PERIODICA POLYTECHNICA SER. CHEM. ENG. VOL. 38, NOS. 3-4, PP. 199-208 (1994)

THERMODYNAMIC AND STRUCTURAL INCOMPATIBILITY IN GLUTEN CONTAINING PROTEIN SYSTEM[1]

Radomir LÁSZTITY

Department of Biochemistry and Food Technology
Technical University of Budapest
H-1521 Budapest, Hungary

Abstract

Model experiments were made with the ternary systems gluten (glutenin, gliadin)-casein-water and gluten (gliadin, glutenin)-sodium alginate-water. It was found that at low concentrations (1–2%) of gluten proteins the casein-gluten solutions are homogeneous. At higher concentrations, as a result of thermodynamic incompatibility, the solution separates into two phases. The same results were obtained with gluten-alginate-water systems. The solubility of gluten proteins may be extended using ionic polysaccharides.

Myosin-gluten gel systems had lower strength than pure myosin gels due to structural incompatibility of the system.

Keywords: gluten, protein, thermodynamic incompatibility, structural incompatibility.

Introduction

If we are speaking about gluten we are generally thinking about the main protein components of wheat and wheat flour, playing a decisive role in producing high quality wheat bread or pasta products.

The correlations between the protein content, protein composition, properties of gluten complex and the rheological properties or wheat flour doughs were thoroughly investigated. Although our knowledge concerning events and correlations at molecular level should be based on deeper information, some basic conclusions are generally accepted:

- An insoluble protein matrix is an essential prerequisite of the formation of a cohesive dough.
- Sufficient amount of the matrix should be present to form a continuous phase in the presence of starch and water.
- The ratio of low- and high-molecular weight components has a significant effect on the rheological properties of a dough.
- All factors influencing the protein–protein interactions change the rheological properties of a dough.

[1]The research project is financed by OTKA (Hungarian Foundation for Basic Research.)

There is no doubt about the necessity of research on molecular basis of wheat quality, new breeding methods and further development of wheat processing technology. Nevertheless, some new trends in the use of plant proteins and particularly of gluten proteins suggest that investigation of gluten proteins might be extended with some new topics.

In the last decades a continuously growing quantity of so-called *vital gluten* has been produced. As a vegetable food protein, wheat gluten today ranks second to soy-based protein in terms of volume of product produced. In 1980, the world production of wheat gluten was approximately 90000 tons; by 1986 it grew to 247,000 tons. It was expected that the output will increase to over 340,000 tons by the end of 1992 (HESSER, 1993). A comparison of wheat gluten production by major geographical areas is shown in *Table 1*.

Table 1
Comparison of wheat gluten production by major geographical producing areas – 1980–1986–1991

Country	Year/Amount (Tons) 1980	1986	1991
Australia	24,000	40,000	45 ,000
North America (Can./USA/Mex.)	30,000	47,000	58 ,000
Europe	29,500	132,300	194 ,000
(EC)*	(25.000)	(118,300)	(180,000)
(Non-EC)	(4,500)	14,000	(14,000)
Japan	3,000	6,700	10 ,000
South America	2,000	7,000	16 ,000
Totals	88,500	247,000	323 ,000

*EC=European Community

Historically wheat gluten has been used by bakeries for improvement of bread quality or at higher addition levels in high protein or starch-reduced breads. More recently vital gluten has become a frequent component of breads made from high extraction flour or breads supplemented with dietary fibre. It is now also used in a wide variety of other foods (see *Table 2*) such as breakfast cereals, pasta, fortification of beverages, meat and poultry products, imitation cheeses, sea food analogues, n and pet foods.

Among the non-food uses, application in biodegradable plastics, as co-binder in paper coatings, filler in urea-formaldehyde resin adhesives,

Table 2
Comparison of worldwide end-use history for wheat gluten by percentage

Category	Year/per cent 1980	1991
Baking	77	61
Milling	4	13
Meats	0	6
Pet Foods	10	9
Cereals	3	4
N	0	1
Pasta	0	1
Cheese analogues	0	1
Seafood analogues	0	1
Other animal feeds	4	1
Devitalized	1	1
Others	1	1
	100%	100%

component in synthetic, edible sausage skins and cosmetics are to be mentioned.

Chemical modification of gluten (or its components) may open new possibilities of utilization both for food and non-food purposes.

The extended field of application is connected with the fact that gluten proteins will be included in new systems (from the points of view of chemical composition, colloidal properties and texture). The study and knowledge of the behaviour of gluten in such systems is a prerequisite of an effective use and optimal processing. In this paper some considerations and preliminary experiments in this field will be discussed.

The about hundred types of polypeptides of gluten complex are combined by different secondary bonds (disulfide-, hydrogen- and hydrophobic) to a network called functional gluten. It was shown that by changing the number and distribution of secondary bonds, particularly disulfide bonds, products of quite different rheological properties may be obtained from the same polypeptide chains (LÁSZTITY, 1984). It is also possible to produce a mixture of polypeptides of wheat storage proteins and polypeptides (or hydrolysates) of other proteins and after reoxidation to obtain a 'mixed' high molecular weight protein. It is also well known that via 'plastein' reaction specific amino acids may be attached to polypeptides which influences the amino acid composition and the functional properties. Finally, different chemically modified (hydrolysed, acylated, desamidated, esterified, etc.) derivatives may be also produced.

The reactivity (possibility of interaction) of gluten proteins is highly dependent first at all on the amino acid composition (see *Table 3*). A low amount of amino acids with basic side chains and high degree of amidation of carboxyl groups limits the possibilities of ionic interactions with charged proteins or other components (ionizable polysaccharides, phytic acid, etc.).

Table 3
Functional groups in the gluten proteins (mmol/100 g protein)

Group	Amino acids	Gliadin	Glutenin
Acidic	Glutamic acid Aspartic acid	27	36
Basic	Lysine Arginine Histidine Tryptophan	39	52
Amide	Glutamine Asparagine	309	266
Thiol and disulfide	Cysteine Cystine	12	12
Total ionic	Acid+ basic	66	87
Total polar	Hydroxy + amide	381	365
Total nonpolar		390	301

The number of groups capable of hydrogen bonding or hydrophobic interaction is high. Which of them will dominate, depends on the molecular structure, on the distribution of amino acids of different type and on the medium in which the protein is situated.

Materials and Methods

Casein (Reanal Ltd.) with an average molecular weight of 35 kDa, vital gluten, gliadin and glutenin were used as protein preparations. Sodium alginate (Reanal Ltd.) was the polysaccharide component of the systems. A Britton Robinson buffer solution was used for adjusting the desired pH values.

The standard temperature was maintained by a water bath thermostat (± 0.2 °C). The phase separation was followed visually until further changes were not observed. In some cases the phase separation was helped by centrifugation (15 min, 1400 g).

After phase separation, a sample was taken by micropipette from both phases. After dilution (1:1000) the protein concentration of the solution

was measured by the Lowry method and those of polysaccharides by the phenol-sulphuric acid method (DUBOIS et al., 1956). The ratio of two proteins (in systems containing more protein components) was determined as proposed by TOLSTOGUZOV (1987).

The materials and methods used for investigation of myosin non-meat protein systems are included in the section 'Results and discussion'.

Results and Discussion

Gluten Proteins in Protein Water and Water Polysaccharide Systems

Model experiments were made with two types of systems: gluten (glutenin or gliadin) – casein – water and gluten (gliadin or glutenin) – sodium alginate – water. As it is known from the solubility profile of gluten proteins, the best solubility may be observed in acidic or basic pH range. It was found that at relatively low concentrations of gluten and gliadin or glutenin the system is homogeneous. At higher concentrations, the phenomenon of thermodynamic incompatibility (POLJAKOV et al., 1986) occurs also in this system. In a wide concentration range the solution separates to two phases having different concentrations of the two proteins. A typical phase diagram of the gliadin–casein system is shown in *Fig. 1*.

In another series of experiments the systems alginate or pectin – water – gluten proteins were investigated. The phenomenon of thermodynamic incompatibility was also observed in a relatively wide range of acidic and basic pH values. Another observation of practical importance is the change of solubility profile of proteins in the presence of ionisable polysaccharides. As an example, nephelometric titration curves are shown for glutenin in the *Fig. 2* in the absence and presence of alginate. A shift of the nephelometric peaks in the direction of acidic pH may be observed and the solubility is extended to the low acidic and neutral pH region.

Gluten Proteins – Myosin Systems

In the framework of the present study protein model systems and frankfurter type sausages were also investigated.

Wheat gliadin and glutenin, soy glycinin, sunflower globulin, actomyosin and myosin were used in model experiments. For the preparation of sausages beef meat, fat, non-meat proteins such as vital gluten, soy isolate, or sunflower seed isolate were used.

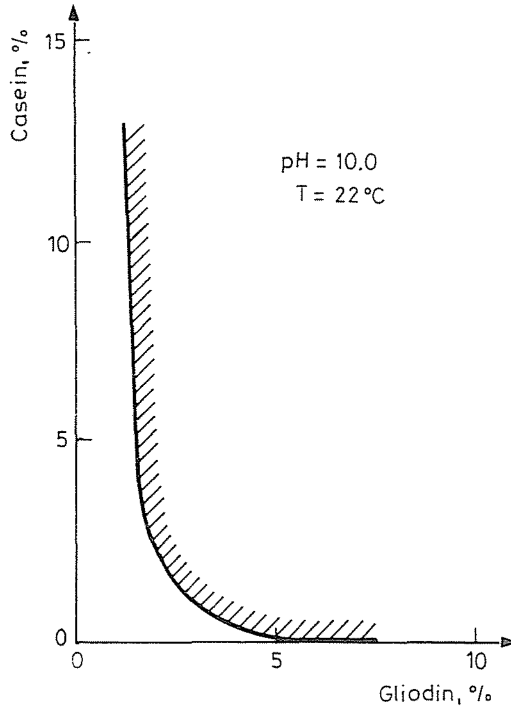

Fig. 1. Typical binodal (phase diagram) of casein–gliadin–water ternary system

In model experiments a suspension containing 2.2 g of myosin or actomyosin, 0.5 g of sodium chloride, 0.05 g of $Na_4P_2O_7$ and 7.25 g of water was prepared and filled in test tubes (26 mm Ø, 12 mm height). To study the effect of plant protein preparations 0.2 g, 0.4 g and 0.6 g of myosin or actomyosin were substituted in the suspension mentioned above by gliadin, glutenin, glycinin or sunflower seed globulin.

The suspensions were heated at 80 °C for 40 min. Sausages were prepared according to the following recipe: Beef meat 95.3, fat 40.0 g, NaCl 3.3 g, nitrite 0.2 g, $Na_4P_2O_7$ 0.6 g, water 59.6 g.

To study the effect of plant protein preparations, 2.0 g of the meat protein were substituted by vital gluten, soy isolate or sunflower seed isolate.

For the characterization of textural properties of model systems the test pieces (26 mm Ø, 12 mm height) were tested in an Instron Type 1140 apparatus using double-compression test with constant rate (25%) of deformation. The tests were made in triplicate and the texture profile curves were evaluated.

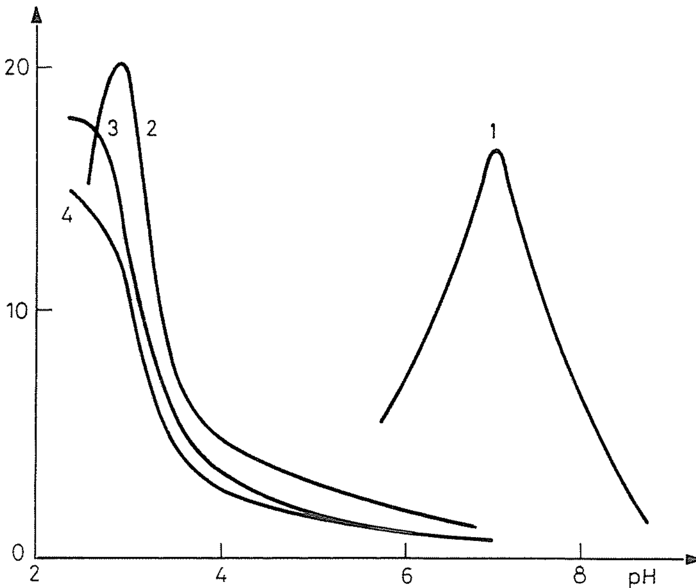

Fig. 2. Nephelometric titration curves of alkaline gliadin – sodium alginate solutions containing different amounts of sodium alginate. 1–0; 2–0.028%; 3–0.05% 4–0.08%

The results of the measurement of textural properties of model systems are summarized in *Table 4*. On the basis of the data in the table it can be stated that samples containing only myosin as protein component possess the best texture properties. Substitution of myosin with other proteins always results in a decrease especially in hardness and elasticity to a smaller or greater extent but changes in cohesivity are small. Glutenin results in a practically unchanged hardness and elasticity. The substitution of part of myosin with soy glycinin and sunflower seed globulin results in a slight decrease in texture parameters.

No significant effect of the quantity of sulfhydryl groups on the texture properties was observed. Desamidation of gliadin caused only a slight decrease in cohesivity and hardness of the samples. The results permit the conclusion that the role of disulphide and hydrogen bonds is limited in the formation of gel during heat treatment.

In *Table 5* textural parameters of sausages prepared with addition of different protein preparations are shown. Myosin addition resulted in a slight improvement of the textural properties as seen from the data in the

Table 4
Textural properties of the model systems

Sample	Myosin	Acto-myosin	Myosin-gliadin	Myosin-glutenin	Myosin-glycinin	Myosine-sunflower seed globulin
Work of 1st compression A_1/mm^2	1350	1180	1220[1] 1170[2] 1040[3]	1310[1] 1290[2] 1270[3]	1210[1] 1150[2] 1030[3]	1150[1] 1030[2] 970[3]
Work of 2nd compression A_2/mm^2	240	210	205[1] 195[2] 190[3]	190[1] 185[2] 176[3]	187[1] 179[2] 1172[3]	185[1] 175[2] 169[3]
Hardness (H)	73.4	73.3	60[1] 58[2] 55[3]	72.5[1] 71.5[2] 70.2[3]	69.5[1] 68.2[2] 67.9[3]	64.2[1] 63.8[2] 61.9[3]
Elasticity (E)	22.0	16.0	18.0[1] 16.0[2] 13.0[3]	20.2[1] 19.3[2] 18.8[3]	17.9[1] 17.5[2] 16.8[3]	17.6[1] 17.3[2] 16.5[3]
Cohesivity (C) (A_2/A_1)	0.180	0.178	0.1682[1] 0.166[2] 0.182[3]	0.145[1] 0.143[2] 0.137[3]	0.154[1] 0.155[2] 0.166[3]	0.160[1] 0.169[2] 0.17[3]
Gumminess (G) ($C \times H$)	13.2	13.0	10.08[1] 9.628[2] 10.01[3]	10.51[1] 10.25[2] 19.61[3]	10.703[1] 10.571[2] 11.27[3]	10.272[1] 10.78[2] 10.77[3]
Chewiness (Ch)($G \times E$)	190.6	208.7[2]	181.44[1] 154.048[2] 130.13[3]	212.302[1] 197.825[2] 180.668[3]	191.58[1] 184.99[2] 189.336[3]	180.78[1] 186.494[2] 177.705[3]

1–2.0 g myosin + 0.2 g added protein
2–1.8 g myosin + 0.4 g added protein
3–1.6 g myosin + 0.6 g added protein

table. A deterioration of textural quality of the product was observed in the case of other protein preparations.

Knowing the important role of gluten in dough formation and in determining its rheological properties the disadvantageous effect of vital gluten and of gluten components on the rheological properties of myosin – gluten gels seems to be surprising. However, investigation of the microstructure of gels may provide an explanation. It could be observed that in the continuous network of myosin the denatured gluten is present in dispersed form, as filler. In the initial mixture (suspension) the gluten – gluten interaction

Table 5
Textural properties of sausages prepared with addition of different protein preparations

Sample	Control	Control + soy isolate	Control + sunflower seed isolate	Control + vital gluten	Control + myosin
Work of 1st compression A_1	1192	1135	970	950	1220
Work of 2nd compression A_2	202	170	160	150	205
Hardness (H)	73.3	68.6	70.0	60.0	70.0
Elasticity (E)	15.0	15.0	13.5	15.0	18.0
Cohesivity (C) (A_2/A_1)	0.178	0.164	0.165	0.157	0.168
Gumminess (G) ($C \times H$)	13.04	11.27	11.55	9.47	11.76
Cheminess (Ch) ($G \times E$)	195.6	169.0	155.9	142.1	211.68

is greater than the myosin – gluten interaction. Due to the relatively lower quantity end very low solubility, gluten is not capable of forming a continuous network. During heating a thermotropic gelation of myosin occurs as a result of denaturation and mainly hydrophobic interactions, and a continuous myosin network is formed. Gluten cannot strengthen the gel, but may disrupt on some places the network causing some decrease in elasticity.

References

1. HESSER, J. M.: Wheat Gluten: A Comprehensive Examination of the Production and Usage of this Vital Functional Protein. In: 'Cereal Science and Technology Impact on Changing Africa' Taylor, J. R. N., Randall, P. G. and Viljoen, V. H. (Eds.), Selected papers from the *ICC International Symposium, CSIR*, Pretoria, 1993. pp. 249–268.
2. LÁSZTITY, R.: The Chemistry of Cereal Proteins, Boca Raton, 1984, pp. 46–69.

3. POLJAKOV, V. I. — POPELLO, I. A. — GRINBERG, V. JA. — TOLSTOGUZOV, V. B.: Thermodynamic Compatibility of Proteins in Aequeous Medium. *Die Nahrung*, Vol. 30, pp. 365–368, 1986.
4. TOLSTOGUZOV, V. B.: Noviye formy belkovoy pishtchy, Agropromizdat, Moscow, 1987.