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FTIR ANALYSIS OF A CRYOPRECIPITATED SOY PROTEIN

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ABSTRACT: ***The effects of heating on the NaOH-CP structure, in the presence of varying concentration of NaCl and reducing agents, were examined by Fourier transform infrared (FTIR) spectroscopy. FTIR spectroscopy revealed that at 25 °C the NaOH-CP protein showed four bands at 1633, 1650, 1667, and 1689 cm^{-1}. At 85 °C, the temperature associated with gelation, the four bands decreased in the intensity with the simultaneous increase of a band at 1644 cm^{-1} as result of the protein unfolding, furthermore two new bands at 1616 and 1685 cm^{-1} appeared. The latter two bands are attributed to intermolecular β-sheet formed during gelation. No major changes were observed in the amide I bands upon heating to the solutions up to 100 °C in the presence of 0.5 or 1.0 M NaCl. In the presence of SDS as function of increasing temperature reveals that a 50 mM of SDS stabilizes the secondary structure against denaturation on the other hand 100 mM of SDS major changes were observed in the amide I above 50 °C.***

Kew words:- ***Soybean proteins, FTIR, Secondary structure.***

INTRODUCTION

Soy is a popular component of the Asian diet and more recently has found increasing acceptance in western diets due to the Food and Drug Administration claim of linking the consumption of soy protein with a lower of heart disease (FDA 1999). Additionally soy products are being recognized as having potential roles in the prevention and treatment of chronic diseases,

most notably cancer (Fukutake *et al* 1996, Applelt and Reicks 1999),osteoporosis, and kidney disease and to decrease plasma triacylglycerol and cholesterol levels in humans (Urade *et al* 2003, Vittadini and Vodovotz 2003).

Structurally, soy protein is classified based on sedimentation characteristics; the conventional nomenclature for soy protein fractions is the 2S. 7S, 11S and 15S fractions (Yamauchi *et al* 1991), the major fractions are the 7S fraction (7S globulin or conglycinin) and 11S fraction (11S globulin or glycinin).

Very little information on the structural conformation of β-conglycinin can be found in the scientific literature. β-conglycinin is a compactly folded protein with high degree of unstructured regions. The α-helix, β-structure and random coil contents of the secondary structure are 5, 35, and 60%, respectively (Lewis and Chen 1979). However, the secondary structure of glycinin was predicted to be 25 % α- helix structure, 25 %.β-Sheet structure, 42 % turns and 8 % unordered forms on the basis of molecular modeling its amino acid sequence (Argos *et al* 1985). Recently, Abbott *et al* (1996) reported Fourier transform infrared (FTIR) spectra of glycinin as 24 % α- helix structure, 30 % β-Sheet structure, 31 % turns, and 12 % unordered. Their data also indicated that glycinin has the same secondary structure in solution and in hydrate solids. While, Dev *et al* (1988) concluded that the secondary structure of 11S consisted mainly of β–sheet, β–turns and unordered structure with very little α–helix structure.

The ordered structure of glycinin can be affected by several factors. Alkali treatment decreased ordered structure, especially the β-Sheet; adding ethanol to the alkali denatured glycinin increased the ordered structure (particularly α- helix) and decreased the random coil contents (Ishino *et al* 1980). Acetylation of glycinin decreased the β-sheet and increased the random coil content at 90% acetylation (Yamauchi *et al* 1979). The observed similarity in the amounts of α–helix and β–sheet secondary structures may indicate that their interior conformations are very similar or highly conserved (Marcone 1999). The present work was conducted to study the secondary structure of cryoprecipitated soy proteins and to investigate the effect of heating temperature, ionic strength, and reducing agents on the secondary structure of that protein.

MATERIALS AND METHODS:

Sodium Hydroxide Extraction / Cryoprecipitation

Proteins were extracted using the procedure of Davidson *et al* (1979) with some modifications. The defatted soybean meal (100g) was mixed with dilute NaOH (1L, 0.02 %, pH 10.7) and allowed to stand for 1 h at room temperature with intermittent stirring. The mixture was centrifuged (12000 x g) for 10 min. The extract was refrigerated (4 ^{o}C) for 18 h and the proteins which

precipitated were recovered by centrifugation (12000 x g) for 10 min followed by lyophilization. This isolate was designated as sodium hydroxide cryoprecipitate (NaOH-CP)

Preparation of Protein Samples for FTIR Analysis

FTIR analysis was used for determination of NaOH-CP secondary structure. NaOH-CP (12 % w / v) was prepared by dissolving the protein in deuterium oxide (D_2O) or H_2O. To study the effect of ionic strength, the protein was dissolved in D_2O solutions containing 0.5 and 1.0 M NaCl. To study the effect of denaturing agents, NaOH-CP (12 % w ⁄ v) were prepared using 50 and 100 mM sodium dodecyl sulfate (SDS), and 0.1and 0.5 % 2-mercaptoethanol (2-MeSH).

Infrared spectra were recorded with a 8210E Nicolet fourier transform infrared spectroscopy (FTIR) spectrometer equipped with a deuterated triglycine sulfate detector. The spectrometer was purged with dry air from Balston dryer (Balston, Haverhill, MA, 018350723, USA). Samples (7 µl) were held in an IR cell with a 25µm path length (for samples prepared in D_2O) and 12µm (for samples prepared in H_2O) between two CaF_2 windows. The IR cell was placed in a temperature-controlled cell holder. The temperature was increased in 5 ^oC increments and the cell allowed to equilibrate for 10 min before recording the spectrum, for the cooling cycle, the temperature was decreased in 10 ^oC increments and the cell allowed to equilibrate for 10 min before recording the spectrum. The second derivative spectrum was obtained as described by Cameron and Moffatt (1987).

RESULTS AND DISCUSSION

FTIR of NaOH-CP

Figure 1a shows a plot of stacked second derivative spectra of the amide I band in the infrared spectra of NaOH-CP dissolved in D_2O. Figure 1b shows a plot of stacked second derivative spectra of the amide I band in the infrared spectra of NaOH-CP dissolved in H_2O. The second derivative spectra were generated from the spectra of NaOH-CP after subtraction of the absorption due to water from each spectrum. At 25 ^oC the amide I band in the infrared spectrum of NaOH-CP in D_2O (Figure 1a) showed four peaks at 1633, 1650, 1667, and 1689 cm^{-1}. In H_2O similar peaks were observed, except that the frequency of the band at 1654 cm^{-1} in H_2O shifted to 1650 cm^{-1} in D_2O, this shifting due to hydrogen-deuterium exchange. The band at (a) 1633 cm^{-1} has been assigned to β-sheet structure (Susi and Byler 1988, Dong *et al* 1990, Herald and Smith 1992); at (b) 1650 cm^{-1} has been assigned to α-helix structure (Susi and Byler 1988, Mitchell *et al* 1988, Holloway and Mantsch 1989, Dong *et al* 1990); at (c) 1667 cm^{-1} has been assigned to the turn structure (Herald and Smith 1992); and the band at (d) 1689 cm^{-1} has been assigned to β-turn structure as reported by other researchers (Surezwiz *and Mantsch* 1988, Arrondo *et al* 1988, Susi and Byler 1988) or to the high

frequency component band of an antiparallel β-sheet (Surewiz and Mantsch 1988). Abbott *et al* (1996) reported that based on FTIR spectroscopy, the secondary structure of glycinin was 33% β–sheet, 25% α–helix, 31% turns and 12 % unordered structure, Dev *et al* (1988) had previously reported that the secondary structure of glycinin mainly of β–sheet structure, β–turns and unordered structure with very little α–helix. Since both studies were carried out in H_2O it was difficult to employ resolution enhancement techniques to ascertain the position of the amide I bands precisely leading to possible miss assignment of the unordered and α-helical structures. It is evident that the secondary structure of NaOH-CP in D_2O or H_2O that the major secondary component is β-sheet with a smaller amount of α-helix and turn structures.

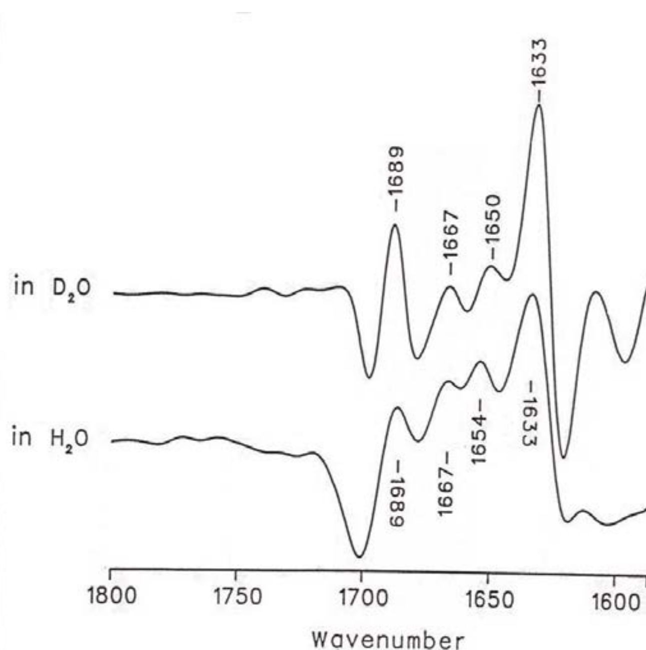

Figure 1:- Stacked plot of the second derivative infrared spectra of NaOH-CP, a:D_2O, b: H_2O

Effect of Temperature on Secondary Structure

Heating the NaOH-CP solution (12 % w / v in D_2O) from 25-70 °C had no significant effect on the secondary structure (Figure 2). Above 70 °C the 1650 cm^{-1} band attributed to α-helix decreases. Heating the protein above 85 °C (temperature associated with gelation) resulted in a substantial

decrease in the intensities of the 1633, 1689 and 1667 cm^{-1} bands and an increase in the intensity of a shoulder at 1644 cm^{-1} attributed to random coil (Nagano *et al.* 1994) formed as a result of protein unfolding. The appearance of the 1644 cm^{-1} coincides with the appearance of two additional bands at 1685 and 1616 cm^{-1} attributed to intramolecular β-sheet formed as a result of re-association of unfolded peptides segments which lead to formation of a gel and aggregate structures (Clark *et al.* 1981, Ismail *et al.* 1992 and De Las Rivas 1997). These results are in contrast to those reported by Chen *and Barber* (1990) who observed that the amount of α-helix of glycinin was increased from 7.8 to 28 % as the temperature of protein solution increased from 25 to 90 ^{o}C employing FTIR spectroscopy. While Wang and Damodaran (1991) reported that at high protein concentration (10 % protein) thermal treatment results in extensive denaturation of the secondary structure in soy 11S (glycinin). They attributed that at high concentration, the propensity of intermolecular interactions apparently leads to aggregation and network formation and this prevents refolding and regaining of secondary structure. Nagano *et al.* (1994) investigated the secondary structure changes of glycinin during heat-induced gelation employing FTIR spectroscopy. They reported that bands at 1618 and 1680 cm^{-1} attributed to β-sheet increased with the formation of heat–induced gel of glycinin attributed the bands at 1618 and 1680 cm^{-1} to intermolecular hydrogen-bonded antiparallel β-sheet structure resulting from protein aggregation. Cooling cycle of the heated solution of NaOH-CP showed no changes in the secondary structure of the heated protein (Figure 3) this suggests that the denaturation temperature of NaOH-CP is irreversible.

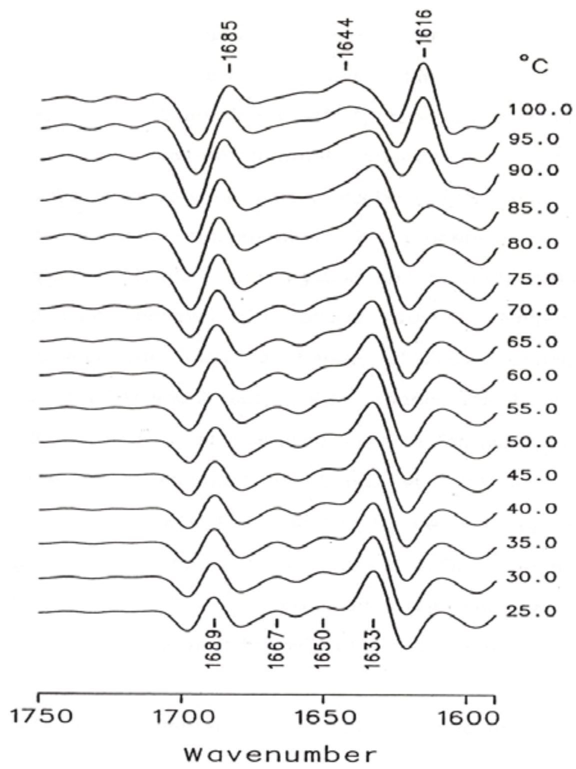

Figure 2:- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature

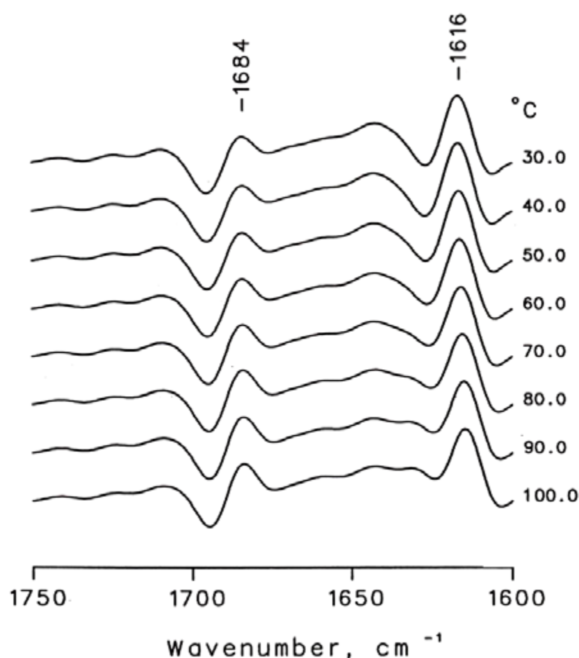

Figure 3:- Cooling cycle of NaOH-CP in D_2O

Effect of Ionic Strength on Secondary Structure

The FTIR spectra in the amide I absorption region of NaOH-CP in the presence of 0.5 and 1.0 M NaCl solutions recorded as a function of increasing temperature between 25-100 °C are shown in Figure 4 and Figure 5 respectively. No major changes were observed in the amide I bands upon heating to the solutions up to 100 °C in the presence of 0.5 M or 1.0 M NaCl. Using FTIR, Boye (1995) reported that increasing NaCl concentration up to 2 M lead to increase the thermal stability of β–lactoglobulin. A notable exception in the small drop in the relative intensity of the band 1650 cm^{-1} (α–helix) to the 1633 cm^{-1} band. This suggests that the α-helix component of glycinin is thermally labile. Elevating the heating temperature of NaOH-CP solution from 25 to 100 °C in the presence of 0.5 M and 1.0 M NaCl did not cause the appearance of the two bands at 1620 and 1684 cm^{-1} attributed to protein aggregation. These results indicate that NaCl appear to stabilize the protein structure aggregate formation possibly through non-specific ion

effects of NaCl on electrostatic interaction between charges groups on the protein (Lèger and Arntfield 1993). Cooling cycle of NaOH-CP (Figure 6) in presence of 1 M NaCl showed the same secondary structure of heating cycle of this protein which indicates that higher heating temperature was required to distrupt the native protein structure at higher ionic strength.

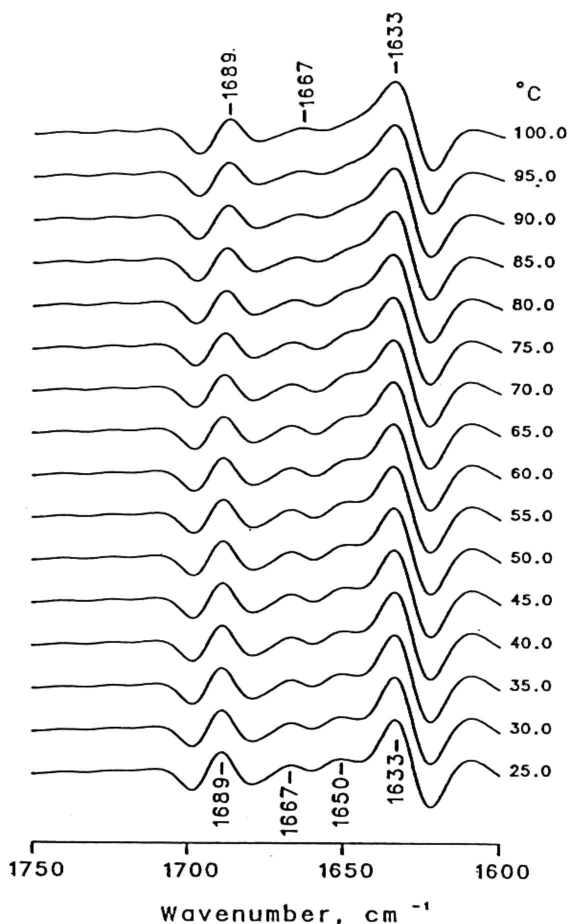

Figure 4 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 0.5 M NaCl

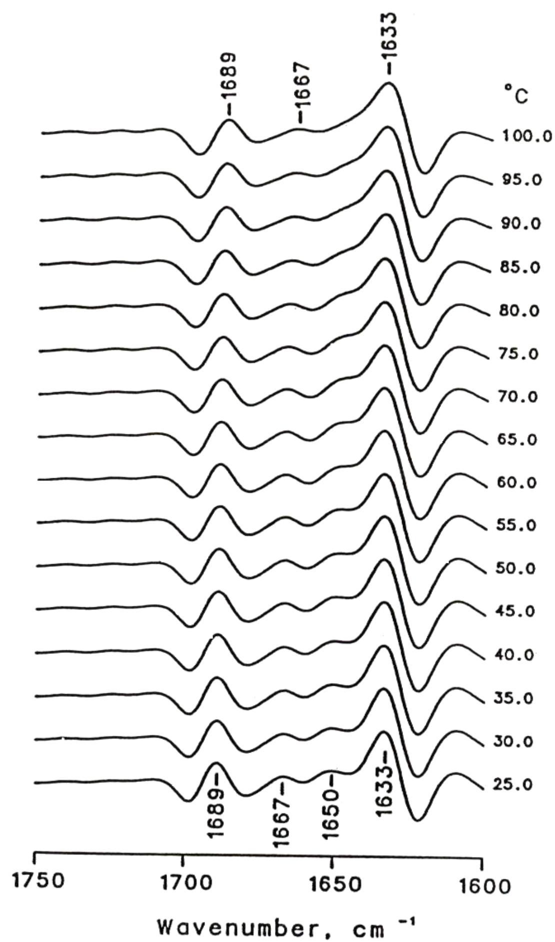

Figure 5 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 1.0 M NaCl

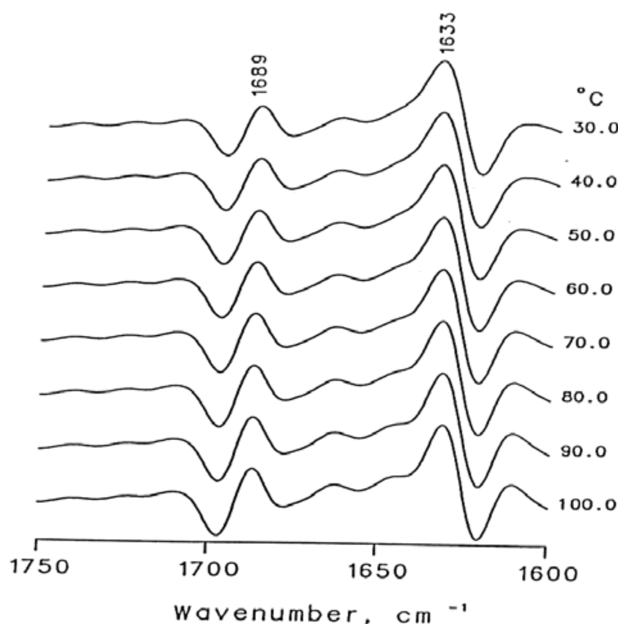

Figure 6:- Cooling cycle of NaOH-CP in D_2O in the presence of 1.0 M NaCl

Effect of Reducing Agents on Secondary Structure

Examination of the FTIR spectra of NaOH-CP in the presence of SDS as a function of increasing temperature reveals that a 50 mM concentration of SDS stabilizes the secondary structure of NaOH-CP against denaturation or aggregation Figure 7. It is of interest to note that the α-helical structure also remains intact. They suggested that SDS at low concentration could form a bridge between cationic groups of protein and ionic groups of SDS, which seem to stabilize the protein structure. The aggregation bands at 1616 and 1684 cm^{-1} did not appear with increasing temperature in the presence of 50 mM SDS, indicating the absence of antiparallel β–sheet formation. These results are in a good agreement with Boye *et al.* (1996) who reported no aggregation bands were observed with bovine serum albumin in the presence of 50 mM of SDS.

In the presence of 100 mM SDS major changes in the amide I bands above 50 °C (Figure 8). A drop in the relative intensity of the 1633 cm^{-1} to the 1650 cm^{-1} band is observed. The bandwidth of amide I bands significantly increase above 70 °C along with the appearance of a weak band at 1616 cm^{-1} attributed to aggregate formation. A broad band centered at approximately

1644 cm^{-1} (unordered structure) is also observed above 70 °C. These results demonstrate that and the increase in SDS concentration favors the unfolding of the protein more than aggregate formation. Steinhardt (1975) reported that high concentrations of SDS favor a decrease in the enthalpy due to the non-specific binding of detergent to the protein facilitating its denaturation.

The spectra in Figure 9 and Figure 10 show the combined effects of 2-mercaptoethanol (2-MeSH) and heating on the secondary structure of NaOH-CP. NaOH-CP in the presence of 2-MeSH appear to loss its secondary structure form aggregates at approximately 80 °C in 0.1% 2-MeSH and 75°C in 0.5 % 2-MeSH.

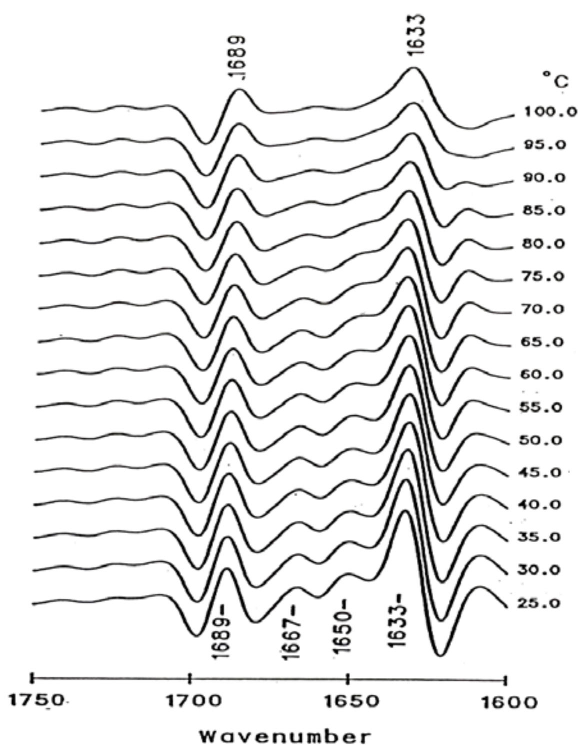

Figure 7 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 50 mM SDS

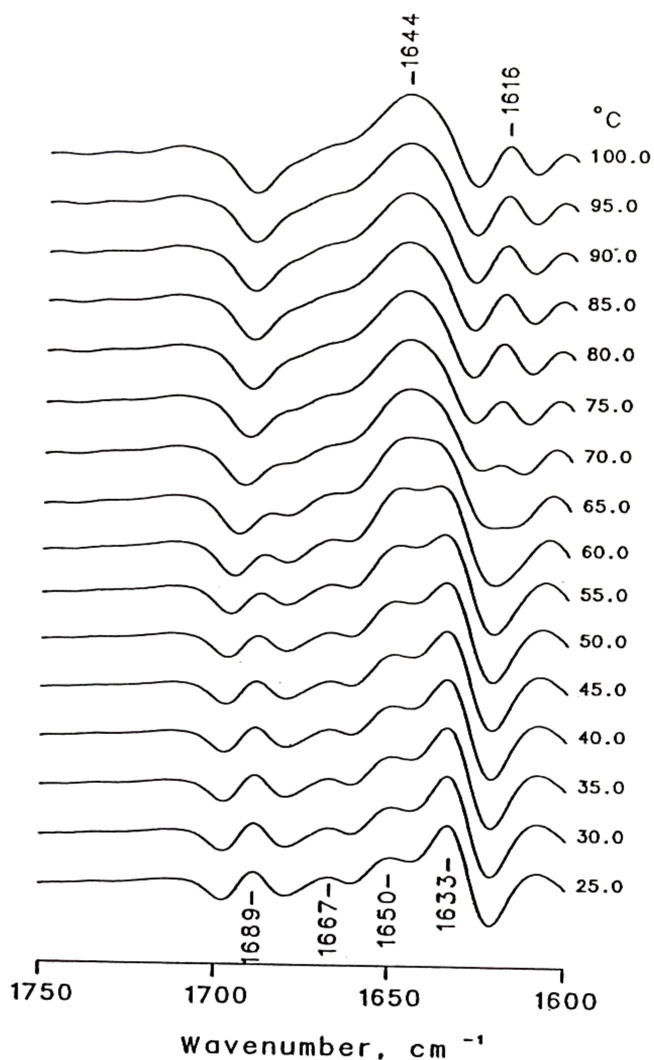

Figure 8 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 100 mM SDS

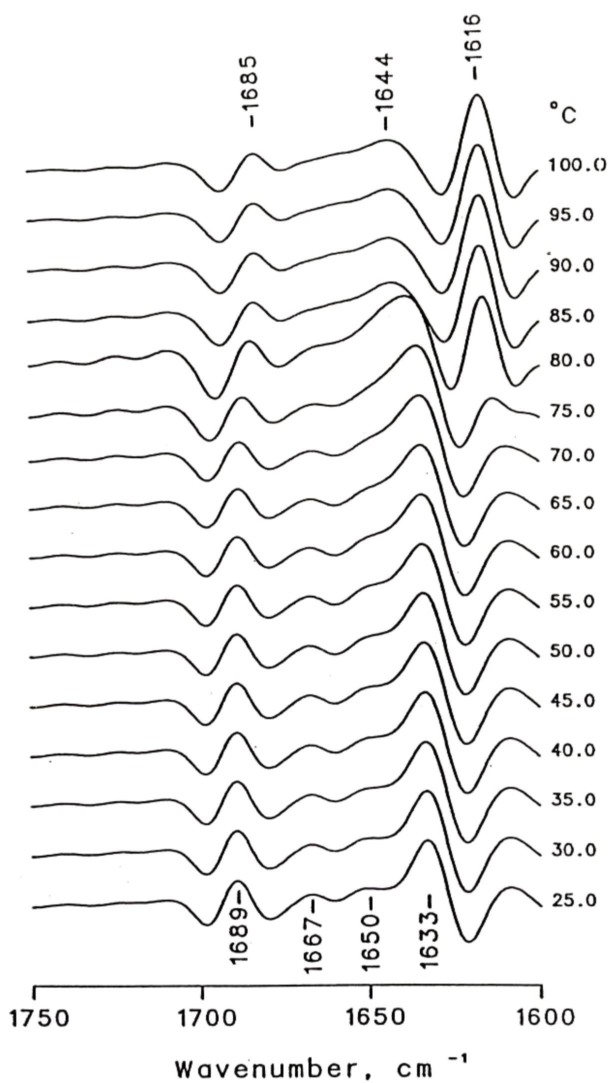

Figure 9 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 0.1% 2-MeOH

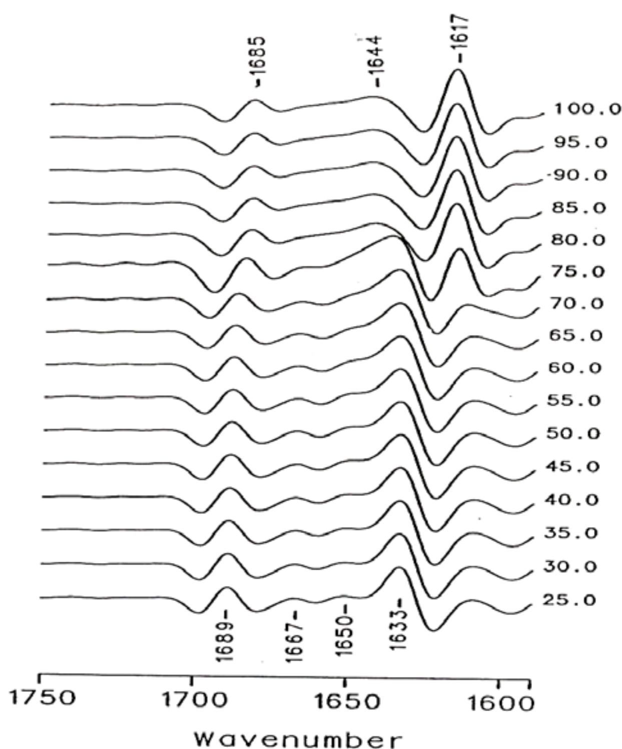

Figure 10 :- Stacked plot of the second derivative infrared spectra of NaOH-CP in D_2O as function of increasing temperature in the presence of 0.5% 2-MeOH

Conclusion:-

Heating temperature, ionic strength, and reducing agents were used to investigate the secondary structure of a cryoprecipitated soy proteins using FTIR spectroscopy. FTIR spectroscopy shows that glycinin has high amount of β–sheet structure, Heating temperature from 25 - 70 °C had no significant effect on the secondary structure while heating temperature above 80 °C had significant effect on the secondary structure. FTIR spectroscopy indicates that NaCl stabilize the structure of glycinin against denaturation while SDS and 2 MeOH appear to disrupt the secondary structure especially at temperature higher than 80 °C

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تحليل بروتينات فول الصويا المرسبة بالتبريد باستخدام مطياف متوسط المدى للاشعة تحت الحمراء

رمضان الصالحين عبدالقادر

قسم علوم وتقنية الأغذية – كلية الزراعة – جامعة عمر المختار – البيضاء – ليبيا

المخلص العربي

تأثير درجة الحرارة في وجود تركيزات مختلفة من كلوريد الصوديوم بالإضافة إلى العوامل المختزلة على بروتين فول الصويا المرسبة بالتبريد تمت دراستها باستخدام مطياف FTIR. بينت النتائج انه عند درجة حرارة ٢٥ م° فان بروتين فول الصويا قيد الدراسة اظهر أربع حزم عند cm^{-1} ١٦٣٣، ١٦٥٠، ١٦٦٧ و ١٦٨٩. عند درجة حرارة ٨٥ م° فان هذه الأربع حزم انخفضت في كثافتها مع زيادة في كثافة الحزمة cm^{-1} ١٦٤٤ والتي تظهر نتيجة لدنترة البروتين بالإضافة إلى زيادة كثافة هذه الحزمة فان هناك حزمتين تم ظهورهما عند cm^{-1}١٦١٦ و ١٦٨٥.

بينت الدراسة عدم حدوث أي تغيرات معنوية على التركيب الثانوي للبروتين الثنائي حتى درجة ١٠٠ درجة مئوية في وجود كلوريد الصوديوم بتركيز ٠.٥ و ١ مولر.

أظهرت نتائج هذه الدراسة أن وجود SDS بتركيز ٥٠mM كان له تأثير على تثبيت البروتين ضد درجة الحرارة بينما تركيز ١٠٠mM من نفس المادة أحدثت تغيراً واضحاً على تركيب البروتين عند درجة حرارة أعلى من ٥٠ م°.