Technical

The Binding of Model Shortenings During Mixing of Mechanically Developed Bread Doughs from Fresh and Stored Flours[1]

B.M. BELL and **N. FISHER**, Flour Milling and Baking Research Association, Chorleywood, Rickmansworth, Herts. WD3 5SH, England

ABSTRACT AND SUMMARY

Model fats were prepared from defined binary mixtures of saturated and unsaturated triglycerides. Their effect on the volume of loaves prepared by the Chorleywood Bread Process (CBP), from fresh and storage-deteriorated flour, was similar to that given by a good bakery shortening. The use of radioactively-labeled glyceryl tripalmitate in fresh flour doughs enabled its distribution between 'free' and 'bound' lipid fractions of dough to be determined. Further studies which were made with the triglyceride of margaric acid, present only in trace amounts in wheat, showed that as the amount of saturated triglyceride added was increased, the amount entering the 'bound' fraction remained small and constant, whereas levels in the 'free' lipid rose at similar rates in doughs made from fresh and stored flour. Evidence is given that 'free' rather than 'bound' saturated triglyceride is responsible for increasing the volume of CBP bread made from fresh flour. Unsaturated triglyceride was 'bound' by stored flour doughs to a greater extent than by fresh flour doughs. A liquid saturated triglyceride could be used to replace the unsaturated triglyceride in a model fat. The poor effect of shortening on the baking properties of storage-deteriorated flour could be overcome by adding more fat, either as such, or by increasing the level of its liquid constituent. Results are discussed in relation to the apparent requirement for hard fat in making bread by the CBP from fresh flour.

INTRODUCTION

Although the mechanism of the improving action of fat (shortening) in breadmaking, which can result in increases in loaf volume of up to 25%, is still uncertain, the importance of some physical properties of the shortening has been recognized for many years (1). In practice, an effective shortening is one which contains a proportion of fat which is still solid at dough-processing temperatures; this has been shown in both conventional (1) and mechanical dough development (2,3) processes, such as the Chorleywood Bread Process (CBP). However, this fat does not necessarily remain solid once mixed with the flour lipid in the dough, nor is melting point the sole critical factor determining the effectiveness of a fat as an improver (4). Fat is an essential ingredient of doughs made by the CBP. A level of 0.7% based on flour weight and a solid to liquid ratio of 1:7 in the fat are recommended to meet the fat requirements of most flours, although less would often be sufficient (3).

In the special case of flours subjected to long-term storage, addition of this amount of shortening does not always increase loaf volume and may even reduce it slightly. Loaf volume may be considerably improved, however, by a large increase in shortening level. The anomalous response of the stored flour to shortening, part of a wider study of the changes occurring in flours during storage, is being examined partly in order to understand the deterioration of such flours and partly to help clarify the effect of added fat on the baking properties of fresh flours.

As a step towards elucidating the mechanism of action of shortening in breadmaking, the distribution of triglycerides between 'free' and 'bound' lipid fractions of dough has been studied by determining their extractability in non-polar and polar solvents, respectively (5). Studies of lipid binding in mechanically developed doughs have been reported previously (2,6-10), some of which have attempted to distinguish between the binding of shortening and that of flour lipid (2,9,10). The effect on lipid binding of shortenings of different composition and of long-term storage of flour has been investigated in this work.

Shortenings contain a number of lipid constitutents of varying polarity and fatty acid composition. To simplify the approach, model fats were prepared from a solid saturated triglyceride and a liquid unsaturated triglyceride. A model fat of this type (11) has been shown to be as effective in breadmaking as a good commercial shortening. Tracer techniques or gas chromatography were used to study the partition of the components of such model shortenings between 'free' and 'bound' lipid fractions of the dough.

MATERIALS AND METHODS

Flours

Recently milled and long-stored commercial flours were compared in these studies, pending a planned comparison between a flour stored at ambient temperatures for several years and its true control held at low temperature in an inert atmosphere.

For the preliminary work with radioactive glyceryl tripalmitate (GTP), a breadmaking flour ('Baker's Pride,' J. Rank Ltd., treated with improvers at the mill: protein 11.9%, moisture content 13.8%) was used. In the main study, another improver-treated breadmaking flour ('Democrat,' J. Rank Ltd.: protein 11.2%, moisture content 13.7%) was compared with an untreated bread flour (protein 12.6%, moisture content 12.5%) which had been stored in moisture-proof bags for 6 yr at ambient temperatures and had deteriorated badly in baking quality.

[1] This work, part of a collaborative study with the Food Science Laboratory of the Ministry of Agriculture, Fisheries and Food, was briefly described at the AOCS Meeting, Philadelphia, October 1974.

Fats

Bakery fat (shortening): 'Covo' (van den Berghs and Jurgens Ltd., Craigmillar Division: slip point 43 C), was used throughout this work. The fatty acid composition, determined by gas liquid chromatography (GLC) was palmitate 26.0%, stearate 8.7%, oleate 45.5%, linoleate 19.8%.

Triglyceride components of the model fats: GTP (GLC gave laurate 1.9%, myristate 5.5%, palmitate 92.6%), was purchased from BDH Chemicals Ltd., Poole, England, or synthesized by a slight modification of the method of Wheeler et al. (12) in which reagents were heated to 140-150 C for about 15 hr until a product free from partial glycerides was obtained. ^{14}C carboxyl-labeled GTP (Radiochemical Centre, Amersham, England: 97% pure; 0.18 mg) was mixed before use with 24.82 mg non-radioactive commercial GTP.

Glyceryl trioleate (GTO): A commercial grade of GTO (BDH) contained palmitate 13.7%, stearate 7.5%, oleate 52.4%, linoleate 26.3%. For the later work, GTO (oleate 99.4%, palmitate 0.4%, stearate 0.2%; Sigma London Chemical Company Ltd., Kingston upon Thames, England) was used and was shown by thin layer chromatography (TLC) to be pure triglyceride.

Glyceryl trimargarate (GTM): GTM was synthesized (12) from 98.5% pure margaric acid and shown by TLC to be pure triglyceride.

Glyceryl tricaprylate (GTC): GTC (Sigma: caprylate 99.5%), was a liquid saturated triglyceride similar in melting point (8 C) to GTO (mp -5 C).

Model fats were prepared by dissolving a mixture of the two constituents in chloroform or in chloroform: methanol (2:1, v/v), and then evaporating all traces of solvent, first by rotary-film vacuum evaporation below 40 C, then by vacuum desiccation. GTO, when used alone, was treated in the same manner. All the model fats were premixed with the flour and incorporated in the dough at a level of 0.7% w/w flour, unless otherwise stated.

Fats are described as 'effective' if they increased loaf volume significantly compared with an unsupplemented control and as 'ineffective' if they did not have this effect.

Doughs and Bread

Doughs were made in a 'Minorpin' mixer (Henry Simon and Co. Ltd., Stockport, Cheshire), which had been modified to mix CBP doughs so that the work input of 40 $kJ.kg^{-1}$ was achieved within 5 min. The mixing bowl was water-jacketed to control the final dough temperature. The dough ingredients were flour, 28 g; yeast, 0.6 g; salt, 0.5 g; ascorbic acid, 2.1 mg; shortening or model fat, where included, 200 mg; water, as required by the predetermined water absorption of the flour.

For lipid analysis, duplicate doughs were frozen immediately after mixing, freeze-dried, finely ground, and finally dried over phosphorus pentoxide. Similar doughs mixed in triplicate were proved at 43 C and baked in miniature tins for 20 min at 220 C, giving one loaf from each mixing. Loaf volumes were measured by seed displacement after cooling for 30 min. The order of mixing was randomized. Duplicate samples of bread crumb were prepared for lipid analysis in the same way as the doughs.

Solvent Extractions

Petrol, bp below 40 C, chloroform, methanol, and butan-1-ol ('R' Grade, May and Baker, Ltd., Dagenham, England) were redistilled prior to use. Water-saturated butan-1-ol (WSB) was prepared by stirring butan-1-ol with excess water at 40 C for about 2 hr, and equilibrating the phases overnight at room temperature (ca. 20 C).

'Free' and 'bound' lipids: 'Free' lipid was extracted by percolation of ~6 ml/g petrol through a short column (4 cm x 2 cm diameter; dimensions not critical) of the dried, powdered dough, using ca. 10 g samples. After all the petrol had been removed, 'bound' lipid was extracted with WSB and purified by the Folch method.

Determination of Triglycerides

Measurement of radioactivity: Radioactivity was determined in lipid samples dissolved in a toluene-based scintillation mixture (13), using a Packard 'Tricarb' Liquid Scintillation Counter, and the measurements were converted into counts/min at 100% efficiency in the total 'free' or 'bound' lipid.

Total fatty acid determinations: 10-20 mg samples of 'free' and 'bound' lipids were saponified with 1.0 N KOH, and the unsaponifiable matter was extracted. The free acids were recovered and weighed.

Gas chromatography of methyl esters: Extracted lipids were transesterified (14), and the methyl esters were separated in a Pye Argon Chromatograph on a 120 cm x 0.4 cm column of 10% diethylene glycol succinate on 80-100 mesh kieselguhr. Butylated hydroxytoluene was used to protect the methyl esters from oxidation during preparation and analysis. Peak areas were measured using a Chromalog II integrator (Electronic Instruments Ltd., Chertsey, England). Margarate and oleate were calculated as percentages of the total methyl esters. The distribution of GTM between 'free' and 'bound' lipid fractions was calculated directly, and that of GTO from the difference in oleate in the lipid of doughs with and without added fat. These results, calculated from the total fatty acid contents of each kind of extract, are expressed as mg/100 g of the dried dough.

RESULTS

Model fats prepared from 1:7 mixtures of GTP or GTM with GTO, used at 0.7% w/w with fresh commercial flour, gave loaves of similar volume to those obtained using the same level of shortening. GTO employed alone was ineffective. A 1:15 mixture of saturated and unsaturated triglycerides, ineffective with the 'Baker's Pride' flour, was subsequently shown to be effective with the 'Democrat' flour, illustrating the considerable variation in the requirements of different fresh flours for solid fat.

It was shown using labeled GTP, that 86% remained 'free' and only 14% became 'bound' in the mixing of fresh flour doughs. Although most of the effective triglyceride remained in the 'free' lipid, a sufficient amount became 'bound' to make it uncertain whether the 'free' or 'bound' triglyceride, or both, exerted the beneficial influence on the gas-retaining properties of the dough. Further evidence was sought to show if the loaf volume was related to the amount or concentration of the saturated triglyceride in the 'free' or 'bound' lipid fractions.

The volumes of loaves baked from doughs made from fresh and stored flours containing model fats with different proportions of GTM and GTO and with GTO alone are shown in Table I. Fresh and stored flours were tested on separate days. The volumes of loaves baked on the two days, from fresh flour and bakery fat, were similar.

With the 'Democrat' flour, all the GTM/GTO mixtures were equally effective, and increased loaf volume as much as bakery fat, but GTO was ineffective. With the stored flour, however, addition of any of the model fats tended to reduce loaf volume. The loaf volume given by the 1:7 mixture was not significantly different from that of the other loaves containing model fats. Shortening, used at the same level in doughs made from this and other storage-deteriorated flours, has also caused loaf volume reductions

on other occasions.

The proportions of 'free' and 'bound' lipid in corresponding unbaked doughs and bread are also shown in Table I. Each fraction contains both flour lipid and model fat. There is a striking decrease in the ratio of 'free' to 'bound' lipid in doughs made from the stored flour compared with those made from fresh flour. In separate studies, a decrease in 'free' dough lipid with increasing flour storage time has been observed in three flours as they aged over a period of 2½ yr, but in contrast to work elsewhere (15,16) no decrease in the 'free' lipid of the flours has been observed.

Changing the composition of the model fat had no effect on lipid binding with either the fresh or the stored flour. The addition of fat had little or no influence on the level of the 'bound' lipid in dough made from fresh flour. Although the proportion of 'bound' lipid increased with the stored flour when fat was added to the dough, there is evidence from other storage studies in this laboratory that this is not always the case.

Contrary to the findings of others (2,9,17) using different methods of breadmaking or of 'bound' lipid estimation, the pattern of lipid binding in bread containing model fats was very similar to that found in dough.

Study of the binding of GTM during doughmaking (Fig. 1) confirmed that the proportion which became 'bound' was small and remained approximately constant and was similar for the fresh and stored flours within the range of the levels examined. The concentration of GTM (w/w dried dough) remaining 'free' increased as more was added to the dough, irrespective of the age of the flour. However, the concentration of GTM in the 'free' lipid of the stored flour doughs was higher than that in the 'free' lipid of the fresh flour doughs because of the smaller amount of 'free' lipid in the former (see also Table I).

Binding of GTO (Fig. 2) was quite different in the two flours. Much less remained 'free' in the doughs from stored flour than in those from fresh flour. Slightly more flour lipid was extractable from doughs which contained fat than from those which did not. Although the addition of fat did slightly affect the binding during dough-mixing of those flour lipids containing oleate, it was calculated that the small differences involved fall well within the error of the GTO determination.

The results of these determinations suggested that the 'free' lipid is more important to the expansion of the dough during baking than the 'bound' lipid. A baking test indicated that supplementation of a dough with a small amount of GTM, such that the plateau concentration in the 'bound' lipid of fresh flour doughs (9.5 mg/100 g dried dough; Fig. 1) was reached with a little also entering the 'free' lipid fraction, did not increase loaf volume. A further baking test with 1:39 and 1:7 GTM/GTO mixtures (Table II) confirmed this observation. This result supported the overriding importance of the saturated fat remaining in the 'free' lipid fraction of the dough.

The CBP baking performance of stored flours had earlier been shown to be improved greatly by addition of more fat; the stored flour used in this work required six times the normal level. The high concentration of GTM in the 'free' lipid of the stored flour doughs suggested that stored flour might also give improved loaf volume if extra oil, rather than extra fat, were added. A baking experiment with model fats containing GTP and GTO showed that loaf volume could indeed be increased as much by adding GTO only as by increasing the amounts of GTP and GTO simultaneously (Fig. 3).

The presence of unsaturated triglyceride in the model fat was shown to be unnecessary to its volume-improving action (Fig. 4), as 0.7% of a 1:7 GTP/GTC mixture was as effective in this respect as the same level of the similar

TABLE I

The Effect of Increasing the Proportion of Glyceryl Trimargarate (GTM) in the Model Fat on Loaf Volume and on Lipid Binding in Dough and Bread Using Fresh and Stored Flours

Model fat (0.7% w/w flour)	Fresh flour						Stored flour					
	No fat	GTO[a] only	GTM/GTO 1:15	GTM/GTO 1:7	GTM/GTO 1:3	With Fat	No fat	GTO only	GTM/GTO 1:15	GTM/GTO 1:7	GTM/GTO 1:3
Mean loaf volume (ml)[b]	107	105ns	119**	121**	121**	122**	109	98**	100*	105ns	99*
Lipid binding in doughs[c]											
'Free' lipid	0.56	1.35	1.32	1.35	1.35		0.13	0.63	0.62	0.64	0.58
'Bound' lipid	1.13	1.22	1.25	1.24	1.23		1.67	1.96	1.94	1.95	1.95
Total lipid	1.69	2.57	2.57	2.59	2.58		1.80	2.59	2.56	2.59	2.53
Lipid binding in bread[c]											
'Free' lipid	0.57	1.40	1.41	1.32	1.30		0.06	0.57	0.50	0.50	0.49
'Bound' lipid	1.10	1.24	1.22	1.22	1.18		1.71	2.11	2.02	2.00	2.03
Total lipid	1.67	2.64	2.63	2.54	2.48		1.77	2.68	2.52	2.50	2.52

[a]GTO = glyceryl trioleate.

[b]Each value is the mean of three replicates. The statistical significance of differences between loaf volumes with and without added fat is indicated as follows: ns = not significant, * = $p<5\%$, ** = $p<1\%$.

[c]Percentages, w/w dried dough or bread.

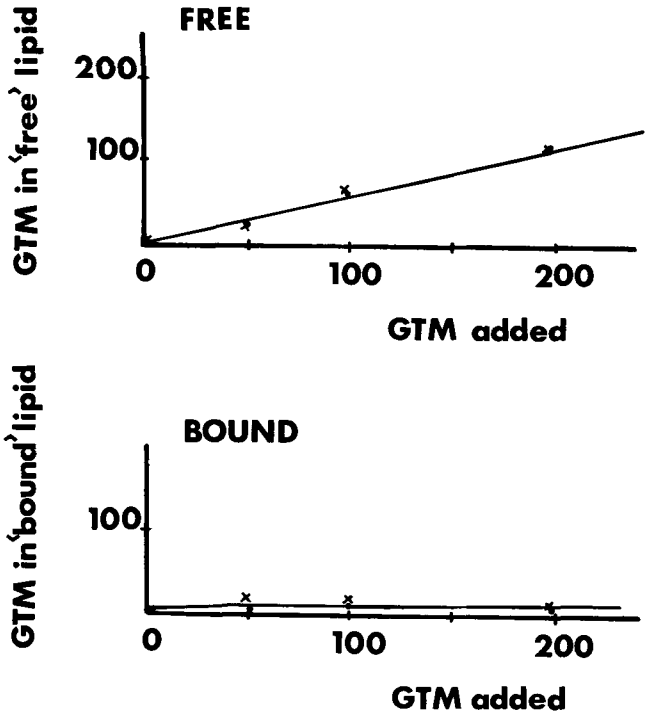

FIG. 1. The binding of glyceryl trimargarate (GTM) during mixing in doughs made from fresh and stored flours. GTM (mg/100 g dried dough) on both axes. • = Fresh flour dough; x = stored flour dough.

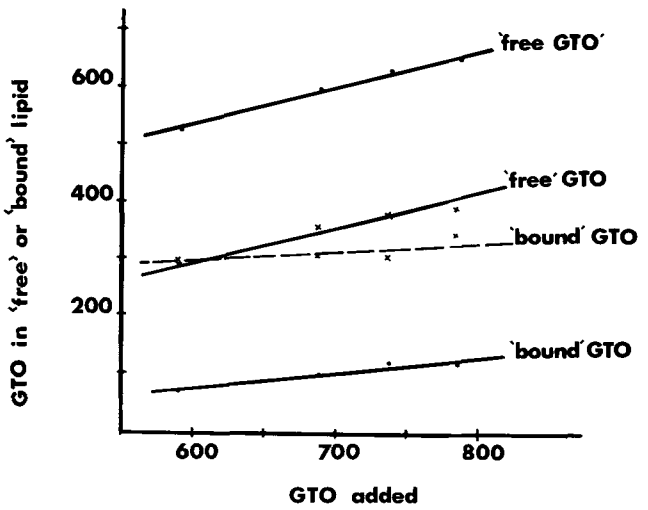

FIG. 2. The binding of glycerol trioleate (GTO) during mixing in doughs made from fresh and stored flours. GTO (mg/100 g dried dough) on both axes. • = Fresh flour dough; x = stored flour dough.

GTP/GTO mixture. GTC alone gave no improvement.

DISCUSSION

Model fats, each prepared from two pure triglycerides, were as effective in increasing the volume of loaves made from fresh flour as a good quality shortening of more complex composition and gave equally poor results with deteriorated stored flour. The surface activity and other characteristics of the partial glycerides present in shortenings need not therefore be involved in the explanation of their volume-improving effects.

As with shortenings, the presence of a solid component in model fats is a good indication that they will increase loaf volume, although solidity is not necessarily the property which determines its effectiveness. As the triglycerides are mutually soluble, the solid phase of the model fats is not exclusively saturated triglyceride.

The effective constituent of the model fat must be prepared from a fatty acid of adequate chain length. GTC, a fully saturated liquid triglyceride prepared from octanoic (caprylic) acid, did not increase loaf volume when used alone. It could, however, be used with GTP in model fats, confirming that unsaturated fatty acids are not essential to the action of shortening in breadmaking, and that saturated fat is not always beneficial if its physical properties are unsuitable [see also (2,3)].

Pure straight-chain hydrocarbons of a certain minimum chain length, although of a different chemical composition to fats, have been shown to be capable of increasing loaf volume; a minimum chain length of 22 carbon atoms corresponding to a melting point of 44 C was required (4).

Castor oil is one known exception of an oil which increases loaf volume in doughs made by conventional breadmaking processes (18); it has been shown during the present study also to be effective in the CBP. The texture of CBP

TABLE II

The Effect of a Further Reduction in the in the Proportion of Glyceryl Trimargarate (GTM) in the Model Fat on the Volumes of Loaves from Fresh Flour

Model fat (0.7% w/w flour)	GTO only	GTM/GTO 1:39	GTM/GTO 1:7
Mean loaf volume (ml)	105	106	115
Difference in volume from loaves with GTO only[b]	---	1 ns	9**

[a]GTO = glyceryl trioleate.

[b]Each value is the mean of three replicates. The statistical significance of the differences from loaves containing GTO only is indicated as follows: ns = not significant, ** =p<1%.

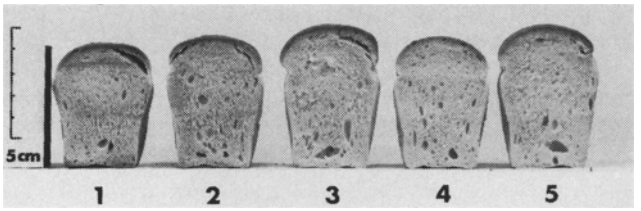

FIG. 3. Loaves baked from deteriorated stored flour containing model fats at normal and increased levels. Levels of fat addition (% w/w flour): loaf 1, 0.7%; loaves 2 and 4, 2.1%; loaves 3 and 5, 4.3%. Loaves 1, 2, and 3 contain 1:7 GTP/GTO. In loaves 4 and 5, the level of GTP is the same as in loaf 1 and only GTO is increased. (GTP = glyceryl tripalmitate; GTO = glyceryl trioleate.)

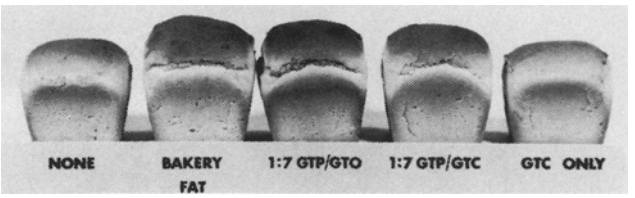

FIG. 4. The effect of replacing the GTO in the model fat by GTC on the volumes of loaves baked using fresh flour. (All model fats added at the level 0.7% w/w flour.) The significances of the differences in loaf volumes between loaves baked with and without fat were bakery fat, $p < 1\%$; GTP/GTO, $p < 1\%$; GTP/GTC, $p < 1\%$; GTC alone, ns. The differences between loaves containing bakery fat and GTP/GTO and between those containing GTP/GTO and GTP/GTC mixtures were not significant. (GTO = glyceryl trioleate; GTC = glyceryl tricaprylate; and GTP = glyceryl tripalmitate.)

bread was, however, judged to be inferior to that obtained using a commercial shortening, which itself was over 99% liquid at a proof temperature of 43 C. The hydroxyl group of ricinoleic acid would give castor oil opportunities for structural interactions not possible with glycerides of unsubstituted fatty acids.

The possession by a lipid of a suitable melting point does not of itself ensure its effectiveness as a bread improver. Mixtures of GTP (mp 65 C) with GTO improve loaf volume, whereas similar mixtures of palmitic acid (mp 64 C) with GTO are ineffective. The lack of effect may, of course, be due to interactions involving the free carboxyl group in palmitic acid (unpublished work).

Study of the binding of the saturated and unsaturated constituents of the model fats supports the hypothesis that 'free' saturated lipids of the molecular weight range predominating in lipids from natural sources are beneficial in baking [compare (10)]. In addition, the results suggest that it is important for the apparently ineffective constituent of the model fats, and the flour lipid also, to remain in the 'free' state. The traditional name 'free' lipid is not intended to imply that this fraction is unable to interact with other dough constituents but only that any associations formed are broken during the drying of the dough or during petrol extraction. In fresh flour doughs, results for the 1:7 GTM/GTO, the model fat closest in composition to shortening, showed that when it was used at the normal level of 0.7% flour, there was nonselective binding of the two triglycerides in agreement with earlier work on shortening (9).

The presence in the 'free' lipid of stored flour doughs of the same *amount* of GTM as in the corresponding fraction of the fresh flour doughs, and the resulting higher *concentration* of GTM in the depleted 'free' lipid of the stored flour doughs, showed that the deterioration in baking quality of the flour was not due to its inability to retain solid fat in the 'free' state.

Poor dispersion of fat can adversely affect the baking properties of dough (3) and less of the solid constituent may be required when the fat is added as an emulsion rather than as a solid (18). However, if the poor baking performance of old flour were simply due to the fat not dispersing well in the dough, an emulsifier such as glyceryl monostearate (GMS) should overcome this, but GMS has been found to have little effect.

When baking with either fresh or stored flours, loaf volume may be increased considerably by the addition of 4.3% (w/w flour) of GTO without any saturated triglyceride, although with fresh flour this is not as effective as the normal 0.7% of shortening. Thus, the idea that the barrier to gas transfer in dough consists simply of a close-packed triglyceride film at a lipid-water interface is untenable. This view is supported by work showing that fat did not completely line the gas cells of bread (11). However, the free dough liquor is a complex solution of water-soluble proteins, starch, sugars, and salts, accompanied by an immiscible lipid phase (20). In the lining of the gas cell walls of the dough, these other water-soluble constituents may associate with the saturated fat to form the barrier which retards the escape of gas or perhaps to strengthen the cell walls and delay their rupture (21).

The most obvious difference in composition between fresh and stored flours is the increase in fatty acid resulting from the lipolysis of the flour lipid during storage (16,22-24), which may induce additional binding of GTO and flour lipids. Lipolysis of bakery fat does not occur appreciably during mechanical development of dough (7). Little storage-induced change in flour protein has been found during the course of a study of the effect of prolonged storage on three flours (25), and it does not appear possible to account for increased binding of the model fats on this basis.

In the preceding work the accepted method of dividing the dough lipids arbitrarily into only two fractions, based on the use of dehydrated dough and two solvents of widely different polarity, was employed. It is arguable that the only truly free lipid is that which can be separated mechanically from the dough, for example during gluten-washing or by ultracentrifuging (20); this lipid is only a small proportion of the fraction extractable by nonpolar solvents such as petrol.

Basic studies of the influence of lipids on carbon dioxide transfer through the gas cell walls in the hydrated dough are needed particularly at the end of final proof and during the initial expansion under the influence of heat. Current concepts of lipid binding thus appear to require refinement if their relevance to bread-baking and particularly to the effects of shortening are to be established on a firm basis.

The composition of the added fat cannot be considered in isolation from that of the lipids of the flour used to make the dough. The fatty acids of the glycerides are mostly common to both, although present in different proportions, and extensive interaction between shortening constituents and the more readily available flour lipids must occur during mixing. The weak associations of these lipids with protein and starch may well determine the cohesion, physical strength, and gas-retaining properties of the expanding dough.

ACKNOWLEDGMENTS

Collaboration and financial support were provided by the Ministry of Agriculture, Fisheries and Food. Technical assistance and advice were given by the staff of F.M.B.R.A.

REFERENCES

1. Baker, J.C., and M.D. Mize, Cereal Chem. 19:84 (1942).
2. Baldwin, R.R., R.G. Johansen, W.J. Keogh, S.T. Titcomb, and R.H. Cotton, Cereal Sci. Today 8:273 (1963).
3. Chamberlain, N., T.H. Collins, and G.A.H. Elton, Ibid. 10:415 (1965).
4. Elton, G.A.H., and N. Fisher, J. Sci. Food Agric. 19:178 (1968).
5. Olcott, H.S., and D.K. Mecham, Cereal Chem. 24:407 (1947).
6. Fisher, N., B.M. Bell, and C.E.B. Rawlings, J. Sci. Food Agric. 24:147 (1973).
7. Mann, D.L., and W.R. Morrison, Ibid. 25:1109 (1974).
8. Wood, P.S., N.W.R. Daniels, and R.N. Greenshields, Ibid. 25:1045 (1974).
9. Daniels, N.W.R., J.W. Richmond, P.W. Russell Eggitt, and J.B.M. Coppock, Ibid. 17:20 (1966).
10. Daniels, N.W.R., J.W. Richmond, P.W. Russell Eggitt, and J.B.M. Coppock, Ibid. 20:129 (1969).
11. Standing, M.A., Ibid. 24:984 (1973).
12. Wheeler, D.H., R.W. Riemenschneider, and C.E. Sando, J. Biol. Chem. 132:687 (1940).
13. Dobbs, H.E., Anal. Chem. 36:687 (1964).
14. Daniels, N.W.R., D.L. Frape, P.W. Russell Eggitt, and J.B.M. Coppock, J. Sci. Food Agric. 14:883 (1963).
15. Sinclair, A.T., and A.G. McCalla, Can. J. Res. 15C;187 (1937).
16. Greer, E.N., C.R. Jones, and T. Moran, Cereal Chem. 31:439 (1954).
17. Chiu, C., and Y. Pomeranz, J. Food Sci. 31:753 (1966).
18. Fisher, E.A., and C.R. Jones, National Association Review, April (1932).
19. Baldwin, R.R., S.T. Titcomb, R.G. Johansen, W.J. Keogh, and D. Koedding, Cereal Sci. Today 10:452 (1965).
20. Mauritzen, E.R., and P.R. Stewart, Aust. J. Biol. Sci. 18:173 (1965).
21. Matsumoto, H., Bakers' Dig. 47(5):40 (1973).
22. Barton-Wright, E.C., Cereal Chem. 15:521 (1938).
23. Cuendet, L.S., E. Larson, C.G. Norris, and W.F. Geddes, Ibid. 31:362 (1954).
24. Morrison, W.R., J. Sci. Food Agric. 14:870 (1963).
25. Shearer, G., A. Patey, and D.J. McWeeny, Ibid. 26:337 (1975).

[Received May 3, 1976]