GELATION ENHANCEMENT OF GLUTEN PROTEIN USING THE MAILLARD REACTION AND HIGH TEMPERATURES

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ABSTRACT

Wheat Gluten (WG) gels were prepared by autoclaving dispersions in the presence of Glucose, Sucrose and also with out any sugar. In the Glucose systems, the pH decrease was a consequence of the Maillard reaction. The Maillard gels showed less syneresis had a higher breaking force and more elastic, as determined by stress relaxation, than the conventional gels. The differences were attributed to formation of additional covalent crosslinks due to the Maillard reaction, as evidenced by greatly reduced solubility in sodium dodecyl sulfate + 2-mercaptoethanol.

Keywords: Wheat gluten, Maillard reaction, Maillard gel, Conventional gel, Reducing sugar, Protein solubility.

Introduction:

Three key properties of food to which consumers respond – color, flavor and texture – are all influenced by the Maillard reaction, but the last of these – texture – has received the least attention[1,2]. It has established unequivocally that protein crosslinking can profoundly affect the texture of food, especially bread and croissants[3-5] .Furthermore, the Maillard reaction has been shown to effect protein crosslinking on a timescale relevant to food processors[6].

Sugars can have two different effects on protein gelation. First, sugars can act to stabilize proteins to heat denaturation.[7-11]This mechanism would affect gel strength by increasing the onset temperature of heat denaturation and altering bond formation during gelation. The second effect of sugars on proteins is the Maillard reaction. Reducing sugars, when heated, can react with available amines on the protein and form covalent cross-links within the protein network[12]. These covalent cross-links then changed the rheological properties of the protein gel. The Maillard reaction can also cause a pH reduction due to the production of acidic side products[12]. Decreased pH can cause changes in the gelation process, thus causing changes in the rheological properties of gels.

For the food scientist, the most significant and obvious effects of the Maillard reaction are the development of color, flavor, and aroma[13]. The type of sugar used in the system affect the rate of the Maillard reaction. Pentose sugars (ribose) react more readily than hexoses (glucose), which, in turn, are more reactive than disaccharides (lactose). The reaction rate with these sugars depends on the amount of open-chain aldehyde or ketone present as a reactant[13]. Lysine tends to be the amino acid that results in the most color development with the Maillard reaction. Therefore, foods containing proteins that are rich in lysine residues are likely to brown readily.

Researchers have concentrated on the effects of the Maillard reaction in protein solution or during protein gelation[12, 14, 15, 1]. Hill *et al.*[12] concluded that there is a correlation between the extent of the Maillard reaction (evidenced by color) and the strength of bovine serum albumin gels. The effectiveness of the reducing sugars on promoting gelation followed the reactivity of reducing sugars (lactose< fructose< mannose< xylose). Armstrong *et al.*[14] found that there was a decrease in pH for gels in which the Maillard reaction occurs. They also observed that gels in which the Maillard reaction had occurred were less soluble than gels without the Maillard reaction, suggesting the Maillard reaction caused additional non-disulfide covalent cross-links in the gels. Cabodevila *et al.*[15] concluded that the presence of such additional non-disulfide covalent cross-links might be expected to alter both the rupture strength and viscoelastic properties of the gels.

Gluten proteins are responsible for elasticity and the extensibility of the dough. Data from this work would provide some insight into the effect of wheat gluten protein substitution on the viscoelastic property of the gel and could usefully be used for development of bakery products e. g., breads, cakes, biscuits and crackers. We found no reports of previous work on use of the Maillard reaction to enhance wheat gluten protein gelation.

The purpose of the following research was to investigate the effects of glucose and sucrose on the rheological properties of wheat gluten (WG) gels. It is unknown if the pH decrease caused by the Maillard reaction or the addition of covalent cross-links to the gels by the Maillard reaction is causing the changes in the rheological properties of the gels. Solubilities in sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (2-ME or β-ME) were measured to identify differences in bonding between the two types of gels. By understanding the mechanisms of gluten protein gelation under this aqueous model system, greater utilization of wheat gluten protein ingredients in foods with elevated sugar levels can be obtained.

MATERIALS AND METHODS:

Materials: Wheat Gluten with 80 % protein, D- glucose (99%) and sucrose (99.5%) and bovine serum albumin (BSA) from Sigma Chemical Co. Ltd. (St Louis, Mo., U.S.A.), glass wool from Merck (Germany). All other chemicals used were of analytical grade.

Gel preparation: Gluten protein gels were prepared following the method described by Cabodevilla *et al.*[15], with some modifications. Aqueous dispersions (10.0 % wheat gluten; and 0 to 9.0 % glucose or sucrose, w/w) of WG or WG + glucose or sucrose were placed in tin cans (7 cm i. d. × 11 cm height) and then sealed. Gelation was carried out by heating the sealed cans in a steam atmosphere in a laboratory size autoclave (Hirayama HA-240M) at 121 °C for 30 min .Tin cans were then cooled rapidly to room temperature in tap water and kept at 4 °C for at least 18 h before analysis.

"Conventional gel" → [(wheat gluten + distilled water) and (wheat gluten + sucrose + distilled water)]
"Maillard gel" → (wheat gluten + glucose + distilled water)

Evaluation of gels:

pH and Color Measurement: The original solution and gel pH were determined using a laboratory pH meter (691 Digital pH meter model- Metrohm Herisau, Switzerland).

Color of the gels was measured using CR-300 Minolta Chroma meter which was standardized using standard white and black tiles. CM-3500d system with L* a* b* was used to determine extend of browning (L* = 0, black to L* = 100, white).

Syneresis: Syneresis of the canned gels was determined according the method described by Mwasaru and Muhammad[16], with some modifications. To determine excluded water the canned gels were centrifuged at 1500 rpm for 15 min, using a Kubota 5100 centrifuge machine (Kubota Corporation, Tokyo, Japan), weight of liquid separated was measured and the extent of syneresis was expressed as the percentage of liquid separated per total weight of the gel in the centrifuge tube, i.e.

Syneresis (%) = [liquid separated (g)] / [total weight of the gel (g)] × 100 (1)

Gels, (1.0 – 1.5g) equilibrated to room temperature, were placed on glass wool (1.8 – 2.0g) held in the middle position of a 50 ml centrifuge tube. Measurements were carried out at least in triplicate.

Gel protein solubility: Gels were homogenized by pressing through a copper sieve (linear size of aperture, 2.8 mm). Samples of 0.5 g of the homogenized gel were extracted in 10 mL of 2% sodium dodecyl sulfate (SDS) and 2% β - mercaptoethanol (β-ME).The extraction time was about 14 h (overnight). Any undissolved was removed by centrifugation at 3000 rpm for 30 min using a Kubota 5100 centrifuge machine (Kubota Corporation, Tokyo, Japan). The supernatant was filtered through a Whatman number 4 filter paper. The concentration of the protein in the supernatant was determined by the Lowry method[17].

Rheological Analysis by Stress Relaxation (SR) Test: For food samples, nature of viscoelasticity was shown by SR curves of gels, which was determined by compressing the cylindrical gel samples (1.7 cm diameter, 1.2 cm height) by a cylindrical probe (1 inch diameter) to 25 % deformation level using Texture Analyzer XT-2 version 1.05 (Stable Micro System, Goldaming, Surrey, UK). A-D Exchanger, Which linked to microcomputer, transmitted signal voltan. The parameter setting and operation of the instrument were accomplished through a PC with Texture Expert software version 1.0. The force and probe calibration procedure for the system were followed before actual tests. The text mode was set to 'texture profile in compression'. Pre-test speed, test speed and post-test speed were 1.0 mm/sec, 10mm / sec, and 5 mm / sec, respectively. Stress relaxation was followed for 60 sec. Three replicates from three separate samples were determined at room temperature 25 ± 1° C.

Break Strength: Gel samples were compressed until fracture occurred, as identified by a peak in the force–time curve, which was taken as the gel break steength[14, 15].

Stress Relaxation Under Compression: The relaxation curves obtained were then normalized and linearized using the method previously applied to food and gels by Peleg[18]. The equation employed was:

$$\frac{F_0 t}{F_0 - F(t)} = K_1 + K_2 t \qquad (2)$$

Where F_0 and F (t) are the initial and momentary force and K_1 (s) and K_2 (dimensionless) are constants. Also calculated was the asymptotic residual modulus (E_A), which is defined by Nussinovitch *et al.*[19] as follows;

$$E_A = \frac{F_0}{A(\varepsilon)\varepsilon}\left[1 - \frac{1}{K_2}\right] \qquad (3)$$

Where ε is the imposed strain and A (ε) is the corresponding cross-sectional area of the relaxing specimen, i.e.

$$A(\varepsilon) = \frac{A_0 L_0}{L_0 - \Delta L} \qquad (4)$$

Where L_0 is the height of the original gel, A_0 the cross-sectional area and ΔL the degree of compression.

Statistical Analysis: A SPSS software version 10 for Windows from Illinois, USA was used to perform a one-way ANOVA. Least significant difference (LSD) was used to determine a statistical significant difference at $P \leq 0.05$.

RESULTS AND DISCUSSION:

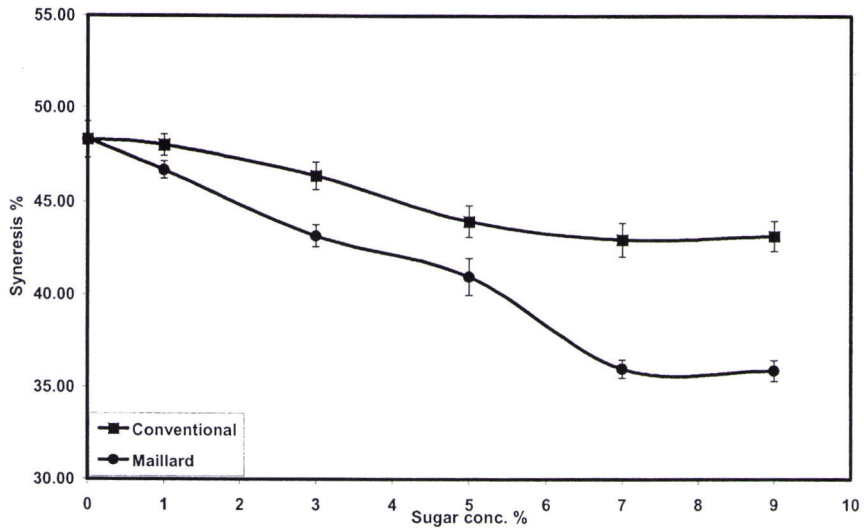

Figure 1: Percentage syneresis for Conventional [(WG + DW) and (WG + sucrose + DW)] and Maillard (WG + glucose + DW) gels as a function of sugar concentration & retorted at 121° C for 30 min.

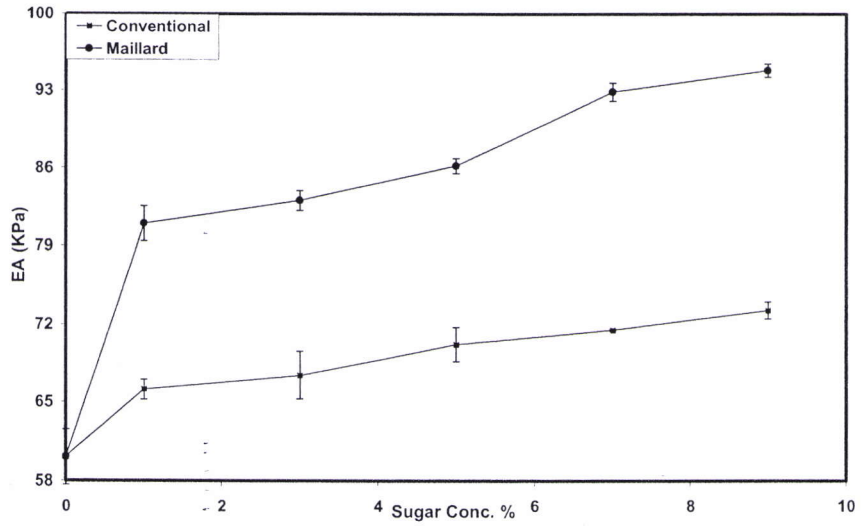

Figure 2: The asymptotic residual modulus (E_A) measured at 25% deformation as a function of sugar concentration. Details as in Figure 1.

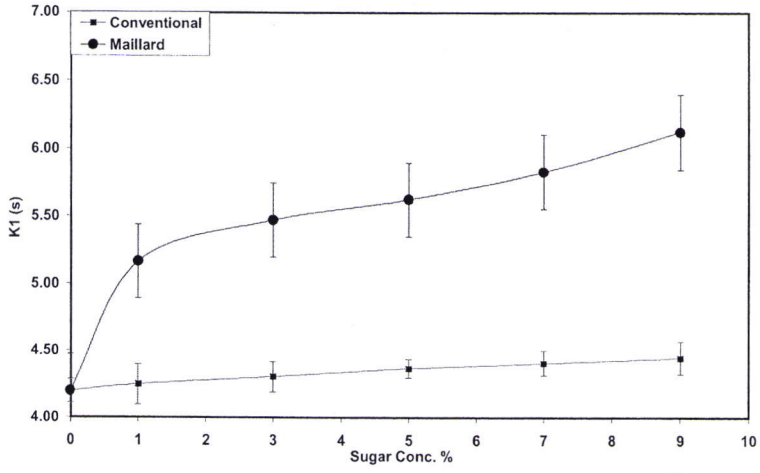

Figure 3: The gel elasticity parameter K_1 (intercept) as calculated by Peleg[18] as a function of sugar concentration. Details as in Figure 1.

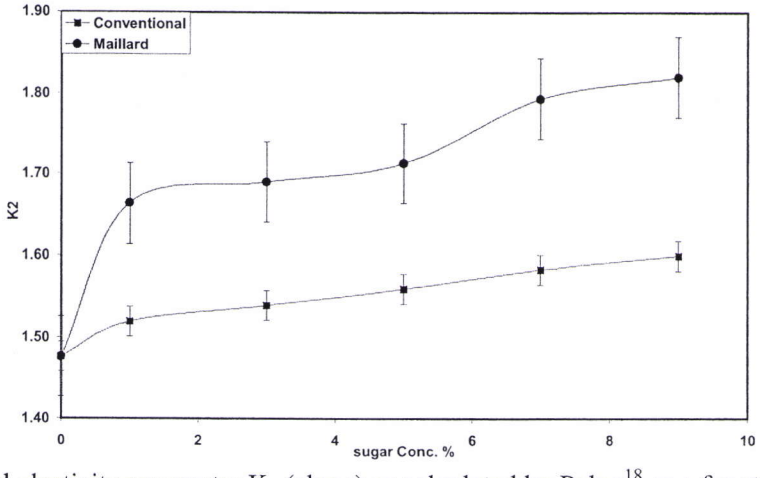

Figure 4: The gel elasticity parameter K_2 (slope) as calculated by Peleg[18] as a function of sugar concentration. Details as in Figure 1.

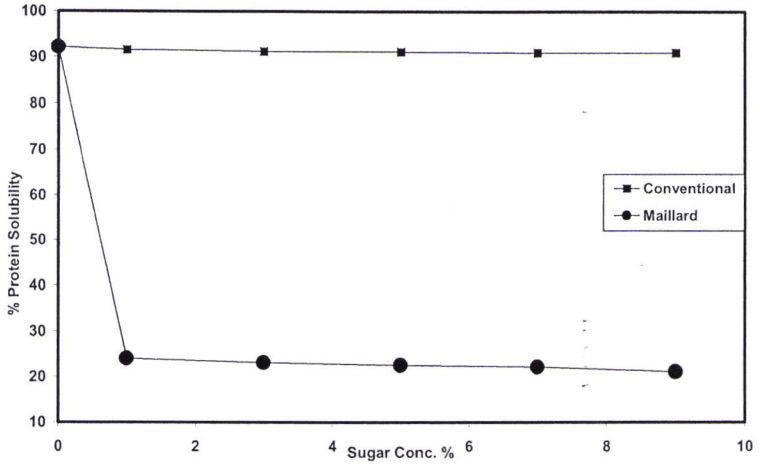

Figure 5: The percentage of protein solubilized from gels in a mixed solvent containing 2% β-ME + 2% SDS as a function of sugar concentration. Details as in Figure 1.

TABLE: 1, Comparison of the pH; gel break strength and color (L*) values of "MAILLARD" (WG + glucose + DW) and CONVENTIONAL [(WG + DW) and (WG + sucrose + DW)] GELS containing 10% WG and retorted for 30 min at 121° C.

Sugars	% Sugars	pH	Gel Break Strength (N)	Color(L*)
Glucose	0	5.67	11.80	60.95
	1	5.55	12.46	60.84
	3	5.41	12.56	58.39
	5	5.34	12.76	54.40
	7	5.26	12.92	52.31
	9	5.08	12.97	48.62
Sucrose	0	5.67	11.80	60.95
	1	5.62	11.89	62.54
	3	5.65	11.93	62.21
	5	5.64	11.98	61.65
	7	5.63	12.02	60.63
	9	5.60	12.03	60.32

Table 1 illustrates that the inclusion of different amount of different sugars has a marked effect on the gelling properties of Wheat Gluten (WG) when heated at 121° C for 30 min. The presence of glucose promotes gelation and there is a correlation between the extent of Maillard reaction, as indicated by a color change from cream to dark brown and a pH decrease. It was also noticeable that the gels with the lowest pH were the darkest[15, 20]. In the absence of sugar, the initial pH of 5.62 was increased by 0.05 units (in lieu of decrease).For the gels contain sucrose the initial pH did not decrease by more than 0.10 units on heating. However, the pH's of the "Maillard gels" decrease by 0.61 units (for 9 % glucose gel). Thus, pH's for the "Maillard" and "Conventional gels" were comparable[15].

The "Maillard gels" showing significantly lower ($p < 0.05$) syneresis than the "Conventional gels" (Fig. 1). For "Maillard gels" syneresis decreased as sugar concentration was increased but for "Conventional gels" the syneresis remained fairly constant. The higher water holding ability of the "Maillard gels" probably reflected the higher net charge of the Maillard net work; and a reduction would occur in number of positively charged amino groups presents, resulting in a reduction in the isoelectric point of the protein[15]

The dependence of gel strength on sugar concentration for the different kinds of gels tested was apparent (Table 1). The breaking force (gel break strength) of the "Maillard gels" increased as related to sugar concentration than for "Conventional gels". The minimum gelling concentration was much higher for gels not containing a reducing sugar.[12] Armstrong *et al* [20] were found that soya and pea protein isolates needed a level of 15-20 % to form a gel, but the inclusion of 3 % xylose reduced the critical value to less than 8 %.

The asymptotic residual modulus E_A showed a similar sugar concentration relationship to that of breaking force (Fig. 2). Both the breaking force and E_A were significantly lower ($p < 0.05$) for the "Conventional gels" compared with the "Maillard gels" prepared under comparable conditions. These results indicate that the "Maillard gels" contain covalent crosslinks; are more viscoelastic than the "Conventional gels". Similar results were found by Armstrong *et al.* and Cabodevilla *et al.*[14, 15]

The relation of K_1 and K_2 on sugar concentration was also shown (Fig. 3 & Fig. 4). These parameters can be considered as a measure of gel elasticity[18]. They may have values between 1 and ∞. For an ideal elastic solid, $K_1 = K_2 \rightarrow \infty$, whereas for nonelastic liquid, $K_1 = K_2 \rightarrow 1$.

The compression test was done at large deformations which could be outside the linear viscoelastic region. The decay in stress with the time reflects the rearrangement and/or rupture of crosslinks within the gel structure. Increasing the degree of covalent bonding within the network, as a result of formation of "additional Maillard crosslinks", reduced this tendency and hence resulted in a more elastic gel[15]. Thus, it could be thought that higher K_1 and K_2 values (Fig. 3 & Fig. 4) for "Maillard gel" may represent additional crosslinking of the protein network. Similar results were found by Armstrong *et al.*, and Cabodevilla *et al.*[20, 15]

Supporting evidence for the increased level of covalent crosslinks in "Maillard gel" was provided by the studies of solubility in SDS and β-ME. Since β-ME solubilizes disulphide linkages and SDS disrupts non covalent interactions[14]. When the solubility in SDS + β-ME were compared (Fig. 5), almost all the "Conventional gel" was solubilized, while only about 21% of the protein in the "Maillard gel" became soluble. Materials not solubilized can be thought of as having crosslinks additional to hydrogen bonding, electrostatic interaction and disulphide bridging i.e., nondisulphide covalent bonds, presumably formed as a consequence of the Maillard reaction.[14, 15, 20]

CONCLUSION:

When subjected to food sterilization temperatures the gelation behavior of Wheat Gluten may be substantially improved by incorporation of glucose. The improvement could have been a consequence of both pH decrease and formation of "additional covalent crosslinks". It seems plausible that "Maillard crosslinks" occurred in wheat gluten gel network.

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References:

(1) Easa, A. M., Hill, S. E., Mithchell, J. R., and Taylor, A. J. Bovine serum albumin gelation as a result of the Maillard reaction. *Food Hydrocolloids*, **1996**, *10,* 199-202.

(2) Pellegrino, L., van Boekel, M., Gruppen, H., Resmini, P., and Pagani, M. A. Heat-induced aggregation and covalent linkages in beta-casein model systems. *International Dairy Journal*, **1999**, *9,* 255-260.

(3) Gerrard, J. A., Brown, P. K., Fayle. S. E. Maillard crosslinking of food proteins I: the reaction of glutaraldehyde, formaldehyde and glyceraldehydes with ribonuclease. *Food Chemistry*, **2002**, *79,* 343-349.

(4) Gerrard, J. A., Fayle, S. E., Wilson, A. J., Newberry, M. P., Ross, M., and Kavale, S. Dough properties and crumb strength of white pan bread as affected by microbial transglutaminase. *Journal of Food Science*, **1998**, *63,* 472-475.

(5) Gerrard, J. A., Newberry, M. P., Ross, M., Wilson, A. J., Fayle, S. E., and Kavale, S. Pastry lift and croissant volume as affected by microbial transglutaminase. *Journal of Food Science*, **2000**, *65,* 312-314.

(6) Fayle, S. E., Gerrard, J. A., Simmons, L., Meade, S. J., Reid, E. A., and Johnston, A. C. Crosslinkage of proteins by dehydroascorbic acid and its degradation products. *Food Chemistry*, **2000**, *70,* 193-198.

(7) Ball, C. D.; Hardt, D. T.; Duddles, W. J. The influence of sugars on the formation of sulfhydryl groups in heat denaturation and heat coagulation of egg albumin. *J. Biol. Chem.*, **1943**, *151,* 163-169.

(8) Back, J. F.; Oakenfull, D.; Smith, M. B. Increased thermal stability of proteins in the presence of sugars and polyols. *Biochemistry*, **1979**, *18,* 5191-5196.

(9) Arakawa, T.; Timasheff, S. N. Stabilization of protein structure by sugars. *Biochemistry*, **1982**, *21,* 6536-6544.

(10) Garrett, J. M.; Stairs, R. A.; Annett. R. G. Thermal denaturation and coagulation of whey proteins: effect of sugars. *J. Dairy Sci.*, **1988**, *71,* 10-16.

(11) Jou, K. D.; Harper, W. J. Effect of disaccharides on the thermal properties of whey proteins determined by differential scanning calorimetry (DSC). *Milchwissenschaft*, **1996**, *51,* 509-512.

(12) Hill, S. E.; Mitchell, J. R.; Armstrong, H.J. The production of heat stable gels at low protein concentration by the use of the Maillard reaction. In *Gums and Stabilizers for the Food Industry 6*; Phillips, G. O., Williams, P. A., Wedlock, D. J., Eds.; 479-482,Oxford University Press: New York, **1992**.

(13) Ames, J.M. Control of the Maillard reaction in food systems.*Trends Food Sci Technol.,* **1990,** *1,*150-154.

(14) Armstrong, H. J.; Hill, S. E.; Schrooyen, P. and Mitchell, J. R.A comparison of the viscoelastic properties of conventional and Maillard protein gels. *J. Text. Stud.* **1994,** *25,* 285-298.

(15) Cabodevilla, O.; Hill, S. E.; Armstrong, H. J.; De Sousa, I. and Mitchell, J. R. Gelation enhancement of soy protein isolate using the Maillard reaction and high temperatures. *J. Food Sci.,* **1994,** *59,*872-875.

(16) Mwasaru, M. A.; Muhammad, K. Effect of surfactant HLB on some gel properties of pigeonpea and cowpea starches. *Starch / Stärke,* **2001,** *53,* 155-161.

(17) Lowry, O. H.; Roseborough, N. J.; Farr. A. L.; Randal, R. J. Protein measurement with the folin phenol reagent. *J. Biol.Chem.,* **1951,***193,* 265- 275.

(18) Peleg, M. Characterization of the strees relaxation curves of solid foods.*J. Food Sci.,* **1979,** *44,* 277-284.

(19) Nussinovitch, A.; Kaletunc, G.; Normand, M. D. and Peleg, M. Recoverable work versus asymptotic relaxation modulus in agar, carageenan and gellan gels. *J. Text. Stud.* **1990,** *21,* 427-438.

(20) Armstrong, H. J.; Hill, S. E. and Mitchell, J. R. Enhancement of the Gelation of Food macromolecules using the Maillard reaction and elevated temperatures. . In *Maillard Reactions in Chemistry, Food and Health;* Labuza, T. P.; Reineccius, G. A., Monnier, V., O'Brien, J., Baynes, J., Eds.; 159-163, The Royal Society of Chemistry: Science Park, Cambridge, U. K., **1994**.