\vartheta_S > 0.7$ (in this case ϑ_S refers specifically to the fraction of CS). It can be concluded that for $\vartheta_S >$ 0.7 the non-gelling effect of CS predominates when mixed either with WPI or corn starch.

It was of interest to observe the effect of incorporation of salt and sugar to mixed WPI/CS gels since food gels usually have these solutes added (Figure 3). Addition of sucrose (5%) and salt (2%) did not alter the dependence of E with ϑ_S observed in Figure 2 and led to higher E values. This further reinforcing effect can be ascribed to a reduced availability of water by the presence of these solutes and a concomitant increase in the effective concentration of both biopolymers (Labuza, 1975). Further work needs to be done on the effect of these two solutes in the mechanical and rheological properties of these mixed gels.

Dynamic experiments

Gelation kinetics were studied by small-amplitude oscillatory rheometry experiments at constant temperature (85°C) and monitoring the increase in the storage modulus (G'), the elastic (solid-like) component of the viscoelastic properties (Figure 4). Data showed a rapid initial increase in modulus representative of the transition from sol to gel. At longer times the rate of increase of G' was slower, reaching an apparent steady value (pseudo-plateau) in the semi log plot. Values of G' were almost one order of magnitude higher than those of G", the loss module (not shown), typical of true gels. G' values reached at the pseudo-plateau region followed the same behavior with ϑ_s as E values of the axial compression tests, e.g., the highest G' corresponded to ϑ_s=0.2, and values for ϑ_s>0.5 were very low. These data suggest that structural features of mixed WPI/CS gels responsible for mechanical and rheological properties are already established at the moment of gelation and are not related to cooling or aging (retrogradation) phenomena.

Muhrbeck and Elliason (1991) measured values of the storage moduli of cassava starch/ bovine serum albumin (BSA) gels as a function of protein fraction (1 - ϑ_s) at 10% TS, heated to 90°C and cooled to 25°C. As reported here, gels with ϑ_s > 0.6 showed low G' (< 0.1 kPa) and when the protein fraction increased (ϑ_s decreased) the modulus increased sharply. Unfortunately, no values were reported by these authors in the range $0<\vartheta_s<0.25$.

Thermal properties of gels

DSC scans were performed to find out the effect on gelation of the presence of one biopolymer on the thermal transitions of the other. Preliminary thermograms (not reported) of a WPI solution (10% TS) showed that the peak of the denaturation endotherm shifted from 82.2 to 74.3°C as pH increased form 4 to 6. At pH 6.5 no denaturation peak was found, coincidental with absence of gelation reported in Fig. 1.

Thermograms of WPI/CS solutions of 10% TS and pH 5.75 are presented in Figure 5. The pure CS solution (ϑ_s = 1.0) showed a single endothermic peak at 66.0°C, coinciding with early DSC work of starch-water mixtures in excess water where only one endotherm represented the starch "gelatinization" process (Lelievre, 1992). This value is the same as the midpoint gelatinization temperature (T_g) reported by Zobel (1988) for tapioca (cassava). A broad denaturation peak between 66 and 79°C was evident for the pure WPI solution (ϑ_s = 0.0) corresponding to a "denaturation" temperature (T_d) for WPI of 74.5°C, close to the value of T_d = 78.8°C (extrapolated at 0°C/min heating rate) reported by Bernal and Jelen (1985) for a whey protein concentrate at pH 5.5. As shown by these same authors, T_d decreases by increasing pH

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or reducing the heating rate and is dependent on the relative proportions of β-lactoglobulin (T_d = 75.9 - 81.2°C), α-lactalbumin (T_d = 61.0 - 61.5°C) and serum albumin (T_d = 71.9-74.0).

Only the denaturation peak near T_d of pure WPI predominated for ϑ_s=0.1 and single gelatinization peaks close to T_g of pure CS were notorious for solutions of $\vartheta_s \geq$ 0.8. Hence, the behavior of the thermal property of these blends may be regarded as typical of pure WPI or pure CS solutions, respectively. Instead, solutions of $0.1<\vartheta_s<0.8$ showed two peaks: a low-temperature peak around 64.8-65.3°C and a high-temperature peak between 73.8-74.3°C, as if both, gelatinization of starch and denaturation of proteins, occurred unaffected by the presence of the other component. A special feature of blends with ϑ_s = 0.2 and 0.3 was a well developed T_d peak and a smaller but noticeable T_g peak. In solutions with a higher proportion of CS (e.g., $0.3<\vartheta_s<0.7$) the gelatinization peak outweighed the contribution of the denaturation peak, which correlated well with the poorer gelling properties of these mixtures.

Microstructural aspects

The microstructure of gels as studied by scanning electron microscopy is presented in Figures 6 and 7. In the single component WPI gel proteins aggregated into almost spherical units 0.2-0.3 μm in diameter which in turn packed into clusters forming the network (Figure 6A). This basic microstructure is typical of gels from whey protein (Aguilera and Kinsella, 1991) and β-lactoglobulin (Stading et al., 1993) formed under similar conditions of pH, concentration and heating rate.

The WPI/CS gel at ϑ_s = 0.2 showed that both components formed independent and well-developed networks of an interpenetrating nature. This microstructural feature is in opposition to the suggestion by Muhrbeck and Eliasson (1991) that the predominant network in a mixed gel is that formed first during heating, in this case CS for $T_g < T_d$, and supports the argument that kinetic aspects of gelation as well as phase separation may be determinant in the development of microstructure. The CS mesh appeared as smooth and fine strands independent of the WPI particle-type matrix, and the presence of remnants of starch granules could not be found, as if they disintegrated completely during heating. The appearance of the CS starch network was quite different from the fine fibrillar structure of pure amylose gels revealed in micrographs prepared by a Pt-C replica technique (Guenet, 1992). Mixed gels of ϑ_s = 0.5 and 0.8 (Figure 6C and 6D) also exhibited independent WPI and cassava starch networks and no remnants of starch granules. In these gels the extent of the WPI matrix was reduced and frequently disrupted by the CS network, in agreement with the poorer mechanical and

rheological properties exhibited. At $\vartheta_s = 0.8$ the microstructure was typical of a phase-separated or composite gel network rather than of an interpenetrating one (Aguilera, 1992) with portions of gelled WPI entrapped in the weaker CS network.

Figure 7 presents the microstructure of WPI/cassava starch and WPI/corn starch gels at $\vartheta_s = 0.2$ prepared under the same conditions. While interpenetrating WPI and CS networks were clearly observed in the WPI/CS gel (Fig. 7A), the mixed gel containing corn starch was of the filled-type, with collapsed starch granules acting as embedded particles in a predominantly WPI gel (Fig. 7B). Also noticeable was the lack of adhesion between the surface of shrunk corn starch granules and the WPI matrix and the absence of an independent amylose network. These two features may account for the poorer mechanical properties of this gel relative to the WPI/CS gel (Fig. 1). Further work is necessary to extend these results to other gelling conditions.

Relation of thermal and mechanical data with microstructure

Gelation of mixed biopolymer solutions leading to structure formation is still not fully understood and remains mostly on a conjectural basis. The process appears to be heavily dominated by phase-separation phenomena as it is known that most biopolymer systems are thermodynamically non-compatible. Brownsey and Morris (1988) have classified two-component mixed gel networks as Type I, when a soluble polymer is entrapped inside the network of the other, and Type II, where both biopolymers form a gel network. In this latter case the spatial distribution of the two components will depend on the degree of mixing/demixing during gelation.

It can only be speculated at this point that amylose gelation in CS at low ϑ_s is possible by rapid solubilization from disintegrated cassava starch granules, demixing and phase separation into a polymer rich phase. Partial support to this hypothesis may come from ample evidence by Russian scientists that most protein/polysaccharide systems form phase-separated solutions (Braudo et al., 1986). Phase separation is likely to occur during most gelation processes (Guenet, 1992). Specifically, there are suggestions that amylose gelation in particular, occurs via aqueous two-phase separation into polymer-rich and polymer-deficient regions leading to turbid gels (Miles et al., 1985; Clark and Ross-Murphy, 1987). Formation of a continuous network of CS at low and not at higher ϑ_s may be interpreted by the excluded volume effect as proposed by Braudo et al. (1986). As the presence of whey proteins in solution increases, water may become a poorer solvent for starch (which gelatinizes first),

increasing its effective concentration in one of the microphases and eventually reaching the concentration threshold for gelation and network formation.

There are a number of physicochemical differences between corn and cassava starches that may help explaining their different gelling behavior (Balagopalan et al., 1988). Cassava starch has a slightly lower gelatinization range than corn (59-70°C versus 62-73°C). CS granules swell on hydration to a diameter almost 3 times larger than corn starch granules and continue to swell after pasting (Wurzburg, 1986; Vasanthan and Hoover, 1992). Paste viscosity of a cassava starch slurry as followed in a Brabender amylograph peaks at around 75-80°C and upon cooling it readily breaks down (Eggleston et al., 1993). Corn starch solutions instead, develop into a low viscosity paste on heating but show a high peak viscosity on cooling (retrogradation). Also, amylose is released faster from cassava starch granules than from corn starch granules (Vasanthan and Hoover, 1992).Sols of corn starch having a relatively high level (ca. 22-28%) of moderately-sized amylose molecules form opaque gels on cooling while cassava starch which has a lower amylose content (ca. 18%) but bulkier amylose molecules only thicken to a translucent paste on cooling but do not form gels (Wurzburg, 1986).

CONCLUSIONS

As revealed by SEM and thermal analysis, a commercial whey protein isolate and cassava starch mixed in solutions at 10% TS and pH 5.75 appear to form a mixed gel with independent networks when heated at 85°C. Elastic moduli (E) and storage moduli (G') of mixed gels were maximum at $\vartheta_S = 0.2$, where both biopolymers formed continuous, interpenetrating networks and gelatinization of CS and denaturation of WPI occurred to a noticeable extent. Kinetics of development of the storage modulus suggests that physical and structural characteristics of mixed gels develop already during heating and not on cooling. Basic microstructures may be induced in mixed WPI/starch gels by varying the relative proportions of the two components and the nature of the starch fraction. These microstructures are responsible for several mechanical and rheological properties of mixed gels.

ACKNOWLEDGMENTS

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FIGURE 1. Effect of pH at 10% total solids (TS, inset) and TS at pH 5.75, on the elastic moduli (E) of pure gels of WPI, cassava and corn starch, formed by heating the solutions to 85°C for 30 min and cooling.

FIGURE 2. Effect of starch fraction (w/w) on the elastic moduli (E) of mixed gels (10% TS, pH = 5.75, T_{gel} = 85°C): (Δ) WPI/cassava starch; ( ) WPI/corn starch gels; (o) Cassava starch/corn starch.

FIGURE 3. Effect of addition of sucrose (5%) or salt (2%) on the dependence of composition on the elastic moduli (E) of mixed gels (10% TS, pH = 5.75, T_{gel} = 85°C): (Δ) WPI/cassava starch; (□) NaCl added; (o) sucrose added.

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FIGURE 7. SEM micrographs of mixed gels ($\vartheta_S = 0.2$). A) WPI/cassava starch gel, and; B) WPI/corn starch gel. Arrows point at separation between starch granules and matrix. WP = whey protein isolate network; S = starch network; SG = starch granules.

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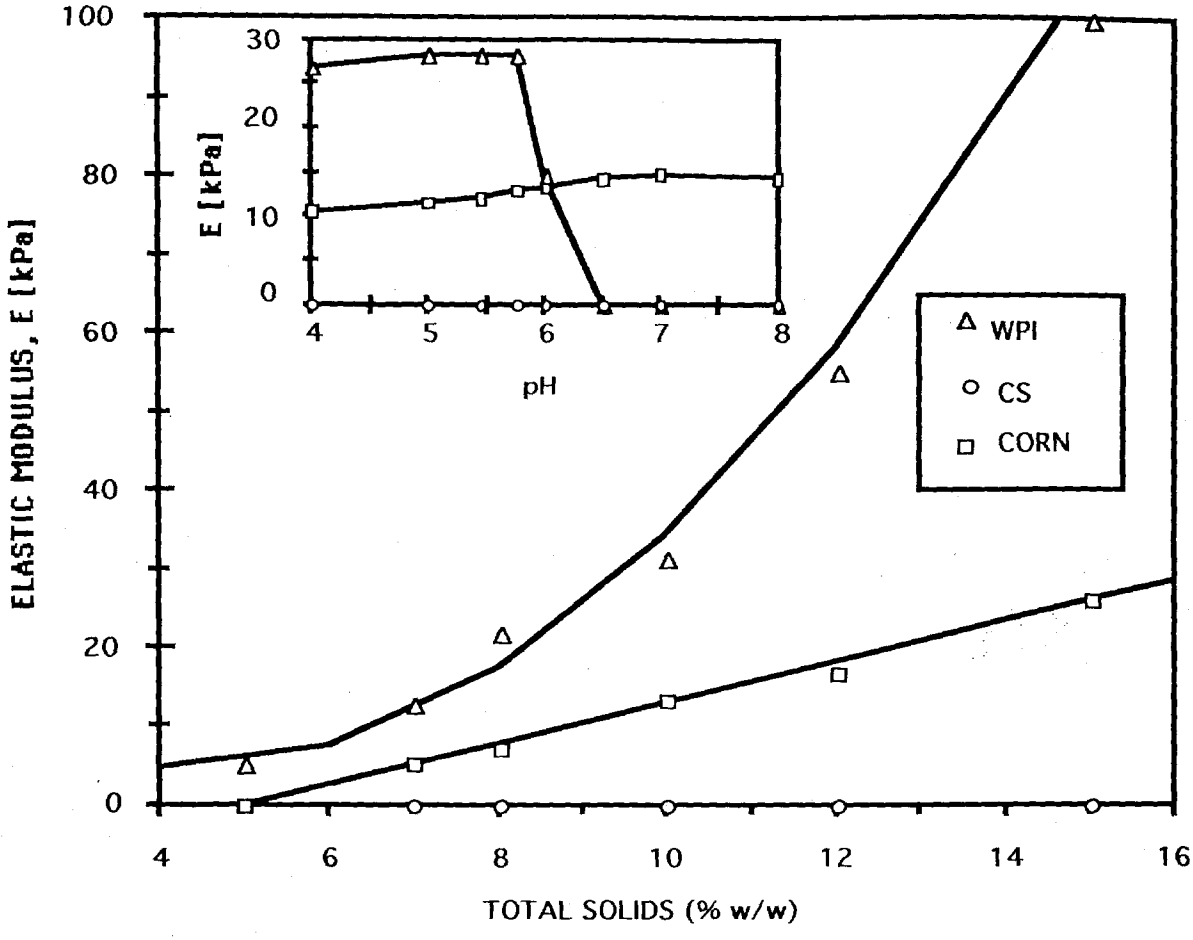

Fig.1- **Axial compression tests** for pure gels.

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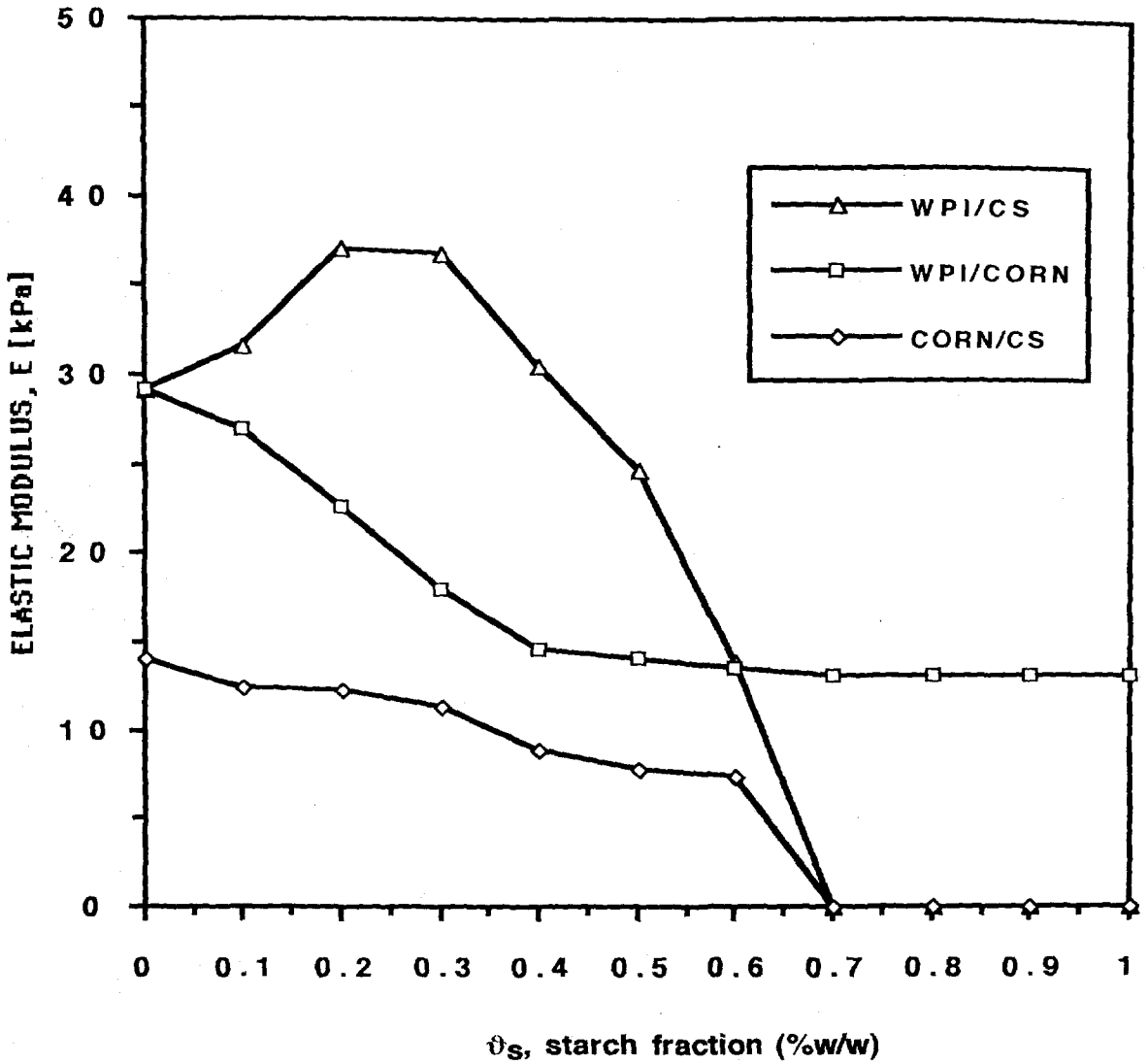


Fig.2- **Effect of starch fraction ϑ_s** [starch/(WPI+starch] on the elastic modulus E of mixed gels (10% TS, pH 5.75, T= 85°C).

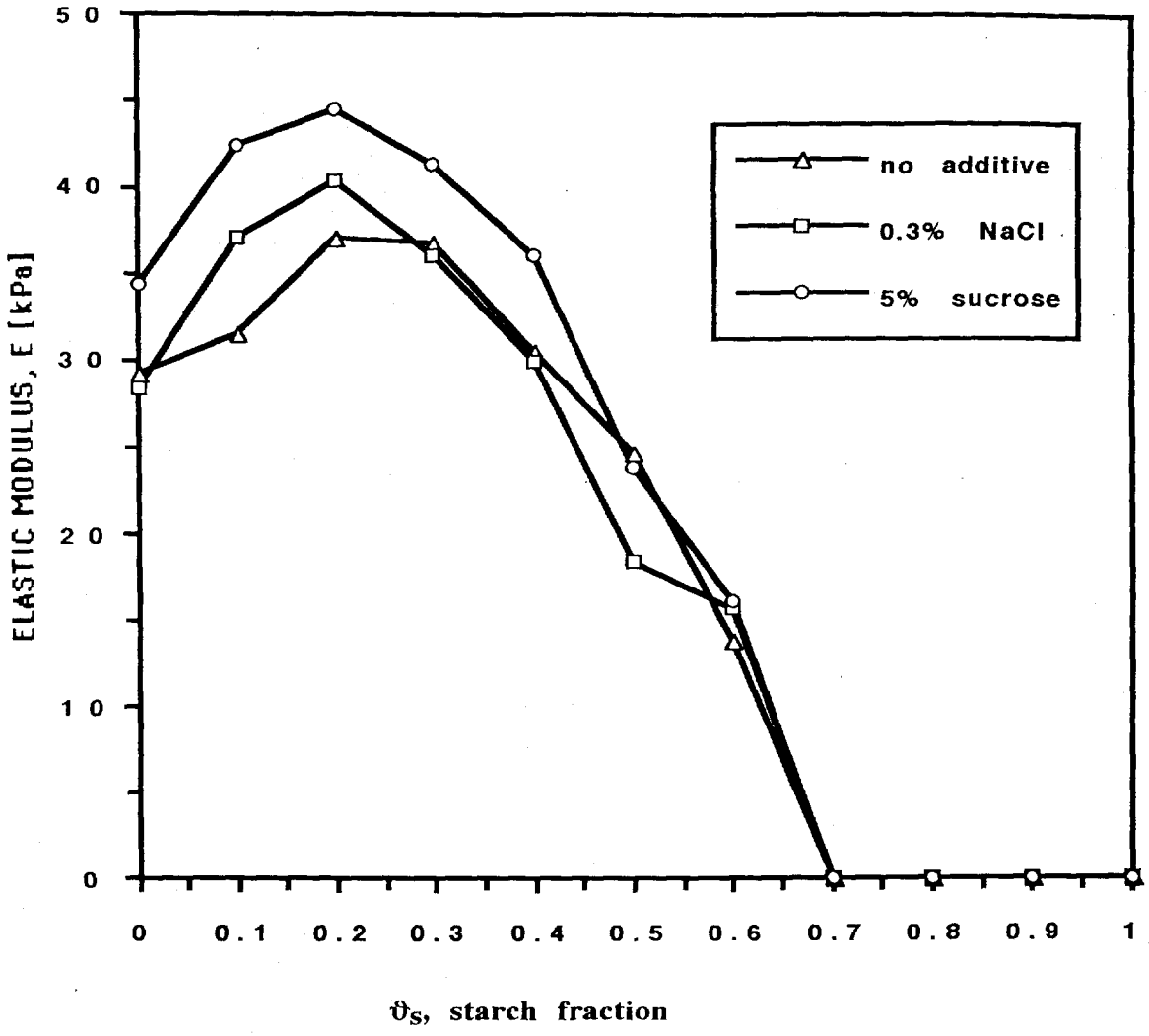


Fig.3- **Effect of starch fraction (ϑ_S) and addition of sucrose and salt** on the elastic modulus (E) of mixed WPI/CS gels. (10% TS, pH 5.75, T= 85°C).

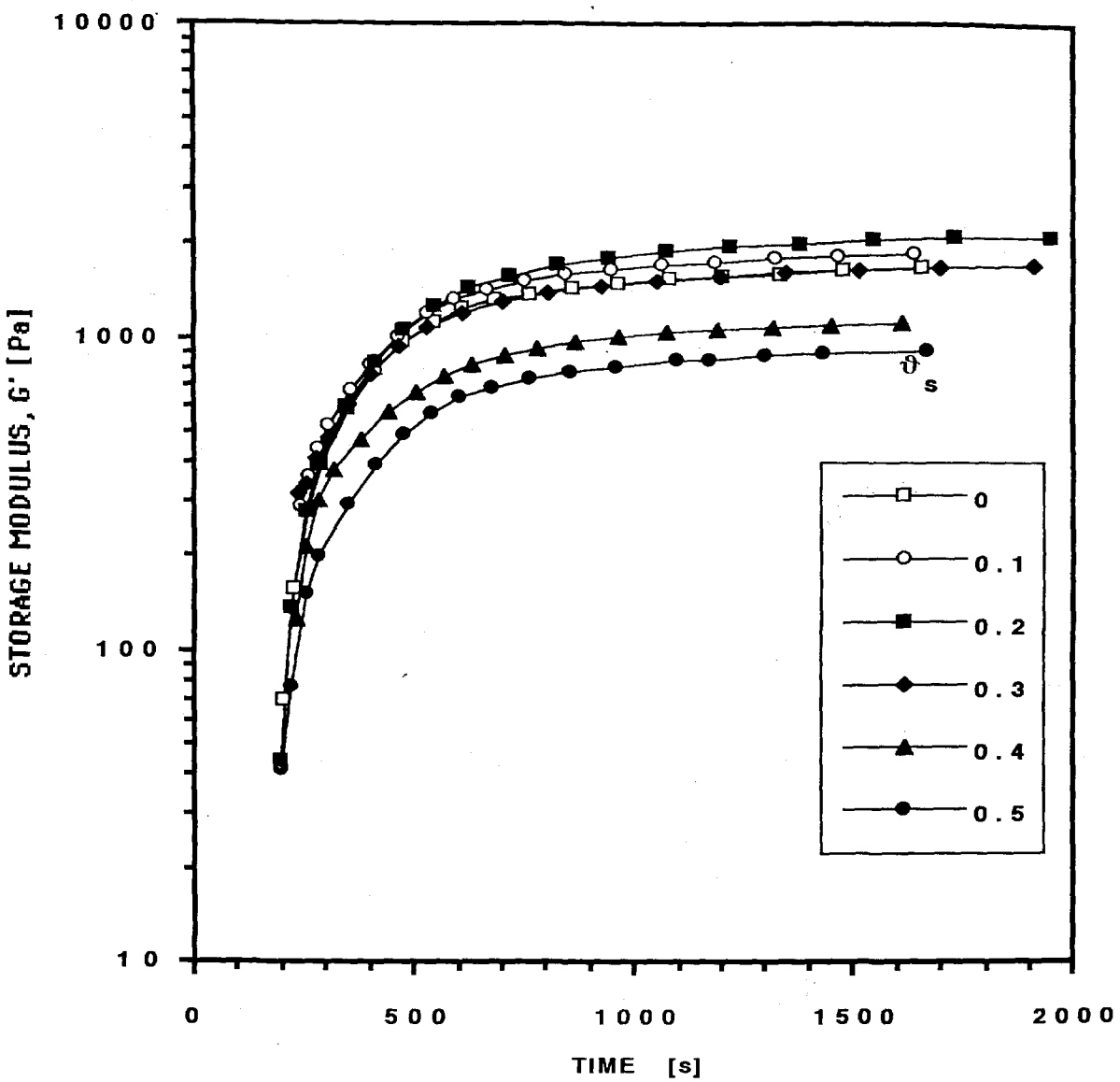


Fig.4- **Effect of starch fraction (ϑ_s) on the storage modulus (G')** of mixed WPI/CS gels. (10% TS, pH 5.75, T= 85°C).

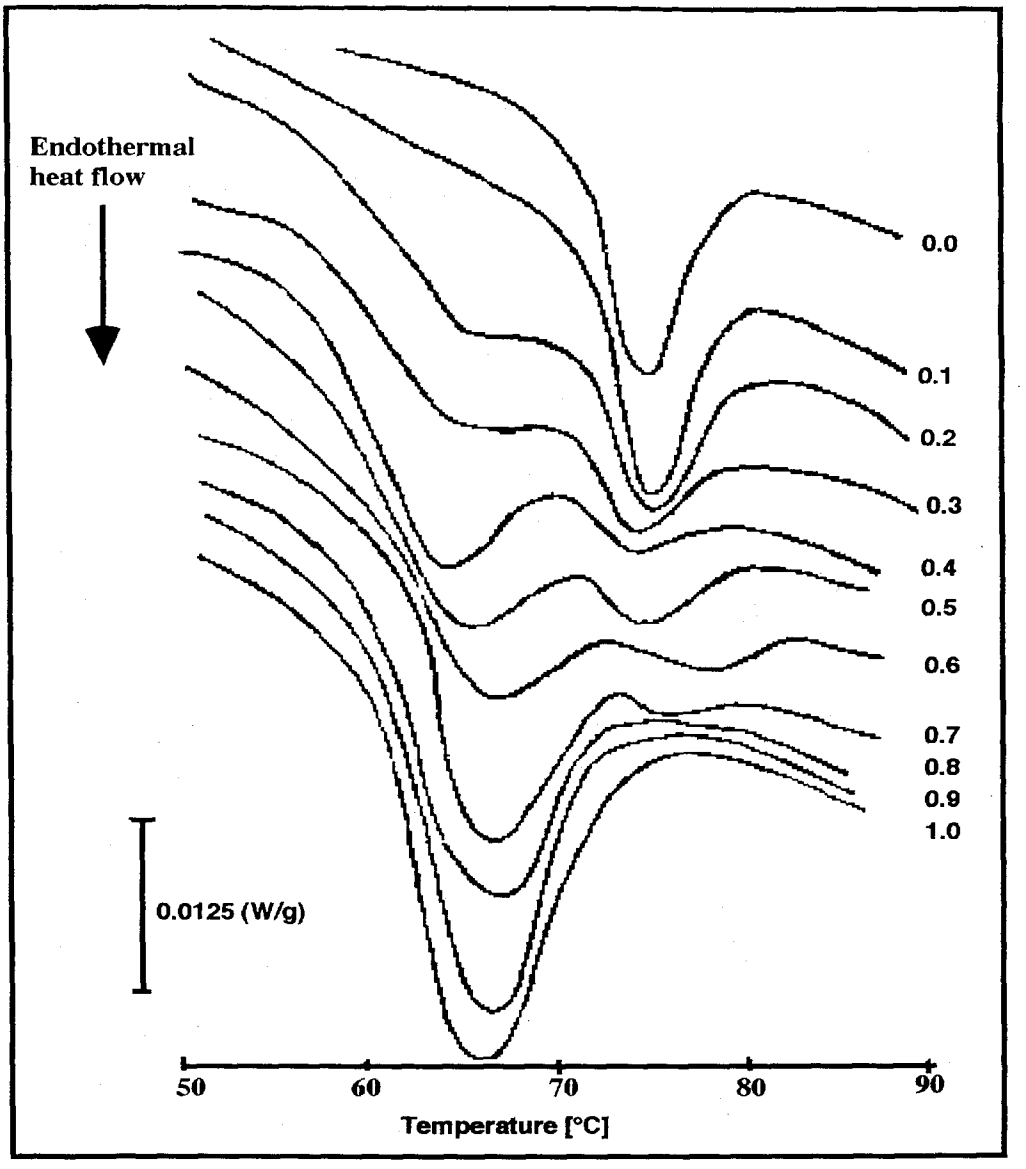
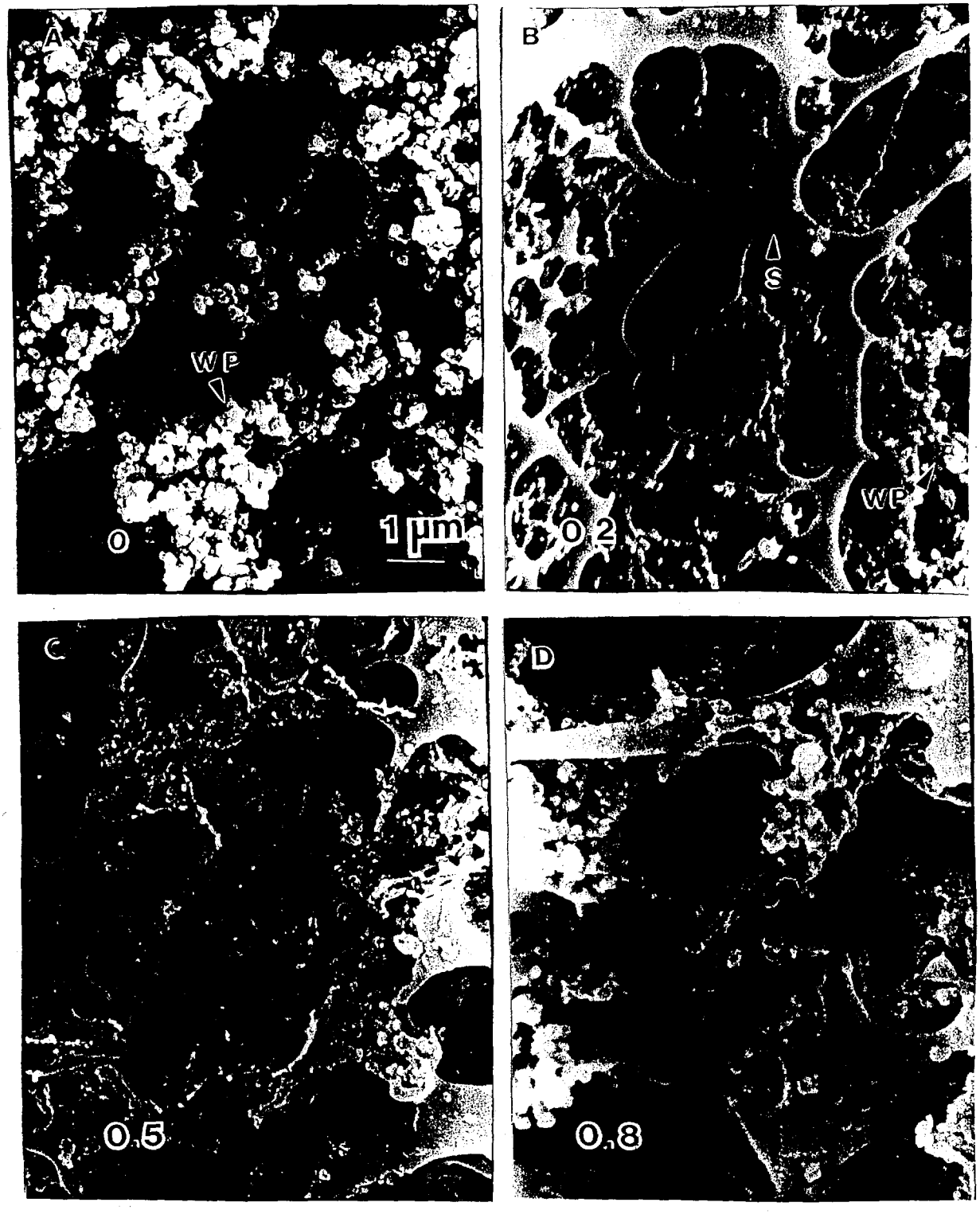


Fig.5- **Tracings of DSC thermograms** for WPI/CS blends (10% TS, pH= 5.75)



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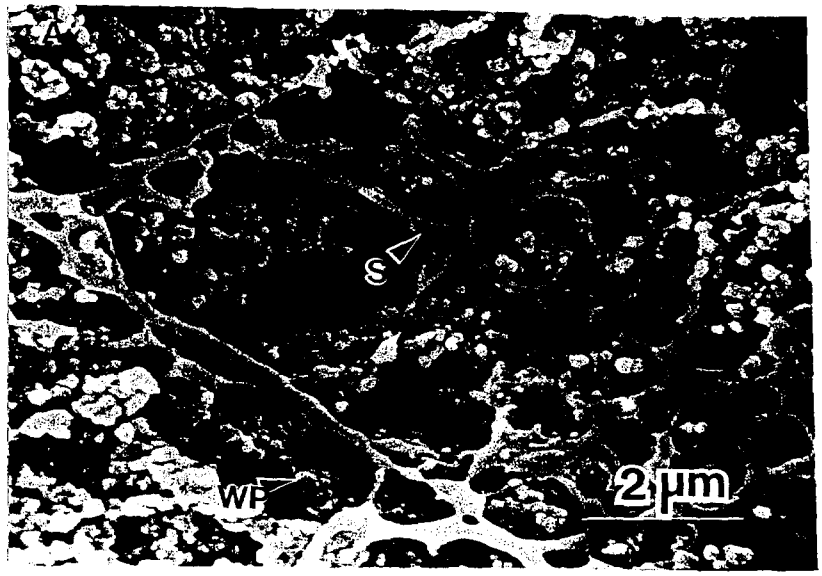
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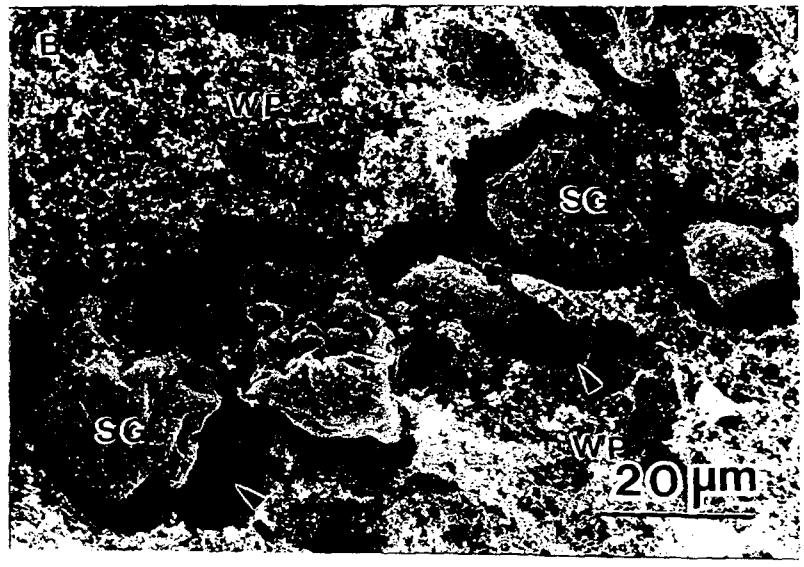
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MICROSTRUCTURAL AND IMAGING ANALYSES AS RELATED TO FOOD ENGINEERING

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Microstructural and Imaging Analyses as Related to Food Engineering

Introduction

In a classical experiment of fluid mechanics Reynolds observed that when a dye was injected to water flowing through a glass tube a critical discharge velocity was reached at which the dye stream suddenly broke up and was diffused throughout the tube (Reynolds, 1883). This study, leading to the fundamental observation of the laminar-turbulent flow transition, has had enormous practical importance in engineering and immortalized Reynolds and his famous number.

In our era of expanding technology many times we must observe, analyze, model and explain phenomena occurring at the micron (1 μm = 0.001 mm) or submicron level. In food processing and engineering several answers are rooted at this level, in the interior of otherwise non-transparent, heterogeneous objects (Aguilera and Stanley, 1990). Sometimes it is the arrangement of microelements which is determinant for a good physical model, appropriate transport equations and boundary conditions, as in extraction of solutes from cellular materials. Often it is the chemical composition of particular organelles or architectural arrangement which is relevant for engineering purposes, as in milling of wheat kernels. More recently, so called physical property-microstructure relationships are needed to interpret the mechanical, thermal or rheological behavior of complex biomaterials. This special role played by microstructure in physical and transport phenomena of biological materials is a distinguishing element of food engineering *vis-a-vis* chemical engineering.

Although significant efforts have been made to develop a "food materials science" (the best example being the application of polymer physics to amorphous food components and macromolecular gels) microscopy has not been fully exploited by engineers as compared to spectroscopy analysis or computerized mathematical algorithms. This is probably so because food microstructure and imaging use techniques developed for biology, medicine and material science, most of which rely on expensive and sophisticated equipment. Only few food science and engineering groups in academia have access to microscopy facilities on a routine basis. Last but not least, microstructural information needed for engineering analysis is usually less elaborated than for pure science, thus it is not a challenge to microscopists.

With the advent of powerful microscopy and non-destructive imaging techniques, image-capturing technologies and computer-aided analysis the possibility now exists

to observe and probe into submicron areas, gather quantitative data and analyze it with fast and powerful computers. A century after Reynolds, engineers can now emulate him and plan experiments for direct observation of the physical phenomena but orders of magnitude smaller. The aim of this presentation is to present some modern microscopy and imaging techniques and their application in food processing.

Examples of Applications of Microscopy to Food Engineering

Microscopy has become established as one of the most useful tools available to the food scientist. Its growing importance is confirmed by the publication of six articles during 1995 directly related to this technique in *Trends in Food Science and Technology*.

Foods are highly heterogeneous materials at the microstructural and ultrastructural levels. Typical sizes of important microstructures and molecules in food phyto-, myo- and lactosystems as well as the resolution level of different microscopy techniques are presented in Figure 1.

Light microscopy (LM) is a widely used technique for studying the microstructure of foods and multiple applications in food processing and engineering can be found in Aguilera and Stanley (1990). Its most used versions (bright field, polarizing and fluorescence microscopy) perform better with thin specimens and many times involve chemical processing (fixation, staining, etc.) (Kalab et al., 1995; Vaughan, 1979).

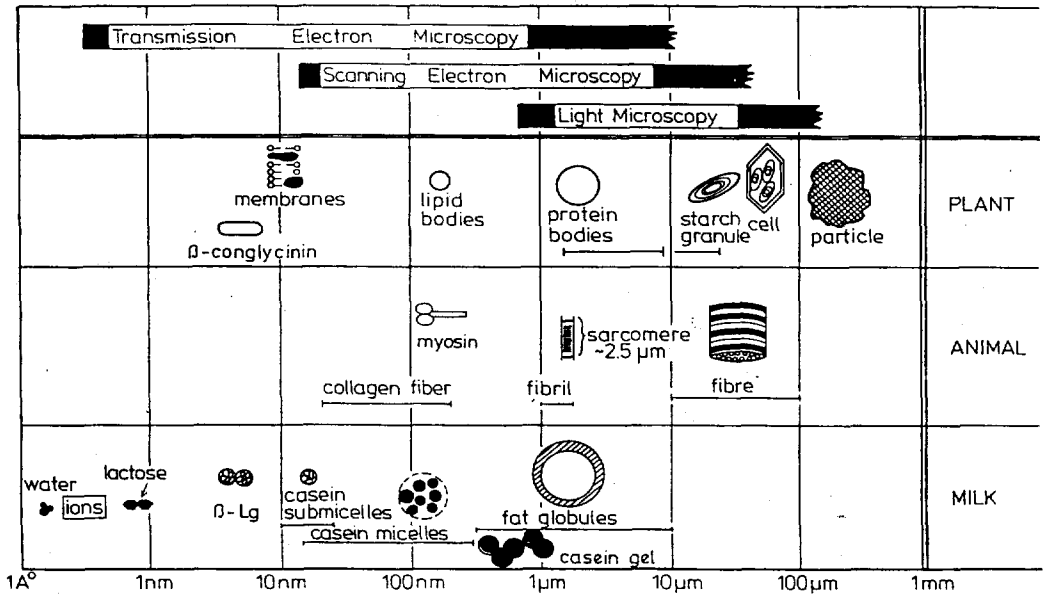

Figure 1. Examples of microstructural elements and molecules in food systems

Our laboratory has used simple LM techniques to visualize microstructural differences in comminuted pieces of fatty fish leading to cell separation according to their terminal velocities (Rutman et al., 1988) and to correlate extraction rates and microstructural modifications in red peppers (Aguilera et al., 1987). Our colleague Dr. Dave Stanley used fluorescence microscopy to demonstrate that cell wall thickening and intercellular fusion is a major microstructural change in hard-to-cook beans (Hincks and Stanley, 1987).

In electron microscopy (EM) the area to be analyzed is irradiated with a finely focused electron beam that replaces photons. The types of signals produced when the beam impinges a sample include transmitted and backscattered electrons, secondary electrons, Auger electrons, x-rays and photons of various energies. Figure 2 shows some typical interactions between the electron beam and the sample in an electron microscope. EM is typically operated at a pressure below 10-4 Pa (10-6 torr) to minimize collisions of the focused primary beam and molecules of the residual gas and to keep a low pressure at the thermal tungsten filament.

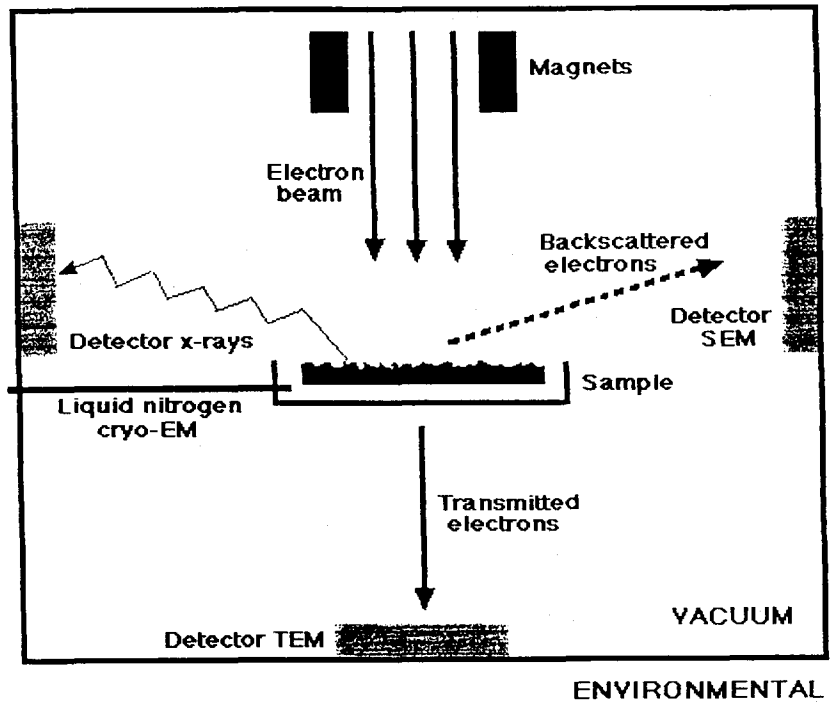

Figure 2. Schematic representation of interactions between the electron beam and the sample in electron microscopy

In biological samples the original water has to be removed by drying (generally freeze-drying or critical point drying) or solidified (freezing). No matter how gentle this process may be structural modifications ("artifacts") are likely to occur. Another requirement of EM is that nonconducting samples be metallized so to eliminate or reduce the electrical charge that builds up in the specimen when impinged by the beam of high energy electrons. The net result is that the original food sample is rarely observed in EM.

Traditional scannning electron microscopy (SEM) uses either low energy secondary electrons ejected from the specimen surface or higher energy backscatter electron reflected from the specimen to reproduce specimen topography. Both signals are typically collected by a scintillator detector requiring high vacuum environment. The resolution of commercial SEM equipment is quoted on the order of 2 to 5 nm. An excellent reference on SEM in the book by Goldstein et al. (1992).

We have also used simple SEM techniques to study fractionation of legumes into starch- and protein-rich fractions based on fine milling and air separation (Aguilera et al., 1984). The architecture of bean cotyledons (large starch granules embedded in a protein/structural polysaccharide matrix) leads itself after milling to large intact starch granules and smaller protein-rich particles (Figure 3). Note the absence of interfacial bonding between starch granules and the matrix which together with the brittleness of the latter results in a marked binodal distribution of particles after fine milling.

Figure 3. Architecture of a bean *(Phaseolus vulgaris)* cotyledon showing large starch granules embedded in a protein/polysaccharide matrix. (mag=1,000x)

Starch granules are then separated from the protein fraction in an air-classifier based on their larger terminal velocities. Fracture characteristics, particle sizes and distributions, as well as cross contamination of the two streams may be easily followed and quantified by SEM and image analysis.

Mechanisms of movement of solutions and their accompanying solutes in intact plant tissue is a phenomenon of major importance in early stages of drying, during solid-liquid extraction and osmotic dehydration and in impregnation with low molecular weight solutes (salts and sugars). We have revised these mechanisms to analyze and model the aqueous extraction process of lupin proteins from the intact seed (Aguilera, 1989). The complexity of water (liquid) movement in plant cells as revealed in this latter study is shown in a diagram in Figure 4. Given the architecture of cell walls and membranes, micropores (plasmodesmata) piercing through them and the intrinsic tortuosity and porosity of intercellular spaces it is highly unlikely that processes like aqueous extraction or osmotic drying can be modeled by applying a simple second Fick's law of diffusion to a homogeneous material. Computer-solved equations of "black boxes" always result in diffusion coefficients varying with concentration or with time, in order to fit the data!. Prof. LeMaguer has studied in detail mass transfer phenomena in structured systems (see paper in this book)

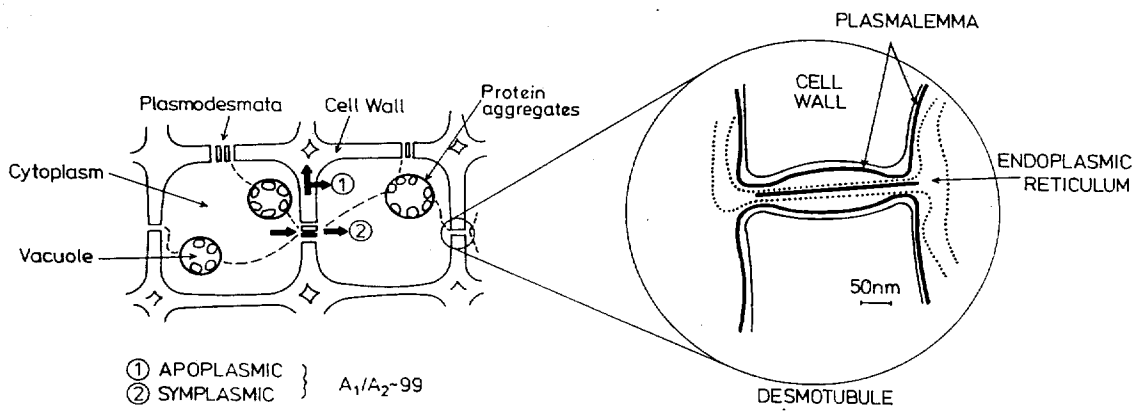

Figure 4. Plasmodesmata and fluid transport in intact plant cells

Highly hydrated food samples, e.g., water-oil emulsions, icecream or those that have delicate structures like gels can be observed directly in the SEM in their frozen state after extremely fast freezing (Cryo-SEM). Etching or sublimation of ice (at around -90C for a few minutes) is effected to reveal underlying structural features. If combined with modern field emission SEM that provides high resolution and operate efficiently at low beam energies, less damage is introduced to the sample. Alternatively, a very stable metal replica of the surface can be prepared after fracture and etching and examined in a regular TEM or SEM (Figure 5). These techniques can be used, for example, to study the effect of homogenization in emulsion formation, demixing phenomena of complex solutions, crystal growth in icecream during storage or formation of mixed gels.

FUTURE PROSPECTS

The study of foods as materials and particularly the role of microstructure in food processing and engineering will expand rapidly in the future, fostered by advances in other areas such as experimental biology, medicine and computer science (image recognition).

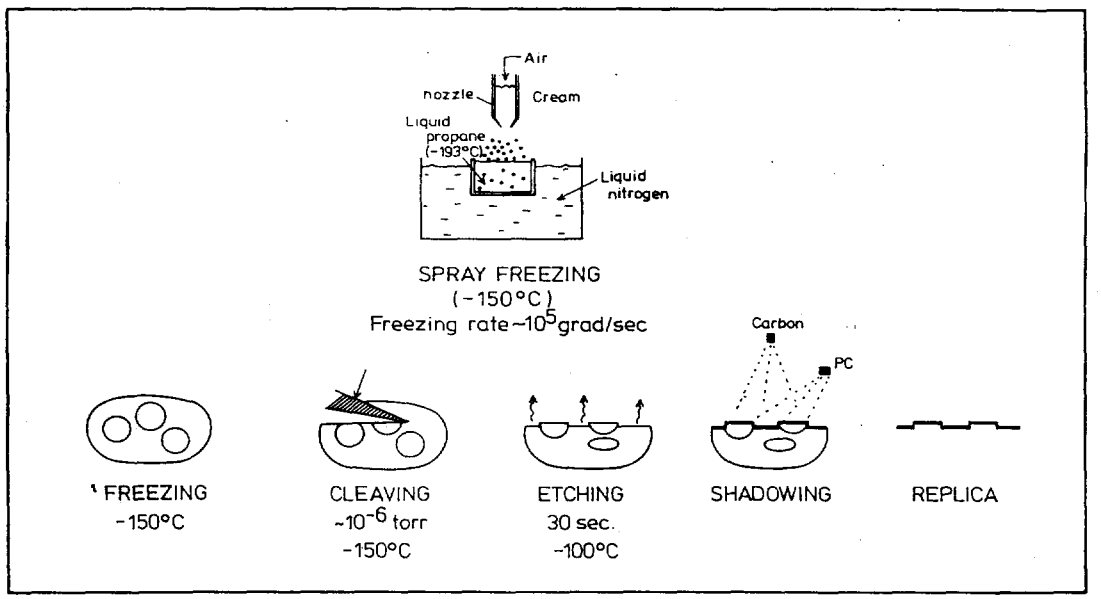

Figure 5. Steps in replica preparation of an emulsion after fast freezing, fracture and etching.

The need of better phenomenological models to predict the effect of variables and process optimization coupled to new computational methods and fast computers will also increase the use of microscopy and imaging techniques. Due to space limitations only a few developments in the microscopy/ microstructure horizon are discussed below. The dedicated reader may benefit from glancing through some of the journals and books cited in the references.

Some Advanced Microscopy/Imaging Techniques

Confocal laser scanning microscopy (CLSM) overcomes some of the limitations of LM relative to specimen thickness and sample preparation using a focused scanning laser to illuminate a "slice" in the interior of a specimen. Only the information from that focal plane is able to pass a confocal aperture and produce an image. Successive scans at different depths can be collected, processed by computer software and reconstructed into a three-dimensional model. Alternatively, a series of time resolved images can be dynamically integrated to produce a moving image showing changes in microstructure. In the fluorescence mode the laser beam is also used to excite a specific dye so that localization of chemical components can be achieved. Details of this technique and applications to foods can be found in Brooker (1995). Into the future is the adaptation of confocal imaging techniques to the study of cells of intact organs *in situ* (Petroll et al., 1994).

Soft x-rays microscopy can also be used to image living cells and tissue in their natural hydrated state. Since the degree of absorption for different components varies markedly with wavelength, images can exhibit considerable contrast.

Scanning probe microscopy (SPM) and scanning tunneling microscopy STM) are generic names for a high resolution technique in which a probe is scanned close to the specimen surface and some interaction between the probe and specimen has a strong dependence on the separation. Related names are scanning tunneling optical microscopy (STOM), scanning force microscopy (SFM) and atomic force microscopy (AFM). Among the advantages are the simplicity of sample preparation that is close to LM and the potential to resolve down to atomic size. This new form of microscopy is particularly suited to study foods at the molecular level, for example, the fine structure of hydrated gels, biopolymers in solution, cell walls and interfacial phenomena (Kirby et al., 1995; Miles and McMaster, 1995).

Coupling Microscopy and Element Analysis/Molecule Localization

There is increasing need of techniques for *in situ* identification and even quantification of ions or molecular species inside cells or food tissues. The interaction of the electron beam with a small localized area of the specimen causes the generation of many signals that may be used simultaneously to create an image and to derive data of the chemical composition. Chemical analysis in SEM is performed by measuring the energy and intensity distribution of the x-ray signal generated by a focused electron beam on the uncoated sample. The energy-dispersive (EDS) detection system has the ability to display the entire elemental spectrum. Using this technique we have confirmed that the core or protoplast of dormant spores of *B. subtilis* is high in calcium ions which are supposed to complex with dipicolinic acid (Figure 6). It is postulated that Ca dipicolinate (up to 15% dw of spore) provides a stabilizing medium

similar to that of the "glassy state" in other organisms but at higher moisture contents (ca. 25%).

Fourier Transform Infrared (FT-IR) spectroscopy is a method of determining the chemical composition of organic materials by measuring their absorption of infrared light of different wavelengths. A scanning microspectrophotometer has been reported to do measurements of composition of selected regions of a specimen as small as a few microns, specifically, layers of wheat bran (Whitworth and Edwards, 1995). The fluorescence microscope has also been used on a routine basis for carbohydrate analysis (Fulcher et al., 1994).

The distribution of water reflects important physiological processes in the cell, including those of organisms that have been severely dehydrated to a desiccated state (< 0.1 g water/g dry matter). Under these conditions, organisms (e.g., nematodes, seeds of plants, fungal spores and cysts of certain crustaceans, among others) have unusual resistance to high or low temperatures and to exposure to UV light and X-rays while key biological structures such as membranes, nucleic acids and proteins seem to be structurally and functionally preserved (Aguilera and Karel, 1996). A "glassy" state provided by accumulation of sugars (most notably trehalose and sucrose) has been postulated to play a key role in the preservation of life under these conditions. Since water is known to depress the glass transition temperature (Tg) of biopolymers, existence of a true glassy state in specific organelles depends on the localized abundance of water, rather than on average moisture contents.

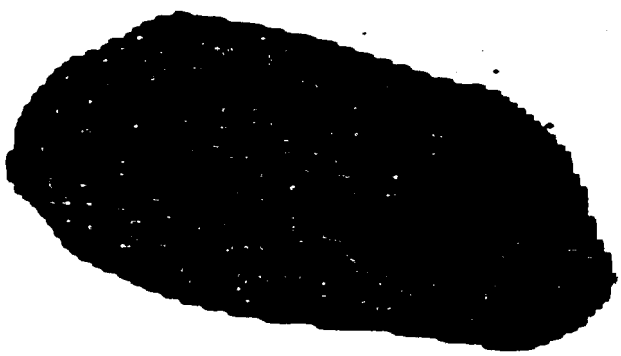

Figure 6. Primary color overlay of the compositional map obtained by x-rays spectral analysis of a spore of *B. subtilis*: red, calcium; green, chlorine. Note high concentration of calcium in the core.

A technique based on electron energy-loss spectroscopy (EELS) in the scanning transmission microscope (STEM) has been developed to map subcellular distributions of water in frozen hydrated biological cryosections. The accuracy of method for subcellular compartments is estimated at 3.5% and the limiting spatial resolution to around 80 nm for 5% precision (Sun et al., 1995).

In a recent paper Kalab et al. (1995) have summarized the use of antibodies that bind to specific sites in individual proteins, glycoproteins and polysaccharides (immunolabelling). Colloidal-gold probes manufactured in very precise sizes and coated with a secondary antibody then bind to primary antibodies with the result that TEM images now have tagged the target macromolecules with distinctive black dots. Applications of this technique range from (semi)quantitative, site- and molecule-specific chemical analysis to a tool to follow kinetics of interfacial adsorption/desorption of biopolymers in emulsions or the kinetics of extraction or infusion of macromolecules. As in the case of water, engineers will have for the first time access to actual data of concentrations as a function of space inside biological tissue, and an opportunity to apply diffusion equations at the microlevel.

"Miniaturization" of Experiments

The idea is to transfer experiments from the bench into a stage mounted in a microscope so that the effect of variables on the process can be visualized and phenomenological aspects followed in real time. The main problems are reproducing the original experimental conditions inside the microscope (e.g., temperature, pressure, RH, etc.) and those relative to having a non representative sample. An interesting example comes again from biology where several cryostages have been developed to study the effects of low temperature on cells and tissue (Echlin, 1992; McCaa and Diller, 1987; Diller and Cravalho, 1971). There are now commercial equipment that allow heating and cooling (freezing) of thin samples or solutions, at controlled rates and attached to TV monitors, VCR devises, etc. We have been following the formation of a mixed thermotropic gel starch/whey protein isolate in a "hot stage" microscope. Figure 7 shows two states of a starch granule being heated at 10C/min in a hot stage microscope

Environmental scanning electron microscopy (ESEM) allows examination of surfaces of uncoated "wet" specimens by allowing maintenance of a saturated pressure atmosphere in the chamber and thus the presence of liquid water. ESEM uses a vacuum gradient (differential pressures) along the beam path to maintain high vacuum conditions at the filament while permitting chamber pressures to be varied up to approximately 20 torr. Such a vacuum system can maintain a six-decade pressure differential between the electron gun and the specimen chamber. The gas in the chamber (oxygen, nitrogen, water vapor) suppresses charge buildup and it is ionized by secondary electron derived from the specimen. Electrons from ionizing gas molecules precipitate cascade multiplication of the imaging current that is monitored by a detector (Danilatos, 1993). The partial pressure of the gas combined with a temperature controlled stage means that even liquid water can be maintained under operating conditions. Sample preparation is eliminated thus reducing or eliminating important types of artifacts. Experiments such as phase transformations can be

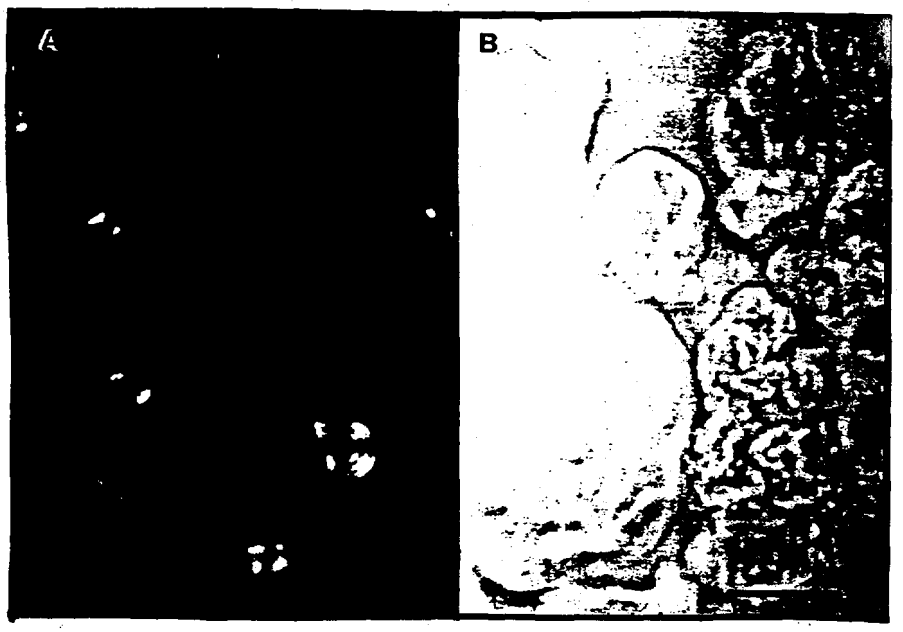

Figure 7. Photographs taken during a simulated run of gelation of cassava starch in a hot-stage microscope. A:Raw starch dispersion at 40°C; B: Gelatinized starch at 70°C. Marker = 20 μm

followed dynamically and recorded on a videotape or the microstructure of food powders as taken from a commercial sample can be directly viewed (Figure 8).

Figure 8. ESEM photomicrograph of a dairy creamer produced by spray drying as viewed in a JEOL 5300LV microscope.

Coupling Physical Property Analysis and Microscopy

Development of a food materials science requires understanding the physical properties/function of biopolymeric microstructures. Several physical techniques borrowed from polymer science have been successfully implemented to characterize their rheological behavior, thermal transitions and mechanical properties.

For example, quality characteristics of many fruits and vegetables can be directly related to mechanical properties of cell walls. Thus, the capability of mechanically testing cell walls in compression and tension while visualizing strained areas and fracture patterns may lead to better mechanical models and the application of mathematical techniques (e.g., finite elements) to predict textural properties. Similarly, running oscillatory dynamic during heating of a thermosetting protein while observing transitions at the macromolecular and microstructural level may find important applications in product development.

Use of Non-invasive Image Techniques in Processing and Quality Control

Magnetic resonance imaging (MRI) initially developed for medical applications (tomography) is a non-invasive technique for viewing sections through living tissue by means of large oscillating magnetic fields. Nuclei, such as ^1H (proton) behave as charged bodies with angular momentum and a magnetic moment. Application of an external magnetic field causes the magnetic moments to precess in the direction of the applied external field at a typical frequency (ω, the Larmor frequency). The return of the system to equilibrium (relaxation) provides information on the physical state of the nuclei. The signal generally decays exponentially with two time constants: T_1, the spin lattice relaxation time and T_2, the spin-spin relaxation time. Images are produced when pulsed linear magnetic field gradients produce a frequency variation across the sample that can be converted into spatial coordinates. Magnetic resonance microscopy extends MRI into applications with a spatial resolution of about 100 μm or less. Examples of applications of NMR imaging to food engineering are: study of velocity profiles during extrusion processing; determination of temperature gradients during heating/freezing, following moisture diffusion during drying; studying crystallization phenomena in fats: determination of bruising and other internal injuries in fruits, etc. (McCarthy, 1994). The possibility exists that vision detectable information be used for large scale, in-line quality control as in fruit packaging.

Another example of and advanced non-destructive microtechnique is the use of acoustic microscopy (AM). AM provides unique possibilities for probing mechanical properties of cells and tissues (Bereiter-Hahn, 1995).

Computer-Mediated Image Processing/Quantification and Analysis

It is important to quantify many of the features revealed by microscopic examination of foods: sizes and shapes of cellular components, thickness of cell walls or particle networks in gels, pore size and size distributions in gels, relative proportions of various phases, etc. Quantitative image analysis is the extraction of information from data that are in the form of images or pictures. An excellent introductory book to image analysis in biology is Glasbey and Horgan (1995).

A modern microscopy facility, be it light or electron microscopy, consists of the following peripheral hardware as part of a computerized image analysis system:

◊ An image capture device to create images form samples in the microscope (e.g., a video camera).
◊ A display monitor to look at images as they are being captured by the device.
◊ A recording device (e.g., a VCR) where images can be stored and played back.
◊ A central processing unit which transform the original image into a digitized form
◊ Peripheral storage media to store digitized images at low cost (e.g., optical disks).
◊ A printer or other device to produce permanent copies of images

A large number of image analysis software are now available that allow for image editing, feature measurement, greyscale morphological operations, zooming and scrolling, and processing with text and pointers into an integrated figure for presentation (Figure 9).

The determination of the kinetics of caking of amorphous powders is one example of the application of image analysis to food engineering. Caking is a deleterious phenomenon by which a low-moisture, free-flowing powder is first transformed into lumps, then into agglomerates and ultimately into a sticky material, resulting in loss of functionality and lowered quality (Aguilera et al., 1995). Samples of powders kept at constant RH and temperature may be observed at different times by SEM, their images digitized and enhanced in their important features (e.g., areas) so that numerical data are extracted and analyzed statistically. Thus, morphological aspects like bridge formation and structural modifications due to flow can be assessed as a function of time and kinetics constants determined as a function of relevant parameters.

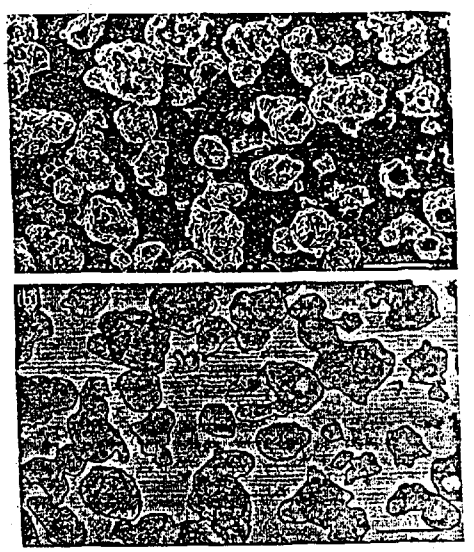

Figure 9. A) Original SEM photomicrograph of a mixed gel. B) After processing by image analysis

Acknowledgments

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[illegible] RELATED [illegible] CHEMICAL CHANGES IN FOODS

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Glass transition characterizes the change of an amorphous solid to a highly viscous, supercooled fluid. Foods in the dehydrated state are often glasses and the amorphous state is also typical of the unfrozen phase of frozen foods. Glass transition occurs at a material specific glass transition temperature (T_g) range concomitant with a drastic increase of molecular mobility and free volume. Dehydrated and low-moisture foods, including sweets, exhibit stickiness, caking, and structural collapse, which result from a decrease of viscosity above T_g. These changes are time-dependent with relaxation times determined by the temperature difference ($T-T_g$) above the glass transition.

T_g values of food materials decrease with increasing water content. Water adsorbed during storage may depress the T_g below ambient temperature. Such plasticization promotes physical and chemical changes including loss of crispness, which is detrimental to the quality of many cereal foods and snacks. Temperatures above T_g may also allow time-dependent crystallization of amorphous components, which e.g. lactose crystallization in dehydrated milk products, contributes to deteriorative changes such as lipid oxidation and nonenzymatic browning.

Stability of frozen foods is related to the glass transition temperature of the maximally freeze-concentrated solute (T'_g) and the onset temperature of ice melting (T'_m) in the maximally freeze-concentrated state. Temperatures above T'_g allow ice recrystallization and may affect rates of deteriorative reactions. Ice melting above T'm increases unfrozen water content, dilutes solutes, and depresses the rate controlling T_g, which may facilitate deterioration of frozen foods.

State diagrams, which show material physical state as a function of temperature and water content, can be used to predict the probability of deteriorative changes in processing and storage. They also provide a valuable tool for the control of food processing to improve product quality in processes including agglomeration, extrusion, dehydration, and freezing.

KEY WORDS--glass transition, food stability, water content

51-2

THE EFFECT OF ORDER-DISORDER TRANSITIONS AND CHEMICAL REACTIONS ON CEREAL PROTEIN RHEOLOGY

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We have studied the glass transition using mechanical spectrometry and differential scanning calorimetry of zein, gliadin and glutenin as a function of moisture content; all 3 cereal proteins were plasticizable by water with the first few percentages of water dramatically decreasing glass transition temperatures of all three proteins. This suggested that their hydrophilic domains play an important part in rheological properties in limited water content. The plasticization effect can be predicted by the Gordon and Taylor equation.

Measurement of G', G" as a function of temperature using small amplitude oscillatory measurements suggested that all 3 proteins undergo crosslinking reactions in the temperature range 70°C to 160°C resulting in the formation of a network structure usually attributed to disulfide linkages similar to the vulcanization of rubber. The number of crosslinkages per mole calculated in the temperature range studied supported that a network structure was being formed. Above 160°C all 3 proteins show characteristic temperature softening. The key differences between zein and gliadin/glutenin were in the temperature of initiation of 3 phases formed: entangled polymer flow phase, network reaction, and softening which were approximately constant with moisture content in the case of gliadin while they significantly varied with moisture content in the case of zein. Also the beginning of the network reaction and softening of the network in zein occurs at a much higher temperature than in gliadin/glutenin. Phase diagrams characterizing behavior as a function of moisture content and temperature were developed for each protein.

We also found that the Bird Carreau model was successful in predicting rheological properties of glutenin in steady flow in the shear rate range of 10^{-5} to 10^{3} sec^{-1} and oscillatory flow in the frequency range of 10^{-3} to 10^{1} rad/sec.

KEY WORDS--protein, rheology, glass transition

WLF VS ARRHENIUS KINETICS FOR PREDICTING THE EFFECT OF TEMPERATURE ON CHEMICAL REACTION RATES AFFECTING FOOD STABILITY

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Through the application of some principles of food polymer science, the understanding of the chemical stability of foods with respect to both moisture and temperature may be better understood if one can account for systems which are not at true equilibrium. This application requires the knowledge of the glass transition temperature vs moisture relationship which is known as the state diagram. The supporters of this new paradigm note that at any given moisture content, a system below the glass transition temperature (T_g) is supposedly very stable to reaction whereas above T_g, the viscosity of the system at the molecular level decreases in an exponential fashion and thus chemical reaction rates are enhanced. This new paradigm is quoted as being better than the concept of a monolayer moisture content as calculated from the BET or GAB isotherm. The criticism lays in part that an isotherm requires true thermodynamic equilibrium and foods are never at equilibrium. If indeed the polymer science approach is correct, the proponents then state that reaction rates no longer follow the classical Arrhenius relationship but rather follow WLF kinetics.

This paper will review these principles and then present both our own and literature data for chemical reactions. These results show that in fact, reactions can occur above and below the T_g line and most cease at the monolayer moisture which is in some cases far below T_g. Because of the limited temperature range, data fit both WLF and Arrhenius plots although a kink in the latter suggests a T_g is present. The concept is best understood as a system in which the ratio of the chemical reaction rate constant to the diffusion rate to the reaction site is controlling. If diffusion is slow then WLF controls, if diffusion is fast then Arrhenius holds. Examples of these extremes will be given.

KEY WORDS--WLF equation, Arrhenius equation, food stability

51-4

GELATION OF WHEY PROTEINS

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Gel formation is an important event in food technology since many polysaccharides and proteins have the ability to form expanded networks retaining an aqueous phase. Whey proteins can form fine-stranded or particle gels and the transition from one type to another can be controlled by pH. Particle gel formation from whey protein solutions at pH<6 proceeds through a pregel state of almost uniform aggregates in the micron range. Gelation or formation of an infinite network of protein aggregates is achieved at temperatures above 75°C and concentrations higher than 4%. The kinetics of gel formation beyond the gel point has been studied as a function of temperature and concentration by measurements of small deformation compression and fracture stresses and by small amplitude dynamic testing at constant frequency.

Structural characterization of the pregel and gel states by electron microscopy (SEM, TEM and cryo-techniques) allow for direct observation of the network but may involve formation of artifacts. Application of non-invasive NMR techniques to intact gels allow for estimation of mean pore sizes from experimental data of the transverse relaxation time (T2) through the application of mathematical algorithms.

Gelation of whey proteins in the presence of other components (fat, starch, other proteins) affect the gelation process in still unpredictable ways, leading to complex structures. Rheological data and microstructures of mixed systems will be presented and discussed.

KEY WORDS--gelation, whey protein, microstructure

STRUCTURE-PROPERTY RELATIONSHIPS IN WHEY PROTEIN/STARCH MIXED GELS

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Protein and starches occur naturally in cereals and legumes or are combined to produce desirable food textures (e.g. egg/wheat, milk/corn starch). Recent availability of refined sources of both biopolymers justify a study of mixed protein/starch systems to develop new foods or improve existing ones.

Whey protein isolate (WPI), corn starch (CS) and cassava or yuca starch (YS), where studied as mixed gels at 10% total solids (TS) and pH 5.75. Gels were prepared by heating WPI/starch solutions with varying weight fractions of starch, ϕ_S (e.g. $0<\phi_S<1$) at 85°C for 30 min followed by cooling.

Compression stress (σ) at 5% deformation (within the elastic range) was determined for pure gels and for mixed WPI/starch gels.as a function of pH and ϕ_S. For similar TS, pure WPI gels were stronger than CS gels in the concentration range 5-15% (w/w); YS did not form gels under any condition. Mixed WPI/starch gels had optimum σ around pH 5.5-5.8.

Addition of YS in the range $0<\phi_S<0.5$ reinforced mixed WPI/YS gels. A maximum σ, 1.6 times higher than that of a pure WPI gel, occurred at $\phi_S=0.2$. WPI/CS gels followed typical mixing rules in the range $0<\phi_S<0.5$. WPI/starch solutions did not form gels for $\phi_S > 0.7$.

DSC scans showed that mixed gels presented independent peaks for gelatinization of starch and denaturation of whey proteins, suggesting phase-separated gels.

The microstructure of mixed gels as studied by scanning electron microscopy revealed independent networks for WPI and starch fractions. Whey proteins aggregated into almost spherical units 0.2-0.3 µm in diameter packed in a string-of-beads arrangement while YS gelled as a fine sponge. WPI/YS gels at $\phi_S= 0.2$ showed well-developed intermeshing networks of WPI and YS. Swollen and collapsed starch granules embedded in an amylose gel was typical for CS containing gels. Non-gelling WPI/starch solutions ($\phi_S > 0.7$) failed to form a continuous WPI network.

Heating mixtures of WPI and two types of starches at 10% TS resulted in a wide array of textures varying from strong gels to pastes.

57-1

CATHEPTIC DEGRADATION OF PACIFIC WHITING SURIMI MYOFIBRILS

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Pacific whiting (*Merluccius productus*) has been used successfully for surimi production due to its bland taste, color and low price compared with that of commonly used Alaskan pollock. Pacific whiting surimi, however, presents a significant technical challenge in proper gelation because of its proteolytic degradation of the myofibrillar components. The degradation was observed to be the highest at temperatures around 55°C, resulting in a serious loss of surimi gel strength. Crude preparation of this enzyme was shown to have the maximal activity at 55°C and pH 5.5.

When activities of the most active cysteine proteinases (cathepsins L, B, and H) were compared, cathepsin B showed the highest activity in fish fillets, and cathepsin L in surimi. Washing during surimi processing selectively removed cathepsins B and H but not cathepsin L. Cathepsin L showed the maximum activity at 55°C in both fish fillets and surimi, indicating its activity is mainly responsible for degradation of muscle tissue and surimi gel.

Autolytic analysis of surimi proteins showed that myosin was the primary target, while actin showed only limited hydrolysis during 2 hr incubation. A purified Pacific whiting proteinase was incubated with various components of fish muscle protein. The proteinase was capable of hydrolyzing purified myofibrils, myosin, actin, and insoluble and heat-denatured stroma from Pacific whiting. This result contributed to the growing body of evidence that the purified proteinase from Pacific whiting muscle is cathepsin L.

Keywords --- cathepsins, Pacific whiting, surimi

EFFECTS OF COMPOSITIONAL AND PHYSICAL CONDITIONS ON THE COLORS OF ALASKA POLLOCK AND PACIFIC WHITING SURIMI GELS

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Color, like texture and flavor, is an important quality aspect of surimi and surimi seafoods. A variety of commercially available surimi seafood has its own unique color and appearance. The objective of this study was to investigate the effects of the compositional and physical conditions on the gel colors of Alaska pollock (*Theragra chalcogramma*) and Pacific whiting (*Merluccius productus*) surimi.

Prepared gels were applied to color analysis using a Minolta CR300 Colorimeter. CIE L*(black(0) to light(100)), a*(red(60) to green(-60)), and b*(yellow(60) to blue(-60)) values were measured to evaluate the effects of moisture contents (73.5, 76, 78.5, 81%), cooking temperatures (90°C, 25/90°C), sample sizes (18, 30mm diameter), light conditions (light, dark room), freeze/thaw (3,6,9 cycles), test temperatures (5, 25°C), and fish species (pollock, whiting) on colors during measurement. Ten samples were used each treatment. All data were statistically analyzed.

Water addition affected L* and b* values very significantly. The higher the moisture, the higher the L* value and the lower the b* value. Gels with higher moisture content looked whiter. Cooking temperature did not affect gel colors significantly when additional water was added. Small samples looked lighter (high L* value) and more greenish hue (lower a*). Light condition at room during measurement did not affect colors at all. As gels were abused by freeze and thaw during frozen storage, L* value decreased significantly: greater reduction with higher moisture content, while a* and b* values were remained relatively constant. Test temperature affected L* values, not a* and b*. Samples set at room temperature for 2 hrs were lighter than samples kept in refrigerator. However, a* and b* remained almost unchanged. Pollock surimi gel is significantly whiter than whiting gel: pollock gel had higher L* and lower b* values.

KEY WORDS- color, surimi gel, physical/compositional factors

57-3

EFFECTS OF WASHING AND LOW TEMPERATURE FROZEN STORAGE ON PROPERTIES OF ALASKA POLLOCK SURIMI

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Surimi has become a major protein commodity in recent years. Predominately made from Alaska pollock, this myofibrillar protein paste is plagued by low recoveries and the need for cryoprotectants and other additives to maintain its unique gelling properties. The recovery of surimi from round fish is about 18% where as that of mince is about 50%. This study investigates the effects of washing, refining and frozen storage temperature as ways to enhance full utilization of the muscle protein from Alaska pollock.

Four different treatments were compared during storage at -20°C and -40°C. Freshly caught fish were processed at a local plant using a traditional surimi line. Fillets were high graded and surimi made from small or damaged fillets and trim. Mince samples were taken prior to the first wash tank, one set had 4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphoshate added as cryoprotectants. Surimi samples were taken just before and after addition of the same cryoprotectants at the end of the line. All samples were then formed into 10 kg blocks and frozen to -30°C in a plate freezer before being transferred to the final storage temperatures. Control samples of all four treatments were stored at -70°C.

Samples were tested after three, seven, fourteen, thirty and 180 days frozen storage. Tests included: Punch and torsion tests of functional properties, color, salt extractible protein profiles by SDS-PAGE, and scanning electron microscopy of ultrastructure. Functional test samples were prepared with addition of 2.5% salt, sucrose and sorbitol to 4%, and sodium tripolyphosphate to 0.3%.

Mince samples were slightly darker with an L value of 71.6 as compared to 76.0 for the surimi samples. Since the mince samples did not give consistent punch test values, the torsion test proved the most useful functional test. Stress values for surimi started at 71.6 kp with cryoprotectants and 50.1 kp without cryoprotectants. Mince values were all less than 20 kp. In all cases samples stored at -40°C were more stable than the corresponding samples at -20°C. After one month frozen storage the strain values indicated the following descending order of functional quality: Surimi(-40), surimi (-20), surimi(-40, no cryo), mince(-40, cryo), surimi(-20, no cryo), mince(-20, cryo) and mince(no cryo, either temperature).

KEY WORDS--surimi, functional properties, frozen storage

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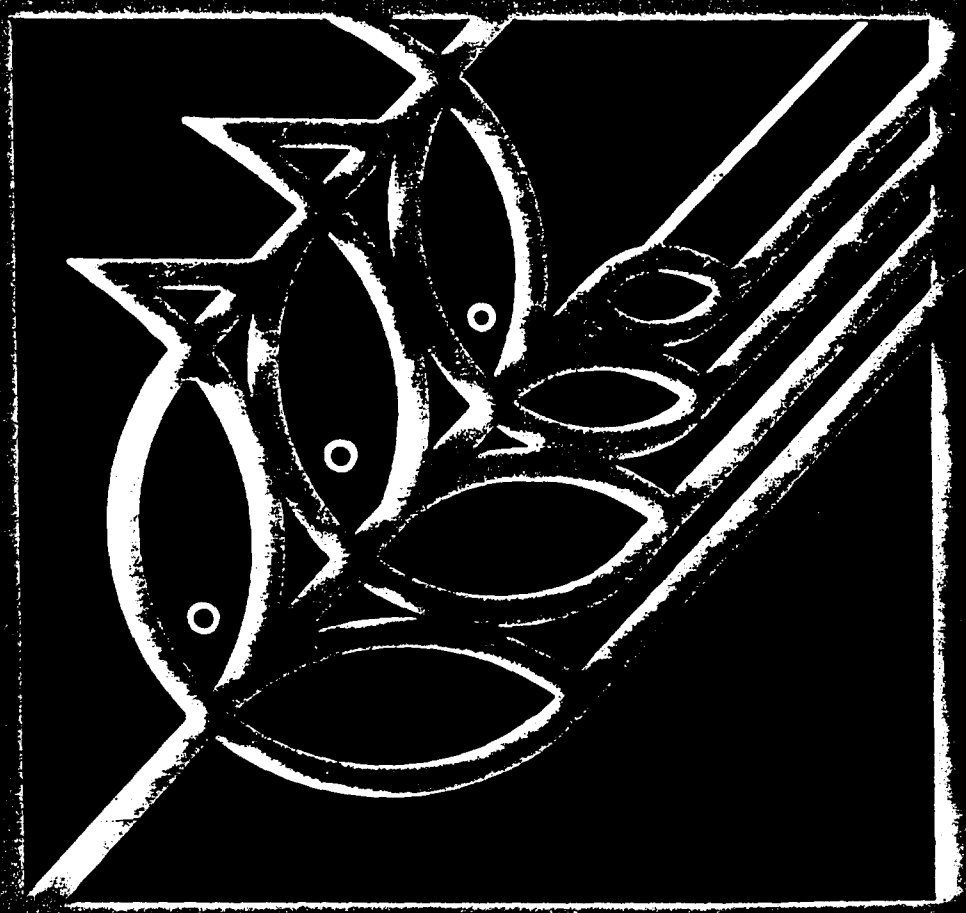

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VIÑA DEL MAR ○ CHILE

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PROGRAMA GENERAL XI CONGRESO NACIONAL DE CIENCIA Y TECNOLOGIA DE ALIMENTOS

DOMINGO 15 DE OCTUBRE

19:00 hrs. **Ceremonia Inaugural**
Salón de Honor Casa Central de la Universidad Católica de Valparaíso

* Conferencia del Dr. Santiago Funes Representante Regional Adjunto de la FAO para América Latina y el Caribe y Representante de la FAO ante el Gobierno de Chile.

* Cocktail de bienvenida.

LUNES 16 DE OCTUBRE

08:00 hrs. Inscripciones

08:45 hrs. Mesa Redonda: "Innovaciones Tecnológicas para la Industria de Alimentos".

10:15 hrs. Café

10:45 hrs. Continuación Mesa Redonda.

12:00 hrs. Conferencia "Modelling, Simulation and optimization as a Tool to Improve Food Processing Plant Efficiency".
Dr. Paul Singh, Universidad de California (Davis), U.S.A.

13:00 hrs. Almuerzo Libre

14:30 hrs. Sesiones Temas Libres (6 salones simultáneos).

16:15 hrs. Café y Presentación Temas Libres modalidad Poster.

17:00 hrs. Continuación Sesiones Temas Libres.

EFECTO DE LA ADICION DE k-CARRAGENATO EN EL PROCESO DE GELIFICACION DE SURIMI DE JUREL (*Trachurus murphyi*) CON DISTINTO CONTENIDO DE KCl.

AGUILERA, J. y ORTIZ, J.

DEPARTAMENTO DE INGENIERIA QUIMICA Y BIOPROCESOS. FACULTAD DE INGENIERIA. PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE. AV. V. MACKENNA 4860-SANTIAGO.CHILE.

Para mejorar la funcionalidad de proteínas en alimentos marinos como surimi, se están utilizando polisacáridos naturales gelificantes que provocan un impacto en las propiedades reológicas y en la textura de alimentos gelificantes, siendo aún poco conocidos los mecanismos e interacciones involucradas.
Los objetivos de este trabajo fueron examinar el efecto del polímero k-carragenato en el proceso de gelificación de surimi de jurel (*Trachurus murphyi*) con distinto contenido de KCl. Además, determinar la temperatura de desnaturalización de las proteínas de surimi, junto con la textura y la microestructura adquirida por los geles mixtos surimi-carragenato.
El efecto sinérgico de k-carragenato en la gelificación de surimi con distinto contenido de carragenato (0; 0,5; 1 y 2%) se determinó midiendo el módulo de almacenamiento (G') durante el calentamiento (10°-80°C) y posterior enfriamiento (80°-10°C) de la mezcla mediante reología dinámica de barrido (DSR) y la fuerza del gel mixto por el ensayo de deformación (10%) en texturómetro Instron. La temperatura de desnaturalización de la proteína de surimi se determinó mediante calorimetría diferencial de barrido (DSC), y la microestructura de los geles mixtos por microscopía electrónica de barrido (SEM). El análisis reológico indicó claramente las etapas de suwari, modori y kamaboko. Además el polímero ejerce efecto sinérgico en la rigidez del sistema a medida que se incrementa su concentración. Este efecto se produce durante la etapa de enfriamiento, luego que ha gelificado la proteína de surimi, y se traduce en geles de alta rigidez y poca sinéresis. Por otra parte, la gelificación de surimi con contenido fijo de carragenato (2%) sólo fue potenciada a una concentración máxima de KCl=0,5%. Las temperaturas de desnaturalización en la mezcla (42°C y 65°C) posiblemente corresponden a actomiosina, y un tercer pico (58°C) a la fusión de carragenato. La microscopía reveló que no hay asociación uniforme entre el polisacárido y la proteína.

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I CONGRESSO IBERO-AMERICANO DE ENGENHARIA DE ALIMENTOS

"Equipamentos e Processos para a Indústria de Alimentos"

I Seminário Brasileiro sobre Propriedades de Engenharia em Alimentos

PROGRAMA E RESUMOS

FEA/UNICAMP - CYTED - SBCTA - ITAL
5-9 de novembro de 1995
Campinas, São Paulo
BRASIL

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Entidades patrocinadoras:

FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo.
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FINEP	Financiadora de Estudos e Projetos.
ALACCTA	Associação Latino-americana e do Caribe de Ciência e Tecnologia de Alimentos.
IUFoST	International Union of Food Science and Technology.
FWP EFCE	Food Working Party - European Federation of Chemical Engineering.

Entidades que apoiam o Congresso:

ABIA	Associação Brasileira de Indústria de Alimentos
Empresas	Indústrias do setor de Alimentos do Brasil.
USP-PCP	Prefeitura do Campus de Pirassununga da Universidade de São Paulo.
FTPAT	Fundação Tropical de Pesquisas e Tecnologia "André Tosello".

Direcciones / Address / Endereços para contatos:

I Congresso Ibero-Americano de Engenharia de Alimentos
FEA-Unicamp, Caixa Postal 6121
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FAX: (0192) 39 15 13
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No Exterior use (192)

FAX: 55 (192) 39 15 13
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39 70 23

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Conferencias Plenarias / Plenary Conferences / Conferências Plenárias

Título Title of lecture Título	Conferencista / Universidad Lecturer / University Palestrante / Universidade
História e Futuro da Engenharia de Alimentos.	**Marcus Karel** Rutgers - The State University of New Jersey / USA
Propriedades de Engenharia dos Alimentos: Estado da Arte.	**M. Anandha Rao** Cornell University / USA
Micro-estrutura de Alimentos.	**José Miguel Aguilera** Pontifícia Universidade Católica de Chile / Chile
Reologia de Alimentos.	**Luiz Durán Hidalgo** Inst. de Agroquimica y Tecnologia de Alimentos / Espanha
Propriedades térmicas na Esterilização de Alimentos.	**Alfredo A. Vitali** Inst. de Tecnologia de Alimentos / Brasil
Isotermas de sorção em substâncias alimentares.	**Constantino Suarez Fernandez** Universidad de Buenos Aires / Argentina
Nova abordagem das operações com alimentos fluidos estruturados.	**Pedro Fito Maupoey** Universidad Politécnica de Valencia / Espanha
Modelos de transferência de massa em alimentos estruturados.	**Marc Le Maguer** University of Guelph / Canada
Cinética das mudanças físicas em Alimentos.	**Theo G. Kieckbusch** Universidade Estadual de Campinas / Brasil
O estado físico e a qualidade dos Alimentos.	**Stéphane Guilbert** École Nationale Superiéure Agron. de Montpellier / França
Alimentos minimamente processados. Tendências atuais.	**Jorge Welti Chanes** Universidad de las Américas-Puebla / México
Metodologia do projeto de "frutas minimamente processadas".	**Maria Soledad Tapia de Daza** Universidad Central de Venezuela / Venezuela
Mudanças estruturais produzidas no processamento mínimo de frutas.	**Stella Maris Alzamora** Universidad de Buenos Aires / Argentina
Análise de variáveis chave no tratamento de alimentos por pulsos elétricos de alta intensidade.	**Gustavo V. Barbosa-Cánovas** Agroengineering Dept. - Washington State Univ. / USA
Salga de queijos com impregnação à vácuo.	**Amparo Chiralt Boix** Universidad Politécnica de Valencia / Espanha
Identificação e monitoramento de pontos críticos no controle "on line"de processos de alimentos com simulação em computador.	**Arthur A. Teixeira** Agric. and Biol. Eng. Dept. - Univ. Florida / USA
Técnicas de visão e inteligência artificial na classificação de matéria prima para a produção de presunto cozido.	**Antonio Mulet Pons** Universidad Politécnica de Valencia / Espanha
Desenvolvimento de programas para uso didático em Engenharia de Alimentos.	**Enrique Ortega Rodriguez** Universidade Estadual de Campinas / Brasil
O ensino de Engenharia de Alimentos: Perspectiva Latino-Americana.	**Bonifacio Efrén Parada Arias** Inst. Politécnico Nacional / México
O ensino de Engenharia de Alimentos: Perspectiva Européia.	**Augusto Eduardo Guimarães de Medina** Escola Superior de Biotecnologia / Portugal
O ensino de Engenharia de Alimentos: Perspectiva Norte-Americana e Asiática.	**R. Paul Singh** University of California-Davis / USA
Perspectiva Brasileira para o Ensino de Engenharia no Século XXI.	**Marcius Fantozzi Giorgetti** Escola de Engenharia de São Carlos - USP / Brasil
Materiais plásticos de alta barreira no projeto de envases para alimentos.	**Ramón Catalá Moragrega** Inst. de Agroquimica y Tecnologia de Alimentos / Espanha
Alguns problemas e soluções para a embalagem de alimentos produzidos com a técnica de processamento mínimo.	**Rubén J. Hernandez** Michigan State University / USA
Desenvolvimento de envases e embalagens para alimentos em Ibero-América.	**Cecilia Rojas de Gante** Inst. Tec. y de Est. Superiores de Monterrey / México
Novos desafios para a Embalagem de Alimentos: Novas técnicas de ensino, Meio Ambiente e Mercados Regionais.	**Luis F. C. Madi** Inst. de Tecnologia de Alimentos / Brasil

Domingo 5 - Coquetel de Boas Vindas, Inscrições e Entrega de Materiais
Local: Clube Semanal de Cultura Artistica. Rua Irmã Serafina 937, Centro, Campinas.
Horário: 18:00 - 23:00

Quadro resumo do Programa do I Congresso Ibero-Americano de Engenharia de Alimentos:

Dia Tema Hora	Segunda 6 ***Propriedades Físicas dos Alimentos.***	Terça 7 ***Análise cinética, termodinâmica e estrutural das mudanças durante o Processamento dos Alimentos.***	Quarta 8 ***Cálculo de Equipamentos e Informática Aplicada à Engenharia de Alimentos.***	Quinta 9 ***Envases e Embalagem de Alimentos***
8:00 **8:30**	Cerimonia inaugural **História e futuro da Engenharia de Alimentos:** M. Karel (EUA) Sessão **A1**: **Propriedades de Engenharia dos Alimentos: Estado da Arte.** M. A. Rao (EUA)	Sessão **B1**: **Transferência de massa, mudanças físicas e estruturais durante o processamento de alimentos sólidos.** P. Fito Maupoey. (Espanha) M. Le Maguer (Canadá) T. Kieckbusch (Brasil) ~~S. Guilbert (França)~~ M. le Meste	Sessão **C1**: **Cálculos de operações e processos de alimentos usando computador: Estado da Arte.** A. Teixeira (EUA) A. Mulet (Espanha) E. Ortega (Brasil)	Sessão **D1**: **Tópicos de atualidade em envases e embalagem de alimentos** R. Catalá (Espanha) R. Hernandez (EUA) C. Rojas (México) L. Madi (Brasil)
10:30	**A2 Temas livres** Trabalhos selecionados entre os resumos enviados ao Congresso	**B2 Temas livres** Trabalhos selecionados entre os resumos enviados ao Congresso	**C2 Workshop: programas de cálculo computacional** Expositores (computador e poster)	**D2 Temas livres** Trabalhos selecionados entre os resumos enviados ao Congresso
14:00	**A3** Mesa redonda **Dados disponíveis e necessidades de caracterização de alimentos.** J. Miguel Aguilera (Chile) ~~L. Durán (Espanha)~~ A. Rao ? A. Vitali (Brasil) C. Suarez (Argentina) P. Singh ?	**B3** Mesa redonda **Ciência e Engenharia do Processamento Mínimo de Alimentos.** J. Welti (México) M. S. Tapia (Venezuela) S. Alzamora (Argentina) G. Barbosa-Canovas (EUA) A. Chiralt (Espanha)	**C3** Mesa redonda **O Currículo de Engenharia de Alimentos em Ibero-América** E. Parada (México) A. Medina (Portugal) M. Giorgetti (Brasil) R. P. Singh (EUA)	**Visitas** (opcionais): Faculdade de Engenharia de Alimentos (FEA) da Unicamp Instituto de Tecnologia de Alimentos (ITAL) de Campinas
17:00	**Poster I:** Trabalhos selecionados entre os resumos enviados ao Congresso **Reuniões de redes e de projetos CYTED**	**Poster II:** Trabalhos selecionados entre os resumos enviados ao Congresso **Reunião da rede ALFA FOOD ENGINEERING**	**Poster III:** Trabalhos selecionados entre os resumos enviados ao Congresso **Reuniões de redes e de projetos CYTED**	

TEMA A:

"PROPRIEDADES FÍSICAS DE ALIMENTOS"
"REOLOGIA: A1 - ORAL"

Autores e Títulos	Instituição	Código
Caracterización física y reológica de cremas de leche batidas **Autores:** M.M. Camacho; N. Martinez-Navarrete; A. Chiralt	Univ. Politécnica de Valencia Depto. de Tecnologia de Alimentos ESPANHA	A1-01 10.30 h
Reología de puré de maçã **Autor:** Lozano, D.C., Rao, M.A.; Gasparetto C.A.	Insl. Tecnológico de Orizaba Univ. Veracruzana - MEXICO Cornell University - USA DEA / FEA / UNICAMP - BRASIL	A1-02 10:50 h
Study of mixtures of calcium carbonate and corn syrup as a shear-thinning standard. **Autores:** Miranda Maldonado, M.L.; Steffe, J.F.	Univ. de Chile Depto. de Ciencias. de los Alimentos y Tecnol. Quimica CHILE	A1-03 11:10 h
Desarrollo y caracterización de geles mixtos proteína-polisacáridos. **Autores:** Aguilera, J.M.; Ortiz, J.; Rojas, G.; Jorquera, H.; Baffico, P.	Pontificia Univ. Católica de Chile Depto. de Ingeneria Quimica y Bioprocesos -CHILE	A1-04 11:30 h
Reología de pure de frutas tropicales (mango, papaya, durazno y banana) y su relación con distintos factores de conservación. **Autores:** Guerrero, S.; Gerschenson, L.N.; Alzamora, S.M.	Univ. de Buenos Aires Depto. de Industrias Fac. de Cs. Exactas y Naturales ARGENTINA	A1-05 11:50 h
Propiedades reológicas de emulsiones carnicas para producir vienesas **Autores:** Castro, E.; Osorio, F	Univ. de Chile Depto. de Ciencias de los Alimentos y Tecnol. Quimica Univ. Santiago de Chile Depto. Tec. Agropecuária CHILE	A1-06 12:10 h

TEMA A:

"PROPRIEDADES FÍSICAS DE ALIMENTOS"
"PROPRIEDADES TERMODINÂMICAS E DE TRANSPORTE: A2 - ORAL"

Autores e Títulos	Instituição	Código
Efecto del contenido inicial de azucares sobre las isotermas de sorción de vapor de agua de manzana y papaya **Autores:** A. Argaiz; A. Lopez-Malo, E. Palou; P. Corte; J. Welti	Univ. de las Americas-Puebla Depto. de Ing. Quimica y Alimentos - MEXICO	A2-01 10.30 h
Predição da pressão de vapor de ácidos graxos saturados, insaturados e ésteres graxos através do método de contribuição de grupos **Autores:** Torres, M.B.; Meirelles, A.J.	FEA - UNICAMP Campinas, SP - BRASIL	A2-02 10:50 h
Determinación de los coeficientes de difusión de ácido láctico y NaCl mediante optimización del modelo ternario del proceso de salado de queso. **Autores:** Gerla, P.E.; Rubiolo C.A.	Universidad de la República Fac. de Ingeniería Montevideo - URUGUAY INTEC - Universidad Nacional del Litoral - CONICET ARGENTINA	A2-03 11:10 h
Difusión en placas finitas: condición para flujo unidireccional **Autores:** Rovedo C.O.; Suarez C.; Viollaz P.E.	Univ. de Buenos Aires Depto. de Industrias Fac. de Ciencias Exactas y Naturales - ARGENTINA	A2-04 11:30 h
Determinación de la difusividad efectiva de la sacarosa en papaya (Carica papaya L.) inmersa en soluciones azucaradas. **Autores:** Fernandez, D.; Velezmoro, C.; Zapata, C.	FEA - UNICAMP Campinas, SP - BRASIL	A2-05 11:50 h
Agregación y desnaturalización termica de aislados protéicos de soja pH ácido por efecto salino **Autores:** Puppo M.C.; Añon M.C	UNLP-CONICET-CIDCA Centro de Inv. y Desarollo en Criotecnologia de Alimentos ARGENTINA	A2-06 12 10 h

TÍTULO:

Study of mixtures of calcium carbonate and corn syrup as a shear-thinning standard

Autor(es):

Miranda-Maldonado, M.L.*; Steffe, J.F.**

Instituição:/Endereço:

*Universidad de Chile, /, Vicuña Mackena #20, Santiago, Chile

**Michigan State University, Department of Agricultural Engineering / East Lansing, MI 488224

RESUMO

A rheological study to determine the behavior of calcium carbonate corn-syrup mixtures was done using mixing viscometry techniques. Graphic representations demonstrated that the temperature effect at each concentration for calcium carbonate corn-syrup mixtures is fairly well represented with a power law model, showing a general trend that the lower the temperature the higher the apparent viscosity. Along the same line, typical graphs for different conditions showed that the higher the concentrations yield higher the apparent viscosities. Changes in the flow behavior index (n) and consistency coefficient (K) were evaluated as functions of temperature and concentration. Temperature had little effect on the flow behavior index. An Arrhenius model was used to explain the decrease in the consistency coefficient with temperature increases, at a content concentration. Several exponential models were used to demonstrate the influence of temperature and concentration, both separately and combined, on shear stress-shear rate relationships. From the combined effect of temperature and concentration on consistency coefficient, it is possible to say that the consistency coefficient decreases as the temperature increases at a constant concentration. On the other hand, at a constant temperature, the consistency coefficient decreases as the concentration goes down. Flow behavior index showed a not very clear trend when it was described as a function of temperature. The calcium carbonate corn-syrup mixtures did not show thixotropic behavior, but did exhibit a problematic settling phenomenon. These mixtures did not exhibit shear-thinning behavior with changes in the calcium carbonate concentration under studied conditions. These mixtures can be described as a non suitable shear-thinning standard fluid for the food industry, however, can be used as a reference fluid when high viscosities for reference fluids are needed for similar composition systems.

TÍTULO:

Desarrollo y caracterización de geles mixtos proteína-polisacáridos

Autor(es):

Aguilera, J.M.; Ortiz, J.; Rojas, G., Jorquera, H., Baffico, P.

Instituição:

Departamento de Ingeniería Química y Bioprocesos/ Pontificia Universidad Católica de Chile

Endereço:

Vicuña Mackenna 4860, Santiago, Chile

RESUMO

La mayoría de los alimentos semi-sólidos son geles mixtos de biopolímeros alimentarios. Este trabajo presenta resultados de un proyecto de tres años relacionado con geles mixtos de hidrocoloides (alginatos, carragenatos y almidones) y proteínas de origen lácteo o de pescado. El trabajo muestra como evaluar las propiedades mecánicas de geles por compresión axial en el rango elástico y el efecto de la proporción de cada componente en el módulo elástico. Se presentan cinéticas de formación de geles estudiadas por reometría oscilatoria dinámica siguiendo el desarrollo del módulo de almacenaje (G) através del tiempo. Transiciones térmicas importantes como gelatinización de almidones, denaturación de proteínas y fenómenos de "modori" y "suwari" en geles de pulpas de pescado/carragenato se determinan a través de calorimetría diferencial de barrido. Por último, se presenta la verificacion de microestructuras formadas en distintos geles mixtos por microscopia electrónica. Se discutirá la potencial aplicación de estos resultados en la formación de nuevos alimentos compuestos.

1993 IFT Annual Meeting Technical Program: Book of Abstracts

July 10–14, 1993 McCormick Place Chicago, Illinois, USA

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KINETIC AND MICROSTRUCTURAL ASPECTS OF GELATION OF A WHEY PROTEIN ISOLATE

J. M. AGUILERA, J. E. Kinsella and M. McCarthy

Dept. of Chemical Engineering, Catholic University, Santiago 22, Chile and Department of Food Science, University of California, Davis.

Gelation of whey proteins involves an initial denaturation step followed by aggregation of individual protein particles into a network and depends, among other variables, on concentration, temperature and curing time. The kinetics of gelation of whey protein isolate (WPI) solutions (3 to 15%, w/w) were followed by oscillatory rheometry (parallel plate geometry, 1 mm gap, 0.1 Hz and strain = 0.02), compression strength (at 20% deformation) and fracture force measurements at temperatures of 80, 85 and 90°C. The value of the complex shear modulus (G*) rose rapidly with time and a quasi-stationary value after 30 min curing time increased with concentration. Tan delta (G"/G') decreased rapidly with curing time to an asymptotic value that was independent of concentration and decreased with temperature. The inverse of the gelling time varied logarithmically with concentration. Compression and fracture strength of self-standing gels followed a kinetic pattern similar to G* but were only detected for gels having a concentration > 4% and curing times over 6 min. Gels studied by cryo-stage SEM revealed a decrease in pore size as concentration of WPI increased, but minor changes in the size of the aggregates forming the network.

KEY WORDS -- Gelation, whey protein, kinetics

The Summer National American Chemical Society Meeting in Washington D.C.

Measurement of the Porous Microstructure of Hydrogels by Nuclear Magnetic Resonance

M. M. Chui[1], M. J. McCarthy[3], J. Aguilera[2], R. J. Phillips[1]

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University of California, Davis, CA 95616

[2]Department of Chemical Engineering,
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University of California, Davis, CA 95616-8598

ABSTRACT

Characterization of the microstructure of hydrogels can lead to better understanding of properties such as texture and permeability. Nuclear magnetic resonance is a non-invasive tool to investigate the microstructure of porous media, and it has been used extensively to measure pore size and its distribution in sandstones and clays. The microstructure of sandstones is traditionally characterized by bounded compartments which are generally modeled as spheres or cylinders. In this investigation, we use NMR relaxation techniques and a cell fiber model to characterize the microstructure of polymeric hydrogels.

Regards, Mike

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1 6

UNIVERSITY OF BRISTOL

Dr. J.W. Goodwin
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Cantock's Close
Bristol
England BS8 1TS

9th November 1994

Dr. J.M. Aguilera,
Pontificia Universidad Catolica de Chile,
College of Engineering,
PO Box 306,
Santiago 22,
Chile.

Dear Dr. Aguilera,

Thank you for submitting an abstract to Faraday Discussion 101 on Gels to be held in Paris in September next year. The Scientific Committee has now met and we received between four and five times as many abstracts as we have spaces in the programme. It is with very great regret that I have to tell you that we were, therefore, unable to include your work for an oral presentation but we recognise the importance of it and would like to invite you to present it as a poster and then to take part in the discussion.

Yours sincerely,

J.W. Goodwin

J.W. Goodwin
Chairman of the Scientific Committee

Abstract Title:

Rheological properties of mixed and filled protein gels

X CONGRESO NACIONAL DE CIENCIA Y TECNOLOGIA DE ALIMENTOS

II SIMPOSIO INTERNACIONAL DE ALIMENTOS PARA REGIMENES ESPECIALES

SOCHITAL

ORGANIZA: SOCIEDAD CHILENA DE TECNOLOGIA DE ALIMENTOS

COORGANIZA: UNIVERSIDAD DE LA FRONTERA
Departamento de Ingeniería Química
Instituto de Agroindustria

"Mejoramiento Continuo de la Calidad como Factor de Desarrollo de la Industria Alimentaria en Chile"

PROGRAMA GENERAL

RESUMENES DE TEMAS LIBRES

PUCON, CHILE, 11 AL 13 DE NOVIEMBRE 1993

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PROPIEDADES MECANICAS DE GELES SURIMI-CARRAGENATO

RAFFO, M.A. y AGUILERA J.M.

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE, DEPARTAMENTO DE INGENIERIA QUIMICA.

Se analizó el efecto de las condiciones de calentamiento y adición de K-carragenato, cloruro de sodio (NaCl) y cloruro de potasio (KCl), en las propiedades mecánicas de geles mixtos confeccionados a partir de surimi de jurel.
Los geles fueron sometidos a un ensayo de compresión en el rango elástico (deformación del 10% de la altura de los geles), y comprensión hasta fractura. Al aumentar la temperatura de calentamiento se produjo un incremento de la dureza en compresión elástica, efecto que se estabilizó a los 60ºC, temperatura en que se presentó la mayor dureza (1.12 N). En compresión hasta fractura se observó una disminución de la dureza al aumentar la temperatura de 40 a 60ºC, la cual se cree es causada por el fenómeno modori. Para analizar el efecto de adicionar a la pulpa combinaciones de K-carragenato y NaCl ó KCl, en concentraciones de 0 a 3% (p/p), se calentó las muestras a 80ºC por 40 min. La edición de K-carragenato y NaCl produjo un incremento en la dureza en compresión elástica y hasta fractura, siendo el carragenato el que promovió el mayor efecto. La mayor dureza observada en compresión elástica fue de 3.93 N en una muestra sin NaCl y 3% de carragenato; en compresión hasta fractura el máximo, para una muestra de 3% de carragenato y 2% de NaCl, alcanza los 70.55 N. Se concluye entonces que es posible reemplazar el cloruro de sodio por K-carragenato y obtener geles de pulpa de pescado con buenas propiedades mecánicas.

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THESIS

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Pontificia Universidad Católica de Chile
Escuela de Ingeniería
Departamento de Ingeniería Química

ESTUDIO DE PROPIEDADES MECANICAS DE GELES MIXTOS

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Memoria preparada bajo la supervisión de la Comisión integrada por los profesores:

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Quienes recomiendan que sea aceptada para completar las exigencias del Título de Ingeniero Civil de Industrias con mención en Química.

Santiago de Chile, 1992

RESUMEN

El objetivo principal de esta memoria fue determinar como se comportaban los geles mixtos Iota-carragenato-gelatina y gelatina-concentrado de fruta frente a un estímulo de deformación.

En la primera parte de la memoria se estudió el gel I-carragenato-gelatina a distintas concentraciones. Se hizo gelificar utilizando tres concentraciones de iones de calcio: 0.8, 5 y 10%. Las muestras de geles se sometieron a un 20% de deformación y se obtuvo el esfuerzo desarrollado por el gel. Se realizaron pruebas de pH para determinar si éste era una variable que afectaba el esfuerzo de compresión del gel mixto.

Posteriormente, se estudió el gel gelatina-concentrado de damasco y piña. Las muestras se deformaron 20 y 80% y se obtuvo el esfuerzo desarrollado por ambos geles.

Sólo se encontró un comportamiento sinergístico en geles mixtos (I-carragenato)-gelatina con 0.8% de iones de calcio. Los geles mixtos con 10 y 5% de iones de calcio presentaron datos con una alta dispersión. La incompatibilidad termodinámica de la gelatina con el carragenato **puede ser** una de las causas del comportamiento del gel mixto (I-carragenato) - gelatina, en las experiencias realizadas.

Los geles mixtos gelatina-concentrado de damasco o piña, deformados un 20%, presentaron un comportamiento sinergístico, es decir, la gelatina fue reforzada por los concentrados.

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Pontificia Universidad Católica de Chile
Escuela de Ingeniería
Departamento de Ingeniería Química

PROPIEDADES MECANICAS DE GELES DE SURIMI/KAPPA-CARRAGENATO

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Santiago de Chile, 1993

RESUMEN

Los objetivos del estudio son analizar el efecto en las propiedades mecánicas de geles confeccionados a partir de jurel, de las condiciones de calentamiento y adición de κ-carragenato, cloruro de sodio y cloruro de potasio.

El jurel, *Trachurus murphyi*, fue capturado en Valparaiso y procesado en el Laboratorio de Tecnología de Alimentos de la Universidad Católica de Valparaiso, obteniéndose una pulpa de pescado denominada surimi. Este tiene la habilidad de formar geles al calentarse. Los geles fueron sometidos a un ensayo de análisis de perfil de textura, determinando la dureza y cohesividad, en dos tipos de compresión: compresión elástica (deformación del 10% de la altura de los geles); y compresión hasta fractura.

El estudio del efecto de la temperatura (20 a 80 °C) y tiempo de calentamiento (10 a 40 min.) del surimi, se realizó a muestras de pulpa con 2% de NaCl (p/p), observándose que el tiempo de calentamiento, en el rango de estudio, no tiene efecto sobre la dureza y cohesividad de las muestras.

Al aumentar la temperatura de calentamiento se tproduce un incremento de la dureza en compresión elástica, efecto que se estabiliza a los 60 °C, temperatura que presenta la mayor dureza (1,13N); el efecto sobre la cohesividad es moderada, distinguiéndose un incremento al variar la temperatura de 20 a 40 °C, el cual es causado por el paso de un gel suwari a kamaboko. En compresión hasta fractura se observa una disminución de la dureza al aumentar la temperatura de 40 a 60°C, la cual es causada por el fenómeno modori, la dureza

máxima alcanza los 25,1N; el efecto sobre la cohesividad es similar al presentado en compresión elástica.

Para analizar el efecto de adicionar a la pulpa, combinaciones de κ-carragenato y cloruro de sodio (NaCl) o cloruro de potasio (KCl), en concentraciones de 0 a 3% (p/p), se calentó las muestras a 80 °C por 40 min. La adición de carragenato, NaCl y/o KCl producen una mejora en la homogeneidad de las muestras y una disminución del desgarro durante el cortado.

La adición de carragenato y NaCl produce un incremento en la dureza en compresión elástica y hasta fractura, siendo el carragenato el que promueve el mayor efecto. En ambos tipos de compresión se observa que el efecto positivo que genera la adición de cloruro de sodio, tiene un límite de efectividad, que tiene su origen en la precipitación de las proteínas al aumentar la concentración de sal.

Esta precipitación afecta, también, al incremento de la dureza al aumentar la adición de carragenato, el cual disminuye a medida que aumenta la cantidad de cloruro presente en la muestra. La mayor dureza observada en compresión elástica fue de 3,93 N en una muestra con 0% NaCl y 3% de caragenato; en compresión hasta fractura el máximo, muestra con 3% de carragenato y 2% de NaCl, alcanza los 70,55 N. El efecto sobre la cohesividad es moderado.

La adición de KCl produce un efecto negativo sobre la dureza y cohesividad de las muestras, el cual es suficiente para compensar, y en la mayoría de las muestras sobrepasar, el efecto reforzador del carragenato. Por ejemplo, en compresión elástica una adición de 2% de KCl a una muestra con 2% de carragenato, produce una variación la dureza de 3,3 a 2,5N.

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Pontificia Universidad Católica de Chile
Escuela de Ingeniería
Departamento de Ingeniería Química

ESTUDIO DE GELIFICACION MEDIANTE SINERESIS POR CENTRIFUGACION

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Quienes recomiendan que sea aceptada para
completar las exigencias del título de Ingeniero
Civil de Industrias con mención en Química

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RESUMEN

Se estudió la sinéresis exógena en geles de WPI (aislado proteico de suero de leche), mediante el método de centrifugación. En este método, las soluciones de proteína de suero de leche se introducen en un tubo eppendorf (1,5 ml) para ser calentadas a las condiciones definidas por cada experiencia. Luego, los tubos son enfriados y centrifugados, pesándose el sedimento y el sobrenadante.

Se estudiaron los efectos de la concentración, condiciones de centrifugación y calentamiento. Para estudiar la primera variable, los geles fueron preparados por calentamiento a partir de soluciones de WPI al 4, 8 y 12% p/p ajustadas a un pH de 5,45. Dicha variable mostró un rol importante en la sinéresis: a una mayor concentración el porcentaje de sinéresis es menor.

Al aumentar la aceleración centrífuga se observa un rápido aumento de la sinéresis exógena, alcanzándose una menor variabilidad entre los 1500 y 2800xg. Para aceleraciones mayores (9000xg), la sinéresis aumenta en una mayor proporción disminuyendo las diferencias entre las diferentes concentraciones. Por ello, para las experiencias de los demás factores la aceleración de centrifugación utilizada fue de 2800xg.

Las soluciones sobrenadantes presentaron un bajo contenido de sólidos totales (menor de 0,6%) para las condiciones de centrifugación, por lo que la sinéresis se reporta como porcentaje de agua separada respecto del total presente en el gel.

Por otra parte, al incrementar el volumen de la muestra aumenta el porcentaje de sinéresis para el 4 y 8% p/p, por el aumento de masa en compresión; sin embargo, para el 12% el aumento de la masa pareciera reforzar la estructura, no observándose sinéresis.

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El reposo de los geles preparados durante 24 y 48 horas, mostró una disminución en el porcentaje de sinéresis para cada concentración, ya que de esta manera las cadenas del gel tendrían tiempo de reordenarse (topológicamente) y reforzar así la matriz. Cabe destacar, que para los tiempos definidos no se observó sinéresis endógena.

Los efectos de las condiciones de calentamiento (temperatura-tiempo) son complejos. De acuerdo a lo observado, se caracterizaron los distintos estados de la solución al variar las condiciones de calentamiento. Estos estados corresponden a: solución agregada, fluído altamente viscoso, gel blando y gel duro. Cada uno de estos estados tiene asociado un rango bien definido en el porcentaje de sinéresis, comenzando con un alto porcentaje para el primero, hasta alcanzar bajos porcentajes para el gel duro.

Se realizaron experiencias para distintas temperaturas. Para ello se determinó la temperatura de denaturación de las soluciones mediante calorimetría diferencial de rastreo (DSC). De esta manera, se definieron las temperaturas de 65°C, 70°C, 77°C (temperatura de denaturación promedio de la soluciones), 85°C y 90°C.

La incidencia de la temperatura se puede dividir en dos rangos. El primer rango corresponde a aquellas temperaturas por debajo de la de denaturación (65 y 70°C), en que el fenómeno de agregación es más importante que el de denaturación por lo que se obtienen geles de mayor porcentaje de sinéresis. Dichos porcentajes disminuyen lentamente con el tiempo por lo que no se alcanzan los estados de gel en tiempos convencionales para la gelificación de WPI (20-30 min).

Por otra parte, el segundo rango corresponde a temperaturas desde la de denaturación hasta los 90°C. En este rango, el mayor grado de denaturación de las proteínas permite alcanzar los estados de gel en los tiempos convencionales y sólo se producen variaciones en la cinética de gelificación. El porcentaje de sinéresis, en este rango, tiene un comportamiento característico para cada concentración, en el

que se alcanza un mismo valor asintótico en forma casi independiente a la temperatura.

Ajustando el modelo de crecimiento microbiológico de Gompertz al porcentaje de retención de agua (inverso de la sinéresis), se pudieron establecer los parámetros de la curva para cada temperatura y concentración. Esta curva muestra una primera asíntota (alrededor del 0%) para tiempos bajos, para luego aumentar rápidamente la pendiente hasta alcanzar un punto de inflexión. Una vez alcanzado este punto, la pendiente disminuye hasta estabilizarse en una nueva asíntota.

De esta manera, se postula que el punto de inflexión correspondería al punto de gelificación, es decir al punto en el que las muestras presentaron el primer estado de gel (gel blando). El aumento, tanto de la temperatura como de la concentración, desplaza el punto de gelificación hacia tiempos menores y aumentan la pendiente de la curva en ese punto. Esto denota un mayor grado de denaturación en un menor tiempo que a pesar de no implicar diferencias en el comportamiento de sinéresis final, si lo hace en las características reológicas.

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PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA
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ESTUDIO DE GELES MIXTOS PROTEINA/ALMIDON

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Santiago de Chile, 1993

ABSTRACT

Addition of YS in the range $0<\phi_S<0.5$ reinforced mixed WPI/YS gels. A maximum σ, 1.6 times higher than that of a pure WPI gel, occurred at $\phi_S=0.2$. WPI/CS gels followed typical mixing rules in the range $0<\phi_S<0.5$. WPI/starch solutions did not form gels for $\phi_S > 0.7$.

DSC scans showed that mixed gels presented independent peaks for gelatinization of starch and denaturation of whey proteins, suggesting phase-separated gels.

The microstructure of mixed gels as studied by scanning electron microscopy revealed independent networks for WPI and starch fractions. Whey proteins aggregated into almost spherical units 0.2-0.3 μm in diameter packed in a string-of-beads arrangement while YS gelled as a fine sponge. WPI/YS gels at $\phi_S=0.2$ showed well-developed intermeshing networks of WPI and YS. Swollen and collapsed starch granules embedded in an amylose gel were typical for CS containing gels. Non-gelling WPI/starch solutions ($\phi_S > 0.7$) failed to form a continuous WPI network.

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PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

UN ESTUDIO ACERCA DE LA GELIFICACION DE PROTEINAS DEL SUERO DE LECHE

FRANCISCO JAVIER GAJARDO HERNANDEZ

Tesis para optar al grado de
Magister en Ciencias de la Ingeniería

Profesor Supervisor
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ABSTRACT

Protein gels are very important materials for food, biotechnology and biomedical applications nowadays. It is reasonable to expect that, in the near future, the applicability of such materials will be greatly enhanced, particularly if it is possible to specify some of their design characteristics.

This work constitutes a first approach to the design of this kind of material. Specifically, an attempt was made to isolate and identify the fundamental aspects involved in their formation, so that future work may be restricted to the design, scaling-up and control aspects, which are fundamental for their industrial development.

In this work, several phenomenological models for the formation of protein gels available in the current literature had been tested and validated with ad hoc experimental measurements; these link kinetic aspects with macroscopic parameters (such as the storage modulus, G'). Although some of these models have shown an excellent fit to the experimental data, their applicability at the conceptual level remains unclear.

By means of nuclear magnetic resonance (NMR), the effective pore size of protein gels has been calculated, yielding results that compare well with previous work in scanning electron microscopy (SEM). At a highly speculative level, it has also been possible to build phase diagrams for such materials.

Summarizing, although much work remains to be done in this area, a beach head has been established. Moreover, it was possible to demonstrate that an engineering approach is suitable for the study of highly complex materials and phenomena, such as the gelation of globular proteins.

PONTIFICIA UNIVERSIDAD CATÓLICA DE CHILE
ESCUELA DE INGENIERÍA
Departamento de Ingeniería Química y Bioprocesos

ESTUDIO DE LA GELIFICACIÓN DE PROTEINAS DE SUERO Y ALMIDÓN DE YUCA MEDIANTE PRUEBAS DE REOLOGÍA DINÁMICA

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ABSTRACT

In this study the relationship between comcentration, temperature, and gelification time of Whey Protein Isolate (WPI), Cassava Starch (CS), and mixture of both (WPY/CS) was established trough oscillating rheometry. The gelification process took place in circles consisting of 30 minutes of heating followed by 20 minutes of cooling. For the WPI the increase of the concentration and/or temperature of heating led to the yield of stiffer gels with an increased storage modulus (G'). The highest increase in the G' values was observed during the cooling step. The initial heating step is irreversible, while the cooling/heating steps to follow are reversible. The smallest concentration in which gelification in the rheometer was detected was 4% at 90°C. The experimental values which correspond to the heating step were fitted to a first order exponential kinetic model.

The gelification of cassava starch (CS) through heating has a different behavior than WPI. The initially rapid increase of the G' value is subsequently slowing down. The increase in the concentration leads to an increase of the G' values, while a temperature increase results in diminishing G' values. The minimum average gelification concentration in the rheometer was 8% at 70°C. The gelification process also took place in two steps: heating (irreversible) and cooling (reversible) with an increase of the G' value observed during cooling. The experimental values corresponding to the heating step were adjusted to a kinetic model described by two equations of first order in series, the first corresponding to the reversible step and the second to the irreversible step.

For WPI/CS mixtures it was established that small quantities of CS lead to an increase of the stiffness. This was demonstrated in both the oscillating rheology trials as well as in the compression trials. On the other hand when the mixtures were treated at 70°C, only the starch gelifies. With increasing temperature the protein gelifies. This kind of gels are weaker than those which were first treated at 90°C.

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UNIVERSIDAD DE CHILE

FACULTAD DE CIENCIAS QUIMICAS Y FARMACEUTICAS

EFECTO DE κ-CARRAGENATO Y KCl EN LA GELIFICACION DE SURIMI DE JUREL (*T. murphyi*)

TESIS ENTREGADA A LA UNIVERSIDAD DE CHILE
PARA OPTAR AL GRADO DE
MAGISTER EN CIENCIAS DE LOS ALIMENTOS

POR

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SANTIAGO - CHILE
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ABSTRACT

In this study the effect of the addition of different content of κ-carragenan and KCl on the viscoelastic, thermic and structural properties during the gelation of jurel surimi *(T. murphyi)* was established trough dynamic oscillatory rheology, differential scanning calorimetry, assays of compression with 10% deformation in the elastic range and scanning electron microscopy.

Use of the dynamic oscillatory rheology technique for the viscoelastic behaviour present during the gelation of surimi allowed us to differentiate between the three typical stages of the sol-gel process in muscle proteins at surimi known as suwari (40°C), modori (50°C) and kamaboko (>60°C).Moreover, the measurement of the "storage" (elasticity; G') and the "loss" (viscosity; G") modulus detected the changes in the melting temperature (53°C) of κ-carrageenan caused by the addition of KCl.

There was no rheological evidence for the solubilization of carrageenan during heating of the mixture; although the polymer was capable of masking the viscoelastic transitions (suwari, modori, kamaboko) involved in the gelation of surimi protein. The gelation of carrageenan during cooling caused a large reinforcing effect in the rigidity and water holding capacity of mixed surimi-polysaccharide gels, which was confirmed by assays of compression with 10% deformation in the elastic range.

Thermal analysis of the mixture indicated the presence of endothermic peaks for the denaturation of the actinomysin protein (41 and 64°C) and melting of carrageenan (55°C) coincidental with the rheological data.

The addition of KCl produced a destabilizing effect in the surimi-carrageenan mixture, in particular in the polysaccharide structure and in the proteins solubility.

In addition, the rate of heating influenced the gel strength of the pure surimi gel and the mixture; gels heated slowly were more rigid than those heated rapidly.

Micrographs from scanning electron microscopy indicated that the microstructure adquired by the mixture is of an irregular order. When the concentration of polymer is low (0.5%) the protein is the continuous component and the gel is of the type "complexe". When the concentration of carrageenan is high (2%) this is the continuous component and the protein acts as "filled" in the system.This indicates that the water concentration in surimi is a main factor since the protein as well as the polymer competes for the water of the system.

In conclusion, the textural property of surimi appears to be favoured by chemical interactions of crosslinking with the polymer carrageenan during the gelation. Therefore, this polymer is an excellent thickening, and gelling agent, capable of improving the textural properties of surimi derivatives. Carrageenan has thermoreversibility properties and a melting point that can be altered by manipulation of the salt content. Therefore it is advisable to use a low salt content in the preparation of surimi gels containing carrageenan, or if this is not possible, temperatures above 80°C.