Hydration-related Properties of Caseins at pH 2·0–3·0

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ABSTRACT

Casein is very soluble at pH values < 3·5 and acidic casein solutions are also very heat-stable as they can withstand heating at 150°C for 1 h. The viscosity of acidic casein solutions increased logarithmically with increasing protein concentration and decreased logarithmically with increasing temperature up to 70°C. At similar protein concentrations, acidic casein solutions were much more viscous than sodium caseinate solutions.

Sorption isotherms of acidic caseins and sodium caseinate were similar up to A_w values of 0·52. Between A_w values of 0·52 and 0·87 sodium caseinate showed a greater increase in water uptake than acidic caseins, while at A_w values of 0·87–0·97 water uptake of acidic caseins increased more sharply than that of sodium caseinate, uptake being in the order acidic casein pH 2·0 > acidic casein pH 2·5 > acidic casein pH 3·0 > sodium caseinate pH 7·0.

INTRODUCTION

In recent years, milk protein products are being marketed with an emphasis on specific functional properties. There are seven major classes of functional milk proteins: acid casein, rennet casein, caseinates, casein–whey protein co-precipitates, whey protein concentrates and isolates and lactalbumin (Mulvihill & Fox, 1983). The functional properties and uses of milk proteins have been reviewed by Southward & Walker (1980), Morr (1982), Evans (1982) and Fox & Mulvihill (1982, 1983).

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The functional properties of caseinates > pH 6·0 are well characterized but apart from a study on the viscosity of acidic casein solutions by Korolczuk (1982*a*, *b*), no reports appear in the literature on other functional properties of caseins at pH values below their isoelectric point (∼ pH 4·6). Therefore, this study was undertaken to investigate the hydration-related functional properties of acidic casein at pH 2·0, 2·5 and 3·0 and to compare them to those of sodium caseinate at pH 7·0.

MATERIALS AND METHODS

Isoelectric casein

Isoelectric (acid) casein was prepared as follows.

Bulk milk from the University herd was pasteurized in an HTST pasteurizer at 72°C for 15 s and skimmed using an Alfa-Laval Separator (Type 3186 M/60). The skim milk was cooled to 2–4°C and its pH adjusted to 4·6 with 2M-HCl. The acidified milk was maintained at 2–4°C for 30 min and the pH readjusted to 4·6. The temperature of the milk was then increased to ∼ 37°C in a waterbath with gentle stirring during heating. The casein curd was separated from the whey by filtration through cheese cloth and washed 3–4 times with volumes of distilled water equal to the volume of milk. The washed curd was 'dewatered' by pressing the drained curd in cheese cloth and lyophilized.

Sodium caseinate

Commercial sodium caseinate was obtained from Kerry Co-Op. Creameries, Listowel, Co. Kerry, Ireland.

Chemicals

All chemicals used were of reagent grade obtained from BDH Chemicals Ltd., Poole, Dorset, Great Britain, or Sigma Chemical Co. Ltd., Poole, Dorset, Great Britain.

Determination of protein content

The protein content of casein/caseinate preparations was determined by the standard macro-Kjeldahl method (AOAC, 1975).

Determination of solubility

Dispersions (1%, w/v, protein) were adjusted to pH values in the range 2·0–7·5 (at 0·5 unit intervals) using 0·1M-HCl or 0·1M-NaOH. After stirring for 5 min, using a magnetic stirrer, the pH of the samples was rechecked and readjusted, if necessary. The samples were then maintained at 4°C for 24 h after which the pH was readjusted, if necessary. The samples were centrifuged at 1000 g for 20 min at 20°C in a Sorval RC-5B Refrigerated Superspeed Centrifuge with GSA Rotor (Du Pont Company, Instrument Products, Newtown, CT-06740, USA). After centrifugation, the supernatants were filtered through Whatman No. 1 filter paper and the protein content of the filtrates determined by the macro-Kjeldahl method.

% Solubility was calculated as:

$$\frac{\text{water soluble protein in the filtrate}}{\text{total protein in the sample}} \times 100$$

Determination of heat stability

Samples of acid casein (3%, w/v, protein) were adjusted to pH 2·0, 2·5 or 3·0 at 20°C with 2M-HCl and held for 1 h at that temperature. The pH was then readjusted and the heat coagulation times (HCTs) at 140°C determined in a thermostatically controlled oil bath as described by Davies & White (1966).

Determination of viscosity

The viscosity of casein/caseinate solutions, at concentrations ranging from 0·5 to 4% (w/v) protein (at 0·5 unit intervals) for acidic casein and 2 to 12% (w/v) protein (at 2 unit intervals) for sodium caseinate, was determined using a Brookfield Synchroletric viscometer (Model LVT with Spindle No. 18) fitted with a small sample adapter (SC4-13R). The pH of individual samples was adjusted to various values (2·0, 2·5, 3·0 or 7·0) using 2M-HCl or 2M-NaOH; the samples were held for 5 min and the pH checked and readjusted, if necessary. The viscosities of the samples were determined over the temperature range 20–70°C. A constant temperature was maintained by circulating water from a thermostatically-controlled waterbath through the jacket of the sample adapter.

The values for the viscosity of water at the different temperatures used to calculate the relative viscosity were taken from Whitaker *et al.* (1927).

Determination of water sorption isotherms

Water sorption isotherms were determined by the method of Spiess & Wolf (1983), modified as follows.

Preparation of caseins at various pH values (2·0, 2·5 or 3·0)
Dispersions (4%, w/v, protein) of acid casein were adjusted to pH 2·0, 2·5 or
3·0 at 20°C with 2M-HCl. The solutions were stirred for 20 min using a
magnetic stirrer and, after readjustment to pH 2·0, 2·5 or 3·0, the samples
were lyophilized.

Preparation and handling of samples
Samples (0·5 g) of dried casein/caseinates (pH 2·0, 2·5, 3·0 or 7·0) were placed
in sample containers and stored over P_2O_5 for 7 days to obtain zero water
content before the sorption process. Samples were then placed in triplicate
over saturated salt solutions (A_w values ranging from 0·11 to 0·97) and
maintained at 25°C throughout the sorption period (12 days).

Following sorption, the average water content of the proteins was
determined (g H_2O/100 g protein) and plotted as a function of A_w.

Determination of water-holding capacity

The water-holding capacity of casein/caseinate was determined by the
method of Quinn & Paton (1979) as follows.

A sample of casein/caseinate, equivalent to 1 g protein, was placed in a
transparent 50 ml centrifuge tube and the tube and contents weighed.
Distilled water was added to the protein in small, unmeasured increments
and the mixture stirred vigorously after each addition. This process was
repeated until the mixture was visibly thoroughly wetted by touching with a
spatula and some free water existed. The tube and contents were centrifuged
at 2000 g for 10 min. The slight amount of supernatant was discarded and
the tube and contents weighed. The weight difference per gram of dry sample
was taken as an approximate measure of the water-holding capacity
(g H_2O/g protein).

RESULTS

Solubility

The typical solubility profile of casein as a function of pH (2·0–7·5) (Fig. 1)
shows minimum solubility at pH 4·5; solubility increases on altering the pH
to either side of the isoelectric point, i.e. casein was very soluble at pH values
< 3·5 and > 5·5, but was more soluble at pH values > 6·0 than at acidic pH
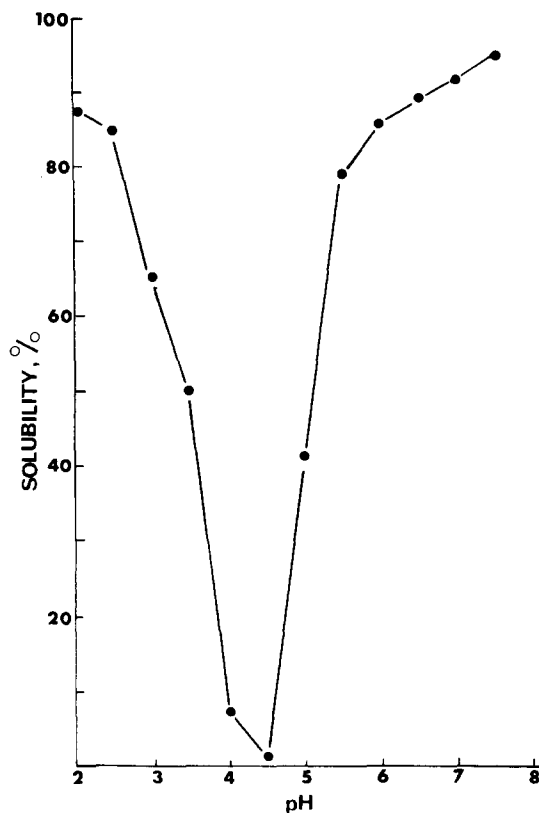(e.g. 2·0).

Fig. 1.   Solubility of acid casein (1%, w/v) as a function of pH.

Heat stability

Acidic solutions of casein were extremely heat stable: 3% solutions at pH 2·0, 2·5 or 3·0 could be heated at 150°C for at least 60 min without coagulation.

Viscosity

Effect of protein concentration and temperature on viscosity
The relative viscosity (ηr) of sodium caseinate solutions decreased logarithmically with increasing temperature (20–70°C) and increased with increasing protein concentration (4–12%, w/v, pH 7·0) (Fig. 2).
    The effects of protein concentration (1–4%, w/v) and temperature (20–70°C) on the relative viscosity (ηr) of acidic casein solutions (pH 2·0) are shown in Fig. 3; viscosity increased with increasing concentration. At
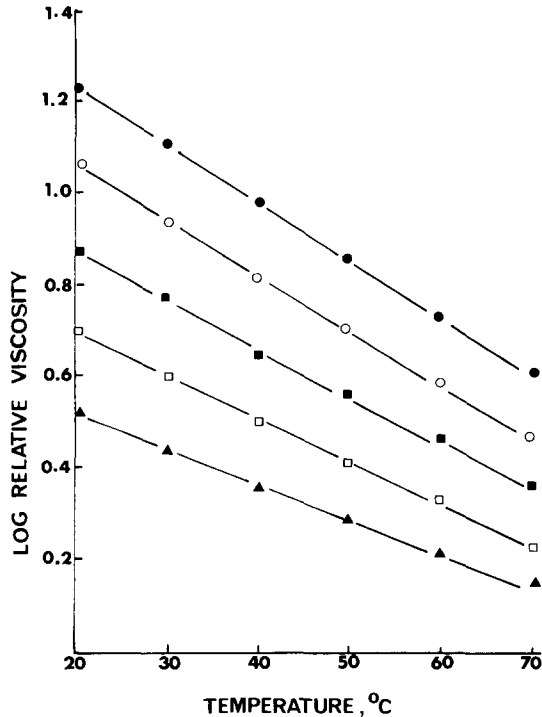
Fig. 2. Effect of temperature on the log relative viscosity of sodium caseinate solutions (pH
7·0) containing 4 (▲); 6 (□); 8 (■); 10 (○); or 12 (●) % (w/v) protein.

protein concentrations of > 5% (w/v), highly viscous solutions with gel-like
appearance at temperatures < 40°C were formed. To determine whether this
was in fact a true gel with a continuous protein matrix and not an extremely
viscous protein solution, a sample of 'gel' was dispersed in excess distilled
water, stirred gently with a spatula and held overnight at room temperature
(20°C). On examination, the 'gel' had dissolved completely to give a clear
solution, indicating that solutions of casein at low pH (< 3·0) do not form
true gels.

The viscosity of the acidic casein solutions decreased sharply as the
temperature was increased from 20 to 40°C and then decreased slightly on
further heating up to 70°C (Fig. 3).

Korolczuk (1982*a*), who studied the effect of protein concentration
(2–16%, w/v) and temperature (25–80°C) on the relative viscosity of acidic
casein solutions (pH 2·4–2·9), found that relative viscosity decreased with
increasing temperature up to 60°C and at higher protein concentrations
viscosity increased between 60 and 80°C. In contrast to Korolczuk's results,
the present data showed a continuous decrease in viscosity with increasing
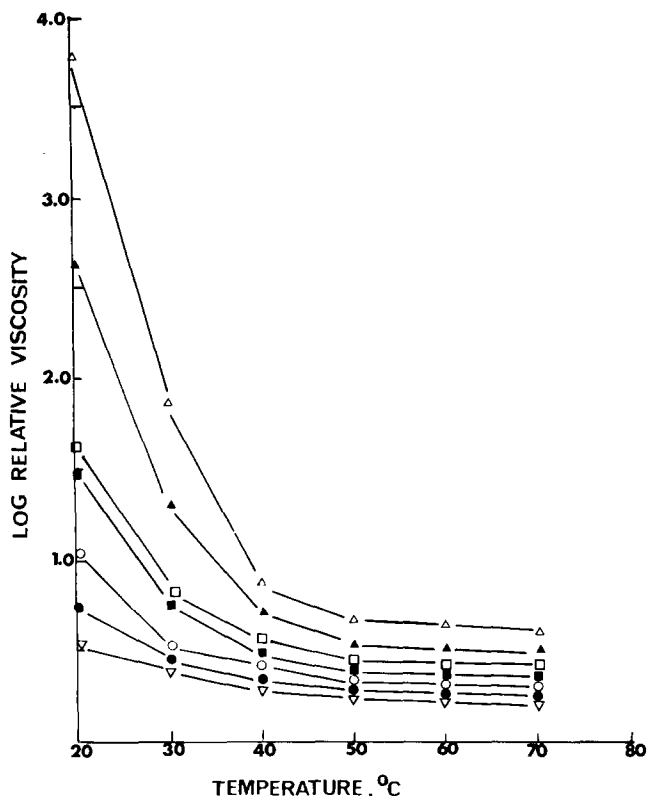temperature up to 70°C; however, viscosities of the acidic casein solutions

Fig. 3. Effect of temperature on the log relative viscosity of acidic casein solutions (pH 2·0) containing 1 (▽); 1·5 (●); 2·0 (○); 2·5 (■); 3·0 (□); 3·5 (▲) or 4·0 (△) % (w/v) protein.

used in the present study were much higher than those reported by Korolczuk (1982*a*) and, as a consequence, solutions of > 5% (w/v) proteins were not studied.

Effect of pH on viscosity

The viscosity of casein dispersed at 4% (w/v) in the acidic range was slightly dependent on pH and was in the order pH 3·0 > 2·5 > 2·0 (Fig. 4). The viscosities of the three acidic casein solutions (pH 2·0, 2·5 and 3·0) were much higher than that of a sodium caseinate solution (4%, w/v) at pH 7·0. These results confirm those of Korolczuk (1982*a*, *b*) who showed that the viscosity and hydration of acidic casein solutions were much higher than those of neutral caseinate solutions.

Water sorption and water-holding capacity

Sorption isotherms of acidic caseins (pH 2·0, 2·5 or 3·0) and sodium caseinate (pH 7·0) are shown in Fig. 5. At A_w values up to 0·52, all the proteins had
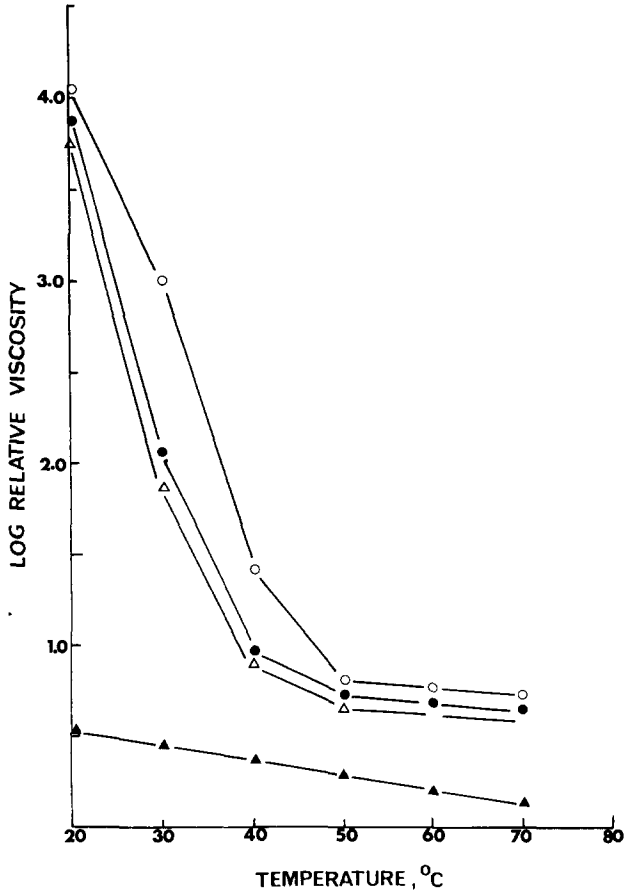
Fig. 4. Effect of temperature and pH on the log relative viscosity of acidic casein solutions at pH 2·0 ($\triangle$), 2·5 ($\bullet$) or 3·0 ($\bigcirc$) and a sodium caseinate solution pH 7·0, ($\blacktriangle$), all at 4% (w/v) protein.

similar sorption isotherms with greatest uptake being shown by sodium caseinate. At A_w values of 0·52–0·87, the three acidic caseins had similar isotherms while sodium caseinate showed a much greater increase in water uptake. The water uptake of the three acidic caseins increased sharply between A_w 0·87 and A_w 0·97 while water uptake by sodium caseinate was much lower over this A_w range. Ruegg & Blanc (1976) reported that, on increasing A_w from 0·9 to 0·95, the water sorption capacity of sodium caseinate (pH 7·0) increased from 31·0 g H_2O/100 g protein to 51·0 g H_2O/100 g protein; in agreement with their findings the present results show that the water content of sodium caseinate (pH 7·0) at similar A_w values increased from 34 g H_2O/100 g protein to 49 g H_2O/100 g protein.

The values for the water-holding capacity of acidic caseins (pH 2·0, 2·5 or
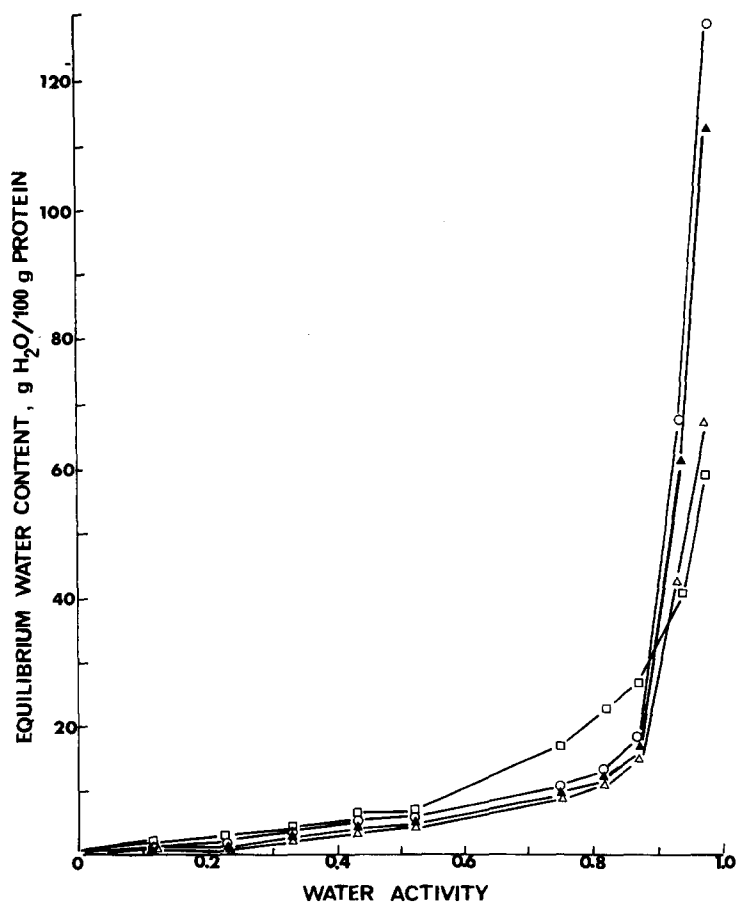
Fig. 5. Water sorption isotherms of casein/caseinate. Each value plotted is the mean of three replicates on 0·5 g samples at pH 2·0 (○), 2·5 (▲), 3·0 (△) or 7·0 (□).

TABLE 1
Water-Holding Capacity (WHC) of Caseins/Caseinate

pH	WHC (g H_2O/g protein)
2·0	3·6 ± 0·3
2·5	3·4 ± 0·5
3·0	3·3 ± 0·7
7·0	3·5 ± 0·4

3·0) and sodium caseinate (pH 7·0) (Table 1) are the average of three evaluations made using the method of Quinn & Patton (1979). The results show that there were no significant differences in the water-holding capacities of acidic casein (pH 2·0, 2·5 and 3·0) and sodium caseinate (pH 7·0).

DISCUSSION

The uses of caseins and caseinates in the food industry are often limited because of their insolubility at, or near, their isoelectric points (pH $\sim$4·6). However, the present study shows that casein is very soluble at pH values < 3·5 and may therefore be used in foods of low pH. Acidic casein solutions are also very heat-stable and in this respect are similar to sodium caseinate, which can withstand heating at 140°C for 1 h when dispersed in water (Fox & Morrissey, 1977; Fox 1982).

The viscosity of macromolecules has been comprehensively reviewed by Yang (1961), Freifelder (1976) and Gruenwedel & Whitaker (1984). Viscosity is dependent on protein type (concentration, molecular size, shape, surface charge) and environmental factors (pH, temperature, ion type and concentration, shear rate) (Bourne, 1982; Kinsella, 1982, 1984). The viscosity of casein/caseinate has been shown to depend on the method of manufacture used in its production (Dolby, 1964; Hayes & Muller, 1961). Hayes & Muller (1961) studied the temperature–viscosity relationship in caseinate solutions containing 11 to 20% protein. A linear relationship was observed when viscosity was plotted on a logarithmic scale against the reciprocal of absolute temperature. Korolczuk (1982a) showed that increasing the temperature of neutral caseinate solutions containing 4 to 17% protein caused a decrease in viscosity while, for acidic casein solutions containing 2 to 16% protein, the viscosity decreased up to 60°C but increased at higher temperatures. Hayes & Muller (1961) reported that a logarithmic relationship exists between viscosity and casein concentration.

The present study shows that the viscosity of acidic casein solutions increased logarithmically with increasing protein concentration and decreased logarithmically with increasing temperature up to 70°C. At similar protein concentrations, acidic casein solutions were much more viscous than sodium caseinate solutions; this has been attributed by Korolczuk (1982a) to lower repulsive forces at low pH values due to the net positive charge of $-NH_3^+$ groups per average casein monomer molecule at low pH being lower than the net negative charge of $-COO^-$ and PO_4^{3-} groups at neutral or high pH values.

Water sorption behaviour of various milk proteins and the effects of environmental factors on sorption isotherms and moisture contents have

been reviewed by Iglesias & Chirife (1982), Duckworth (1983), Spiess & Wolf (1983) and Kinsella & Fox (1986). Casein is one of the major water-sorbing components in dehydrated dairy products at low and intermediate values of A_w. The amount of water sorbed by casein is influenced by its physico-chemical status (Berlin *et al.*, 1968).

In a study on the influence of pH on water sorption by casein, Ruegg & Blanc (1976) found that the water sorption capacity changed with pH, the changes becoming more pronounced at high values of A_w. At an A_w of 0·90 they reported water contents of $\sim 35\%$ for sodium caseinate pH 7·0, and ~ 20–25% for acidic caseins at pH 2·8–4·0. The order of these values agrees with the values found at an A_w of 0·87 in the present study. However, while Ruegg & Blanc (1976) reported an increase in water content with decreasing pH which became apparent at A_w values $> 0·9$ they did not observe the large increase in water uptake at pH 2·0–3·0 at high values of A_w as reported here, possibly because the lowest pH they studied was 2·8; they reported that at an $A_w = 0·98$ and pH values $< 3·5$, equilibrium was not attained in their study.

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