Rheological properties of gelatin gels filled with phospholipids vesicles. Dynamic and uniaxial compression measurements

C. Genot, S. Guillet, and B. Metro

Laboratoire d'Etude des Interactions des Molécules Alimentaires, Institut National de la Recherche Agronomique, Nantes, France

Abstract: The effects of addition of small unilamellar vesicles (SUV) or multilamellar vesicles (MLV) of phosphatidylcholine (PC) on rheological behavior of gelatin gels (approximately 5 % W/W) were studied. Gelation kinetics of pure gelatin gels and gelatin gels containing lipid vesicles as determined by storage modulus (G') vs. time were quite similar and no difference was observed on G' after 20 h. Apparent Young's modulus (E_0) was decreased by 15 % when 5 g/l of lipids (either MLV or SUV) were present in the gel. When the gels were submitted to three successive uniaxial deformations (respectively 25 %, 55 %, and 75 %) at a low rate (2 mm/min), a decrease in the force measured at 20 % or 50 % deformation, as a function of lipid concentration and deformation number was observed when MLV and more specially when SUV were added. Height loss of the gels after two deformations at 25 % and 55 % was the largest for gels containing SUV, and the smallest for pure gelatin gels.

Key words: Gelatin-liposome-gel rheological behavior.

Introduction

Many foods can be described as composite gels in which intrinsic properties of the polymeric network (i.e., protein or polysaccharide matrix) are modified by inclusions, such as starch granules, gas bubbles, oil droplets, or mesomorphic lipid phases. Wheat gluten, for instance, contains 5–10 % of lipids organized as vesicles, the effects of which on the rheological properties of the protein network are not yet elucidated [1]. Many dairy and delicatessen products also present a gel-like structure with inclusions. Moreover, developments of liposomes applications for food industry can be foreseen in the very near future, but the consequences of these inclusions on rheological properties of food are still not known.

In our laboratory, we studied a model system composed of gelatin gel in which phosphatidyl choline (PC) vesicles were inserted. The small and large deformation behavior of these lipid + protein gels were characterized from uniaxial compression experiments and shear dynamic measurements.

Experimental

Material

Gelatin: Powdered edible gelatin (275 Blooms, viscosity 6.67 cP at 60 °C: 4.55 mPa·s, pH: 5.7, isoelectric point: 7.9, ash: 0.6 %, moisture: 11 %, average molecular weight: 178 000) from swine skin was obtained from Rousselot-France.

Lipids: Frozen egg yolk phosphatidylcholine (egg PC) was purchased from Sigma (ref. P 5388). Its purity, checked by HPLC [2], was better than 90 %.

Methods

Preparation and characterization of lipid vesicles: The vesicles were prepared according to the procedure of Huang [3]. Phospholipids (10–300 mg) in CHCl₃ were poured into a round-bottomed glass flask. Solvent was evaporated by rotary evaporation followed by N₂ blowing. 10–50 ml of filtered and degassed NaCl 0.1 M + 0.02 % NaN₃ (Solution A) was then added above the thin lipidic film formed on the vial. Multilamellar vesicles (MLV) were obtained by vortexing the vial for 30 min after a hydration time of 48 h. The size distribution of these vesicles is very large as shown by Counter Coulter measurements (not shown) and transmission electron microscopy (TEM) after freeze fracture (Fig. 1). Small uni-

Fig. 1. Freeze-fracture electron microscopy of MLV of egg PC in NaCl 0.1 M

lamellar vesicles (SUV) were prepared from MLV by sonication for 3 h under nitrogen at 4°C with a 500 W Sonic and Material Inc. pulse sonifier (titanium microtips; output: 4; 40% duty cycle). Following sonication, SUV were centrifuged at 185 000 g for 6 h in order to remove titanium particles, lipid aggregates, and MLV. The translucent supernatant contained 90–95% of SUV (expressed in lipid weight) as shown by size exclusion chromatography of liposomes on Superose 6 and phosphorus determination [4] (Fig. 2) and freeze fracture TEM (Fig. 3).

Preparation of gels: *P* g of dry gelatin was swollen in *V*1 ml NaCl 0.1 M, NaOH 4.5 mM + 0.02% NaN₃ (Solution B) [$V1 = P(1 - 0.11)/(5 \times 2)$] for 30 min at room temperature. The gelatin was then dissolved in a water bath at 55°C for 15 min and *V*1 ml of solution A containing lipid dispersion (0.3 to 5 g lipids per gel liter) were added to obtain a 4.97% gel (dry matter/total water) at pH 7. Some experiments were performed at 4.71%, pH 7 gelatin gels. When dynamic measurements were carried out the progel was directly poured on the apparatus plate. For uniaxial compression measurements, the progel was poured into cylindrical molds (diameter: 30 mm; height: 30 mm) the top and bottom edges of which were raised with a sticky plastic tape. The gels which formed by cooling were allowed to mature for 3 days in controlled room temperature of 20°C. Before measurements, the sticky tape was removed and the excess gel above and below the top and bottom edges of the mold was cut off with a thin steel wire. Thereafter the

Fig. 2. Size exclusion chromatography of SUV of egg PC on Superose 6 (Pharmacia) (30 × 1 cm) equilibrated with NaCl 0.1 M. The column was previously saturated with the lipid dispersion. Flow rate: 0.4 ml/min; NaCl 0.1 M + NaNO₃ 0.02%. The two peaks (1 = MLV and aggregated vesicles; 2 = SUV) were collected separately and lipid content of each fraction was estimated after phosphorus determination [4]. *V*₀ = void volume; *V*_t = total volume

gell was removed from the mold and covered with paraffin oil to avoid dehydration during the experiment.

Dynamic measurements: The gelatin sol introduced on the prewarmed (50°C) horizontal plate of the Carrimed 5001 in cone-plate geometry (cone: 4°; diameter: 2 cm) was cooled to 20°C (ap-

Fig. 3. Freeze fracture electron microscopy of SUV of egg PC

proximatively 1 min). During this time and for the next 30 h, G' and G'' , the dynamic storage and loss moduli were calculated every 10 min. The oscillatory frequency was 0.5 Hz and the amplitude 3 mrad.

Uniaxial compression: Uniaxial compression experiments were carried out using the Instron universal testing instrument 1222. The cylindrical samples were compressed between flat platens at a fixed crosshead speed. The plates of the instrument were wetted with paraffin oil in order to obtain optimal slip conditions and reduce "barrelling" of the gel. Successive deformations on individual samples were 5 %, 25 %, 55 %, and 75 % using a crosshead speed of 2 mm/min. The force $F(N)$ was measured as a function of deformation. The whole experiment was performed in a controlled laboratory temperature of $20 \pm 1^\circ\text{C}$.

Small strain: determination of Young's modulus (YM)

Apparent Young's modulus E_0 ($\text{N} \cdot \text{m}^{-2}$) was defined from

$$E_0 = (F/A) \cdot (H_0/H_0 - H) \quad (1)$$

where $F(N)$ was the strength component parallel to the direction of the deformation, A (m^2) the sample area which was considered to be constant in low deformation range and H_0 and H the heights of the gel before and after compression ($(H_0 - H)/H_0 \cdot 100$: deformation percentage).

E_0 was estimated from the slope of the linear part of the 5 % compression curve.

Large strain

The values of F for 20 % and 50 % deformation during the successive deformations were measured.

Results

As shown in Fig. 4, when lipid vesicles were present in gelatin gel, the initial pure, transparent gel became

turbid, the turbidity being higher when MLV rather than SUV or when increasing vesicles concentration were added.

Dynamic measurements: No significant differences were observed in gelatin gelation kinetics (Fig. 5) or in storage modulus (G') after 20 h of gelation (Table 1) when egg PC small unilamellar vesicles are present in the gels as compared to pure gelatin gels.

The loss modulus was always very low and varied between 0 and 150 Pa during the whole gelation experiments, no difference being observed between pure gelatin gels and gels containing lipid vesicles.

Uniaxial compression

Low strain: Apparent Young's modulus (E_0) of gelatin gels containing egg PC SUV and egg PC MLV (Tables 2 and 3) was decreased by 1 % to 15 % when the vesicle concentration in the gel increases from 0.12 to 5 g/l.

High strain: When three successive deformations (25 %, 55 %, and 75 %, respectively) were applied to the gels containing different lipids concentrations at a deformation rate of 2 mm/min, two phenomena were observed (Fig. 6). First, during the first compression (25 %), a decrease of the force (F) measured at 20 % deformation of the gels containing SUV or MLV as compared to pure gelatin gels occurred. This decrease was more obvious in the case of SUV addition (Figs. 6 a and 6 b). During the second compression (55 %) a similar decrease of F (measured at 20 % and 50 % deformation) after addition of lipid vesicles was observed. Second, when the third compression was

Fig. 4. Cylinders of gelatin gels with (a) and without (b) MLV (3 g/l)

Fig. 5. Gelation kinetic of gelatin gels as shown by storage modulus vs. time. $\blacktriangle$, $\bullet$: gelatin 4.71%; +, $\star$: gelatin 4.71% + 2 g/l SUV of egg PC. (dynamic measurements — Carrimed)

Table 1. Storage modulus (G') after 20 h gelation at 20 °C of gelatin gels without or with small unilamellar vesicles (SUV) of egg phosphatidylcholine (PC) (dynamic measurements, Carrimed)

	Gelatin $G' (N \cdot m^{-2})$	Gelatin + SUV $G' (N \cdot m^{-2})$	lipid concentration (g/l gel)
gelatin 4.97 %	3284	3443	1.18
	3499	2956	1.28
Mean value $\pm \sigma$	3391 ± 152	3199 ± 344	—
gelatin 4.71 %	2239	2160	4.20
	2569	2631	4.20
	2915	2588	4.00
	2151	2356	3.15
	2196	2361	2.52
Mean value $\pm \sigma$	2414 ± 325	2419 ± 192	—

applied to gels containing increasing lipid concentrations (either MLV or SUV), an important decrease of F measured at 20 % and 50 % deformation was observed, compared to F measured on pure gelatin gels during this third compression, and to F measured on the lipid + protein gels during the first and the second compressions (Fig. 6 a–d).

Moreover, after three compressions at 5 %, 25 %, and 55 % the height of cylinders of gelatin, gelatin + MLV, and gelatin + SUV gels was shown to be decreased by 9 %, 14 %, and 17 % respectively.

Table 2. Apparent Young's modulus (E_0) of gelatin gels (after 72 h of gelation) containing multi-lamellar vesicles (MLV) of egg PC. (Instron)

a) 4.71% gelatin gels containing different MLV concentrations prepared the same day from the same gelatin solution

Lipid concentration (g/l gel)	$E_0 (N \cdot m^{-2})$	ΔE_0 (%)
0	7492	0
0.125	7407	0.9
0.25	7382	1.5
0.5	7229	3.5
1	7052	5.9
1.5	6834	8.8
2	7203	3.9

b) 4.97 % gelatin gels prepared different days (in parentheses is indicated the number of E_0 determinations for each concentration)

Lipid concentration (g/l gel)	$E_0 (N \cdot m^{-2})$	ΔE_0 (%)
0	9700 ± 670 (5 × 8)	0
2.6	8720 ± 468 (6)	10.1
3.55	8386 ± 144 (6)	13.5
4.93	9047 ± 92 (4)	6.7
5.55	8230 ± 179 (6)	15.2

$$\Delta E_0 = 100 \times (E_0 (\text{no lipids}) - E_0) / E_0 (\text{no lipids})$$

Table 3. Apparent Young's modulus (E_0) of gelatin gels (after 72 h of gelation) containing SUV of egg PC. (Instron)

a) 4.71 % gelatin gels containing different SUV concentrations prepared the same day from the same gelatin solution

Lipid concentration (g/l gel)	E_0 ($N \cdot m^{-2}$)	ΔE_0 (%)
0	7260	0
0.09	6989	3.7
0.18	7131	1.2
0.37	7015	3.4
0.75	6931	4.5
0.125	7144	1.6
1.5	6947	4.3

b) 4.97 % gelatin gels prepared different days (in parantheses is indicated the number of E_0 determinations for each concentration)

Lipid concentration (g/l gel)	E_0 ($N \cdot m^{-2}$)	ΔE_0 (%)
0	9700 $\pm$ 670 (5 $\times$ 8)	0
1.6	8949 $\pm$ 52 (4)	7.7
3.75	8364 $\pm$ 113 (4)	13.8

$$\Delta E_0 = 100 \times (E_0 (\text{no lipids}) - E_0) / E_0 (\text{no lipids})$$

Discussion and conclusion

Two hypotheses can be assumed to explain the observed modifications in rheological behavior of gelatin gels after lipid vesicles addition.

The first is that gelatin-lipid interactions occurs. According to this hypothesis, previous work [5] showing an increase of gelatin solution viscosity when anionic surfactants were added was interpreted as a cooperative binding effect between surfactant micelles and gelatin molecules. In our case the phospholipid we used is zwitterionic and electrostatic interaction between PC and gelatin molecules may not be involved. Furthermore, lipid-protein interactions should occur in the sol state, when the molecules are more mobile, leading to subsequent constraints in triple-helix gelatin growth. Thus, modification in gelation kinetics, especially in the fast first stage should be observed, contrary to our observations.

The second hypothesis is a filler effect of lipid vesicles. Depending on the filler volume fraction [6, 7], the relative rigidities of the particles and the matrix [6], and on size and shape of the particles [8], a weakening or a strenghtening of the composite gel can be observed. Approximative volume fractions of phos-

Fig. 6. Compression force (N) vs. lipid concentration (g/l) of 30 $\times$ 30 mm gelatin gels (4.97 %) cylinders containing MLV or SUV during successive deformations. (Uniaxial compression - Instron). Crosshead speed: 2 mm/min; $\bullet$: first deformation; $\star$: second deformation; $\blacktriangle$: third deformation. a: gelatin + MLV, deformation 20 %; b: gelatin + SUV, deformation 20 %; c: gelatin + MLV, deformation 50 %; d: gelatin + SUV, deformation 50 %

pholipid vesicles were calculated using the measured average vesicles diameters (MLV: 5 000 nm; SUV: 25 nm), and assuming an encapsulated volume of 2.5 l/mole for MLV and an average number of 3 000 PL molecules per SUV vesicle [9]. The estimated volume fractions of vesicles are 0.25 % for 1 g of PC incorporated as MLV per liter of gelatin gel, and 1.35 % as SUV. The volume fractions of vesicles incorporated in our gels (always lower than 1.25 % for MLV and 4.2 % for SUV) are lower than the volume fractions of fillers whose effects are reported in the literature (from 5 % to 60 %). The weakening of gelatin gels we observed (lowering in E_0 and F) would indicate a very lower rigidity of lipidic vesicles as compared to gelatin gel rigidity.

Further experiments are needed to assert and quantify the relative roles of lipid-gelatin interactions and vesicle filler effect in weakening of the gelatin gels, as well as to study the influence of various parameters (size, volume fraction, affinity of lipid vesicles for gelatin matrix).

References

1. Marion D, Le Roux C, Akoka S, Tellier C, Gallant D (1987) *J Cereal Sci* 5:101-115
2. Stolyhwo A, Martin M, Guiochon G (1987) *J Liquid Chromatogr* 10(6):1237-1253
3. Huang CH (1969) *Biochemistry* 8:344-352
4. Barlett GR (1959) *J Biol Chem* 234:466-468
5. Greener J, Contestable BA, Bale MD (1987) *Macromolecules* 20:2490-2498
6. Brownsey GJ, Ellis HS, Ridout MJ, Ring SG (1987) *J Rheol* 31(8):635-649
7. Richardson RK, Robinson G, Ross-Murphy SB, Todd S (1981) *Polym Bull* 4:541-546
8. Ring S, Stainsby G (1982) *Prog Fd Nutr Sci* 6:323-329
9. Dousset N, Douste-Blazy L (1985) In: Puisieux F, Delattre J (eds) *Les Liposomes. Applications thérapeutiques*, Lavoisier, Paris, pp 41-72

Received October, 1988;
accepted January, 1989

Authors' address:

C. Genot
Institut National de la Recherche Agronomique
B. P. 527
F-44026 Nantes Cedex 03, France