PROTEIN-LIPID INTERACTIONS AND THE FUNCTION OF THE INTERFACIAL PROTEIN FILM IN RAW MEAT BATTERS

ANDRE GORDON*[1], SHAI BARBUT[2] and SANDY SMITH[2]

[1]Grace, Kennedy and Co. Ltd., 64 Harbour St., Kingston, Jamaica, W.I.
[2]Department of Food Science, University of Guelph, Guelph, Ont. N1G 2W1, Canada.

INTRODUCTION

The stabilization of fat and water within comminuted meat products such as frankfurters greatly influences the yield and texture of the final cooked product. Therefore, an understanding of the way in which different components of the system interact to affect product stability is desirable. There are currently two main theories to account for the stabilization of emulsion-type meat products. The first, the emulsion theory, is based on the formation of an interfacial protein film (IPF) around fat globules which stabilizes them during cooking (Hansen, 1960; Jones, 1984). The second is the physical entrapment (gelation) theory which emphasises the ability of the meat proteins to gel on heating (Lee, 1985). This latter theory adequately explains several of the physical properties which are observed with finely comminuted products. However it has been unable to satisfactorily explain some of the features of meat batters, especially those relating to fat stabilization and protein-lipid interactions in meat batters. Consequently, the role which fat encapsulation by the meat proteins plays in batter stabilization is still not well understood although recent studies have indicated its potential importance in meat systems (Koolmes et al., 1989; Gordon and Barbut, 1989).

Theno the Schmidt (1978) and, more recently, Gordon and Barbut (1990 a,b) have shown that fat globules were directly bound to the protein matrix through their IPF. There is also evidence that fat binding by the matrix affects the final texture and microstructure of the product (Schut, 1978; Gordon and Barbut, 1990c). The structure of the interfacial protein film, the occurrence of pores (openings) in the IPF, the mechanism of pore formation and fat release during cooking in batters made with different chloride salts were investigated in a previous study (Gordon and Barbut, 1990b). It was felt that an investigation of the structure of the IPF in raw batters could yield useful information regarding batter stabilization. Hence, this study was undertaken to examine the nature of the IPF in raw batters made with different chloride salts and explore its role in fat stabilization in raw batters.

MATERIALS AND METHODS

Batter Preparation

Five chicken breast meat batters (25% preground pork backfat added) were prepared with iosionic strengths of NaCl (2.5%), $MgCl_2$, $CaCl_2$, KCl and LiCl. The meat batters were made by chopping the meat, salt, water and fat in a bowl chopper for a total of 5 min as described by Gordon and Barbut (1990c). The final temperature did not exceed 12°C in any of the treatments. All treatments were formulated with 6% added water. The chloride salts (Fisher, Ont., Canada) varied among treatments.

Electron Microscopy

The meat batters were prepared for cryo scanning electron microscopy (cryo SEM) according to Gordon and Barbut

(1990b). The cryo system used was an Emscope SP2000A System which was kept at -165°C (± 5°C). Samples were examined by SEM at 10 kV (Hitachi S-570, Tokyo, Japan) and the microscope stage was kept at -165°C with liquid nitrogen.

The procedure of Gordon and Barbut (1990a) was used to prepare samples for transmission electron microscopy (TEM). In summary, samples ($2mm^3$) taken from the interior of the treatments were fixed in 2% glutaraldehyde/1% paraformaldehyde for 2 hr, post-fixed with 1% OsO_4 for 4 hr, dehydrated and embedded in Spurr's resin. Samples were cured in capsules for 16 hr at 60°C and sections (70 μm) were cut, picked up on grids, contrasted with uranyl acetate (10 min) and lead citrate (5 min) and viewed at 80 kV (JEOL 100S).

RESULTS AND DISCUSSION

Structure of the Interfacial Protein Film

Several researchers have demonstrated the existence of a defined interfacial protein film (IPF) around fat globules in uncooked (raw) batters (Borchert et al., 1967; Swasdee et al., 1982; Gordon and Barbut, 1990b). The results of this study provide further evidence that the interfacial film is a universal feature of raw meat batters (Figure 1). The IPF is thought to be formed by myosin (Jones, 1984). However a recent study has indicated that actomyosin as well as several other minor myofibrillar proteins are involved in IPF formation (Gordon and Barbut, 1990d). Therefore, the structure of the IPF around fat globules within a particular meat batter could vary depending on the proteins involved in its formation. Differences between fat globules in terms of IPF structure were found within each batter in all of the treatments (Fig. 1).

The protein coat around some fat globules in cooked meat batters was shown to have a multilayered structure (Gordon and Barbut, 1990a). Some fat globules in all of the raw batters in this study also had multilayered protein coats (Figs. 2 and 3b). This is to be expected since the IPF is formed during raw batter preparation. The fat globule from the LiCl batter (Figure 2) had an interfacial film composed of three protein layers. The stable globule from the $MgCl_2$ batter (Fig. 3b) was an example of a globule which had a dual-layered protein coat. For all IPF-stabilized globules, the outermost protein layer was bound to the protein matrix in some areas on the circumference of each globule. This helped to form a kind of a protein "sheath" which surrounded the globule and was part of the protein matrix (Fig. 4). Hence, it appears that once the IPF has been formed, fat is immobilized by being bound to the protein matrix as well as physically restricted by this protein "sheath".

The differences in electron density of the protein layers in multilayered protein coats may indicate that different proteins were involved in each layer, an observation which would be supported by the findings of an previous study (Gordon and Barbut, 1990d). Differences existed between globules in terms of the electron density of the internal (primary) protein layer which directly coats the fat. A thin, dense primary layer was observed in some globules (Fig. 2) while others had a thicker, more diffuse protein coat (Fig. 3b). These structures may be due to the protein assuming different conformations at the oil-in-water interface (Graham and Phillips, 1979). For most of the globules observed, these seemed to be the dominant structures.

Figure 1b shows two fat globules from the $CaCl_2$ treatment, the smaller of which was completely surrounded by a very thick protein coat which is identical to, and part of, the matrix. The larger globule also had a similar protein film but, in this case, it did not completely enclose the globule. These observations support previous suggestions that insoluble proteins were involved in the stabilization of $CaCl_2$ batters (Gordon and Barbut, 1990c). The proteins which comprised the matrix also

appeared to form the IPF of most globules in this treatment. The IPF was therefore an integral part of the matrix in these batters. Others have suggested that the IPF proteins may be an integral part of the protein matrix (Schut, 1978; Hermansson, 1986). This could result in immobilization and stabilization of fat in raw batters where very little protein extraction is achieved (Figs. 1b and 4). It should be noted that in all meat batters, the soluble proteins are preferentially adsorbed to form the IPF (Schut, 1978; Gordon and Barbut, 1990d).

The results indicate that there are differences between chloride salt treatments in terms of the most common IPF structure formed in raw batters. These differences, when combined with those in the structure of the protein matrix (Gordon and Barbut, 1990c) probably account for the stability and textural differences obtained from raw and cooked chloride salt batters (Barbut and Mittal, 1988; Gordon and Barbut, 1989). It also appears that while soluble proteins are the preferred agents of interfacial film formation in both stable and unstable meat batters, insoluble proteins play a significant role in IPF formation and fat stabilization in some raw batters. This should be considered when interpreting the effects of treatments on meat batter stability with respect to the soluble protein concentration.

Pores and Rupture Holes in the IPF

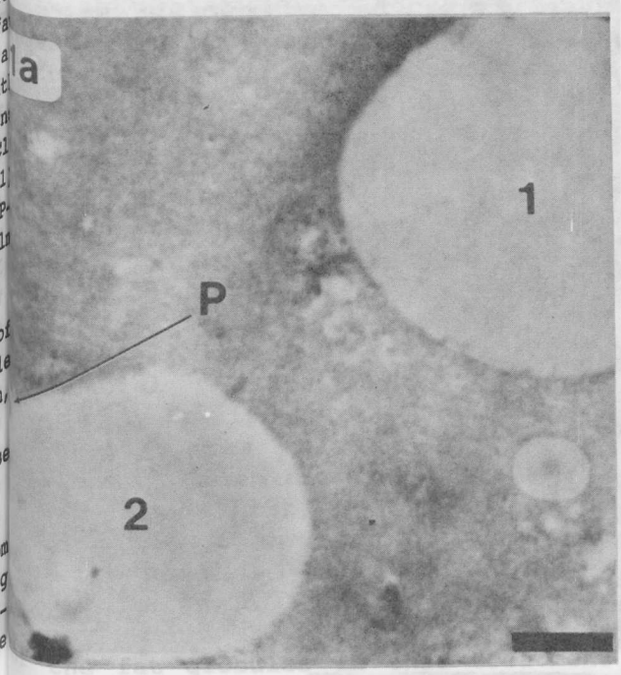
Pores are small openings in the interfacial film around fat globules and have been shown to function in controlled fat exudation during cooking (Jones and Mandigo, 1982; Gordon and Barbut, 1990a). Conversely, ruptures are large holes in the IPF through which extensive fat loss occurs and which are usually associated with eventual collapse of the IPF and fat coalescence (Deng et al, 1981; Gordon and Barbut, 1989). Huber and Regenstein (1988) reported that not enough protein is extracted during comminution to fully coat all of the fat in meat batters. This suggests that pores should be present in raw batters or that incomplete coverage of globule surfaces should occur for at least some globules. Pores were found in the IPF of some globules in all treatments (Fig. 1). However, the number of pores and their distribution depended on the type of protein coat around the globule. Many of the globules in the stable treatments had protein coats with no pores (Fig. 1a) or few, well distributed pores which showed little, if any, fat exudation. Some fat globules with thinner protein coats tended to have more pores and these were generally concentrated in areas where the IPF was of uneven

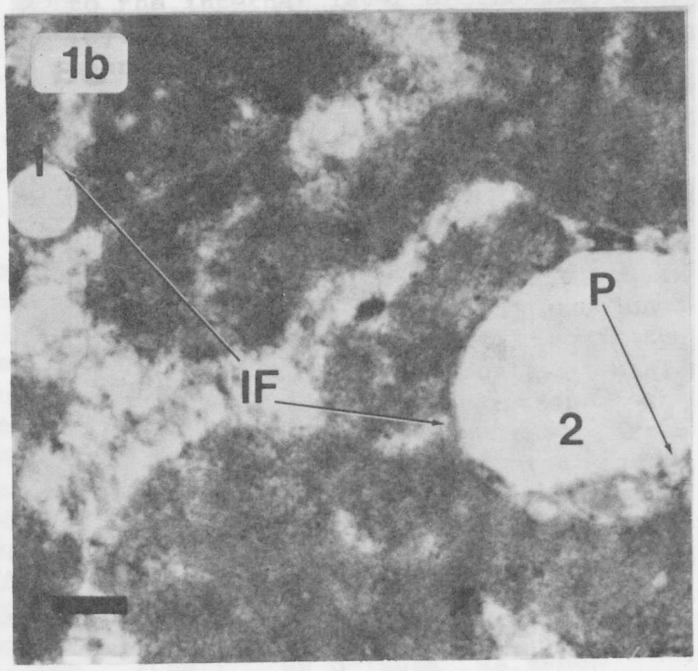
Figure 1: Transmission electro micrograph (TEM) of protein-coated fa globules from two treatments. a globules from the KCl treatment (1) wit a relatively thick IPF and no pores an (2) with a thin IPF; b) from the CaCl treatment: thickly coated globule (1 and a partially coated globule (2). P pore, E -exudation, IF-interfacial fil (Bar=0.5μm).

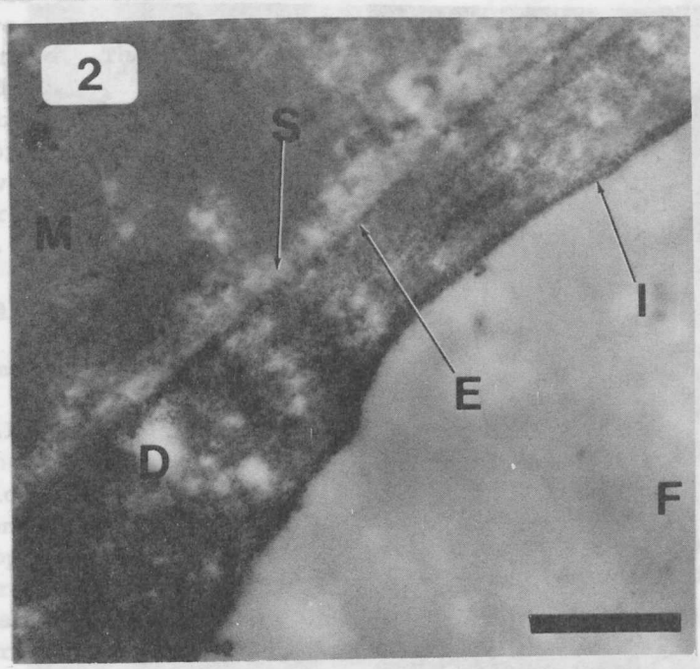
Figure 2: High magnification TEM of the protein coat around a stable globule in the LiCl batter. I-internal protein, E-external protein, F-fat, M-matrix, S-space between IPF and matrix, D-dense middle protein layer (Bar=1.0μm).

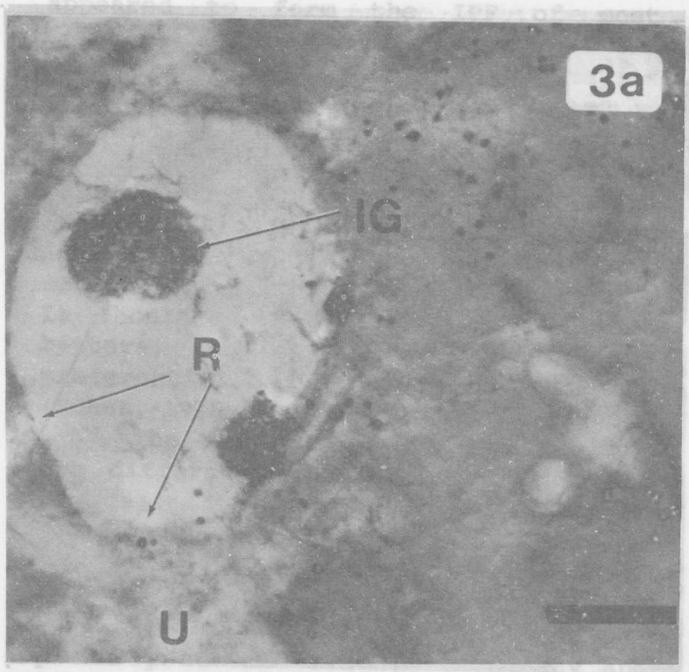
Figure 3: TEM of fat globules from the $MgCl_2$ batter a) field showing unstable fat globule with a protein-coated internal globule; b) stable globule showing internal structure; P-pore, U-unstable fat, R-rupture hole, IG-internal globule, SM-stable, multi-layered globule, I-internal protein coat, E-external protein coat; (Bar=1.0μm).

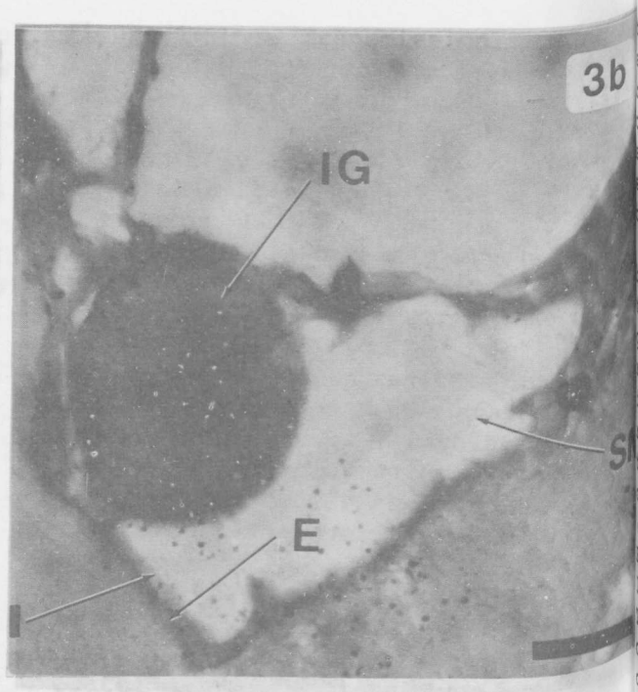
Figure 4: Cryo SEM of a fat globule from the $MgCl_2$ treatment showing the continuity of the protein matrix and IPF. F-fat, M-matrix, E-external protein coat (protein sheath; part of matrix); arrows indicate the junction between "E" and the internal protein layer (Bar=5.0μm).

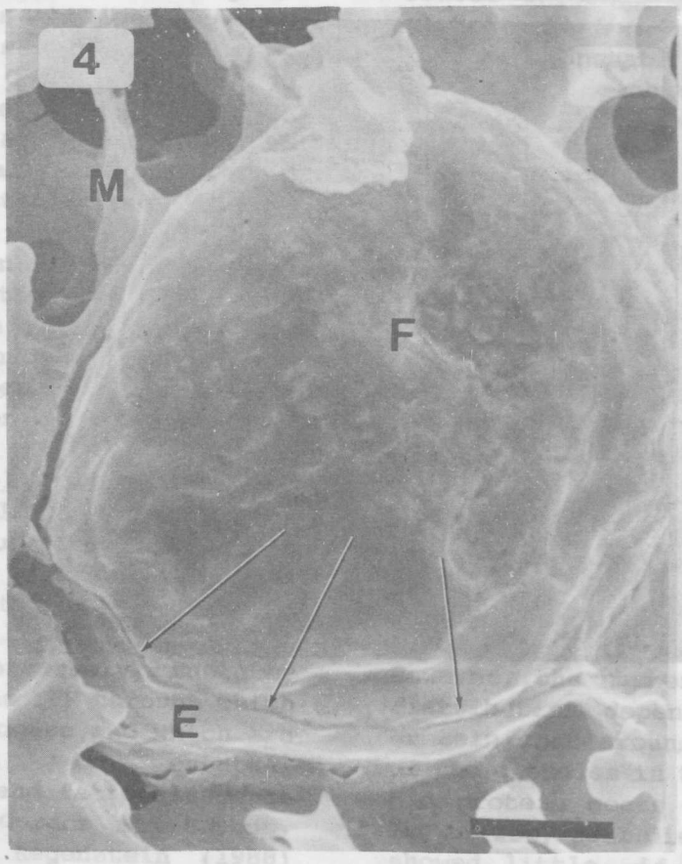

1a
1
P
2


1b
I
IF
P
2


2
S
M
I
E
D
F


3a
IG
R
U


3b
IG
E


4
M
F
E

thickness (Fig. 1a). There were no signs of extensive fat exudation or ruptures in the IPF of globules in any of the stable raw monovalent chloride salt batters. However, some (limited) exudation was evident is some stable batters (Fig. 1a).

In the less stable $CaCl_2$ raw batter, many more pores were present in the protein coat surrounding the globules (Figs. 1b). In addition, ruptures were present in some interfacial films although they were relatively few in number. In contrast, the $MgCl_2$ raw batter which has consistently been shown to be unstable (Gordon and Barbut, 1989; Gordon and Barbut, 1990c,d) had fat globules with large ruptures in their IPF (Fig. 3a). This caused the formation of large pools of fat in this batter. The IPF of many of the fat globules in this treatment also contained pores through which some fat exudation occurred. The differences between $MgCl_2$ and $CaCl_2$ in terms of stability is related to differences in protein extraction and specific ion effects which have been discussed in detail in earlier papers (Gordon and Barbut, 1990 c,d).

Internal Structure of Fat Globules

Gordon and Barbut (1990a) have reported that some of the larger fat globules in most batters had varying kinds of internal protein structures which were continuous with their IPF. This study provided further evidence that this phenomenon is common in meat batters and develops during the chopping of the raw batter. Different types of internal organization were observed, mainly in the larger fat globules, in all treatments; the more common of these are shown in Figure 3. Several globules contained internal structures which appeared to be protein-coated internal globules, all of which were linked to the protein of the IPF (Fig. 3a). Other larger globules had a compartmentalized structure as previous described (Gordon and Barbut, 1990a). In these globules, fat was separated into lobes or compartments by protein which was linked to the internal layer of the IPF (Fig. 3b). Figure 3b also shows a situation where both types of internal structure were present within the same globule. It may be that the internal appearance of these large fat globules depends on the plane of the section through them. If they were sectioned near the surface of an internal globule then the electron dense oval internal structure would be seen. If the globules were sectioned such that the plane passed through the middle of the internal globule, it would appear as an internal compartment. The internal organization of these larger globules probably serves as a buffer against the reduced thermodynamic stability and loss of viscoelasticity by the IPF, especially in globules which are non-spherical in shape.

REFERENCES

Barbut S, Mittal GS. (1988). Rheological and gelation properties of meat batters prepared with three chloride salts. J. Food Sci. 53, 1296-99, 1311.

Borchert LL, Greaser ML, Bard JC, Cassens RG, Briskey EJ. (1967). Electron microscopy of a meat emulsion. J. Food Sci. 32, 419-421.

Deng JC, Toledo RT, Lillard DA. (1981). Protein-protein interactions and fat and water binding in comminuted flesh products. J. Food Sci. 46: 1117-1121.

Gordon A, Barbut S. (1989). The effect of chloride salts on the texture, microstructure and stability of meat batters. Food Microstr. 8, 271-283.

Gordon A, Barbut S. (1990a). The role of the interfacial protein film in meat batter stabilization. Food Structure 9, (in press).

Gordon A, Barbut S. (1990b). Use of cold stage scanning electron microscopy to study meat batters. J. Food. Sci. 55 (in press).

Gordon A, Barbut S. (1990c). The microstructure of raw batters prepared with monovalent and divalent chloride salts. Food Structure (submitted).

Gordon A, Barbut S. (1990d). The effect

of chloride salts on protein extraction and interfacial protein film formation in meat emulsions. IFT Abstracts, 1990 (accepted).

Graham, DE, Phillips MC. (1979). Proteins at liquid interfaces 111. Molecular structures of adsorbed films. J. Colloid Int. Sci. 70, 427-439.

Hansen LJ. (1960). Emulsion formation in finely comminuted sausage. Food Technol. 14, 565-569.

Hermansson AM. (1986). Water and fat holding. In "Functional properties of food macromolecules". Mitchell JR and Ledward DA (eds), Elsevier, London, UK, 273-314.

Huber, DG, Regenstein, JM. (1988). Emulsion stability studies of myosin and exhaustively washed muscle from adult chicken breast muscle. J. Food Sci. 53, 1282-1286, 1293.

Jones KW, Mandigo RW. (1982). Effect of chopping temperature on the microstructure of meat emulsions. J. Food Sci. 47, 1930-1935.

Jones KW. (1984). Protein-lipid interactions in processed meats. In Proc. Ann. Recip. Meat Conf. 37, 52-57.

Koolmees PA, Moerman PC, Zijderveld, MHG. (1989). Image analysis of the fat dispersion in a comminuted meat system. Food Microstr. 8: 81-90.

Lee CM. (1985). Microstructure of meat emulsions in relation to fat stabilization. Food Microstructure. 4, 63-72.

Schut J. (1978). Basic meat emulsion technology. Meat Industry Res. Conf. Proc., 1978, 1-15.

Swasdee RL, Terrell RN, Dutson TR, Lewis RE. (1982). Ultrastructural changes during chopping and cooking of a frankfurter batter. J. Food Sci. 47: 1011-1013.

Theno DM, Schmidt GR. (1978). Microstructural comparison of three commercial frankfurters. J. Food Sci. 43, 845-848.