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Effects of some additives on wheat gluten solubility: A structural approach

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Abstract

The effects of KCl, NaCl and cysteine on the solubility in water of partially hydrolyzed gluten were studied at different pH values. As expected, solubility was shown to depend on solution pH. It decreased to reach a minimal value at the isoelectric pH (pH 6) and then it increased.

In the presence of additives, the same behaviour was observed with an increase in solubilities values. In fact, an improvement of solubility of 28–30% was observed in the case of KCl and cysteine, while NaCl was much less efficient. The solubilisation effect of such additives was found to decrease with the initial concentration of wheat gluten.

The structural investigation, by analysis of the amide I region of the infrared spectra of gluten samples, solubilised in the presence of additives, showed an increase in the proportion of β-turns and extended structures, accompanied by a decrease in the α-helix proportion. Such results confirm and explain the improvement of wheat gluten solubility by enhancing water–protein bonding and reducing protein–protein interactions. Structural changes result in a better accessibility of more water molecules to the protein and in greater hydration and solubilisation effects.

Keywords: Gluten; Hydration; Solubility; Additives; Secondary structure; Infrared spectra

1. Introduction

Wheat gluten proteins are composed of monomeric and polymeric or aggregated prolamines. They are viscoelastic when hydrated and are responsible for the elastic texture of wheat flour dough (Shewry, Tatham, Forde, Kreis, & Miflin, 1986). The limited solubility of wheat gluten in aqueous solvent has been generally attributed to its large molecular size and intermolecular aggregation, arising from strong non-covalent interactions, involving hydrogen bonds and hydrophobic interactions (Weegels, de Groot, Verhoek, & Hammer, 1994). It has been reported that acidic amino acids and ionizable groups of native gluten are important factors in accentuating the potential role of ionic interactions and salt effects in the solubility of these proteins in aqueous solvents (Fu, Sapirstein, & Bushuk, 1996). Improvement of gluten solubility in water by addition of salts is well documented (Fu et al., 1996; Khalid et al., 2003; Kim & Bushuk, 1995; Ragab, Babiker, &

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El Tinay, 2003) but no structural evidence of this effect has been reported.

On the other hand, several studies were devoted to the effect of hydration on the secondary structure of gluten, using spectroscopic techniques such as IR, Raman or circular dichroïsm spectroscopy. Pézolet, Bonenfant, Dousseau, and Popineau (1992), have shown that the solubilisation of gluten proteins results in an important decrease of the amount of β-sheets, accompanied by an increase of the content of β-turns and α-helical conformation. These results were contrasted with those of Belton et al. (1995) who found that increasing hydration appears to influence the conformation of β-sheet and extended chain structures. Such findings are in agreement with Wellner, Belton, and Tatham (1996) who have shown that the secondary structure of the fully hydrated gluten proteins was a mixture of β-turns and extended chains. Popineau, Bonenfant, Cornec, and Pézolet (1994) have noticed, that in the hydrated viscoelastic state, the β-sheet content is higher than that in solution, whereas, the α-helix content is lower.

It appears, from previous work, that hydration yields an increase in water-protein interactions via hydrogen bonds and a rupturing of protein-protein interactions responsible for the backbone distortion into helical structures, which can lead to an increase in extended structure, β-turns and β-sheet.

This paper reports the effects of NaCl, KCl and cysteine on water solubility of wheat gluten and explores (by FTIR spectroscopy) the resulting secondary structure modifications. The objective is to obtain information on the structural changes of the hydrated protein in the presence of such additives.

2. Materials and methods

2.1. Materials

The investigated wheat gluten sample was kindly donated by CHAMTOR, (Bazancourt, France) and identified as partially hydrolyzed gluten (Hydrolysis Degree = 3%). This gluten contained 86% protein (N × 5.6) on a dry basis, and 5% moisture, and was obtained after the action of a commercial protease (Neutrase 0.8 L, Novo Nordisk, Denmark) on native wheat gluten.

Chemicals used (NaCl, KCl, Cysteine, NaOH and HCl) were analytical grade Sigma products.

The following symbols are used for the studied gluten samples: *gluten H*, the original partially hydrolyzed gluten sample; *gluten HN*, the dissolved fraction in presence of 0.2% NaCl; *gluten HK*, the dissolved fraction in presence of 0.2% KCl; *gluten HC*, the dissolved fraction in presence of 0.5% cysteine.

2.2. Determination of the solubility ratio of wheat gluten in the presence of additives

The effect of additives on the solubility of *gluten H* sample was studied as a function of pH. The solubility of the protein at 1% (w/v) in distilled deionised water or in the presence of, respectively, 0.2% NaCl (*gluten HN*), 0.2% KCl (*gluten HK*), and 0.5% cysteine (*gluten HC*), was determined over a pH range from 2.0 to 10.0.

The dispersion was gently shaken at different pH values in a shaker (140 jolts/min) at room temperature for 1 h. Finally the supernatant was recovered after centrifugation (20 min at 1500 rpm) and freeze-dried.

The protein concentrations in the supernatant and in the original samples were determined using the Kjeldhal method (AOAC, 1995).

The protein solubility was calculated as:

$$\text{Solubility}(\%) = [P_{\text{sup}}]/[P_{\text{total}}]^{*}100,$$

where $[P_{\text{sup}}]$ is the protein concentration in g/100 g.

This method is derived from that described by Wang and Zayas (1991).

2.3. Water vapour sorption isotherms

Water vapour sorption isotherms of the above listed samples were established using the microclimate method (De Jong, Van den Berg, & Kokelaar, 1996). Equilibrium relative humidity (ERH) of gas-tight jars were fixed with saturated salt solutions (LiCl 11%, $MgCl_2 \cdot 6H_2O$ 33%, $K_2CO_3 \cdot 2H_2O$ 44%, NaBr 58%, NaCl 75%, KBr 82%, K_2SO_4 97%) (Greenspan, 1977). The adsorption curves were carried out on previously solubilised gluten samples in the presence of additives and then freeze-dried. Gluten was weighted into cupels (1 g) and maintained at 20 °C under different ERH until equilibrium (constant weight) was reached after 7 days. At a given equilibrium relative humidity (ERH), water content was calculated and the sorption curve established as water content = f(ERH).

2.4. FTIR spectra

Samples after dissolution were lyophilized and submitted to equilibrium with humid air (ERH = 96%). Infrared spectra (200 scans) of humid (ERH = 96%) additive-free samples and gluten samples containing additives (0.2% KCl and 0.5% cysteine) were recorded with a resolution of 2 cm^{-1} on a Nicolet® infrared spectrophotometer (Model "Impact 410") using a thunderdome attenuated total reflectance (ATR) accessory. Hydrated thin layer samples were obtained by regulating the relative humidity. The samples were first vigorously mixed and then humidified to pasty state in a gas-tight jar in the presence of saturated K_2SO_4 solutions (ERH = 96%) (AOAC, 1980).

The interpretation of the changes in the overlapping amide I i.r. band components, was made possible by Fourier self-deconvolution using Grams software (Galactic Industries Corporation, USA). Initial band parameters were considered as a basis and used for each new spectrum (Wellner et al., 1996; Belton et al., 1995).

3. Results and discussion

3.1. Effect of additives on the solubility of partially hydrolyzed gluten at variable pH

Variation of solubility, as a function of solution pH for partially hydrolyzed wheat gluten in the absence and in the presence of cysteine, NaCl and KCl, is shown in Fig. 1

The curve describing the effect of pH on the solubility of wheat gluten, in additive-free solution, shows that this parameter increases with decreasing pH to reach a minimum value of about 50% at pH 6, followed by an increase in the region of basic pH. This is not surprising because the minimal solubility of proteins is known to occur at the isoelectric point, corresponding to the neutral global charge of the proteins and the increase of association reactions of polypeptide chains at this point (Drago & Gonzàlez, 2001). On both sides of the isoelectric point, the solubility increases, resulting in the increase of the global charge of the ionized protein. The slightly higher solubility in the alkaline region is due to the presence of a high proportion of acidic aminoacids (Glu, Asp) in wheat gluten proteins which are ionized at alkaline pH (Belton et al., 1995; Tatham et al., 1990).

The curve corresponding to the effect of cysteine shows the same behaviour, with a relatively higher solubility (about 90%) compared to that obtained in the absence of cysteine, even if the pH is equal to the isolelectric point. On both sides of the isoelectric point, the solubility increases to reach 100% at pH 4. Such increase in solubility under the effect of cysteine could be attributed to its mild reducing property (German & Phillips, 1994). Cysteine is known to reduce the SS bonds and to weaken the protein tertiary structure and consequently to improve the wheat gluten solubility (Popineau, Huchet, Larré, & Bérot, 2002).

The curve corresponding to the effect of NaCl shows different behaviour. A slight solubility-enhancing effect was observed at pH 4 and 6. The solubilities were, respectively, 90% and 80%. The solubility is decreased below the reference curve obtained in the absence of additives. Fu et al. (1996) reported that low ionic strength favours the exposure to solvent of polar and hydrophilic residues at the surface of the protein and enhances gluten solubility. However, at alkaline pH, solubility decrease is very likely due to an increase in protein–protein interactions via ionic bonds, which results from the deprotonation of surface polar residues by salt addition. Similar results on the increasing solubility of seed protein isolates at different pH levels at low NaCl concentrations, were reported in recent papers (Khalid, Babiker, & Tinay, 2003).

KCl shows behaviour different from that of NaCl, and comparable to that of cysteine, with higher solubilities at all the tested pH levels which remain above 90%. It seems that, apart from its effect on the ionic strength, KCl acts as a water structure-breaker as compared to NaCl which acts as a water structure maker (Mathlouthi, Hutteau, & Angiboust, 1996). The increase in mobility of water molecules in KCl–gluten solutions affects the water–gluten interactions and the exchange of hydrating water molecules between gluten surface and the aqueous environment. Damodaran (1989) showed that weak concentrations of certain salts result in

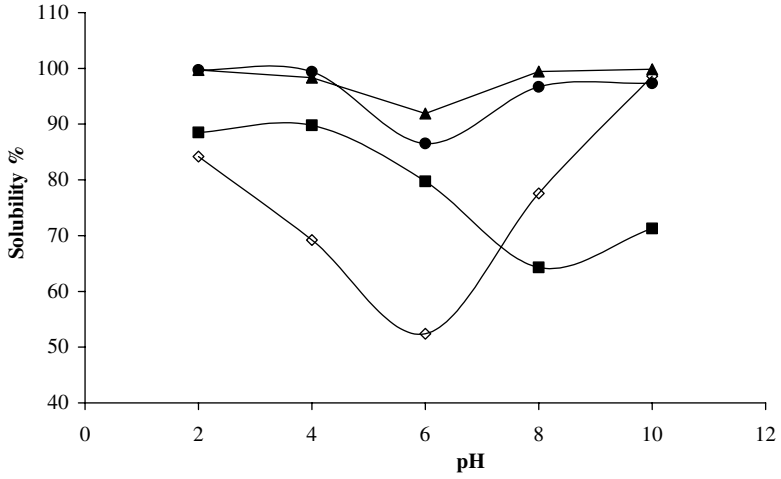

Fig. 1. Effect of pH levels on solubility of partially hydrolysed gluten in absence of additives (gluten H: ◇) and, respectively, in the presence of 0.2% NaCl (gluten HN: ■), 0.5% cysteine (gluten HC: ●) and 0.2% KCl (gluten HK: ▲).

changes in the structure of water. This affects the water–β-lactoglobulin interface and contributes to the exposure of polar residues at the surface of the protein which leads to an enhancement of its solubility.

Oshodi and Ojokan (1997) reported an enhancing of techno-functional properties of bovine plasma protein, by low salt concentrations, especially solubility, water absorption and gelation. The effect of KCl on water absorption of bovine plasma protein gels was reported to be twice that obtained with NaCl at the same concentration (0.5%). The combined effects of salts and pH were studied and the reported results are in agreement with ours. However, no structural arguments were advanced.

In summary, the difficulty in solubilising gluten proteins, arises predominantly from the lack of ionisable groups and the increase of molecular weight. Factors which promote the ionization of residues or the disaggregation of protein molecules, certainly enhance the solubility and consequently improve the functional properties (Bondos & Bicknell, 2003; Singh & MacRitchie, 2001).

As the effect of NaCl seems negligible, only KCl and cysteine were used for the following experiments.

3.2. Dependence of solubility enhancement, by additives, on initial gluten concentration

The effect of additives on solubility was found to depend on initial sample concentration (Fig. 2). A progressive decrease in gluten solubility was observed as initial concentration was increased. The relative decrease in solubility is about 40% for an increase in gluten concentration from 1% to 10%. The same behaviour was observed in the presence of 0.2% KCl and 0.5% cysteine. However, the drop in solubility is particularly important in the presence of cysteine: 70% in the same range of initial gluten concentration. Thus, the solubility-enhancing effect of cysteine is only possible at very low gluten concentration. For KCl, although the solubility remains above 20% for the tested initial gluten concentration range, the drop in solubility is also of the same order as for cysteine (70%). The increase in protein concentration yields more protein–protein interactions and causes the aggregation of proteins, leading to reduction of solubility. At high gluten concentration, the solubilising effect of additives and the interactions responsible for this phenomenon, culminating in hydrophobic interactions between protein molecules, contributed to the aggregation and precipitation (Bondos & Bicknell, 2003).

3.3. Water vapour sorption isotherms

To understand the hydration behaviour of partially hydrolyzed gluten and the effect of additives on such properties, we determined water vapour adsorption isotherms over the whole range of water activities (Fig. 3). From the isotherms, it seems clear that *gluten HK* adsorbs more water vapour, especially for a_w values above 0.9. In this a_w range ($a_w > 0.9$), the *gluten HK* sample adsorbed more than once and half a times its dry weight. For *gluten H* and *gluten HC*, the water content did not exceed, respectively, 0.7 and 0.9 g/100 g (dry basis). The interactions with ionic additives contributed to exposure of polar and ionic groups of the protein at the surface and led to increased interactions with water vapour molecules. Ionic interactions (protein–salt–protein) may promote the formation of a hydrophilic network in which water molecules aggregate and form micro-droplets embedded in the protein (Maréchal, 1997).

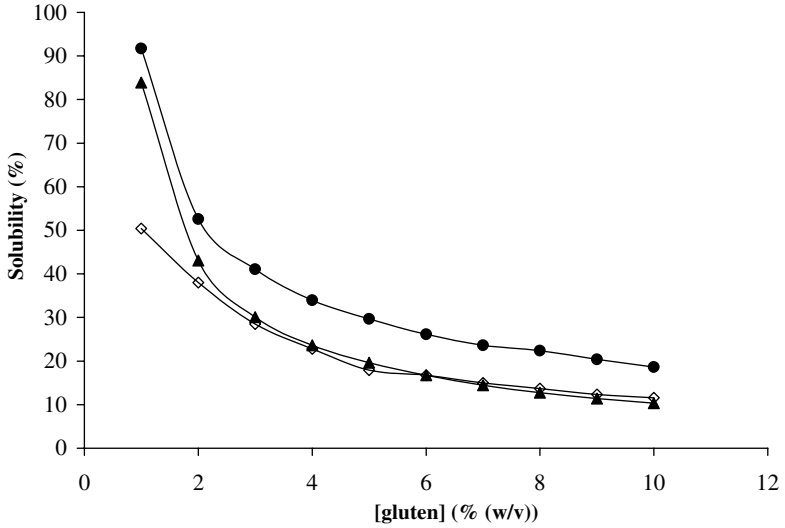

Fig. 2. Effect of initial concentration on the solubility of gluten H (◇), gluten HC (●) and gluten HK (▲).

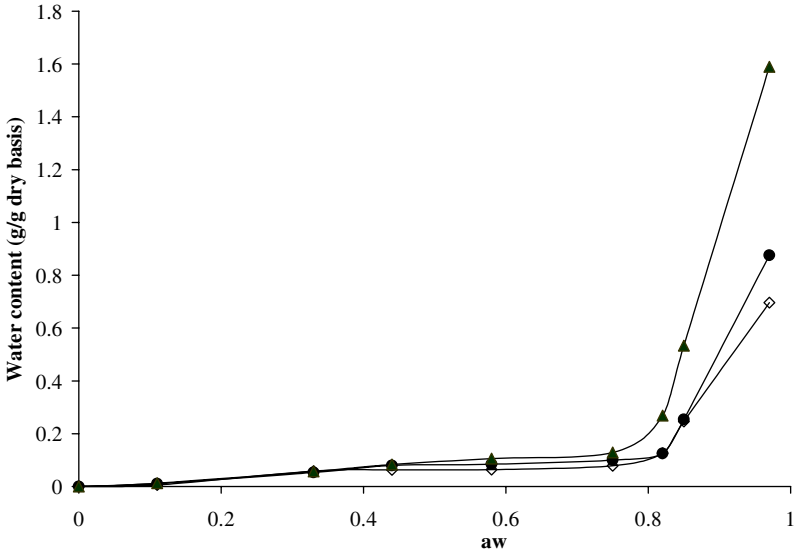

Fig. 3. Water vapour sorption isotherms, at 20 °C, of gluten H (◇), gluten HC (●) and gluten HK (▲)

3.4. Infrared study of the effects of additives on the behaviour of partially hydrolyzed gluten

3.4.1. Effect on hydration properties

FTIR spectra of soluble gluten samples were recorded in the OH stretching region (2800–3800 cm^{-1}) and are shown in Fig. 4. The observed broad absorption band is mainly assigned to the OH stretching mode of water (Servaty, Schiller, Binder, & Arnold, 2001).

In the presence of additives (*gluten HK* and *HC*), the intensity of (OH) increases, especially for *gluten HK*. Servaty et al. (2001) have correlated the intensity of this band with the amount of water absorbed by the protein polymer. A variation in the shape and area of band is observed in the presence of additives (Fig. 4), the area of the OH band being proportional to sample water content (Grdadolnik & Maréchal, 2003; Maréchal, 1997; Servaty et al., 2001; Starzak & Mathlouthi, 2003). These results confirm that gluten is more hydrated, after solubilising by KCl (*gluten HK*), than by cysteine (*gluten HC*) or in the additive-free sample (*gluten H*). This is in agreement with the results of water vapour sorption isotherms. Michels (2000) obtained a perfect correlation between water sorption isotherms, determined by

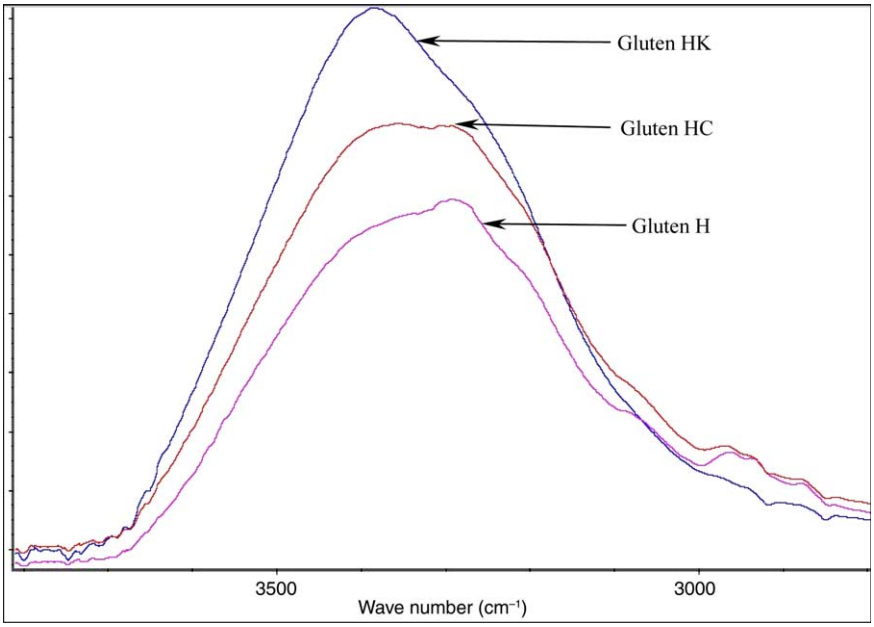

Fig. 4. Infrared spectra in the OH region of freeze-dried and humidified (ERH = 96%) soluble fractions of gluten H, gluten HC and gluten HK.

gravimetric measurements, and those determined by measuring the area of the OH infrared absorption band during the hydration process. The same type of correlation was previously observed by (Rüegg & Häni, 1975).

3.4.2. Effect on the secondary structure

Fourier transform infrared spectroscopy was used to detect structural changes in the secondary structure of proteins and determine the water binding-properties (Table 1). Shifts in frequencies of amide I band (1600–1700 cm^{-1}), as well as those of amide II and amide III, were correlated with the changes in secondary structure of proteins (Byler & Susi, 1986; Surewicz & Mantsch, 1988). Amide I band mainly originates from the C=O stretching vibration (Byler & Susi, 1986; Arrondo et al., 1993) and appears as a broad band, centred at 1650 cm^{-1} and composed of several overlapping component bands due to various protein segments with different structures (Surewicz & Mantsch, 1988).

The deconvolution of amide I band reveals that it is composed of at least five components situated approximately at 1615, 1630, 1650, 1670 and 1687 cm^{-1} (Subirade, Kelly, Guéguen, & Pézolet, 1998. Belton et al. (1995) found, in addition, a shoulder at 1625 cm^{-1}. The relative assignments of these bands, based on previous studies, are summarized in Table 1.

In order to investigate the structural effect of cysteine and KCl on partially hydrolyzed gluten solubility and hydration, amide I infrared absorption bands were Fourier deconvoluted using the same method and parameters as described above. Fig. 5 shows the original infrared spectra in the amide I region of gluten and additive containing gluten. The qualitative analysis of spectra reveals a decrease in the intensities of the amide I bands and in the whole amide region. This supposes that important changes in the secondary structure have occurred as a result of the effects of additives on the protein.

Fig. 6 shows the deconvoluted amide I band for additive-free gluten. The deconvolution showed 5 components centred at wave-numbers in agreement with those listed in Table 1 and discussed previously. However, the information provided directly by the deconvoluted spectra is only qualitative. Surewicz & Mantsch (1988) have observed that the fractional areas (integrated intensities of bands) of the resolved component bands are directly related to the relative populations of the conformational structures represented by these components.

The percentages of secondary structure segments (α-helices, β-sheets or β-turns) have been estimated as the percentage of the corresponding area by ratios to the total amide I band area (Surewicz & Mantsch, 1988). This method was adopted in several studies for the estimation of protein secondary structure from amide I band in infrared and Raman spectra (Dousseau & Pézolet, 1990; Lee, Haris, Chapman, & Mitchell, 1990).

We used this method to quantify the change in secondary structure of gluten in the absence and the presence of additives and the results obtained are listed in Table 2.

(Table 2) shows that hydrated *gluten H* (partially hydrolyzed gluten) contains 34% of β-sheet, 16% of α-helix and 49% of β-turn structures. According to Lee et al. (1990) hydrated native gluten contains approximately equal amounts of β-sheet, α-helix and β-turn structure (28% of β-sheet, 31% of -helix, 27% of β-turn structure) and a proportion of extended structures (15%). Comparison of the secondary structures of native and partially hydrolyzed gluten, allows the conclusion that the partial enzymatic hydrolysis of gluten engenders a noticeable decrease of the α-helix content (from 31% to 16%), with an increase in the β-turn amount from 28% to 49%, accompanied by a weak increase in β-sheet structure. In fact, the enzymatic hydrolysis reduces the size of gluten molecules by rupturing of the peptide chains. This reduces the distortion of the polypeptide backbone, responsible for bearing of the helical structure. In fact, partially hydrolyzed gluten is more soluble than native gluten. This is due to the shortage in α-helix structures which are more hydrophobic, rigid and less accessible to hydrating water.

Table 1
Positions and relative assignments, from earlier studies, of the different components resulting from the deconvolution of the amide Iband.

Component band position	Assignment	Reference study
1615	Intermolecular β-sheets due to protein aggregation	Surewicz and Mantsch (1988) Carrier et al. (1990)
1625	Extended structures	Mantsch et al. (1993) Belton et al. (1995)
1630	Extended β-sheet structures (hydrated)	Byler and Susi (1986); Surewicz and Mantsch (1988); Surewicz et al. (1990, 1993)
1650	α-Helices	Byler and Susi (1986)
1670	β-Turns	Byler and Susi (1986); Surewicz and Mantsch (1988); Mantsch et al. (1993)
1687	β-Sheet structures	Byler and Susi (1986); Surewicz and Mantsch (1988); Surewicz et al. (1990, 1993)

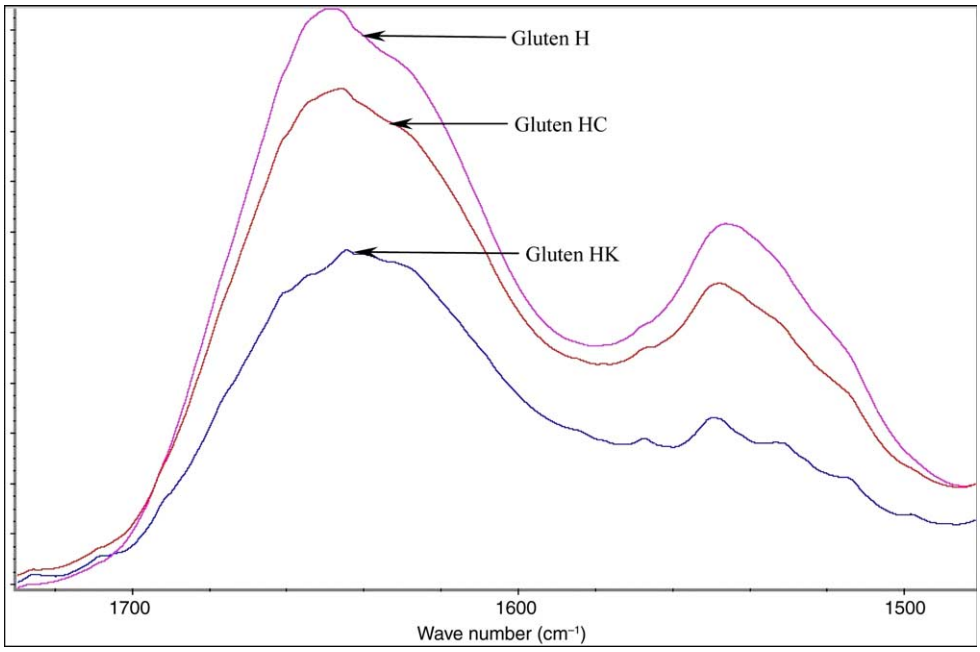

Fig. 5. Infrared spectra in the amide I and amide II region of freeze-dried and humidified (ERH = 96%) soluble fractions of gluten H, gluten HC and gluten HK.

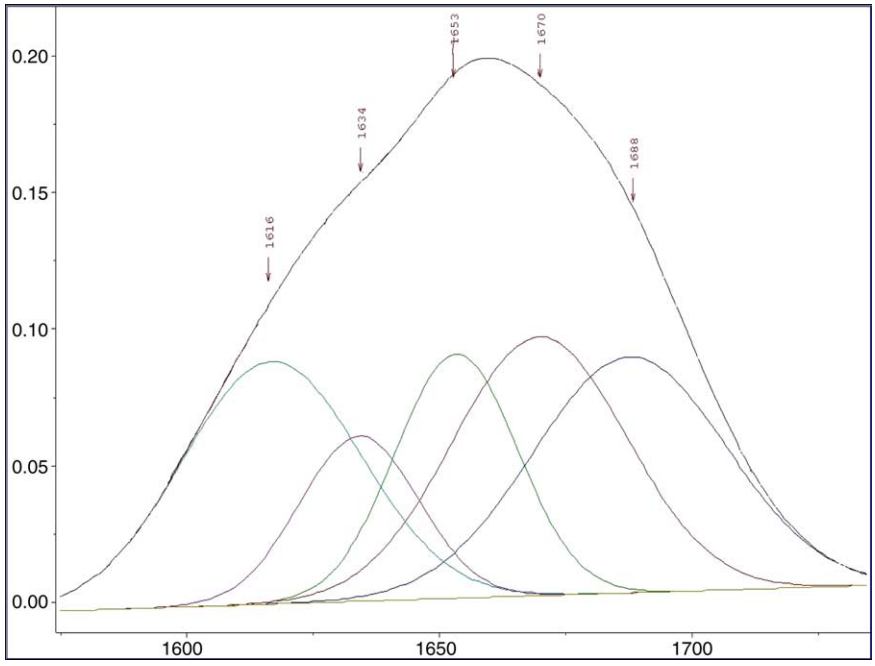

Fig. 6. Deconvoluted infrared amide I band of gluten H.

If we now compare the secondary structure of partially hydrolyzed gluten sample to that solubilised in the presence of additives, we observe a reduction of α-helices, from 16.3% to 7.5%, in the presence of cysteine (*gluten HC*) and to 0.4% in the presence of KCl (*gluten HK*). This is accompanied by an increase in β-turns in the presence of additives. The β-sheet structure shows a certain stability in the presence of cysteine, but it increases in the presence of KCl (from 34% to 42%). The ratio, α-helix/β-sheet, is divided by two in the presence of cysteine (from 0.48 to 0.22) and becomes very weak in the presence of KCl. This ratio expresses the variation of the secondary structures observed in the presence of additives. Structural changes of gluten in the presence of additives, consists in a reduction of α-helix, which is converted into β-turn structures. Such

Table 2
Determined frequencies of amide I component bands, relative assigned structures and their distributions for gluten H, gluten HC and gluten HK.

Assignment	Gluten H		Gluten HC		Gluten HK	
	Frequency (cm^{-1})	Structure distribution (%)	Frequency shift (cm^{-1})	Structure distribution (%)	Frequency shift (cm^{-1})	Structure distribution (%)
β-Sheet	1616	34.2	−4	33.6	5	42.0
	1633		0		8	
	1687		−7		−1	
α-Helix	1653	16.3	−1	7.5	0	0.4
β-Turn	1669	49.5	−8	58.8	−7	57.6
αH/βS	0.48		0.22		0.01	

structural changes are in agreement with the higher hydration and solubility observed in presence of additives during the solubilisation experiments. Our results are also in agreement with previous studies (Wellner et al., 1996) which showed that the hydration of gluten leads to a loss in α-helix structures and an increase in β-turns and extended structures.

4. Conclusion

The above results show that the solubility of partially hydrolyzed wheat gluten is greatly affected by pH, initial gluten concentration and by the nature of the additive. An increase in wheat gluten solubility was observed and found to be proportional to hydration which itself affects the distribution of the secondary structure segments of the protein. The increase in hydration results in a reduction of the α-helix structure and its conversion into β-turns and extended structures. Therefore, additives may be selectively used to improve functional properties of wheat gluten and the spectroscopic evaluation of structural features may be used to evaluate an eventual solubilising effect.

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References

Arrondo, J. L. R., Muga, A., Castersana, J., & Goni, F. M. (1993). Quantitative studies of the structure of proteins in solution by Fourier-transform infrared spectroscopy. *Progress in Biophysics and Molecular Biology, 59*, 23–56.

AOAC international. (1980 and 1995). *Official methods of analysis*. Gaithersburg: Association of Official Analytical Chemists.

Belton, P. S., Colquhoun, I. J., Grant, A., Wellner, N., Field, J. M., Shewry, P. R., et al. (1995). FTIR and NMR Studies on the hydration of a high-Mr subunit of glutenin. *International Journal of Biological Macromolecules, 17*(2), 74–80.

Bondos, S. E., & Bicknell, A. (2003). Detection and prevention of protein aggregation before, during, and after purification. *Analytical Biochemistry, 316*, 223–231.

Byler, D. M., & Susi, H. (1986). Examination of the secondary structure of proteins by deconvolved FTIR spectra. *Biopolymers, 25*, 469–487.

Carrier, P., Mantsch, H. H., & Wong, P. T. T. (1990). Pressure induced reversible changes in secondary structure of poly(L-lysine): an I.R. spectroscopic study. *Biopolymers, 29*, 837–844.

Damodaran, S. (1989). Influence of protein conformation on its adaptability under chaotropic conditions. *International Journal of Biological Macromolecules, 11*, 2–8.

De Jong, G. I. W., Van den Berg, C., & Kokelaar, A. J. (1996). Water vapor sorption behavior of original and defatted wheat gluten. *International Journal of Food Science and Technology, 31*, 519–526.

Dousseau, F., & Pézolet, M. (1990). Determination of the secondary structure content of proteins in aqueous solutions from their amide I and amide II infrared bands. Comparison between classical and partial least-squares methods. *Biochemistry, 29*(37), 8771–8779.

Drago, S. R., & Gonzàlez, R. J. (2001). Foaming properties of enzymatically hydrolyzed wheat gluten. *Inovative Food Science and Emerging Technologies, 1*, 269–273.

Fu, B. X., Sapirstein, H. D., & Bushuk, W. (1996). Salt-induced disaggregation/solubilization of gliadin and glutenin proteins in water. *Journal of Cereal Science, 24*, 241–246.

German, J. B., & Phillips, L. (1994). Protein interactions in foams: Protein-gaz phase interactions. In: N. S. Hettiarachchy, & G. R. Ziegler, (Eds.), *Protein functionality in food systems. IFT Basic Symposium Series* (Vol. 9).

Grdadolnik, J., & Maréchal, Y. (2003). Infrared difference spectroscopy Part II. Spectral decomposition. *Vibrational Spectroscopy, 31*, 289–294.

Greenspan, L. (1977). Humidity fixed point of binary saturated aqueous solutions. *Journal of Food Research, 81*(1), 89–96.

Khalid, E. K., Babiker, E. E., & Tinay, A. H. (2003). El Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. *Food Chemistry, 82*, 361–366.

Kim, H. R., & Bushuk, W. (1995). Salt sensitivity of acetic acid extractable proteins of wheat flour. *Journal of Cereal Science, 21*, 241–250.

Lee, D. C., Haris, P. I., Chapman, D., & Mitchell, R. C. (1990). Determination of protein secondary structure using factor analysis of infrared spectra. *Biochemistry, 29*, 9185–9193.

Mantsch, H. H., Perczel, A., Hollosi, M., & Fasman, G. D. (1993). Characterization of β-turns in cyclic hexapeptides in solution by Fourier transform IR spectroscopy. *Biopolymers, 33*, 201–207.

Maréchal, Y. (1997). Interaction configuration of H2O molecules in a protein (*Stratum corneum*) by infrared spectrometry. *Journal of Molecular Structure, 416*, 133–143.

Mathlouthi, M., Hutteau, F., & Angiboust, J. F. (1996). Physico-chemical properties and vibrational spectra of small carbohydrates

in aqueous solution and the role of water in their sweet taste. *Food Chemistry, 56*, 215–221.

Michels Franck. (2000). Contribution à l'étude des propriétés physico-chimiques des glutens de blé. *Mémoire CNAM – Reims, spécialité Biochimie Industrielle et agroalimentaire*. 5 juillet 2000. p. 145.

Oshodi, A. A., & Ojokan, E. B. (1997). Effect of salts on some of the functional properties of bovine plasma protein concentrate. *Food Chemistry, 59*, 333–338.

Pézolet, M., Bonenfant, S., Dousseau, F., & Popineau, Y. (1992). Conformation of wheat gluten proteins. Comparison between functional and solution state as determined by infrared spectroscopy. *FEBS Letters, 299*(3), 247–250.

Popineau, Y., Bonenfant, S., Cornec, M., & Pézolet, M. (1994). A study by infrared spectroscopy of the conformations of gluten proteins differing in their gliadin and glutenin compositions. *Journal of Cereal Science, 20*, 15–22.

Popineau, Y., Huchet, B., Larré, C., & Bérot, S. (2002). Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science, 35*, 327–335.

Ragab, D. M., Babiker, E. E., & El Tinay, A. H. (2003). Fractionation, solubility and functional properties of cowpea (*Vigna unguicullata*) proteins as effected by pH and/or salt concentration. *Food Chemistry, 84*, 207–212.

Rüegg, M., & Häni, H. (1975). Infrared spectroscopy of the water vapor sorption process of caseins. *Biochimica et Biophysica Acta, 400*, 17–23.

Servaty, R., Schiller, J., Binder, H., & Arnold, K. (2001). Hydration of polymeric components of cartilage-an infrared spectroscopic study on hyaluronic acid and chondroitin sulfate. *International Journal of Biological Macromolecules, 28*, 121–127.

Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Miflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science, 4*, 97–106.

Singh, H., & MacRitchie, F. (2001). Application of polymer science to properties of gluten. *Journal of Cereal Science, 33*, 231–243.

Starzak, M., & Mathlouthi, M. (2003). Cluster composition of liquid water derived from laser-Raman spectra and molecular simulation data. *Food Chemistry, 82*, 3–22.

Subirade, M., Kelly, I., Guéguen, J., & Pézolet, M. (1998). Molecular basis of film formation from a soybean protein: Comparison between the conformation of glycinin in aqueous solution and in films. *International Journal of Biological Macromolecules, 23*, 241–249.

Surewicz, W. K., Leddy, J. J., & Mantsch, H. H. (1990). Structure, stability and receptor interaction of cholera toxin as studied by infrared spectroscopy. *Biochemistry, 29*, 8101–8111.

Surewicz, W. K., & Mantsch, H. H. (1988). New insight into protein secondary structure from resolution-enhanced infrared spectra. *Biochimica et Biophysica Acta, 952*, 115–130.

Surewicz, W. K., Montsch, H. H., & Chapman, P. (1993). Determination of protein secondary structure by Fourier transform infrared spectroscopy. A critical assessment. *Biochemistry, 32*, 389–394.

Tatham, A. S., Drake, A. F., & Shewry, P. R. (1990). Conformational studies of synthetic peptides corresponding to repetitive regions of the high molecular weight (HMW) glutenin subunits of wheat. *Journal of Cereal Science, 11*, 189–200.

Wang, C. R., & Zayas, J. F. (1991). Water retention and solubility of soy proteins and corn germ proteins in a model system. *Journal of Food Science, 56*(2), 455–458.

Weegels, P. L., de Groot, A. M. G., Verhoek, J. A., & Hammer, R. J. (1994). Effects on gluten of heating at different moisture contents. II. Changes in functional properties. *Journal of Cereal Science, 19*, 39–47.

Wellner, N., Belton, P. S., & Tatham, A. (1996). Fourier transform IR solid state of ω-gliadins. *Biochemical Journal, 319*, 741–747.