OPTIMISING FUNCTIONAL PROPERTIES OF PROTEINS FOR PRODUCTION OF MEAT PRODUCTS EMULSIONS

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INTRODUCTION

The use of non meat proteins to finely comminuted meat products has been a common practice, in the last two decades. Despite the remarkable commercial variety, those which source is soy or milk, specially their isolates, are still the most required by meat industry. Working usually the meat industry, with low cost formulations of critical stability, these protein materials have made possible the appearance of new technological solutions, being the pre-emulsification processing the most important, allowing the stabilization of significant amount of fat. Zayas, 1985, studding the influence of this unit operation in the final features of meat products, pointed out an increasing yield and stability.
Several technological parameters influence the characteristics of an emulsion. However, its stability depends mainly, upon the protein functional behavior and the ratio to other major components (fat and water). Earlier studies (Hermansson, 1979; Schimdt, 1981; Acton & al., 1983; Ziegler, 1984; Lin and Zayas, 1987; Lee, 1987) emphasize the gelling ability and water retention capability of proteins as important factors in the control of emulsions stability, ranking bellow the former theory of emulsification (Hansen, 1960; Swift, 1961; Helmer and Saffle, 1963; Meyer, 1964).
The objective of this study is to get useful information about protein functionality in order to optimize pre-emulsions processing concerning its stability For this purpose, were evaluated the influence of pH, time, temperature, NaCl and CaCl2 ionic strength and protein concentration on solubility, free uptake of water and gelling ability of two commercial protein isolates (soy and sodium caseinate).

MATERIALS & METHODS

Reference proteins

Soy protein isolate (SPI) (IPSO-MR) with 85.9% protein, 8.5% water content, 3.8% ashes, pH 7.1 on 1% solution and a sodium caseinate (Na Cas) (BINDOX 050-Low viscosity) with 90.8% protein, 2.7% water content, 4.2% ashes, pH 7.5 on 1% solution were purchased to Vaessen Schoemaker Chemische Industrie B.V.-Holand.

The proximate analysis were determined according AOAC and Kjeldahl methods. For pH values a Metrohm 654 with a combined glass electrode and type I deonized water were used.

Solubility

Solubility tests were performed on 1% protein dispersions. Solubilization processing was carried on with magnetic stirring at 1000 rpm in two steps: firstly at room temperature for 20 min with the aim of incorporating in liquid phase, all protein powder without granule development and secondly at 25 or 50°C, for 30 min in a water bath with the same stirring speed.
The pH of dispersions was adjusted using sodium acetate, phosphate or citrate buffers. Buffer concentration was fixed (10 mM for IPSO-MR; 150 mM for BINDOX 050) according to previous experiments having been chosen the lowest value allowing non significant changes of pH value during the run of the assay. Sodium and calcium chloride were added to the buffers before protein incorporation.
After centrifugation (25000 g - 30 min), supernatant was used for protein determination, being used bicinchoninic acid (Pierce BCA protein assay reagent - 23225) and Coomassie blue G-250 (Pierce Coomassie assay reagent -23200) methods, respectively for SPI and Na Cas. Solubility was determinate by the ratio of supernatant and original dispersion protein contents. Three replicates were done for each sample.

Absorption Capability

Water imbibing capacity (WIC) - The method used by Torgersen & Toledo (1977) was followed with modifications in the equipment, namely water bath thermostatization, the connection tube between the plastic "bacteriological field monitor" and the pipet (Tygon R603, cat.6409-13) and filter paper (Whatman 17 Chr, cat.3017195).
The sample was spread in the filter paper, only 10 min after the removal of excess liquid, to ensure that the run did not start before equilibrium.The assay was finished when readings less than 0,01 ml apart for 30 min. Sample weight (0,1g) was chosen taking into account the three factor experimental compromise: time consuming/filter paper filling/easy protein spreading. The experimental parameters were the same as for solubility. For temperatures other than room temperature, the whole apparatus was set up in a temperature controlled water bath and was allowed to equilibrate with new temperature for at least 15 min before applying the sample. At high temperatures the readings were corrected, due to evaporation, by running a blank. Three replicates were done for each sample.
Oil imbibing capacity- Experiments were made in the same way as water uptake. Instead of whatman 17 Chr, a glass microfiber whatman GF/G filter was used (Kanterewicz, 1987). All the assays were run at room temperature, making use of a commercial soy oil. Four replicates were done for each sample.

Gel Formation

All dispersions (12, 14 and 16%- protein weight on 350 ml of aqueous phase) were prepared by mechanical stirring (ATOMIX) for 90 s, using the lowest speed during protein addition. The adjustment of pH was made with 1N HCl or NaOH. To avoid changes in protein behavior, all acid , alkali and salts were added before the protein addition.
Three 45 g aliquots of each dispersion were weighted into 50 ml plastic centrifuge tubes and stoppered to prevent evaporation. Dispersion air bubbles were removed by gentle centrifugation (680 g x 10 min) for IPSO-MR and vacuum for BINDOX. When the effect of heat treatment was studied, the dispersion was heat treated at 50, 70 or 90°C for 10, 30 or 180 min, and rapidly cooled down over night at 5°C. Rheological evaluation of gels were performed directly in the centrifuge tubes due to the lack of hardness of the product.
Rheological properties of SPI (adhesiveness, cohesiveness and hardness) were evaluated at room temperature by texture profile analysis, with a J.J. Instrument Universal Texturometer, forcing twice in a reciprocating motion, a 12 mm diameter flat bottom rod, through the gel (4cm deep), at 200 mm/min, using a 5N cell.
Due to the observation of a non gelified structure for Na Cas, viscosity measurements were carried out in a Haake Rotovisko RV-20 with MV-I and SV-I systems. The consistency index (K) of the dispersions was calculated using the power low. Three replicates were done for each sample.

STATISTICAL CALCULATIONS

All calculations were made with a personal computer Apple Macintosh II. For the statistics and graphical presentation we used the program StatView II.

RESULTS & DISCUSSION

Solubility

In order to obtain maximum functionality and an easier incorporation, in high water content food systems where gelation and emulsifying activity are extremely important, high soluble proteins are required (kinsella, 1979). The solubility profile, over a large pH range generally gives information about other functional properties. Those profiles, with maximum, minimum and inflection points may in some cases be misleading about the state of the protein structure and its functionality (a low and unchangeable solubility over a broad pH domain is generally a sign of some degree of denaturation, Hermansson, 1979).
The pH solubility curves of Na Cas and SPI are presented in Fig. 1. A typical bell-shaped curve was observed for Na Cas. Minimum solubility in both cases was detected at quite close pH values. Buffer concentration showed a negative effect on solubility of SPI whereas no changes were observed for Na Cas.

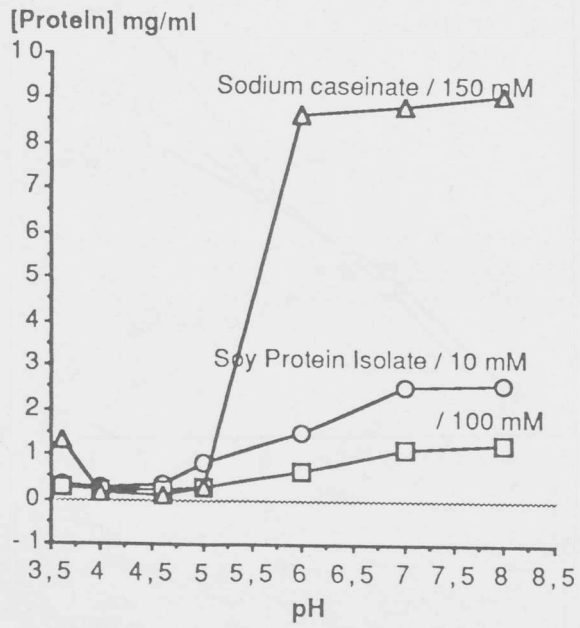
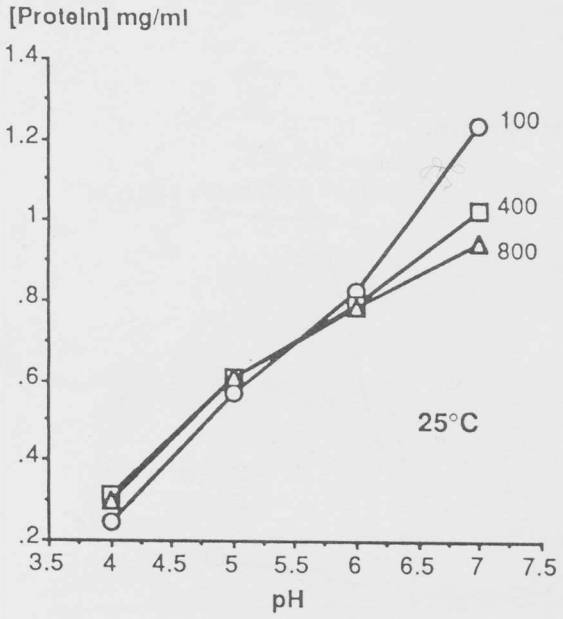


Fig 1- Influence of pH on Na Cas and SPI solubility.

In the same experimental conditions (temperature and buffer concentration- 10mM) SPI solubility was only slightly affected, in the tested pH range by the increase of NaCl ionic strength (fig. 2A). A better solubility was observed when the assays were run at 50°C.(Fig. 2B).Conversely, in Na Cas solubility profile was differently affected by NaCl, being the influence of this factor dependent on prevailing pH and assay temperature. While a small decrease on solubility was seen on the top side of the pH range (6-7), an important improvement was found on pH near the isoelectric point (Fig.3A-B). Changes in protein solubility, caused by the addition of CaCl2 are shown in Fig.4 and 5. In the concentrations tested , CaCl2 had similar effects on SPI solubility when compared to NaCl. Concerning Na Cas, this salt showed a negative effect on solubility over the tested pH range, being its action opposite to the NaCl, at pH 5.
Protein concentration has the same effect on solubility of both proteins. At pH 6, solubility was not significantly improved with increasing protein concentration (Fig.6)

Absorption Capability

Water imbibing capacity- The dynamics of absorption shown by the two proteins has reaveled important differences with development of drifts already observed in former studies. Concerning SPI and in all experimental conditions the absorption rate was limited with a fast initial phase (80 - 90% of maximum value in the first 10 - 15 min), keeping the swollen particles without disintegration for a long period of time. On conversely, Na Cas showed, excepting around pH 4, a progressive and continuous increasing of imbibed water during the assay, being necessary a long period for



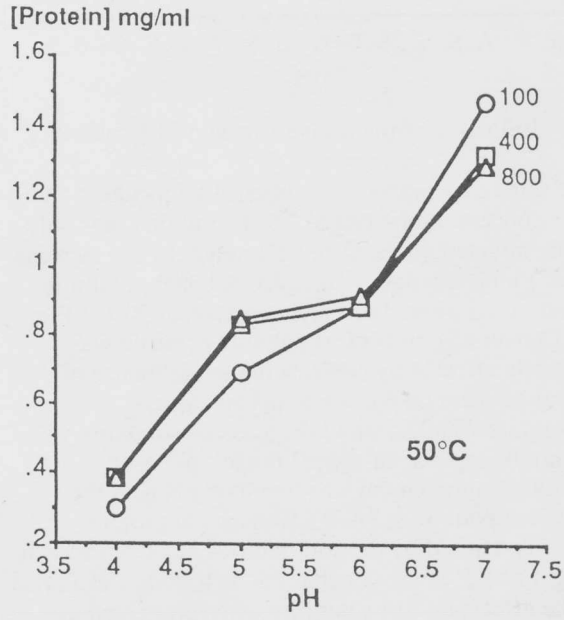
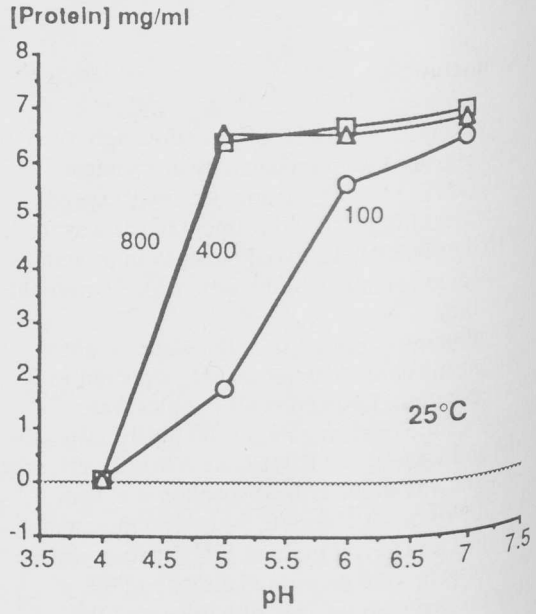


Fig.2A and B- Influence of NaCl ionic strength on SPI solubility at 25 and 50°C.



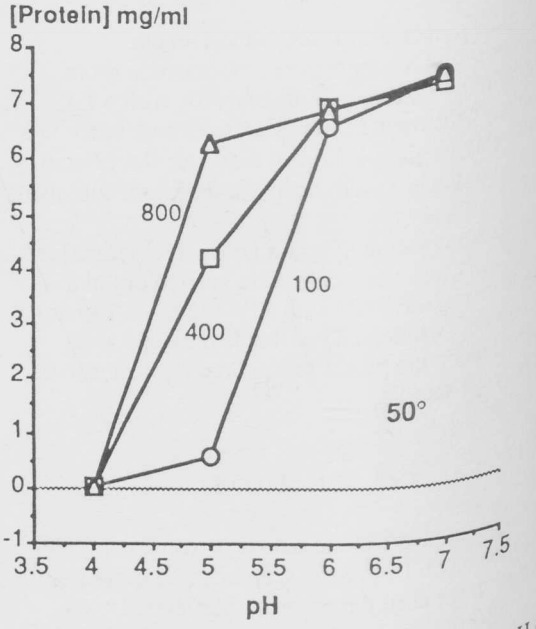


Fig. 3A-B. Effect of NaCl ionic strength and pH on Na Cas Solubility at 25 and 50°C.

complete stabilization (4 hours) with tendency for solubilization of the swelled particles (Hermansson, 1979; Urbansky, 1983). Running the assays with bidestiled water SPI showed higher water imbibing capacity (WIC) (5.3 ml/g 0.02 ml) than Na Cas (4.9 ml/g 0.02 ml).

The influence of pH and buffer concentration on WIC for both proteins are shown in figure 7. Over the tested pH range, values were progressively improved with increasing pH, while buffer concentration had a negative effect. Na Cas showed a behavior highly dependent on pH, being more enhanced between pH 4 and 5.

The effect of various temperatures on WIC of proteins is quite different (fig.8).

Na Cas showed a continual drop from 19 to 50°C being more drastic between 25 and 35°C, whereas no remarkable change were observed for SPI. The influence of the temperature on WIC is not significantly affected by pH. Meat products are highly complex salt systems. The resulting ionic strength establishes the functional behavior of meat and non meat protein ingredients, concerning water holding capacity (Ham,1960). NaCl and calcium salts ionic strengths, are extremely important for different techno logical and sensorial reasons. The influence of those factors (NaCl and CaCl2) on the WIC of Na Cas and SPI have been studied, using for this purpose a Doehlert experimental design. The limits of the technological parameters were fixed as follows: NaCl - 100 mM to 800 mM; CaCl2 - 100 mM to 300 mM.

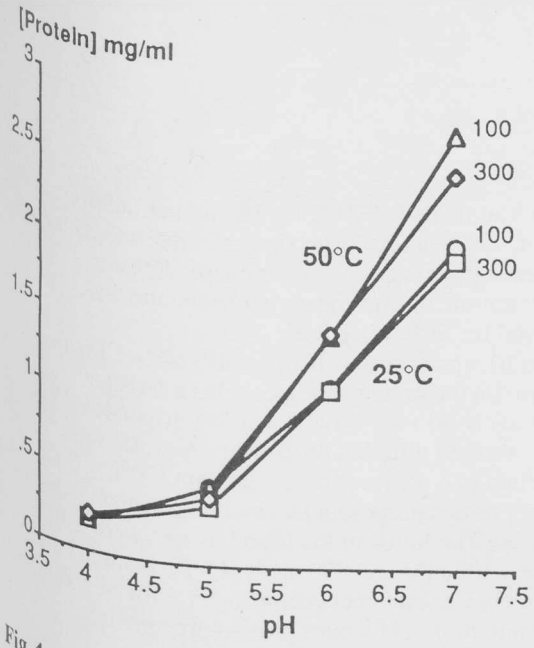


Fig 4. Influence of CaCl2 molarity and pH on SPI solubility at 25 and 50°C.

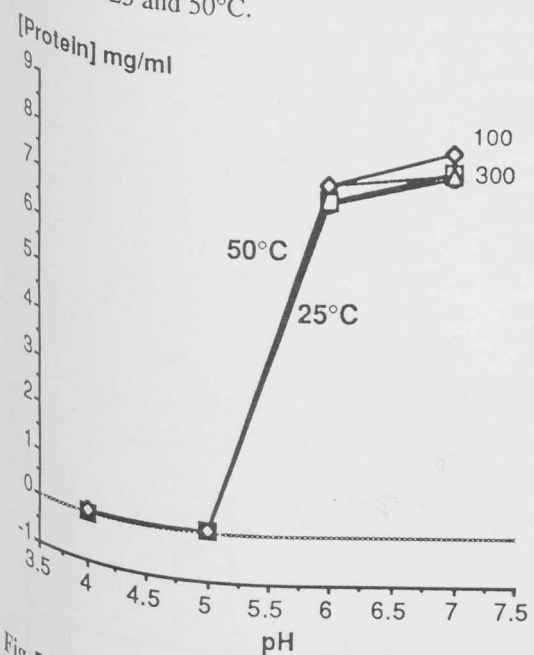


Fig 5. Influence of pH and CaCl2 ionic strength on Na Cas at 25 and 50°C.

The specific aim on testing the CaCl2 was to simulate the use of water with different hardness. According to the results of figure 9 an important interaction between those technological parameters were observed. Around pH 5.5, NaCl and CaCl2 had virtually no effect in WIC, being their action opposite in both sides of the pH domain.

Oil imbibing capacity - Four replicates were done for each sample. Results obtained are as follows: Na Cas - 1.78 ml oil/g; SPI - 1.07 ml oil/g.

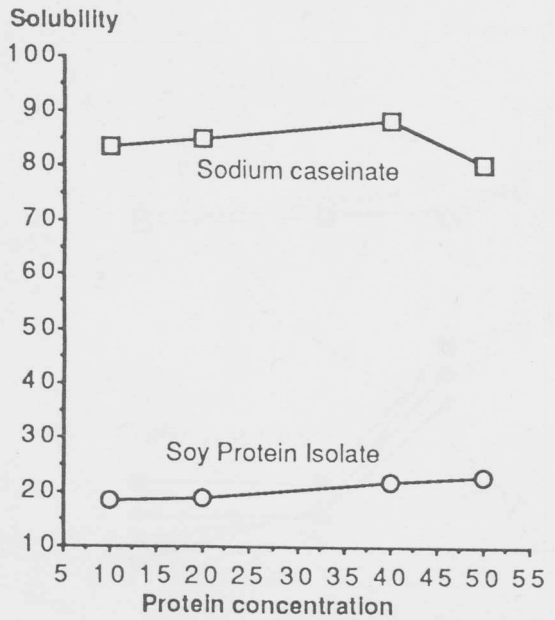


Fig. 6. Effect of protein concentration at 50°C and pH 6 on Na Cas and SPI solubility.

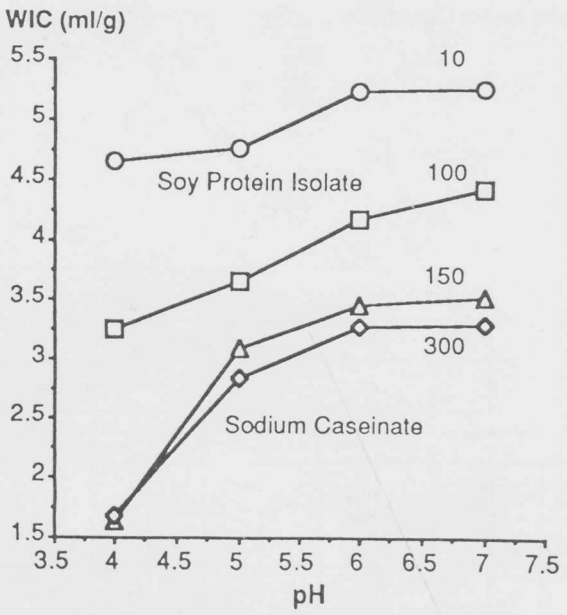


Fig.7- Influence of pH and buffer molarity on Water imbibing capacity of Na Cas and SPI.

Gel Formation

The influence of protein concentration, heat treatment temperature and duration, on Na Cas dispersions consistency index (K) and SPI gel hardness, cohesiveness and adhesiveness are shown in table 1. The results show that the evolution of the rheological parameters is mainly dependent on protein concentration. Despite the improved values obtained after heat treatment, no significant differences were attained for the different temperature and time operational ranges.

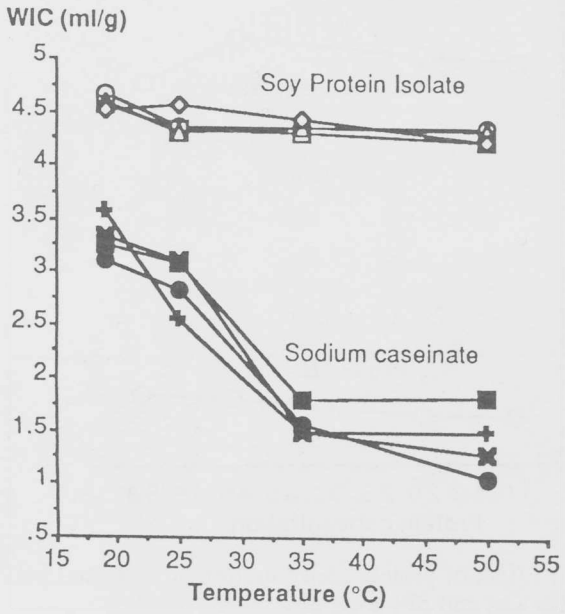


Fig.8- Influence of temperature on water imbibing capacity of Na Cas and SPI.

When heat treated at 70°C for 180 min or at 90°C above 10 min, 12% Na Cas dispersions clearly showed protein flocculating, with a top watery phase. At same concentration, no significant gel formation was observed for SPI dispersions.

Figure 10 represent the effect of pH and NaCl ionic strength on the same rheological characteristics, respectively on 14% Na Cas and SPI dispersions. The results showed different drifts, when samples were submitted to a previous heat treatment (70°C x 30 min) or not. For this purpose a Doehlert experimental design was used. The limits of the tested factors were: NaCl- between 100 mM and 800 mM; pH- between 5.5 and 7.5 for Na Cas , and between 6 and 7.5 for SPI. Below those minimum pH values protein precipitation occurred

According to previous assays, CaCl2 concentration between 100 mM and 300 mM did not affect the rheological behavior of the tested proteins.

		14%										16%									
		room temp.	50°C			70°C			90°C			room temp.	50°C			70°C			90°C		
			10 min	30 min	180 min	10 min	30 min	180 min	10 min	30 min	180 min		10 min	30 min	180 min	10 min	30 min	180 min	10 min	30 min	180 min
BINDOX	K (Pas n)	0.68	0.93	0.84	0.92	0.92	0.96	1.01	0.88	0.91	0.92	3.01	3.01	3.19	2.71	2.72	3.27	2.93	2.87	2.59	3.19
IPSO - MR	Cohesiv. A2/A1	0.81	0.79	0.74	0.72	0.75	0.69	0.65	0.74	0.68	0.62	0.65	0.60	0.64	0.65	0.66	0.67	0.66	0.67	0.65	0.74
	Adhesiv. (Ncm)	0.30	0.41	0.49	0.68	0.49	0.66	0.89	0.67	0.75	0.61	1.44	1.66	1.99	2.34	1.96	2.20	2.40	2.16	2.10	1.43
	Hardness (N)	0.26	0.33	0.39	0.49	0.39	0.51	0.63	0.51	0.61	0.53	0.94	1.04	1.12	1.53	1.14	1.42	1.43	1.42	1.31	0.95

Table I - Effect of heat treatment (temperature and time) on rheological properties of 14 and 16% Na Cas dispersions(consistency index - k) and SPI gels(cohesiveness, adhesiveness, hardness).

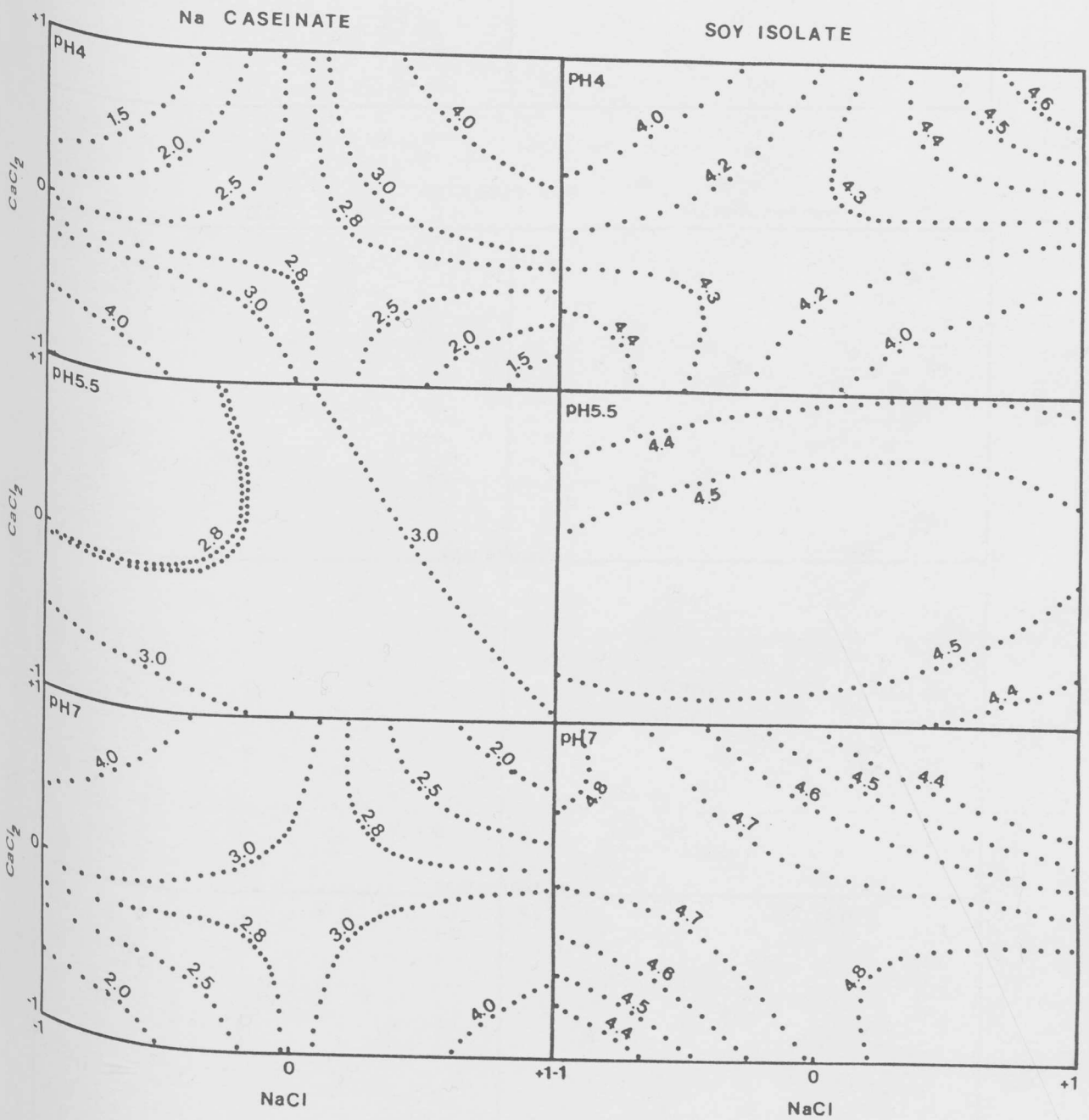


Fig 9 Influence of the solvent ionic strength on water imbibing capacity of Na Cas and SPI powders at pH 4, 5.5 and 7.

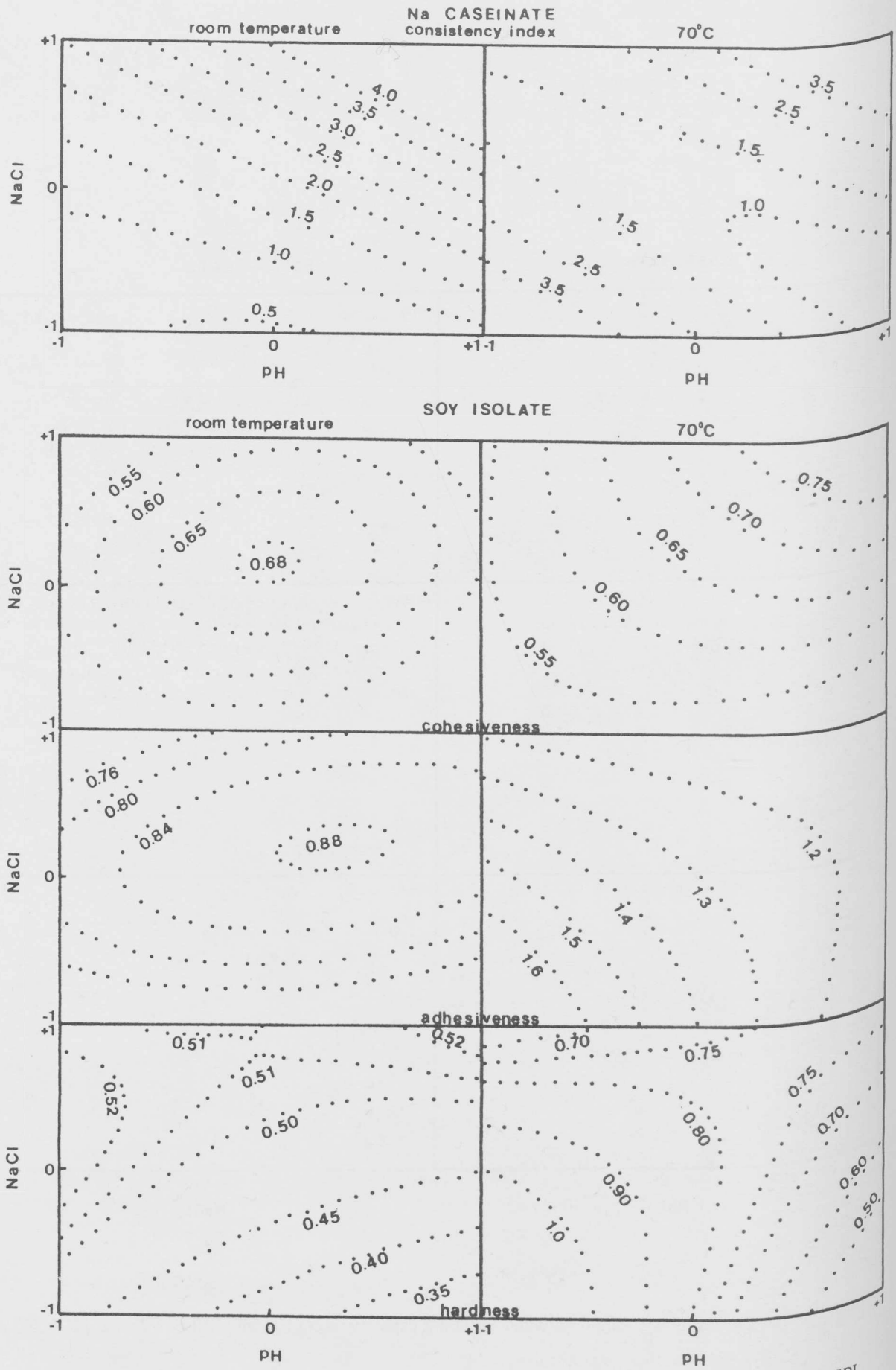


Fig 10 Influence of the solvent pH and NaCl ionic strength on rheological properties of 14% Na Cas and SPI dispersions

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