LIPID INTERACTIONS IN UNWORKED WHEAT FLOUR DOUGHS

by

ROBIN JOHN DAVIES

A

THESIS

Submitted to

THE UNIVERSITY OF ASTON IN BIRMINGHAM

in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy.

Department of
Biological Sciences

September, 1970.

Summary

A method of adjusting flour moisture level without the introduction of mechanical work was developed to study resulting lipid redistribution, changing physical properties and water distribution.

Three flours, two similar and one of different composition, were found to show closely similar qualitative and quantitative patterns of lipid redistribution.

Both extractable and unextractable additional binding of both neutral and polar lipid was produced by work-free hydration, the pattern changing as water distribution changed and unworked dough structures developed. When flour was fully hydrated, all polar lipid was extractably bound and a third of all neutral lipid bound, both extractably and unextractably in equal proportions.

A method was developed to successfully reconstitute solvent extracted flours with the extracted flour lipid and to add to such extracted flours, neutral fractions of extracted flour lipid, both a liquid and solid triglyceride fat and also a flour lipid fraction containing a low proportion of neutral lipid.

These manipulations were shown to produce redistribution of bound lipid. Work free wetting of these reconstituted flours produced increased binding of liquid, neutral lipid.

Critical moisture levels in the development of unworked dough structure were revealed by changes in lipid migration patterns during the course of simple hydration.

Even more neutral lipid binding was produced in mechanically developed doughs although reduced slightly by the presence of added salt.

A differential thermal analyser was used as a calorimeter to show that worked doughs, with different total moisture contents, had the same distribution of freezable and unfreezable water which was unaffected by the presence of salt. No added salt was found in solution in freezable water of these doughs.

The results of this study of simple hydration were discussed in terms of a hypothetical lipid binding site and related to other work with mechanically developed doughs.

Work supervised by

N.W.R. Daniels, B.Sc., Ph.D., F.R.I.C.
Spillers Limited,
Cambridge.

R.N. Greenshields, B.Sc., Ph.D., F.R.I.C.
Department of Biological Sciences,
University of Aston in Birmingham.

To my wife

Cathryn

ACKNOWLEDGMENTS

I would like to express my gratitude to the Directors of Spillers Limited for the opportunity to carry out this research at their Technological Research Station, Cambridge.

I am grateful to Professor J.B.M. Coppock, O.B.E., B.Sc., Ph.D., F.R.I.C., F.R.S.H., F.Z.S., Director of Research, P.W. Russell Eggitt Esq. B.Sc., Ph.D., F.R.I.C., M.R.S.H., Research Controller, and other members of the staff of the Technological Research Station for their encouragement and advice during this work. I would particularly like to thank Dr. P.W. Heaps and T. Webb Esq, for preparation of mechanically developed doughs with different water contents, for advice on the use of the Instron Tester and for many helpful discussions. I thank T. Pratt Esq for statistical advice and Spillers Limited, Central Laboratory for flour analyses.

I am pleased to acknowledge the guidance and encouragement of my two supervisors, Dr. N.W.R. Daniels and Dr. R.N. Greenshields, throughout the course of this work. My sincere thanks are due to Dr. Daniels for his constant advice and, during the writing of this thesis, his helpful criticism and patience.

I am also most grateful to my wife who not only typed this thesis with great accuracy but ensured the completion of its writing.

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INTRODUCTION

Introduction

In view of the considerable technological and academic interest in the role of lipids in modern breadmaking processes, the relationship between lipid and protein in wheat flour was chosen for the study reported in this thesis. The known association produced between lipid and protein when flour moisture content is increased drew attention to the role of water in the relationship. This study will therefore concentrate on the function of water in the lipid and protein relationship in wheat flour.

The approach and object of this work will be introduced by an account of the present understanding of the lipid and protein relationship in flour. This will amplify the technological interest in the subject. Cell membranes have long been the principle subject for the study of such relationships and provide the main source of information. The current state of development of these studies together with appropriate conceptual models will be presented with their relevance to the wheat flour system. Since this work will consider the relationship from the point of view of lipids, the role of lipids in breadmaking will be considered first.

There has been continued interest in the role of flour lipids in breadmaking during the past fifty years. However the precise functions of flour lipid in conventional breadmaking processes have not yet been fully established. Likewise, while there is no critical requirement for added fat in a fermented bread, the improving effects on loaf volume and crumb properties justify its widespread utilisation. Rheological studies

on dough have failed to show any significant effect due to lipids. Generally, studies on the role of lipid in breadmaking have involved adding shortening fats or lipid fractions to flour or fat-extracted flour. Bread has been produced from these ingredients and the effect has been judged in terms of loaf volume and crumb properties. These studies produced many contradictory results and few conclusions as reviews by Cookson and Coppock,(1956) Fisher, (1962) Daniels,(1963) and Mecham (1964) show. These reviews also discuss the many factors which must be considered in studies of lipids in breadmaking. These factors all have significant consequences for bread quality and in many cases were not appreciated by earlier workers. Examples are the use of different solvents, extraction methods, flour types, dough formulae and breadmaking procedures. Recent studies have allowed for these factors and the following conclusions have been reached about the role of lipid in breadmaking.

Lipid that can be removed in a soxhlet extractor with petrol or diethyl ether from freezedried, finely ground material is commonly considered to be 'free'. 'Bound' lipid is either that remaining after petrol extraction and released by acid hydrolysis or that which is removed by a polar solvent system. Examples of such systems are chloroform, methanol and water (Tsen, Levi and Hlynka (1962)) and water-saturated n-butanol (Mecham and Mohammad (1955)).

Free flour lipid was shown to be important to breadmaking properties (Pomeranz et al. (1965)). Free and to a lesser extent bound polar lipid, especially galactosyl

glycerides contribute towards loaf volume and crumb texture. (Daftary et al. (1968)). Yet this polar lipid fraction had a detrimental effect if neutral or bound lipids were not also present (Hoseney et al. (1969)). Free neutral lipid affects the dough rheological properties (Tao and Pomeranz (1968)).

Trace amounts of organic solvents e.g. hexane have a remarkable improving effect on bread quality (Ponte (1968)) if added fat is present in the dough. The dough lipid content was obviously implicated in this effect. Flour lipid is also involved in oxidation reactions during the breadmaking process (Hawthorn, (1961), Tsen and Hlynka, (1962), Bloksma, (1963), (1964), Pomeranz, (1968)).

Less than ten years ago, new breadmaking processes were introduced which have revolutionised the breadmaking industry. These processes, develop dough in powerful high speed mixers for only a brief period, a matter of minutes, allowing the traditional three hour fermentation to be omitted. Two important points emerged when these processes were introduced, both of which were reported by Baldwin et al. (1963), (1965).

Firstly a critical requirement for special added fat was demonstrated. For satisfactory bread quality it was necessary to include fat with a melting point above the final dough temperature in the dough formula. Secondly a high proportion of both flour lipid and added fat became unextractable with petrol as a result of such vigorous dough mixing.

The demonstration of a requirement for hard fat

renewed interest in the role of both flour lipid and added fat in breadmaking. This work also stimulated interest in the phenomenon of lipid binding since for the first time there appeared to be a direct link between the lipid-protein relationship and modern bread technology.

This was not the first account of lipid binding. For example Dill mentioned the difficulty of extracting lipid from gluten in 1925. Similar observations were made by Sullivan and Near (1927), Fisher and Halton (1933), Blish (1936) and McCaig and McCalla (1941).

In 1947, Olcott and Mecham found that when flour was wetted to a moisture level above 30%, the proportion of free lipid was reduced from 70% to 40%. The lipid was found to be bound to the glutenin fraction of flour protein. Although mixing the wetted flour into a dough further reduced the free lipid to 6%, the comparatively simple process of wetting without the introduction of mechanical work produced an equally large effect on lipid binding. It is for this reason that this means of actuating the association was chosen for this study. The wetting effect was confirmed by Wootton in 1966 but otherwise has never been studied in detail.

The work reported in this thesis ~~firstly~~ examined the precise effect of simple hydration on the flour lipid distribution. Consequently it was necessary to develop a method of wetting flour to required moisture levels which avoided other factors known to affect lipid binding. Since these other factors all relate to mechanically worked doughs the avoidance of work was a primary requirement.

Once the effect of hydration on flour lipid distribution was established the constituents of the lipid-protein-water system were manipulated. To achieve this, flours with different protein contents were considered and hence different breadmaking potentials and the flour lipid composition altered. This provided information about the nature of the lipid and protein relationship and the role of water in this relationship when work is excluded.

The objective of this work was to use a knowledge of the system to construct a working theory and model of an unworked dough structure in which the functions of flour constituents are defined.

While it is true that the majority of studies of lipid binding reported in the literature have involved mechanically developed doughs and are therefore not directly relevant to this work; a brief account of the present understanding of lipid binding in wheat flour doughs and the factors which influence it will be discussed to provide a background to the technological significance of this subject.

Other factors which have been shown to be involved in and to affect lipid binding include the atmosphere of the dough mixer, other dough ingredients, baking, trace amounts of organic solvents and fat extraction with solvent and return of extracted lipid. It is impossible to consider these factors independently as some if not all of the effects are interrelated.

The complex process of dough mixing, particularly to levels of total work input used in modern processes

was found by Daniels et al.(1966) to increase bound lipid to 50% of total lipid. Binding was non-selective for saturated free lipid and triglycerides. No preferential binding of linoleic acid could be detected although Baldwin et al. (1963) and Chiu et al. (1966) suggested this in studies with otherwise similar results.

The increase of binding with increase of work input was also shown by Pomeranz et al. (1968) and in greater detail by Daniels et al. (1967). More binding occurred (up to 75% of total lipid) with higher rates of work input and higher total work input levels. Excessive mixing however caused release of bound lipid (Pomeranz et al. (1968)).

Daniels et al. (1967) also demonstrated that mixing chamber atmosphere could influence lipid binding. More bound lipid was detected when oxygen eliminated from the mixer. This was achieved by flushing with nitrogen, mixing at much reduced pressure or by using a 'continuous' type mixer in which air space was minimized. These last two are used commercially.

In a study on the effect of dough ingredients, Chiu and Pomeranz(1966) found common salt to decrease binding. Mecham and Weinstein (1952), Wootton (1966), and Pomeranz et al. (1968) also found this but disagreed on whether it was predominantly neutral (Pomeranz) or polar (Wootton and Mecham and Weinstein) lipid that was released. Chapman (1969) has suggested that electrostatic lipid binding would be adversely affected by increased ionic concentration.

The effect of adding fat (shortening) was found by

Mecham and Weinstein (1952) not to affect lipid binding. Chiu and Pomeranz (1966) found no binding of shortening during dough mixing unlike Baldwin et al. (1963) and Daniels et al. (1966) who did. Pomeranz et al. (1968) found less binding of saturated fat of the type essential to modern breadmaking processes.

As well as having an improving effect on bread quality, addition of organic solvents to dough promotes lipid binding particularly of phospholipids in dough systems containing lard. (Ponte et al. (1964).

Baldwin et al. (1963) Daniels et al. (1966), and Chiu et al. (1966) found that baking further increased binding. Daniels et al. found it to a lesser degree (a further 10%) than Baldwin et al. and Chiu et al. who found more substantial increases (38%). Binding due to baking was greater in doughs which had received more mechanical development. A higher proportion of non-polar than polar lipid was bound by baking than by mixing only. Only traces of polar lipid remained free, after baking, selective trapping of triglyceride occurred.

Chiu et al. (1968) and Pomeranz et al. (1968) and Daniels et al. (1969) found that a large increase of binding occurred when the free lipid of flour was replaced with shortening fat. Since Daniels showed that binding increased in such doughs, mixed in both air and nitrogen, the sensitivity to mixer atmosphere appeared to have been eliminated by fat extraction. This was probably related to the removal of poly unsaturated free flour lipid by solvent extraction and suggests a role of these lipids in

the oxidation processes of dough mixing. Baking released bound neutral lipid from air mixed doughs and more total lipid was recoverable from bread baked from fat-extracted flour.

Daniels et al. (1969) also returned free lipid to fat extracted flour to see the effects of fat extraction on lipid binding properties. An identical binding pattern was found to the original with no extra binding. When free neutral and free polar fractions of flour lipid were returned to fat-extracted flour, more of each fraction was bound when added individually than if both were added together (Pomeranz et al. (1968)) less water saturated n-butanol extracted polar lipid was bound when returned to fat-extracted flour than petrol extracted polar lipid.

The link that is apparent between the role of lipids in breadmaking and lipid binding emphasises the technological interest in the lipid and protein relationship. This relationship, which is the essence of this thesis, can be found as the basis of a variety of important biological systems. These include enzyme reactions, blood coagulation, lipase reactions and serum lipoprotein structure. A further example, the structure of cell membranes has received particular attention and provides the most comprehensive information that is available on the lipid-protein association.

Most major activities of cells occur in, on or through cell membranes, therefore research has concentrated on their physical and chemical structure. Now that protein

structure and synthesis studies have made such progress attention is now turning to the problems associated with cell organisation which are directly related to membrane structure.

The original concept of a cell membrane was one of a physiological barrier, with specific permeability properties, between cell contents and external medium. This concept has been developed as a result of intensive investigation and models for the structure have been proposed. A structure for wheat flour gluten (Coppock (1959)) was based upon the first membrane model and for this reason this early model will be described. This model was proposed by Danielli and Davson in 1943, largely as a result of the similarity between a structure predicted by Gorter and Grendel (1925) and the early electron micrographs of membranes (Danielli and Harvey (1934)).

This model was postulated as comprising three continuous layers, a bilayer of phospholipid 35Å thick sandwiched between two layers of protein each 20Å thick. Electrostatic interaction between the charged groups of the phospholipids and the extended polypeptide chains would produce stabilisation of the membrane structure.

Coppock (1959) suggested that gluten structure may prove to be an 'immature myelin' and consist of alternating protein and phospholipid layers. Such a structure, with interlocking units of various coherence, could be related to variations in gluten strength. This postulate was taken a stage further by Grosskreutz (1961) who considered

the elastic properties of such a configuration in terms of the relative bond strengths involved. The width of the bimolecular leaflets inherent in this structure is about the spacing (47Å) found to be characteristic of wheat grain and flour polar lipid (Traub, Hutchinson and D.G.H. Daniels (1957), D.G.H. Daniels (1958)). However, since polar lipid forms only a quarter of the total flour lipid and the neutral remainder is also involved in lipid binding, it is likely that a more comprehensive model of gluten will be necessary for a complete understanding of the lipid-protein relationship.

A triple-layer membrane structure was reported by many workers using electron microscopy (Sjöstrand and Zetterqvist (1955) Sjöstrand (1953) and Robertson (1958) and interpreted in terms of the Danielli-Davson model. X-ray diffraction also offered confirmation of a continuous bimolecular leaflet structure (Finean et al. (1966)) as did the many studies of myelin-like lipid structures in water. These bilayer forms, closely resembling the structure of myelin form spontaneously in water in a manner reminiscent of the binding of flour lipid by wetting (Olcott and Mecham (1947)). Although these myelin figures form without the presence of protein they indicate a natural conformation of lipid in water.

The Danielli-Davson model was further developed by the incorporation of a 'pore' concept to explain the permeability of membranes to water and small ions by Danielli (1954) and Stein and Danielli (1956). However

this model could not explain the passage of water through the ordered lipid bilayer.

Robertson (1957, 1958, 1959, 1964, 1966) has concluded from many electron microscope results that all cell membranes have a common size, structure and origin. He then proposed a universal unit membrane.

Meanwhile other workers (Green and Tzagoloff (1966), Green and Fleischer (1963) and Benson (1966) were less willing to accept either the bimolecular leaflet or unit membrane concept. They considered that although the early electron micrographs confirmed first concepts of membrane structure, the drastic procedures involved in sample preparation and electron microscopy must make their interpretation difficult. Particularly in view of the affects of denaturation and dehydration on membrane structure reported by Sjöstrand and Barajas (1968) and Lenard and Singer (1968).

Sjöstrand (1963a) led the reappraisal of the bimolecular leaflet and unit membrane concepts. He concluded that these concepts were weakened rather than strengthened by the available evidence. He then reported that membranes appear particulate and proposed a new model (Sjöstrand 1963b, 1965, 1967,a,b, 1968 a,b.) It was suggested that membranes consist of globular particles of lipid in the form of small micelles. A surface layer of protein prevents these micelles from fusing into larger droplets or bimolecular leaflets. The globular micelles will have lipophilic cores with a diameter dependent on fatty acid

chain length (about 40Å). Adjacent micelles will be held together by both hydrogen bonds and electrostatic interactions (Lucy and Glauert (1964a,b.). Such sub-units have been reported by other workers to have sizes near to 50Å Nilsson (1964a,b.) (1965) Blasie et al. (1965) Robertson (1963) Weier et al. (1966) Malhotra (1966) Branton (1966) and Rosa and Tsou (1965).

The globular micelle model has the advantages of being more dynamic and allowing permeability properties to be protein controlled. The focus is changed from lipid to protein as the backbone of membrane structure. Fleischer et al. (1965) (1967) confirmed this by removing 90% of the lipid from mitochondria without changing the structure. The role of lipids is suggested as one of conformation modification and is thus more suited to the biological function of membranes.

Green & Tzagoloff (1966) and Green & Goldberger (1967) observed that if membrane sub-units are released with bile salts, they will realign spontaneously in two dimensions without the presence of external information molecules. Lipid is suggested as an intrinsic part of these repeating units since if absent, the formation of three dimensional structures occurred instead of the two dimensional association found in the presence of lipid (Green and Goldberger (1967) McConnell et al. (1966).

Membrane permeability was ascribed to the presence of pores between globules. The pore size proposed for the model was a radius of 4Å which agreed well with measured values. (3.5Å Paganelli & Solomon) (1957-1958)

4.2Å Goldstein and Solomon (1960-1961) 4.0Å Lindemann and Solomon (1961-1962) 4.25Å, Villegas and Barnola (1960-1961)).

In a recent study Ottolenghi and Bowman (1970) removed phospholipid from membranes with the enzyme phospholipase C. The protein structure that remained after enzyme digestion was not as regular as a unit membrane theory would suggest. Furthermore the possibility of enzyme attack of the phospholipid rules out an outer protein layer. When red blood cells were similarly treated and examined by nuclear magnetic resonance and optical rotatory dispersion (Glazer et al. (1970)) the lipids were drastically altered but the protein substantially unaffected. Conversely, protein conformation was altered by heating the membranes while the lipids remained unaltered. These results suggested that lipid and protein components of membranes show a degree of independence.

The globular micelle concept of membrane structure after the models of Sjöstrand and Lucy is the most satisfactory way of representing the membrane structure that is available at present. In this model, both protein and lipid play important roles yet there is still only limited knowledge of the nature of the interaction between them. Many workers consider that the contribution of hydrophobic interaction should not be underestimated. (Kauzmann (1959) Scheraga (1961) Scheraga et al. (1962) Green & Fleischer (1963) Perutz et al. (1965) Green & Perdue (1966) Green & Tzagoloff (1966) Green et al. (1967)

Since the presence of water is essential to hydrophobic interaction the importance of water to membrane structure and the significance of binding of both neutral and polar lipid solely by the addition of water to flour is emphasised. Likewise the increased binding that occurs when mechanically developed doughs are baked (Baldwin et al. (1963) Daniels et al. (1966)) may be hydrophobic in character since heating strengthens hydrophobic interactions.

It is apparent from this account that lipid binding and the role of lipids in modern bread technology are related and that this relationship involves the lipid-protein-water interaction. Interest in this interaction in biological materials has been considerable and is increasing as there is still much to be understood. It is the intention of this work to contribute to the knowledge and understanding of this subject, particularly in the little studied area involving the changes that take place in lipid distribution during the initial wetting of flour to form a dough; before the introduction of any mechanical work whatsoever.

EXPERIMENTAL

Materials

Flours

All three flours studied were milled commercially yet were obtained unbleached, untreated and without added powders. The hard wheat flours were designated GOL 1 and GOL 2 and the soft wheat flour HIL. Sufficient quantities of each flour for these studies were stored in sealed tins. The grists, milling data and results of analyses are given in Table E:1.

Table E:1.	GOL 1	GOL 2	HIL
Wheats (%)			
Hard Winter	20		
No. 2 Atlantic Manitoban	80	67.5	
Russian		22.5	
Prime Hard Australian		10	
English			75
Australian			15
Plate			10
Flour divide %	100	100	100
Extraction rate %	72.5	72.5	70-72
Moisture %	14.05	12.50	12.60
Protein % (dry wt.basis.N x 5.7)	14.5	15.0	6.9
OIL (by hydrolysis) %	1.76	(1.10)	1.40
Oil (total extractable) %	1.653	1.572	1.090
Colour grade (K-J &Aunits)	2.5	1.4	3.0
Ash (gross)	0.58	0.65	0.4

Solvents.

All solvents used were of analytical quality and in the case of light petroleum and acetone, redistilled immediately before use. The light petroleum used was the fraction distilling between 40°C and 60°C. Only distilled water was used.

Fats.

Free flour lipid

Free flour lipid, for reconstitution of fat extracted flours and addition of extra lipid to flours, was obtained by percolation of light petroleum through flour (GOL 1 or GOL 2) contained in glass tubes (130 x 8cm.) at the rate of 5 litres/Kg. flour.

Petrol was removed from the free lipid solution using a rotary evaporator (Büchi Rotavap RSB/B), with the temperature held below 50°C and a constant bleed of oxygen-free nitrogen through the apparatus.

A trace of quinol was added to the petrol solution as a precaution against oxidation and the solvent free lipid was stored under oxygen-free nitrogen in sealed bottles at -20°C.

Flour free lipid was made up of 75% neutral lipid and 25% polar lipid. The proportions of each fatty acid present in GOL 2 free neutral lipid is given in Section 8, Table 8:8b

Tripalmitin.

A commercial sample from Loders and Nucoline Ltd, Cairn Mills, Silvertown, London E.16.

Coconut Oil

A commercial sample of coconut oil, also from Loders

and Nucoline Ltd. refined, deodorised and with 200 p.p.m. butylated hydroxy anisole antioxidant added.

The fatty acid composition determined by gas liquid chromatography and thermographs of these fats obtained with a Dupont 900 differential thermal analyser are given in Section 8.

Methods: Part One - Preparations.

Flour wetting.

A number of methods for wetting flour were examined to find a method which satisfied the following requirements. The method had to allow a precise control of flour moisture adjustment, give even moisture distribution through the flour and avoid mechanical mixing or any other factor that could affect lipid distribution. The liquid nitrogen technique was the only entirely satisfactory method found.

Wetting by spraying.

A known weight of flour was spread thinly over a metal tray. The appropriate weight of water to give the required moisture level was sprayed evenly over the flour. The wetted flour was then scraped off the tray and freezedried. Flour moisture content was determined before and after spraying.

Equilibration.

Flour samples were placed in sealed vessels over water and the moisture content examined every 24 hours. After two weeks, the moisture level had risen to only 25% and mould growth had started.

Fog.

Flour was allowed to fall repeatedly through a column of dense fog generated by passing steam over solid carbon dioxide. Flour moisture level increased by about 2% with each pass but above 20% the flour particles caked together and could not be fed slowly and evenly through the column.

Addition of ice

Ice was ground with a pestle and mortar at -20°C and added to flour at this same temperature. At -20°C the ice could not be ground to a fine powder because it tended to melt. Even after three weeks storage at -20°C poor distribution of water resulted when the flour was thawed.

Liquid nitrogen technique.

The previous method was improved (Davies, Daniels and Greenshields (1969)) by grinding ice at the temperature of liquid nitrogen. At this temperature (-196°C) ice was sufficiently brittle to be ground to a fine powder. An iron mortar and pestle was used as a ceramic mortar could not with-stand the low temperature.

The procedure finally used in this work was to suspend the flour sample (100g.) in a beaker of liquid nitrogen and add dropwise the necessary amount of water, calculated to give the required moisture level, to liquid nitrogen in the mortar. The ice was then ground to a fine powder at this low temperature and added as a slurry in liquid nitrogen to the beaker containing the flour suspended in liquid nitrogen. The beaker was transferred to a deep freeze at -20°C for the nitrogen to evaporate (this was sufficiently vigorous to mix the flour and ice) and the mixture to equilibrate.

After 24 hours, the sealed beaker was allowed to thaw at room temperature. After two hours, samples were removed for moisture determination and the wetted flour frozen and freezedried.

Fat extraction of flour.

Fat-extracted flour was prepared by percolation of light petroleum through flour packed in a glass tube (130 x 8cm.) The tube had a constriction at the bottom with the outlet covered by fine mesh stainless steel gauze and plugged with absorbent cotton wool. At least 5 litres of petrol were passed through each kilogram of flour and the extraction took about 24 hours. The extracted flour was spread out on sheets of filter paper until all residual solvent had evaporated and then sealed in tins.

Separation of specific fractions from flour lipid.

Neutral lipid rich fraction.

To obtain 7.5g neutral flour lipid with which to reconstitute fat extracted flour, 10g flour lipid obtained by percolation extraction was separated by a scaled up version of the method used in lipid distribution determinations.

Flour lipid was dissolved in chloroform (250ml) and the solution mechanically shaken with 110g activated silicic acid (100 mesh, Mallinckrodt Chemical Works, St, Louis, U.S.A.) The chloroform solution was separated from the silicic acid on a sintered glass funnel (POR 3) and the silicic acid washed with 4 x 50ml aliquots of chloroform. The solution including washings was concentrated using a rotary evaporator and shaken with a further 100g activated silicic acid. The mixture was filtered and the silicic acid again washed with 4 x 50ml portions of chloroform. Chloroform was completely removed from the neutral lipid fraction on a Büchi rotary evaporator with a continuous bleed of

oxygen-free nitrogen. The lipid (yield 7.49g) was stored in a glass bottle, under oxygen-free nitrogen, with a trace of quinol at -20°C.

Neutral lipid deficient fraction.

The acetone-insoluble fraction of flour lipid has a low content of mono-, di- and triglycerides (Fisher (1962)) and is considered to consist mainly of phospholipids and galactosyl glycerides (Cookson, Ritchie and Coppock. (1957)).

Acetone precipitation was therefore used to obtain a fraction of flour lipid that had a low neutral lipid content. Percolation extracted flour lipid (17.4g) was dissolved in a minimum of light petroleum and added to chilled (0°C), freshly distilled acetone (500ml). After 12 hours in a refrigerator at 2°C, the mixture which consisted of a white bulky solid in a yellow solution, was separated in chilled bottles in a centrifuge the acetone solution removed by decantation and the precipitate washed with small amounts of cold acetone.

Complete removal of acetone from the solid by rotary evaporation required particular care. If the product, which was a creamy light solid when free of acetone, was exposed to air or warmed above 10°C it became an intractable brown gum which could not be reclaimed for reconstitution of fat extracted flour. The flask containing the product was kept cool during rotary evaporation with an ice bath. Oxygen-free nitrogen was bled into the evaporator throughout the two hours necessary to completely remove acetone. The material (yield 14%) was stored in a sealed bottle under oxygen-free nitrogen at -20°C. The final product consisted of 57% polar and 43% neutral lipid.

The silicic acid method of neutral and polar lipid fraction separation could not be used as methanol was required to strip the polar lipid from silicic acid. Methanol could not then be removed from the polar lipid without producing the sticky brown gum. Removal of methanol was only part of the problem as removal of too much neutral lipid from the fraction appeared to reduce the stability of the product. This was also found if the acetone insoluble fraction was refined by reprecipitation since a less stable product was obtained when the acetone was removed.

Reconstitution.

In Section 6 the requirements that must be satisfied by a method for adding lipid to flour for lipid distribution studies are discussed. The method used in this study (Davies, Daniels and Greenshields (1970)) was developed from the liquid nitrogen technique for work-free wetting of flour.

The required weight of lipid was added as droplets from a pipette to liquid nitrogen in a mortar. Flour lipid was brittle at the temperature of liquid nitrogen and could be ground to a fine powder. This powder was added as a slurry in liquid nitrogen to flour (fat extracted or untreated) also suspended in liquid nitrogen. The mixture was held in a deep freeze while the liquid nitrogen evaporated vigorously. The sealed container was then allowed to warm to room temperature.

This technique was used to return lipid to extracted flour, add neutral-rich and neutral-deficient lipid fractions, tripalmitin, and coconut oil to extracted flour and to add

extra flour lipid to extracted and unextracted flour.

The effects of fat extraction by percolation and reconstitution were examined by comparisons of lipid distribution and composition (neutral and polar) of the original, fat extracted and reconstituted flours and of the extracted and added lipids.

Dough mixing.

Doughs were mechanically mixed for two studies. The first was to see the effect on lipid distribution of two levels of work, with and without salt. The second was to produce worked doughs from different flours, with and without salt, which had the same work input totals yet had different total water contents. The freezable water contents of these doughs were then measured with a Dupont 900 differential thermal analyser.

In both studies, the doughs were mixed in a modified Brabender DoCorder fitted with a 300g flour Farinograph bowl. A constant dough weight of 470g was used. The same rate of work input was used, 0.2 h.p.min./lb/min., after a dry premix at 35r.p.m. for 2 minutes and a wet premix at the same speed for one minute. The stainless steel-clad mixing bowl was held at 30°C by a water jacket and no efforts to exclude air were made. The calibration factor for this dough weight was 1.50×10^2/lb. To maintain constant work input, the speed of the mixer was matched to the torque registered on the recorder chart. The calibration figures are given in Table E:2.

Table E:2.

Calibration figures for dough weight of 470g.
Rate of work input 0.2 h.p.min /lb /min.

Torque m.kg	Mixer speed. r.p.m.
0.90	167
0.95	158
1.00	150
1.05	143
1.10	137
1.15	131
1.20	125
1.25	120
1.30	115
1.35	111
1.40	107
1.45	103.5
1.50	100

In the first study doughs were mixed to work input totals of 0.4 and 4.0 h.p.min /lb, with and without 2% added salt (sodium chloride R.Q.) on a flour weight basis. Doughs were cut into small pieces and freezedried immediately after mixing.

In the second study (Davies and Webb (1969)) all doughs were mixed to the same total work input, 0.6 h.p. min /lb. The total water content was varied between the limits imposed by introducing the level of work and handling the doughs. The doughs made from GOL 1 had moisture levels ranging from 36.8% to 47.9% and from HIL, the range was 36.9% to 44.75%.

Rewetting and wetting by blending.

In Section 7, the rewetting of freezedried, wetted flours is described. In view of the reported damaging effects of rehydration of freezedried flour (Williams and Hlynka (1968)) the freezedried flours were rewetted to 14% moisture by the liquid nitrogen method before rewetting by Olcott and Mecham's (1947) blending wetting technique. The liquid nitrogen technique should have minimised the damaging effects of heat of hydration and rapid swelling that could be caused by blending wetting.

Blending wetting was performed in a stainless steel M.S.E. Atomix container, flour was added to the appropriate amount of water in the mixer. A Variac transformer was used to keep the mixer speed to a minimum and mixing was stopped as soon as the 'dough' was homogeneous.

Moisture

Moisture contents of all samples were determined by the American Association of Cereal Chemists (Anon (1962)) Vacuum Oven method. Duplicated samples were heated for two hours at 105°C under a vacuum of at least 28in.Hg. Lids of the tins were only put in place at the end of the two hours while the samples cooled in a desiccator. Moisture was expressed as a percentage of water on a wet weight basis.

Preparation of samples for lipid distribution determinations.

All material was freezedried to a moisture content of about 2% using a pilot scale freezedryer (Edwards High Vacuum Ltd, Model 30P1/484) in about 12 hours. Flours with moisture contents below 25% were freezedried in trays covered with porous paper to prevent loss of flour into the pump. Dried material was then ground to fine powder in the ceramic mortar of a Pascall end-running mill. The material was ground to pass through a 0.28 mm. silk screen (5 XX). Reduc-tion to uniform size was necessary for quantitative lipid extraction (Pomeranz (1967)). If material was not analysed immediately it was stored in sealed plastic bags under oxygen-free nitrogen at -20°C.

Lipid distribution.

Standardised techniques were used throughout this study for the determination of free and bound lipids and the separation of these lipids into neutral and polar fractions. The methods were published by Daniels et al.

(1966) (1969) but since they were used to define the lipid distribution they will be described here.

Free

After freezedrying, grinding and sieving, duplicated samples were weighed into soxhlet thimbles (33 x 80mm) and extracted with light petroleum (boiling between 40° and 60°C). in soxhlet extractors for 7 hours. Extracts were dried to constant weight on a rotary evaporator. The yield of free lipid was calculated on a dry weight basis after transfer to a small weighing bottle.

Bound.

Extracted samples were air-dried and when free of solvent bound lipid was determined by a polar solvent extraction adapted from the method of Tsen, Levi and Hlynka (1962). Samples were mixed in a chilled stainless steel container of an M.S.E. Atomix blender for two minutes at half speed with a mixture of methanol, chloroform and water (50:25:20). A further 25 ml chloroform was then added and blended for 30 seconds. Finally 25ml water was added and blended for a further 30 seconds. The homogenate was poured into a 250ml centrifuge bottle and cooled in an ice-bath for 30 minutes. The mixture was then centrifuged at 2000r.p.m. (M.S.E. Major centrifuge) for 30 minutes (1100g.)

Three layers were produced in the bottle, a lower clear chloroform solution separated from the upper aqueous methanol layer by a central solid dough-like layer of extracted, hydrated sample. The top, aqueous layer was poured away and an aliquot of the lower chloroform layer removed with a pipette. This solution was filtered through absorbent cotton wool and dried on a rotary evaporator. The

residue was redissolved in chloroform and dried to constant weight in a small weighing bottle. Total yield of bound lipid was calculated from the lipid content of the aliquot.

Unextractable.

Total free and bound lipid, extracted from flours before any moisture increase, was considered the total extractable lipid. Any loss of total extractable lipid as a result of moisture adjustment was considered to be due to unextractable binding of lipid to form an unextractable fraction.

As a result of wetting, total extractable neutral or polar lipid in some cases exceeded the original values. This suggested the presence of an unextractable fraction in the original flour, a possibility confirmed by greater total lipid values determined by acid hydrolysis than total extractable lipid. When results were expressed as percentage of flour dry weight, such unextractable lipid was assigned a negative value where necessary. When values were expressed as percentages of total lipid present in a flour the sum of the maximum values for total neutral and total polar lipids were taken as 100%.

No effects on lipid distribution could be attributed to either the treatment with liquid nitrogen or freeze-drying.

The extraction of minor amounts of non-lipid material by these extraction procedures was anticipated from the results of studies on the petrol soluble lipoprotein lipopurothionin. (Balls and Hale (1940), Axford, Elton, Fisher

and Redman (1968) and Redman and Fisher (1968)). Non-lipid material was eliminated to some extent by evaporating extracts to dryness, then redissolving in solvent before finally drying to constant weight. Folch washings of extracts was not considered practicable when so many samples were involved. The use of standardised procedures on freezedried material was thought to minimize differences in non-lipid content between samples.

Separation of free and bound lipid into neutral and polar fractions.

This separation was based on a method proposed by Morrison (1963). Duplicated lipid samples (100mg) were dissolved in 50ml $CHCl_3$ and shaken with 10g activated silicic acid for ten minutes on a Microid mechanical shaker. Mallinckrodt 100 mesh silicic acid (Mallinckrodt Chemical works, St. Louis, U.S.A.) was prepared for this purpose by repeated washings with methanol to remove fines and activated by drying in a vacuum oven at 105°C for 4 hours before leaving open to atmosphere for 24 hours.

The chloroform solution (containing hydrocarbous, triglycerides, diglycerides, sterol esters and some free fatty acids) was removed by filtration through a sintered glass funnel (POR 4) using a slightly reduced pressure. The silicic acid was washed with 4 x 25ml portions of chloroform and the combined chloroform extracts evaporated to constant weight on a rotary evaporator to give the neutral lipid yield.

Polar lipids were removed from the silicic acid by five successive washings with 25ml portions of methanol.

The methanol solution of polar lipids including the washings was evaporated to dryness, redissolved in chloroform and dried to constant weight to give the polar lipid yield.

The efficiency of this separation was checked and examined to see which lipid classes were included in the neutral and polar classification.

Firstly the amount of lipid removed by each washing was weighed individually in a separation of free and bound lipid samples into neutral and polar fractions. Then each fraction was examined by thin-layer chromatography (t.l.c.) by two different solvents. Tables E:3 and E:4 show the percentage of total reclaimed lipid removed by each portion of solvent during the separations of free and bound lipids.

Table E:3

Efficiency of neutral and polar separations. Free lipid.

	Volume ml	% of total	Running total %	% of fraction
chloroform	50	49.4	49.4	64.3
	25	16.3	65.7	21.5
	25	5.8	71.5	7.6
	25	3.3	74.8	4.2
	25	2.0	76.8	2.4
methanol	25	15.6	15.6	66.7
	25	5.8	21.4	24.9
	25	1.1	22.5	4.5
	25	0.4	22.9	2.0
	25	0.3	23.2	1.9

Table E:4

Efficiency of neutral and polar separation. Bound lipid.

	volume ml	% of total	Running total %	% of fraction
chloroform	50	9.9	9.9	54.7
	25	3.9	13.8	21.5
	25	2.2	16.0	12.2
	25	1.2	17.2	6.6
	25	0.9	18.1	5.0
methanol	25	67.0	67.0	81.7
	25	8.7	75.7	10.6
	25	3.1	78.8	3.8
	25	1.8	80.6	2.2
	25	1.3	81.9	1.7

For the t.l.c. examination silicic acid (Keiselgel G nach Stahl E) was spread 0.25mm thick on 20 x 20cm plates with a Desaga applicator. Plates were activated at 105°C for 2 hours and stored in a dry-box. The two solvent systems used were a neutral system of light petroleum, diethyl ether and water (60:40:1) and a polar system of chloroform, methanol and water (80:25:2). Samples of each lipid fraction (0.5mg) were chromatographed by both solvent systems and visualised by charring with concentrated sulphuric acid. The resulting chromatograms are shown in Figures E:1. E:2, E:3 and E:4.

Thin layer chromatography plates.

Figures E:1-4 Identification of lipids.

Key

Neutral solvent system.

1. Polar lipids.
2. monoglycerides.
3. 1,2 diglycerides.
4. 1,3 diglycerides.
5. Free fatty acids.
6. triglycerides.
7. sterylesters, hydrocarbons.

Polar solvent system.

a. lysolecithin.
b. lecithin.
c. digalactosyl glyceride.
d. glycolipids.
e. monoglycerides.
f. neutral lipids including diglycerides.

Figure E:1.

Free lipid, neutral solvent system.

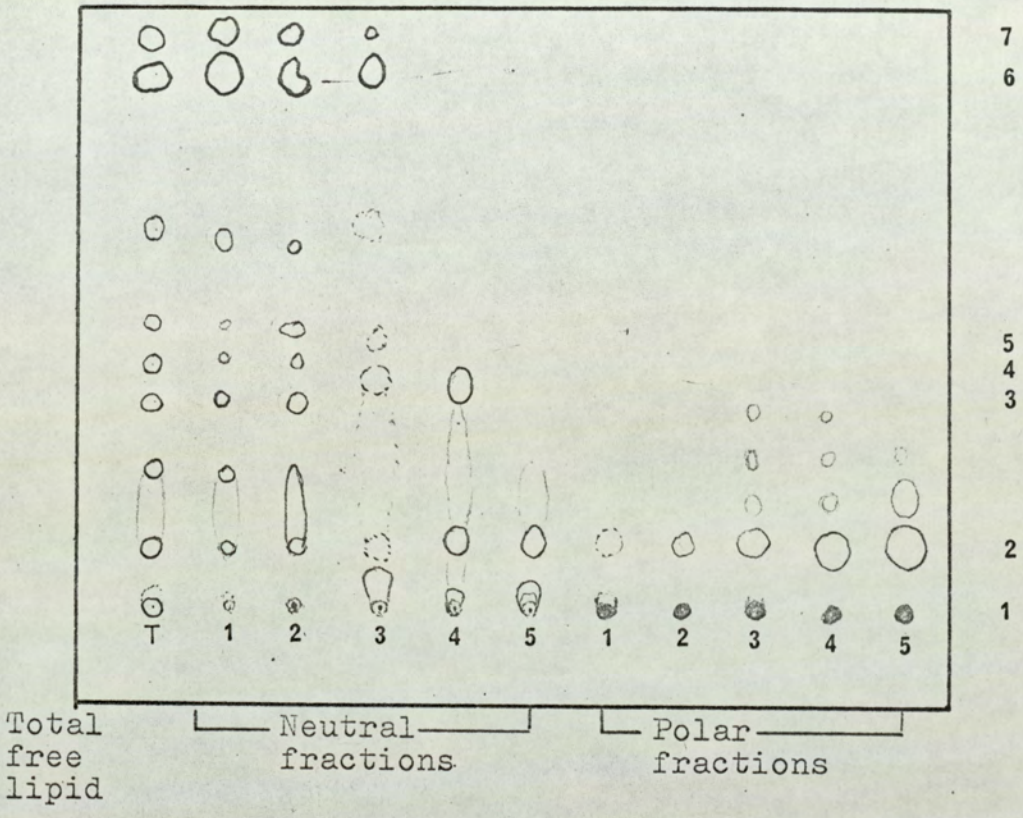


Figure E:2. Free lipid, polar solvent system

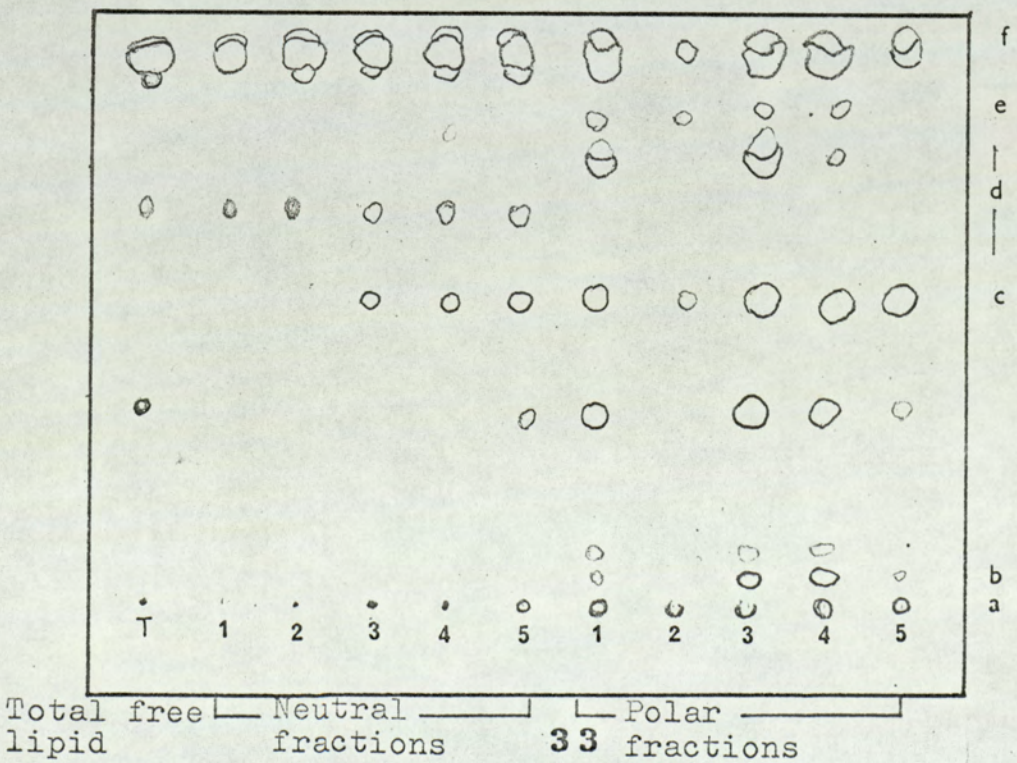


Figure E:3.

Bound lipid, neutral solvent system.

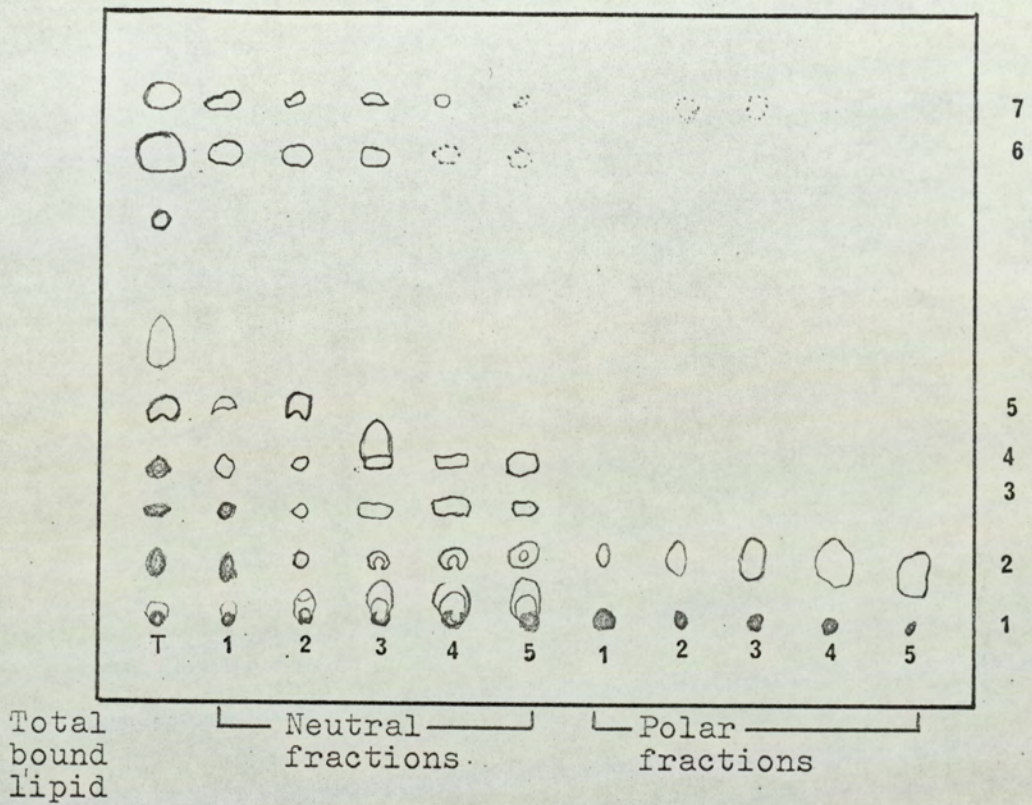
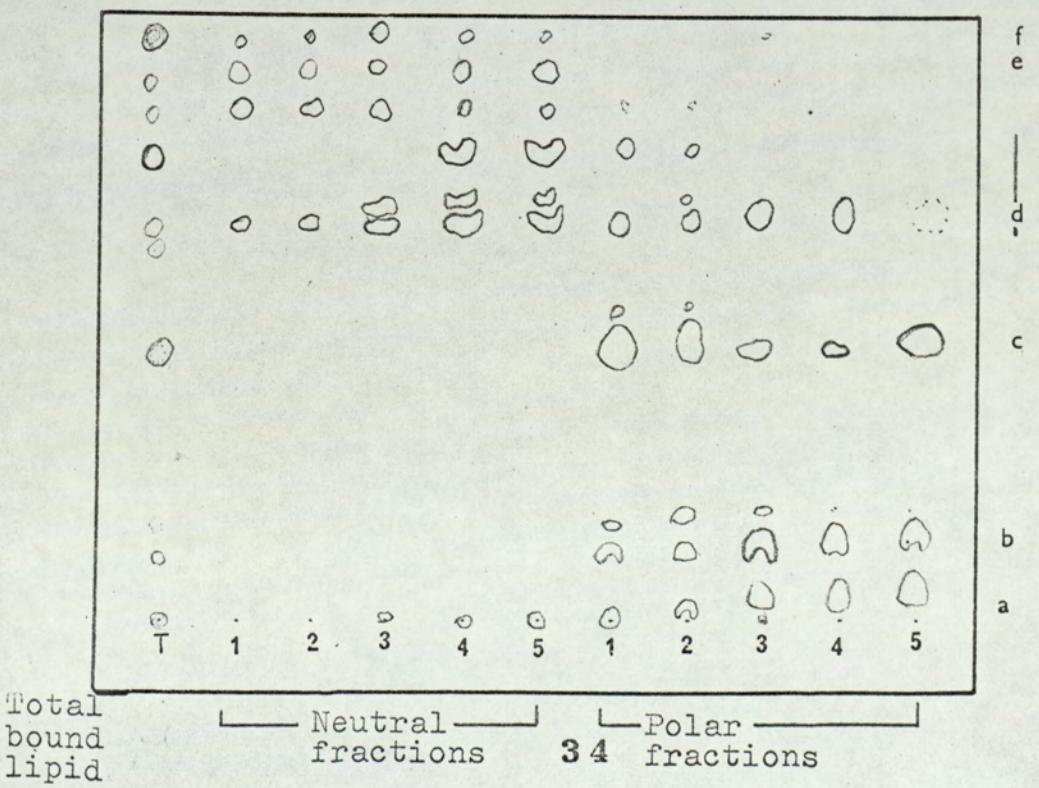


FIGURE E:4. Bound lipid, polar solvent system.



The chromatograms showed that the separation classified hydrocarbous, steryl esters, triglycerides, diglycerides and some free fatty acids as neutral and monoglycerides, glycolipids and phospholipids as polar.

Gas liquid chromatography.

The fatty acid compositions of flour free lipids, coconut oil and tripalmitin were determined by gas liquid chromatography. Details of the techniques and apparatus that were used were reported by Daniels et al. (1966)(1969). The methyl ester derivatives were prepared by the rapid method of Peisker.(1964).

Differential thermal analysis.

A Dupont 900 differential thermal analyser was used with a Disc cell attachment as a calorimeter to determine freezable water in dough and wetted flour (Davies and Webb (1970)). The apparatus was first calibrated to correlate the magnitude of the recorded endotherm of melting ice with the amount of water involved in the ice-water transition at $0^{o}C$. A correlation coefficient E was determined by measuring the areas recorded for the endotherms associated with known weights of water at $0^{o}C$.

$$E = \frac{H.m.}{A.T.} \quad \text{.....(1)}$$

Where H was the heat of fusion of ice (79.71mcal /mg), in the weight of water, m, mg, A, the area of the endotherm in square inches and, T, the sensitivity of the recorder (Y axis) in ^{o}C/in. when the X axis sensitivity, S, was fixed at $10^{o}C$/in. and the heating rate, a, at $10^{o}C$/min.

The value of E used was 17.50 mcal/^{o}Cmin.

When used as a calorimeter, the sample, in a lidded cup, was placed on the reference thermocouple and an empty lidded cup used as reference. Doughs were sealed in aluminium foil immediately after mixing and flour samples (5-10 mg each) were cut from the centre of each and weighed in tared sample pans on a torsion balance (V.D.F. 'United 10') to 0.01mg.

The weight of freezable water in each dough sample (W) was obtained from

$$W = \frac{A.E.T.S.}{H.a.} \quad \ldots\ldots\ldots\ldots (2)$$

Volume shrinkage.

The samples for the photograph of flours wetted to different moisture levels were obtained by cooling aluminium rings of radius 1.75cm and height 4.4cm and filling each completely with unthawed flours wetted by the liquid nitrogen technique. A metal plate was placed over each ring and the flours left to thaw for two hours. For shrinkage measurements, the rings were completely filled again, to compensate for shrinkage, with firstly rapeseed and secondly flour. The volume of the former and the weight of the latter required in each case was measured. For the photograph, the rings were lifted off the 'doughs'.

Load relaxation measurements with an Instron Tester.

An Instron Tester (Table model) was used in studies of load relaxation properties of wetted flours. Unthawed wetted flours of different moisture levels were placed in 2oz flat bottomed bottles (diameter 3.6 cm) to a depth of 1.5cm The bottles were sealed and left at room temperature

for two hours before examination. Each bottle containing a sample was placed on the load cell, the recorder zeroed and an anvil (circular of diameter 2cm) attached to the crosshead lowered onto the sample at a constant speed of o.5 cm/min. When the load reached 180g the relaxation of the material under constant deformation was noted every 6 seconds for three minutes.

Treatment of results.

In all studies in which the effect of wetting on lipid distribution was examined the following treatment of results was adopted. Lipid distributions were determined at specific moisture levels and the means of duplicated results reported. To obtain the Figures, with curves for each fraction, smooth lines of best fit through the values of each determined moisture level of total free, total bound, free neutral and bound polar lipids were drawn. From these lines, values at each 2½% moisture level were taken. Values for free polar and bound neutral fractions at each moisture level were then inferred by difference and plotted. Small adjustments were then made to the lines in order to obtain smooth curves for each fraction which closely followed the results.

Values for unextractable fractions were obtained by difference between the maximum neutral and polar totals and found values. For comparisons within flours the values were also expressed as percentages of total lipid present. this total was made up of the sum of the maximum neutral and polar totals.

Derivation of lipid migration diagrams.

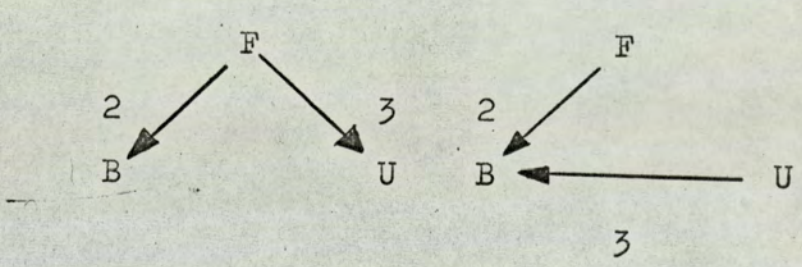
The migrations of neutral and polar lipids were considered separately. The differences between free and bound fractions for increments of moisture level increase (either 2½% or 5% intervals) were considered migrations, any discrepancies between the differences were attributed to the unextractable fraction.

Values were expressed as percentages of total lipid present and the migrations illustrated in triangle form, (a lipid fraction at each corner and arrows to show direction of movement) or as block diagrams.

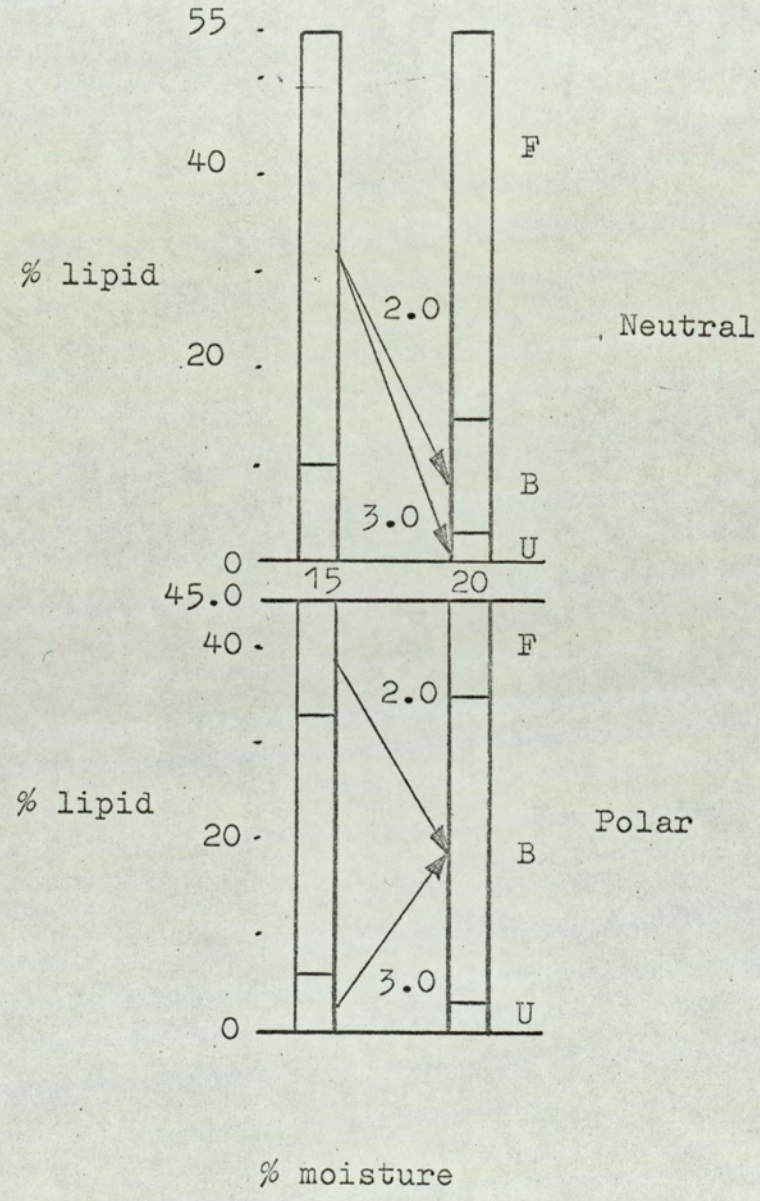
An example is given below to demonstrate the derivation of the Figures.

Moisture content %	Free		Bound		Unextractable.	
	neutral	polar	neutral	polar	neutral	polar
15	45	12	10	27	0	6
20	40	10	12	32	3	3
difference	-5	-2	+2	+5	+3	-3

Moisture level change



Example of lipid migration diagram.



Statistical treatment of results.

Values expressed as percentages of flour dry weight.

Flour	Total	FT	BT	NT	PT
GOL 1	1.650	1.125	0.523	0.958	0.690
GOL 1W	1.422	0.676	0.746	0.742	0.680
GOL 1FE	0.486	0.064	0.422	0.136	0.350
GOL 1FEW	0.438	0.004	0.435	0.119	0.320
GOL 2	1.574	1.016	0.556	0.924	0.648
GOL 2W	1.388	0.716	0.673	0.803	0.586
GOL 2FE	0.436	0.049	0.387	0.128	0.308
HIL	1.090	0.696	0.415	0.708	0.403
HIL W	1.025	0.533	0.492	0.599	0.426
HIL FE	0.318	0.035	0.283	0.109	0.209
HIL FEW	0.324	0.022	0.302	0.104	0.220
GOL 1RT	1.531	1.107	0.424	1.113	0.418
GOL 1RTW	1.417	0.636	0.781	0.906	0.511
GOL 2RN	1.251	0.834	0.417	0.879	0.372
GOL 2RNW	1.193	0.614	0.579	0.832	0.361
GOL 2RXN	1.914	1.535	0.379	1.553	0.361
GOL 2RXNW	1.856	1.148	0.709	1.485	0.372
GOL 2RP	0.785	0.181	0.604	0.306	0.479
GOL 2RPW	0.720	0.120	0.600	0.234	0.486
S.E. mean	0.0228	0.0156	0.0181	0.0216	0.0123
least significant difference.					
P= 0.05	0.054	0.036	0.045	0.051	0.029
P= 0.01	0.072	0.049	0.061	0.069	0.039
P= 0.001	0.097	0.066	0.081	0.092	0.053

Key to flour titles (over page)

Key to flour titles.

Flours GOL 1, GOL 2 and HIL.

W, indicates value at 50% moisture, F.E., indicates fat extracted by percolation extraction, R, indicates reconstituted, T, with total free flour lipid, N, with free neutral lipid, XN, with extra free neutral flour lipid and P, with an acetone-insoluble fraction of flour free lipid.

Table E:6.

Effect of moisture level on lipid distribution of flour GOL 1.

Values taken at 5% moisture intervals. Expressed as percentages of total lipid present (1.718%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	50.7	14.5	6.2	24.1	0.3	3.3
20	50.7	13.6	6.5	23.8	0.0	4.5
25	47.2	10.5	7.6	23.2	2.4	8.2
30	41.3	7.6	10.2	27.3	5.7	7.0
35	37.6	4.7	10.4	31.4	9.2	5.8
40	37.0	3.8	9.7	34.0	10.5	4.1
45	37.0	3.8	9.1	34.6	11.1	3.5
50	37.0	3.8	7.6	36.1	12.6	2.0
55	37.0	3.8	6.4	37.3	13.8	0.8
60	37.0	3.8	5.6	38.1	14.6	0.0
Standard Errors of Means	± 1.85	± 1.29	± 2.28	± 1.72		

Table E:7.

Effect of moisture level on lipid distribution of flour GOL 2. Values taken at 5% moisture intervals. Expressed as percentages of total lipid present. (1.572%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	51.8	12.5	6.4	29.3	0.8	0.0
20	51.5	11.5	7.5	28.2	0.0	2.1
25	49.7	9.1	7.9	27.5	1.4	5.2
30	47.0	6.8	8.0	28.9	4.0	6.1
35	43.8	5.0	8.1	31.9	7.1	4.9
40	41.7	3.5	8.3	34.6	9.0	3.7
45	40.9	3.0	8.6	34.3	9.5	4.5
50	41.5	2.8	8.9	34.0	8.6	5.0
S.E. of Means	± 2.36	± 2.50	± 1.78	± 1.94		

Table E:8.

Effect of moisture level on lipid distribution of flour HIL. Values taken at 5% moisture intervals. Expressed as percentages of total lipid present (1.089%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	51.4	10.6	10.0	27.5	0.5	0.8
20	51.4	10.6	10.4	26.1	0.1	2.2
25	51.4	10.6	10.5	24.6	0.0	3.7
30	50.5	8.8	10.1	25.0	1.3	5.1
35	46.3	6.9	12.6	27.5	3.0	5.5
40	44.1	5.9	13.8	30.3	4.0	2.7
45	42.8	5.3	13.4	32.8	5.7	0.8
50	43.0	4.9	12.3	34.0	6.6	0.0
55	45.9	3.3	11.6	34.2	6.4	0.4
S.E. of Means	± 3.4	± 2.4	± 3.6	± 2.8		

Table E:9.

Effect of moisture level on lipid distribution of fat extracted flour reconstituted with total free flour lipid. Values taken at 5% moisture intervals. Expressed as percentages of total lipid present. (1.662%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	57.2	9.0	8.0	17.6	0.6	7.6
20	54.7	8.9	10.8	18.1	0.3	7.2
25	43.8	7.5	15.3	19.3	6.7	7.4
30	37.6	6.0	17.7	20.8	10.5	7.4
35	35.4	4.9	19.1	22.7	11.3	6.6
40	34.9	3.8	19.1	25.8	11.8	4.6
45	34.9	3.3	17.3	29.5	13.6	1.4
50	34.9	3.3	16.7	30.9	14.2	0.0
S.E. of Means	± 2.24	± 1.68	± 2.39	± 1.83		

Table E:10.

Effect of moisture level on lipid distribution of fat-extracted flour reconstituted with neutral flour lipid at the original level. Values taken at 5% moisture intervals. Expressed as percentages of total lipid present. (1.293%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	60.3	4.6	8.7	24.6	0.0	1.8
20	57.2	6.6	9.6	24.4	2.2	0.0
25	54.1	5.4	10.9	24.1	4.0	1.5
30	51.2	4.5	14.5	24.4	3.3	2.1
35	48.3	3.3	15.6	26.3	5.1	1.4
40	46.7	1.7	16.6	27.2	5.7	2.1
45	46.7	0.9	17.3	27.5	5.0	2.6
50	47.8	0.4	16.6	28.3	4.6	2.3
55	49.3	0.8	14.8	29.2	4.9	1.0
S.E. of Means	± 2.88	± 2.16	± 3.07	± 2.35	-	

Table E:11.

Effect of moisture level on lipid distribution of fat-extracted flour reconstituted with neutral flour lipid at double the original level. Values taken at 5% moisture intervals. Expressed as percentages of total lipid present (1.932%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	74.5	4.7	5.8	14.2	0.8	-
20	74.5	4.4	6.3	14.5	0.3	-
25	73.0	3.9	8.1	14.9	0.0	-
30	70.8	3.2	9.6	15.7	0.6	-
35	68.2	2.6	11.7	16.3	1.0	-
40	65.2	2.0	14.0	16.9	1.8	-
45	63.1	1.4	16.7	17.5	1.2	-
50	61.1	1.0	18.2	18.1	1.7	-
55	59.5	0.5	19.2	18.5	2.3	-
60	58.4	0.1	20.1	18.9	2.4	-
S.E. of Means	± 1.92	± 1.44	± 2.05	± 1.57		

Table E:12.

Effect of moisture level on lipid distribution of fat-extracted flour reconstituted with an acetone-insoluble fraction of free flour lipid. (neutral lipid deficient). Values taken at 5% moisture intervals. Expressed as percentages of total lipid present (0.956%).

	Free		Bound		Unextractable	
% moisture	neutral	polar	neutral	polar	neutral	polar
15	14.5	5.4	11.7	50.1	12.3	6.0
20	13.4	5.7	11.6	50.8	13.5	5.0
25	12.5	5.6	17.5	46.0	8.5	9.9
30	12.1	4.7	25.7	38.8	0.7	18.0
35	11.8	3.9	26.3	39.3	0.4	18.3
40	11.5	3.2	22.5	46.0	4.5	12.3
45	11.2	2.5	19.4	49.8	7.9	9.2
50	10.5	2.1	13.6	51.1	14.4	8.1
S.E. of Means	± 3.90	±2.92	± 4.10	± 3.18		

Figure E:6.

Lipid fraction migration diagram. Effect of moisture level on flour HIL. Expressed as percentages of total lipid present.

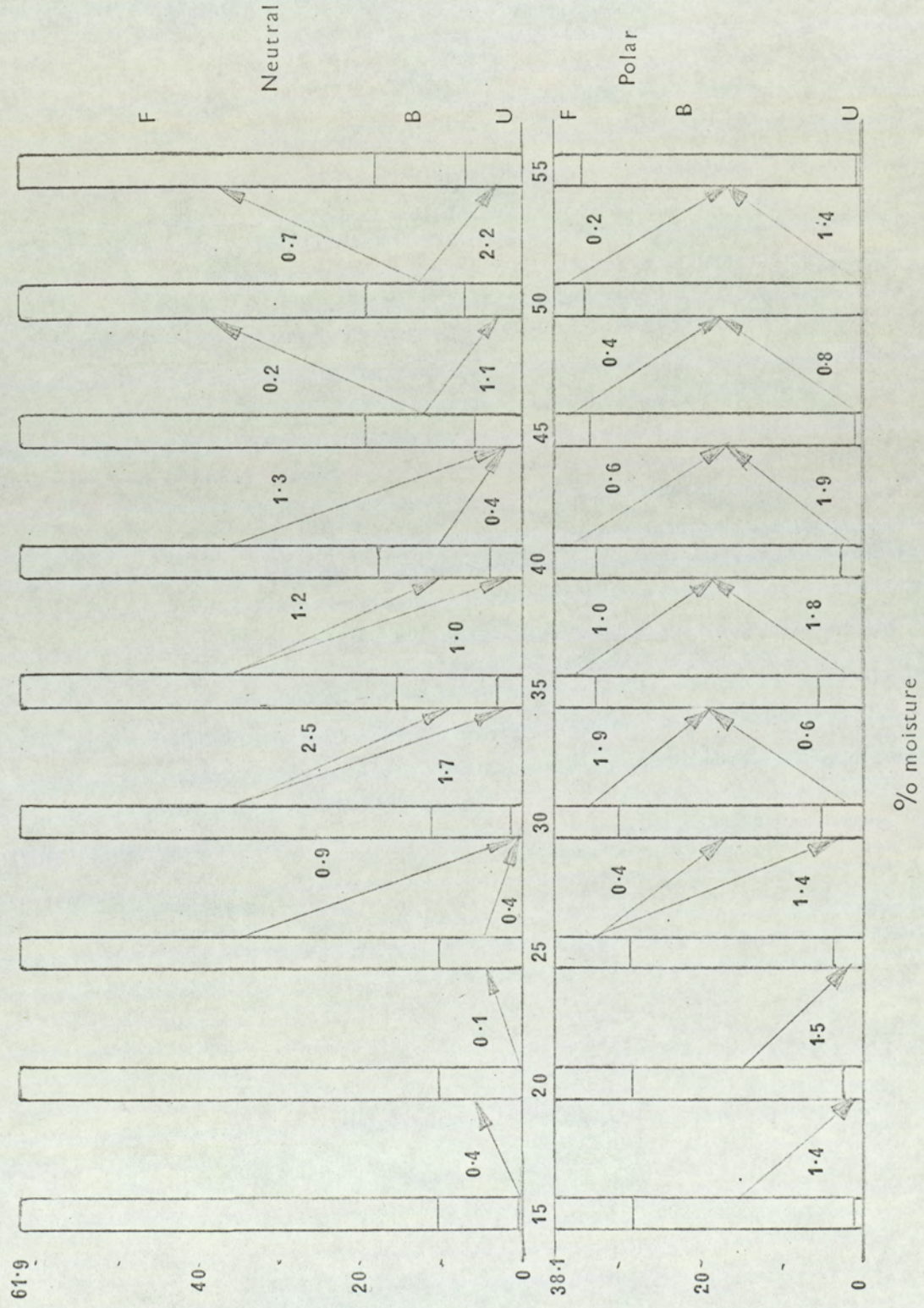


Figure E:7

Lipid fraction migration diagram. Effect of moisture level on flour reconstituted with total free lipid. Expressed as percentages of total lipid present.

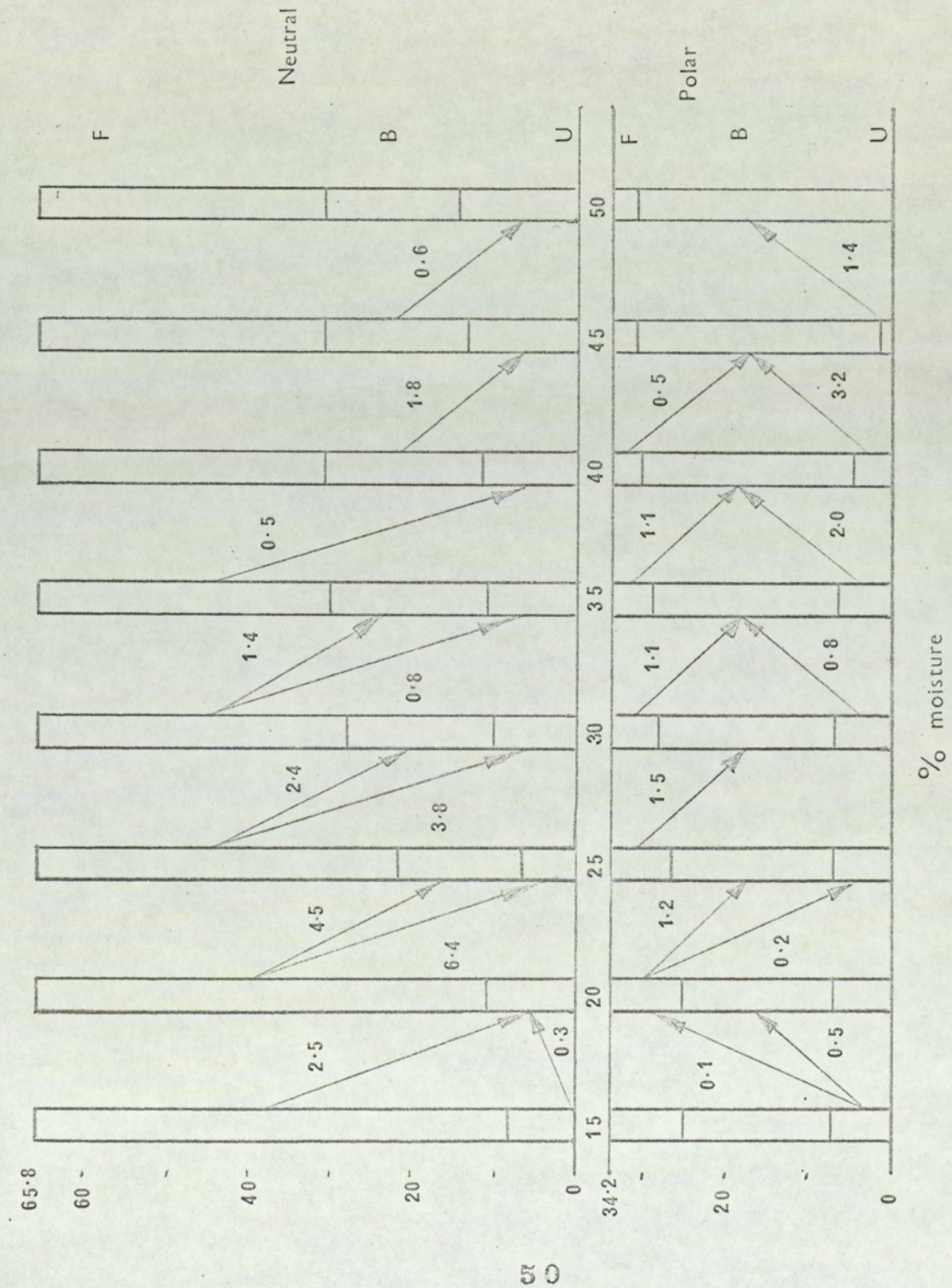


Figure E:8

Lipid fraction migration diagram. Effect of moisture level on flour reconstituted with free neutral lipid. Expressed as percentages of total lipid present.

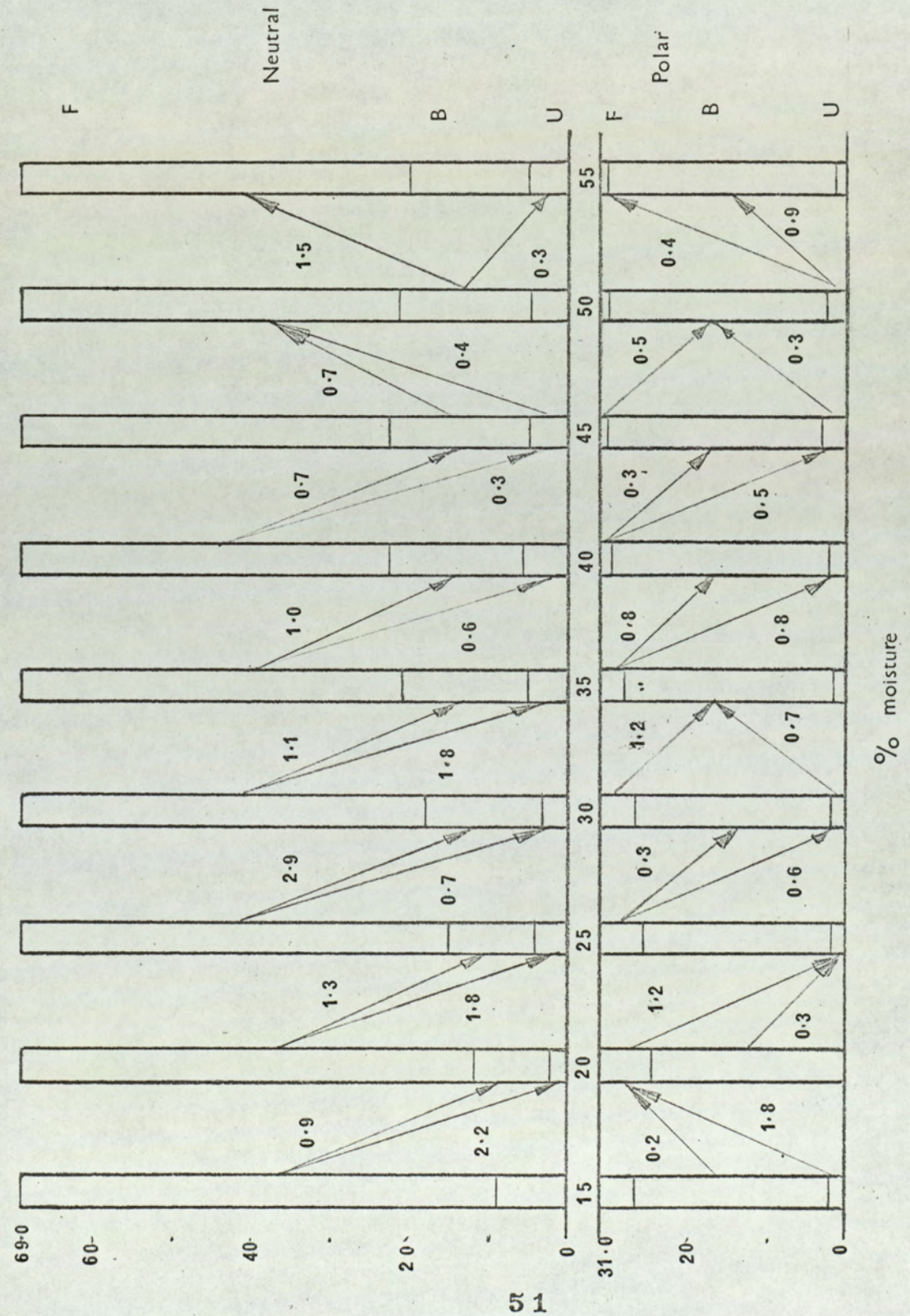


Figure E:9.

Lipid fraction migration diagram. Effect of moisture level on flour reconstituted with extra free neutral lipid. Expressed as percentages of total lipid present.

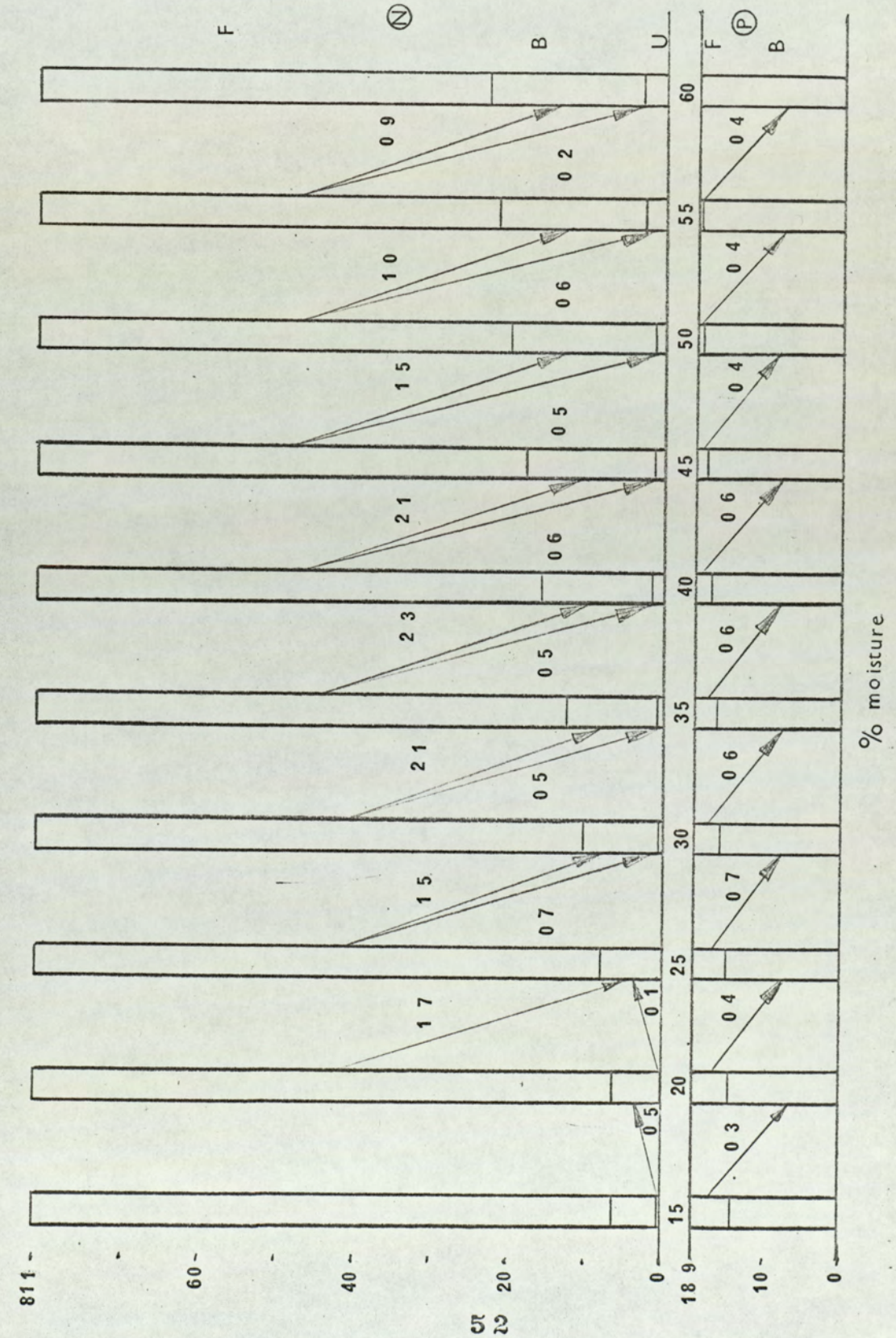
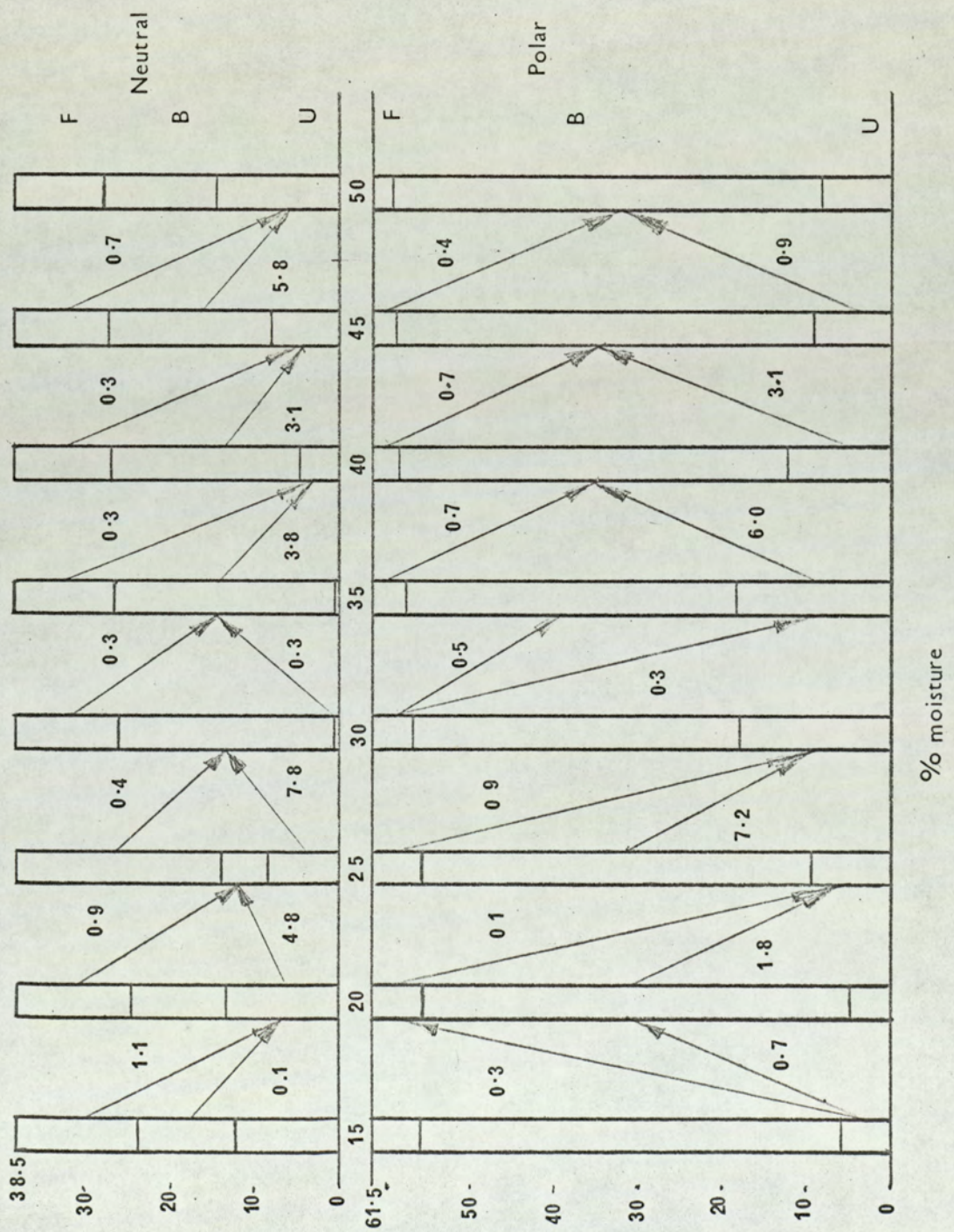


Figure E:10.

Lipid fraction migration diagram. Effect of moisture level on flour reconstituted with a neutral lipid deficient fraction of flour free lipid. Expressed as percentages of total lipid present.



SECTION ONE

Effect of wetting on lipid distribution in flour.

Extraction of Free and Bound Lipid

For the initial stages of the study involving an examination of the effect of wetting on lipid distribution, an untreated, unbleached hard wheat milled from a commercial grist was chosen. The composition of the flour is given in detail in the Materials section, the flour being identified by the abbreviation "GOL 1". The classification of lipid as 'free' and 'bound' in this flour resulted from the methods of extraction which are described in full in the methods section. A quantity of this flour (500g) was freeze-dried and flour samples extracted in soxhlet extractors for seven hours with redistilled light petroleum spirit to remove the free lipid. The weight of lipid obtained after removal of the extracting solvent was noted and reported as a percentage of flour dry weight in Table 1:1. The soxhlet thimbles containing the petrol extracted flour were then air dried to remove any residual solvent and the flour residue extracted with the solvent recommended by Tsen et al. (1962) in the manner they describe. The solvent, chloroform-methanol-water is a very polar solvent and removes much of the remaining 'bound' lipid. The yield of lipid by this extraction was also noted after being redissolved in chloroform and dried down. The free and bound lipids were retained for further fractionation.

Table 1:1 indicates the distribution of free and bound lipid in the flour and shows that compared with the amount of lipid determined by acid hydrolysis, most of the lipid had been removed by the combination of the two extractions.

TABLE 1:1

Flour GOL 1 at 14% moisture.

Yield of lipid expressed as % of flour dry weight.

FREE	BOUND	TOTAL	
1.088	0.530	1.638	
1.132	0.524	1.656	
1.158	0.529	1.687	
1.140	0.534	1.674	
1.140	0.495	1.635	
1.131	0.522	1.653	Means
0.0262	0.0157		standard deviations

Total lipid by A.O.A.C. acid hydrolysis method 1.76%

Extraction of free and bound lipid from wetted flour.

A quantity of the original flour (100g) was then spread in a thin layer over a tray and sprayed with a suitable quantity of water to raise the moisture level to about 30%. The wetted flour was then collected and sealed in a jar while the moisture level was checked. Before the free and bound lipids were extracted as described above, the flour was freeze-dried, ground and sieved. The results of these extractions are shown in Table 1:2.

Table 1:2

Flour GOL 1 moistened to 31% moisture.

Yield of lipid expressed as percentage of flour dry weight.

% moisture	FREE	BOUND
14	1.132	0.526
31	0.890	0.580
31	0.940	0.570
31	0.910	0.575 Mean Total 1.485

This confirms the result of Olcott and Mecham (1947) that as a result of wetting to about 30% there has been a loss of free lipid (0.221%), and further reveals that this was not matched by a corresponding increase in bound lipid (0.058%). This discrepancy suggests that some lipid has apparently disappeared or become so tightly bound that even the Tsen solvent will not extract it. This discrepancy, some 0.168%, is worthy of further consideration since it is not only a significant proportion of the total lipid (10%) but also represents a new, third class of lipid that increases in flour after the addition of water. It suggests that some of the free lipid becomes bound when the flour is wetted and some of this free lipid or possibly some of the bound lipid becomes even more strongly bound, or bound in a different manner. This third class of flour lipid will be referred to as 'unextractable'.

Separation of free and bound lipids into neutral and polar fractions.

The extracted lipids, both free and bound, from the two flour samples were then further separated into two fractions by a technique based on a method proposed by Morrison (1963). As described in detail in the methods section, a chloroform solution of the lipid is shaken with activated silica gel. The silica gel is filtered off and washed with more chloroform. The filtrate and washings are quantitatively collected and dried down to constant weight. The silica gel is then washed with methanol and the washings collected, dried down, dissolved in chloroform and dried to constant weight. The first fraction, not retained by the silica gel in chloroform, contained neutral lipids and the second fraction, polar lipids. Details of which lipid classes are found in these two fractions are given in the methods section but in this study the method of separation used will be to define the fractions. Table 1:3 shows the full results of the separations and permits a fuller account of the effect of wetting to be given.

The effect of wetting on the free lipid was to reduce the free neutral lipid by 0.097% and reduce the free polar lipid by 0.124%. However, the bound neutral lipid only increased by 0.055% and bound polar lipid decreased insignificantly by 0.002%. These results suggest that the effect of wetting on the free neutral lipid is two-fold. About half of that which is lost becomes bound and half unextractable.

Table 1:3

Effect of moisture level on flour lipid fractions. Fractions expressed as a percentage of flour dry weight.

Flour GOL 1

	Moisture Level %		
	14	31	Difference
Free Neutral	0.871	0.774	-0.097
Free Polar	0.260	0.136	-0.124
Bound Neutral	0.104	0.159	+0.055
Bound Polar	0.418	0.416	-0.002
Neutral Total	0.975	0.933	-0.042
Polar Total	0.678	0.522	-0.126
Unextractable Neutral	-	0.042	+0.042
Unextractable Polar	-	0.126	+0.126

The effect on the free polar lipid is for about half of the polar lipid present as free lipid to become unextractable and for none of it to appear in the bound fraction. This treatment of the results assumes that the unlikely event of inter-conversion of neutral and polar lipid does not occur.

The presence of an unextractable lipid fraction in flour and dough has been reported in the literature, although unknowingly in some cases and never in relation to unworked moistened flour. Pomeranz (1967) has reported a 'small but substantial' fraction of lipid in flour which remained insoluble in water-saturated-butanol and chloroform-methanol-water.

Since acid hydrolysis at elevated temperatures was required to release these lipids, nothing could be stated about their nature or form since only the fatty acids were extracted after hydrolysis (Pomeranz et al. (1968)). Inkpen and Quackenbush (1969) also refer to a'bound' lipid fraction which can only be released by acid hydrolysis. These observations have caused comment when the total lipid obtained by acid hydrolysis is more than that extractable with solvent and suggest that an unextractable lipid fraction may exist in flour at 14% moisture and can act as a reservoir to accept or supply lipid material in certain circumstances. The feeding of lipid material into the unextractable fraction has been recorded in the results of several workers although no comment has been made on the phenomenon. The presence of an unextractable fraction is revealed when the total extractable lipid decreases after dough is made from flour (Chiu et al. (1968) Daniels et al. (1969)) or when the decrease of free lipid which results from the mixing of flour into dough is greater than the increase of bound lipid (Tsen, Levi & Hlynka (1962), Chiu & Pomeranz (1966)).

These first experiments show that the effect of wetting flour has some interesting effects on the lipid distribution and is an area worthy of further attention. The next stage is to find a suitable method for obtaining flour wetted to the whole range of moisture levels from 14% to 45% and above, the moisture levels used in breadmaking.

SECTION TWO

Detailed effect of moisture level on lipid distribution in flour.

Requirements of a method for adjusting flour moisture level.

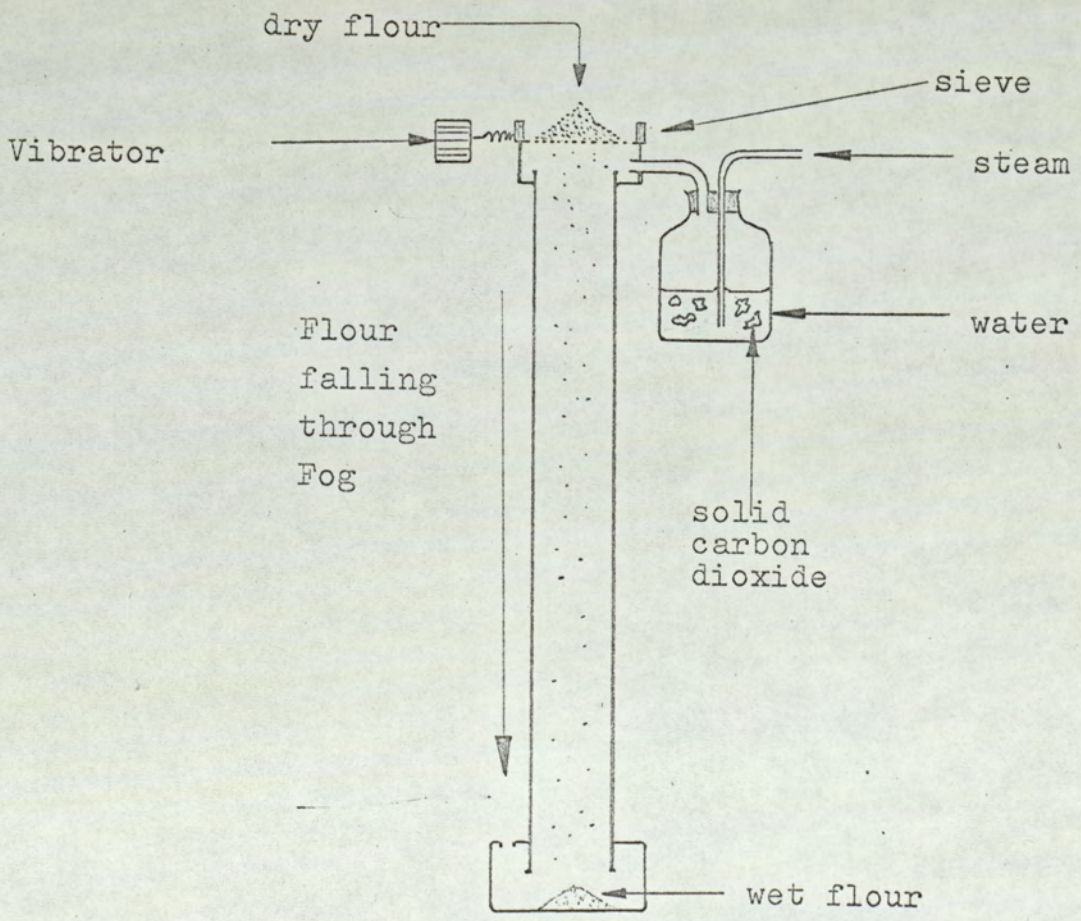
In order to produce flours with a wide range of moisture levels suitable for lipid binding studies the method used must fulfil some demanding conditions. Obviously water must be evenly distributed throughout the flour to avoid localised doughing and the method must not be too time consuming. It would be helpful if the moisture level could be predetermined and it is essential that the lipid distribution be effected in a repeatable way. In addition the method should be adaptable to a practicable scale, should have neither side nor after effects on flour properties, nor incorporate any additional ingredients. Previous work on lipid binding has suggested three further special requirements or factors which should be avoided. The studies of Baldwin et al. (1963), Ponte et al. (1964) (1966), Chiu and Pomeranz (1966), Wootton (1966), and Chiu et al. (1968) have all shown that mixing wheat flour doughs results in lipid binding and Daniels et al. (1966) (1967) (1969a) (1969) have shown that lipid binding is affected by rate of dough mixing, total mechanical work input, atmosphere in the dough mixing chamber and baking. Therefore in any method of wetting flour for lipid binding studies, it would seem advisable to avoid the introduction of mechanical work, heat and an atmosphere of oxygen. It can be concluded that the simple, even distribution of water through flour presents a particularly difficult problem.

Methods of flour moisture adjustment

Attempts to raise the flour moisture content by equilibration over water in a closed vessel failed due to mould growth on the damp flour. Moreover even after two weeks the moisture level had not risen above 25%. The addition of mould inhibitors (e.g. toluene used by Olcott and Mecham (1947)) was avoided in view of the possible effects on lipid distribution. A more effective technique for raising the moisture allowed the flour to fall repeatedly through a column of dense fog generated by passing steam over blocks of solid carbon dioxide. This method seemed more promising and is shown in Diagram 2:1.

Diagram 2:1.

Flour moisture adjustment with fog.



However, above 20% moisture the damp flour particles caked together and were no longer evenly accessible to the fog.

The application of a measured amount of water in a fine spray to a thin layer of flour as was used in the first experiment produced a thin surface skin of dough indicating an unsatisfactory distribution of moisture. The greatest disadvantage of these methods was the lack of fine control over the moisture level that could be obtained. Furthermore the unpredictable nature of the results produced a time lag as a moisture determination was necessary before proceeding further.

Liquid nitrogen technique

The addition of powdered ice to flour at sub-zero temperatures (Olcott and Mecham (1947)) appeared to offer a more precise control of moisture content but had the disadvantage that a long period of storage (one month at -9.4°C) was required to allow the ice to sublime into the flour. Trial experiments showed how difficult it was to obtain a powdered ice fine enough for intimate dispersion in flour by grinding ice in a mortar cooled to -20° C. Invariably, even after prolonged storage below freezing point, when the mixture was brought to room temperature the moisture was found to be unevenly distributed, producing damp clumps in surrounding dry flour.

It was noticed that when ice was ground at -20°C, the ice surface melted under the pressure of the pestle preventing the required breakdown to a fine powder. A considerable improvement in the fineness of grinding was obtained when ice was ground below the surface of liquid nitrogen (-196°C) added to the mortar.

Moreover the addition of the required weight of flour to the finely divided ice suspended in boiling liquid nitrogen produced a mixture in which the ice was intimately mixed with the flour before storage and equilibrium at -20°C.

The procedure finally used in this and all later studies for moisture adjustments in the range 14-60% was to measure the required amount of water into liquid nitrogen held in an iron mortar (a ceramic mortar was unable to withstand the extreme range of temperatures used) and grind quickly to a finely divided powder. This was added to flour (100g) similarly suspended in boiling liquid nitrogen in a beaker and the self mixing slurry transferred to a deep-freeze cabinet at -20°C. Here the nitrogen boiled off and the ice-flour mixture reached equilibrium by sublimation. It was found that at all moisture levels, an equilibrium was reached rapidly and the mixture was ready for use within 24 hours.

Evaluation of liquid nitrogen technique.

In a trial experiment this method of moisture adjustment proved most effective since a particular moisture level could be obtained to within 1% of that required, with an even moisture distribution throughout the flour. This was verified by repeated moisture determinations on the same sample. Table 2:1 shows the results obtained on four samples.

Table 2:1

Variation of moisture within flour samples moistened by the liquid nitrogen technique.

		Flour samples			
Determination	No.	A	B	C	D
	1	21.37	29.54	35.20	46.88
	2	21.44	29.46	34.87	47.05
	3	21.47	30.02	35.30	46.91
	4	21.20	29.41	35.17	46.63
	5	21.62	29.43	34.91	47.17
	6	21.36	29.32	35.27	46.82
Means		21.41	29.53	35.12	46.91
Standard Devs.		0.139	0.250	0.185	0.187

Further confirmation of even moisture distribution was given by the absence of small dough particles in the flour which were seen for example when spray moistening of the flour was tried. The conditions of the technique eliminated completely any question of mechanical development of the wetted flour at all moisture levels and also avoided the possibility of mould contamination.

The possibility that treatment with liquid nitrogen or freeze-drying could have influenced the extractability of lipids was examined by subjecting a sample of flour to the routine described but with the omission of thawing before freeze-drying. As can be seen in Table 2:2 there was no loss of free lipid or increase of bound lipid even though sufficient ice had been added to raise the moisture to 40% indicating that any change in free or bound lipid distribution was due solely to the increase in moisture level of the flour.

Table 2:2

Effect of liquid nitrogen treatment and freeze-drying on lipid distribution.

	Free	Bound
GOL 1 at 14% -mean values	1.131	0.522
GOL 1, ice added equivalent	1.129	0.500
to 40% moisture, freeze-dried without thawing	1.136	0.496
Means	1.133	0.498

Effect of Moisture level on physical properties of the flour.

Across the range of moisture levels, the wet flours or unworked doughs that resulted when the flour-ice mixtures were allowed to thaw showed interesting changes of physical form. This was first noticed when the sealed beakers containing the flour-ice mixtures were removed from the deep-freeze cabinet and allowed to thaw before freeze-drying. After two hours, the wet flour or 'doughs' had pulled away from the sides of the beakers. The higher the moisture level the more the material had shrunk away from the edge. To show this more clearly, flour-ice mixtures were allowed to thaw inside standard open ended cylinders (3.5 x 4.5 c.m.). After two hours, the cylinders were lifted off and the 'doughs' photographed.

Figure 1 shows the appearance of the moistened flours over the range of the moisture levels 14-50%.

The flour at 18% moisture collapsed as it was still as free flowing as it was at 14%. But between 18 and 28% moisture, the flour changed from a free flowing to a sticky powder.

Figure 2:1.

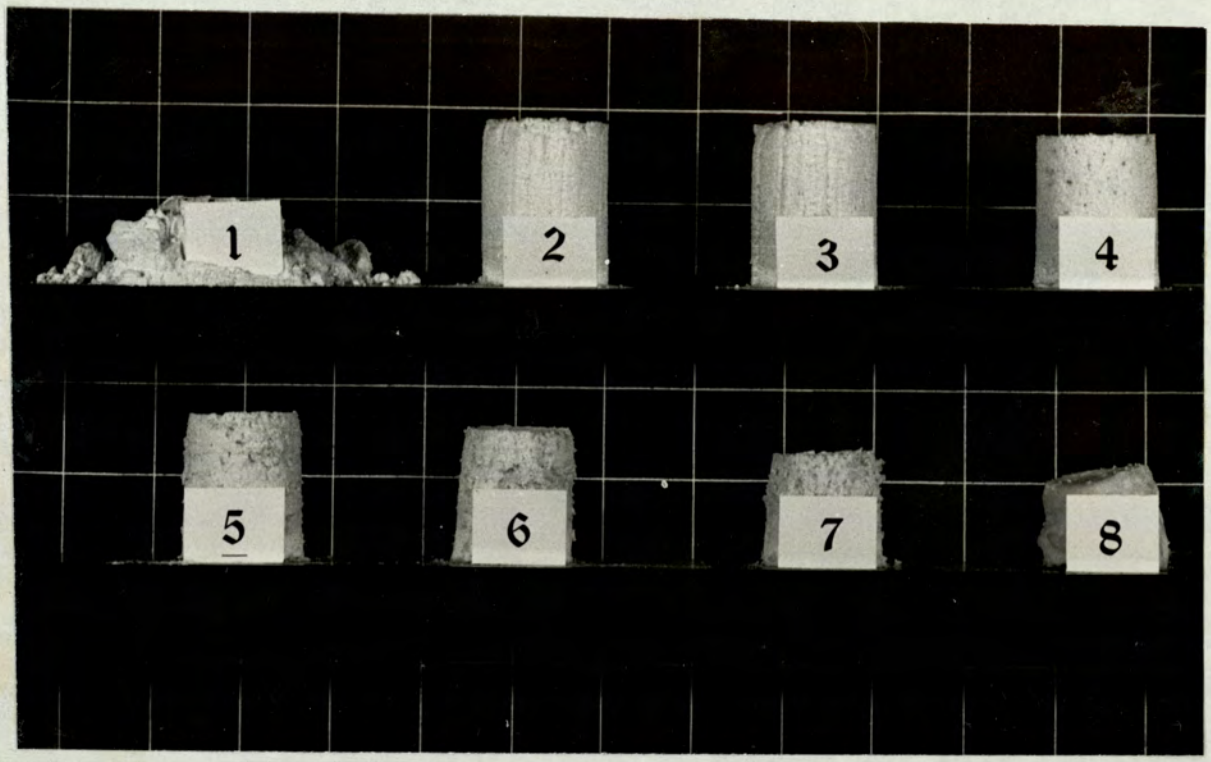


FIG. 2:1 Effect of moisture on the shrinkage of flour cylinders. Moisture levels; (1) 18%, (2) 23%, (3) 28%, (4) 30%, (5) 34%, (6) 36%, (7) 39%, (8) 45%

The onset of this change was at about 20% and it can be seen that the cylindrical form was retained and did not collapse or shrink. Above 28% the moist flour showed for the first time a tendency towards a continuous structure, it no longer appeared to consist of discrete particles and if squeezed gently became dough like. Although the material could hardly be called a dough, the volume significantly decreased when left standing for a period of two hours. The shrinking effect became more pronounced as the moisture level was further raised to the 50% level. At 50% moisture, the dough appeared to have a wet surface after about an hour at room temperature and after two hours had the consistency of a stiff batter.

These physical effects were considered sufficiently interesting to be examined quantitively and the results of this investigation will be described in a later section (page 88).

Effect of moisture level on flour lipid distribution over the range 15-60%.

The effect of moisture on the extractability of lipids from this first flour was studied over the range 15-60%. Samples were prepared at intervals of less than 5% moisture throughout the range and at smaller intervals (about 1%) in the area of particular interest (18-35% moisture).

The result of the lipid extractability study is shown in Table 2:3. This shows the free and bound lipid extracted at each moisture level, the extractable total and the unextractable fraction by difference. These results are plotted in Figure 2:2 and lines of best fit drawn in. The amount of free lipid in the flour remained constant at 1.12% as the flour moisture was raised to 20%. Above 20% and up to 40% the free lipid decreased steadily to give a constant value of 0.70% above 40% moisture. However the amount of bound lipid at 14% moisture, 0.52%, remained constant until the mixture level rose to 25%. At this point bound lipid started to increase until it reached a maximum value of 0.75% at a moisture level of 40% and remained constant thereafter. The free lipid results were in broad agreement with those of Olcott and Mecham (1947) and further indicated that binding increased continuously.

Table 2:3

Effect of moisture level on free and bound lipid distribution. GOL 1 Expressed as % flour dry weight. Mean values.

Moisture Level(%)	Free	Bound	Total	Unextractable.
14.1	1.130	0.520	1.650	0.000
15.8	1.125	0.525	1.650	0.000
16.4	1.120	0.488	1.608	0.042
18.0	1.125	0.520	1.645	0.005
20.0	1.115	0.540	1.655	0.005
21.4	1.075	0.545	1.620	0.035
22.3	1.035	0.527	1.562	0.088
23.5	1.004	0.539	1.543	0.107
24.2	1.004	0.530	1.534	0.116
24.7	1.002	0.515	1.517	0.133
25.5	0.973	0.515	1.488	0.162
26.8	0.999	0.585	1.584	0.066
27.4	0.945	0.585	1.530	0.120
28.2	0.893	0.644	1.537	0.113
29.5	0.897	0.593	1.490	0.160
30.1	0.828	0.640	1.468	0.182
31.5	0.786	0.690	1.476	0.174
31.9	0.808	0.620	1.428	0.222
35.1	0.806	0.705	1.511	0.139
38.9	0.704	0.749	1.453	0.194
46.9	0.723	0.755	1.478	0.172
54.8	0.676	0.746	1.422	0.228
58.4	0.720	0.688	1.408	0.242
Standard Errors of means	± 0.009	± 0.016		

An interesting feature of the results shown in Figure 2:2 was the loss of free lipid between 20 and 25% moisture that was not matched by an increase of bound lipid in the same moisture range. This result suggested that the initial effect of increase of moisture level was the strong binding of a part of the free lipid, so strong as to be unextractable. Then above 25% moisture, free lipid became bound both extractably and unextractably until at about 40% moisture apparent upper limits of the two bound lipid fractions were reached. It would appear that above this moisture level, no further lipid binding would occur as a result of wetting alone.

Effect of moisture level on neutral and polar lipid distribution.

The fractionation of the free and bound lipids into neutral and polar gave a better insight into these changes, over the whole range of moisture levels. When the full results in Table 2:4 are examined together with Figures 2:3 and 2:4 the effect of moisture level on the different lipid fractions can be seen. An account of the derivation of these figures is given in the Methods section (page 39)

Polar Lipids

Looking first at the polar lipid it can be seen that the initial effect was an immediate decrease in free polar lipid. This was not matched by an increase of bound polar lipid as this particular fraction did not start to increase until 25% moisture had been reached.

Figure 2:2. Effect of wetting on total lipid fraction of flour GOL 1. Results expressed as percentages of flour dry weight.

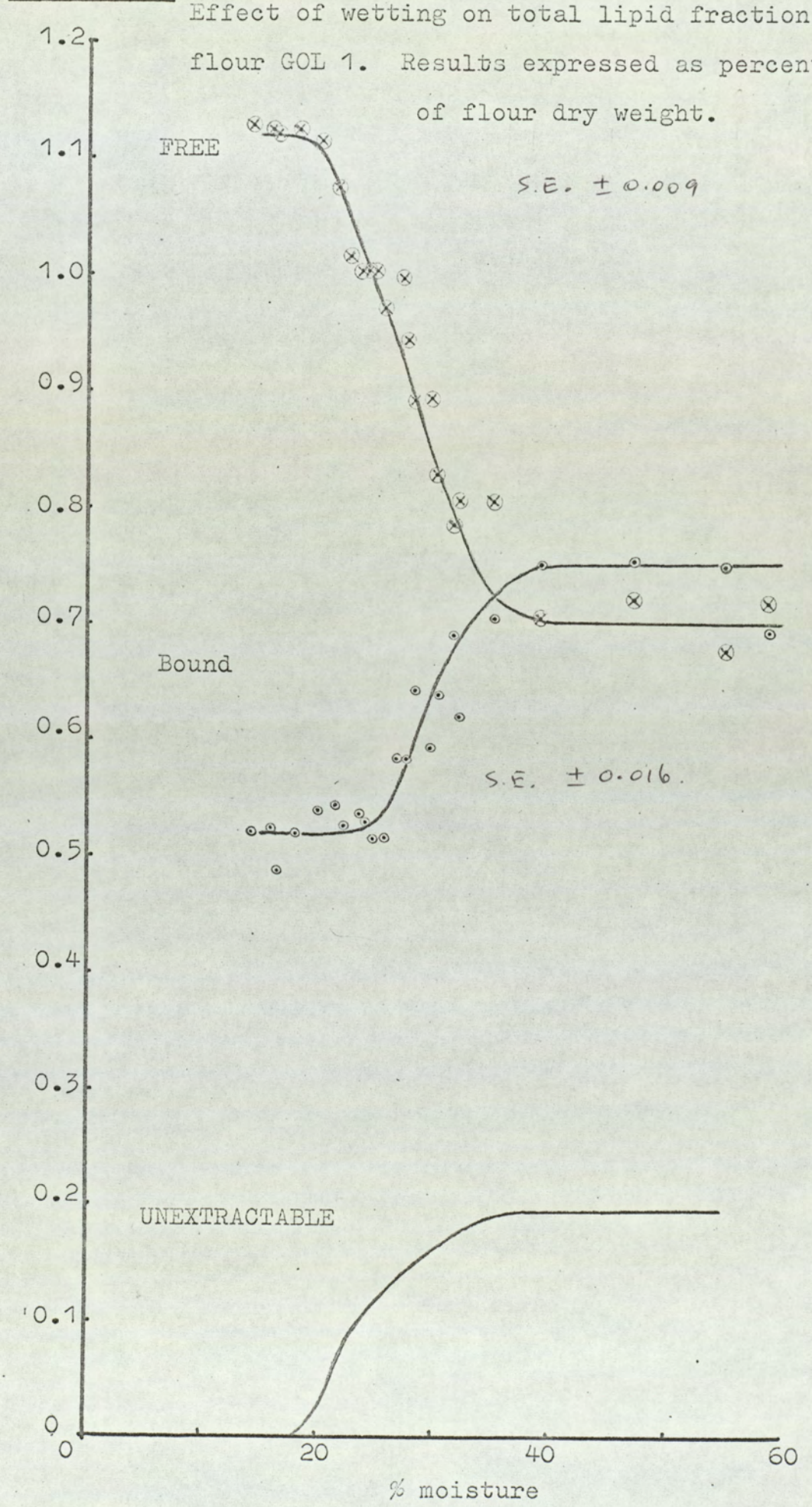


Table 2:4

Effect of moisture level on neutral and polar lipid distribution. GOL 1.

(a) Distribution. Mean values expressed as % of total extractable lipid.

Moisture level (%)	Free		Bound	
	% neutral	% polar	% neutral	% polar
14.1	52.7	15.8	6.3	25.2
15.8	52.7	15.5	6.2	25.6
16.4	54.3	15.4	5.8	24.5
18.0	53.7	14.7	6.3	25.3
20.0	53.2	14.2	6.9	25.7
21.4	53.1	13.3	7.5	26.1
22.3	53.4	12.9	7.8	25.9
23.5	52.6	12.5	8.4	26.5
24.2	53.3	12.3	8.3	26.1
24.7	54.1	12.0	8.5	25.4
25.5	53.6	11.8	8.8	25.8
26.8	52.3	10.7	9.7	27.3
27.4	51.6	10.1	10.2	28.1
28.2	48.8	9.3	11.3	30.6
29.5	50.7	9.5	10.9	28.9
30.1	47.9	8.5	12.0	31.6
31.5	45.8	7.5	13.5	33.2
31.9	48.7	7.9	11.9	31.5
35.1	46.9	6.5	11.9	34.7
38.9	43.7	4.7	12.1	39.5
46.9	44.0	4.9	10.2	40.9
54.8	42.7	4.8	8.2	44.3
58.4	46.1	5.1	5.5	43.3

Table 2:4

Effect of moisture level on neutral and polar lipid distribution. GOL 1. Expressed as percentages of flour dry weight.

(b) Results. Mean values.

Moisture level (%)	Free		Bound	
	% neutral	% polar	% neutral	% polar
14.1	0.870	0.260	0.104	0.416
15.8	0.870	0.255	0.102	0.423
16.4	0.873	0.247	0.094	0.394
18.0	0.883	0.242	0.104	0.416
20.0	0.880	0.235	0.115	0.425
21.4	0.860	0.215	0.123	0.422
22.3	0.834	0.201	0.124	0.403
23.5	0.812	0.192	0.130	0.409
24.2	0.816	0.188	0.130	0.400
24.7	0.820	0.182	0.129	0.386
25.5	0.798	0.175	0.131	0.384
26.8	0.830	0.169	0.152	0.433
27.4	0.790	0.155	0.155	0.430
28.2	0.750	0.143	0.174	0.470
29.5	0.758	0.139	0.163	0.430
30.1	0.703	0.125	0.176	0.464
31.5	0.676	0.110	0.200	0.490
31.9	0.695	0.113	0.170	0.450
35.1	0.710	0.096	0.180	0.525
38.9	0.635	0.069	0.176	0.573
46.9	0.650	0.073	0.150	0.665
54.8	0.607	0.069	0.116	0.630
58.4	0.648	0.072	0.078	0.610

Least significant differences, P=0.001 Neutral 0.092, Polar 0.053

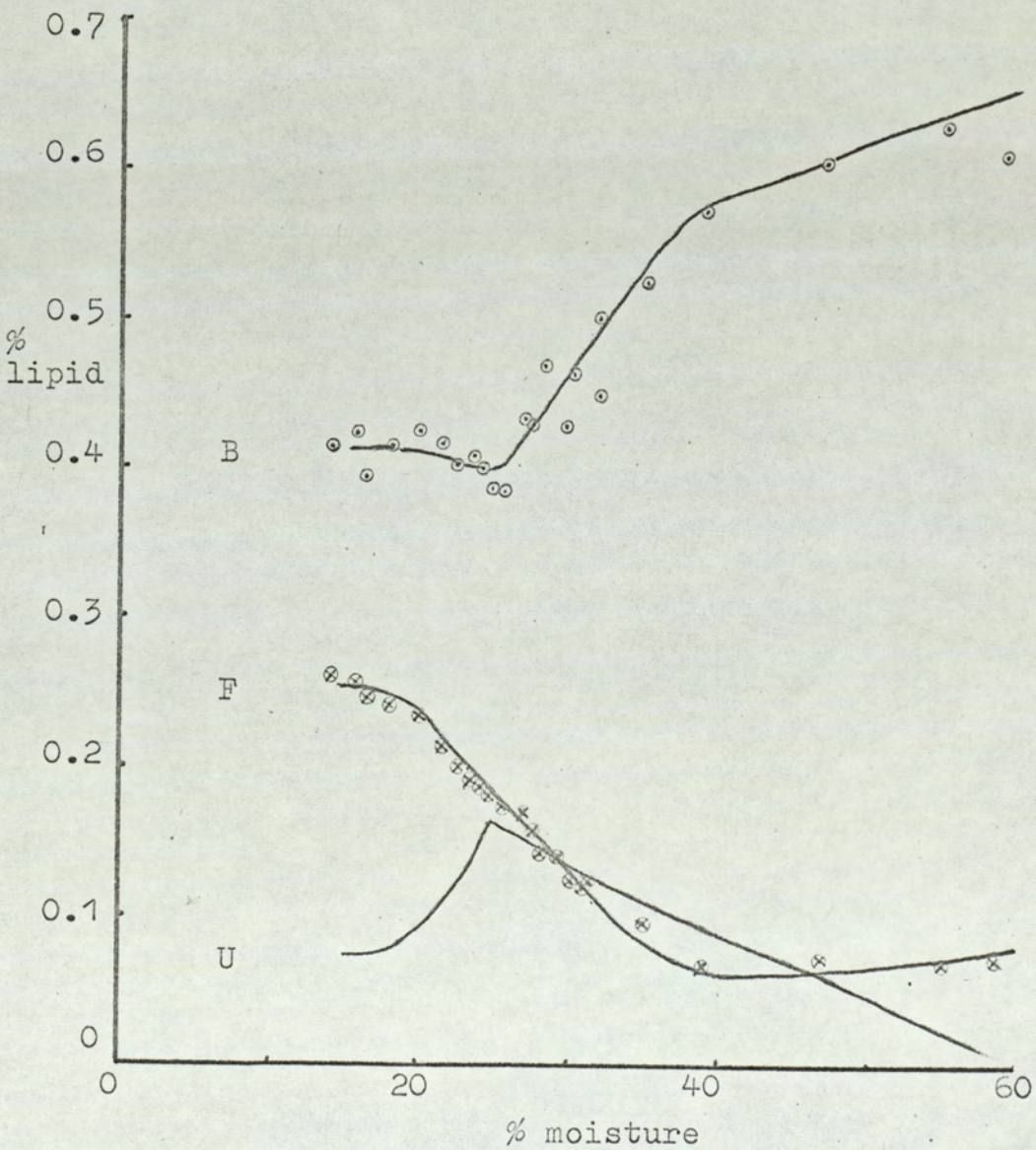
Figures 2:3.

Effect of wetting on polar lipid fractions of flour GOL 1. Results expressed as percentages of flour dry weight.

Standard Errors of Means

Free ± 0·0213

Bound ± 0·0283



This confirmed that polar lipid was unextractably bound in the initial stage of moisture level increase. As the moisture level was further raised beyond 25%, the free polar lipid was transferred to the bound state until at 30% moisture the rate of increase of bound polar lipid was greater than the rate of decrease of free polar lipid. This suggested that above this moisture level, unextractable polar lipid was released and became extractably bound. There was no further decrease in free polar lipid beyond 40% moisture although the release of unextractable polar lipid continued.

Neutral Lipid.

The response of neutral lipid to raising the moisture level is seen (Figure 2:4) to have been initially similar to that of the polar lipids in that the first effect, at 20% moisture, was a loss of free lipid which was not matched by a corresponding increase in the bound neutral fraction. One must conclude that some neutral lipid also became unextractably bound. The bound neutral fraction increased between 25 and 32% moisture but then decreased again so that the net result was a continuous transfer of neutral lipid from free to unextractable between 20 and 40% moisture.

Between 25 and 32% moisture level, some of the neutral lipid was taken from the free fraction by the extractable bound fraction but after 32% this bound fraction decreased to the advantage of the unextractable fraction.

This pattern of behaviour was in contrast to that of the polar lipid which showed no further transfer to the unextractable fraction either from the free or bound fractions above 25% moisture.

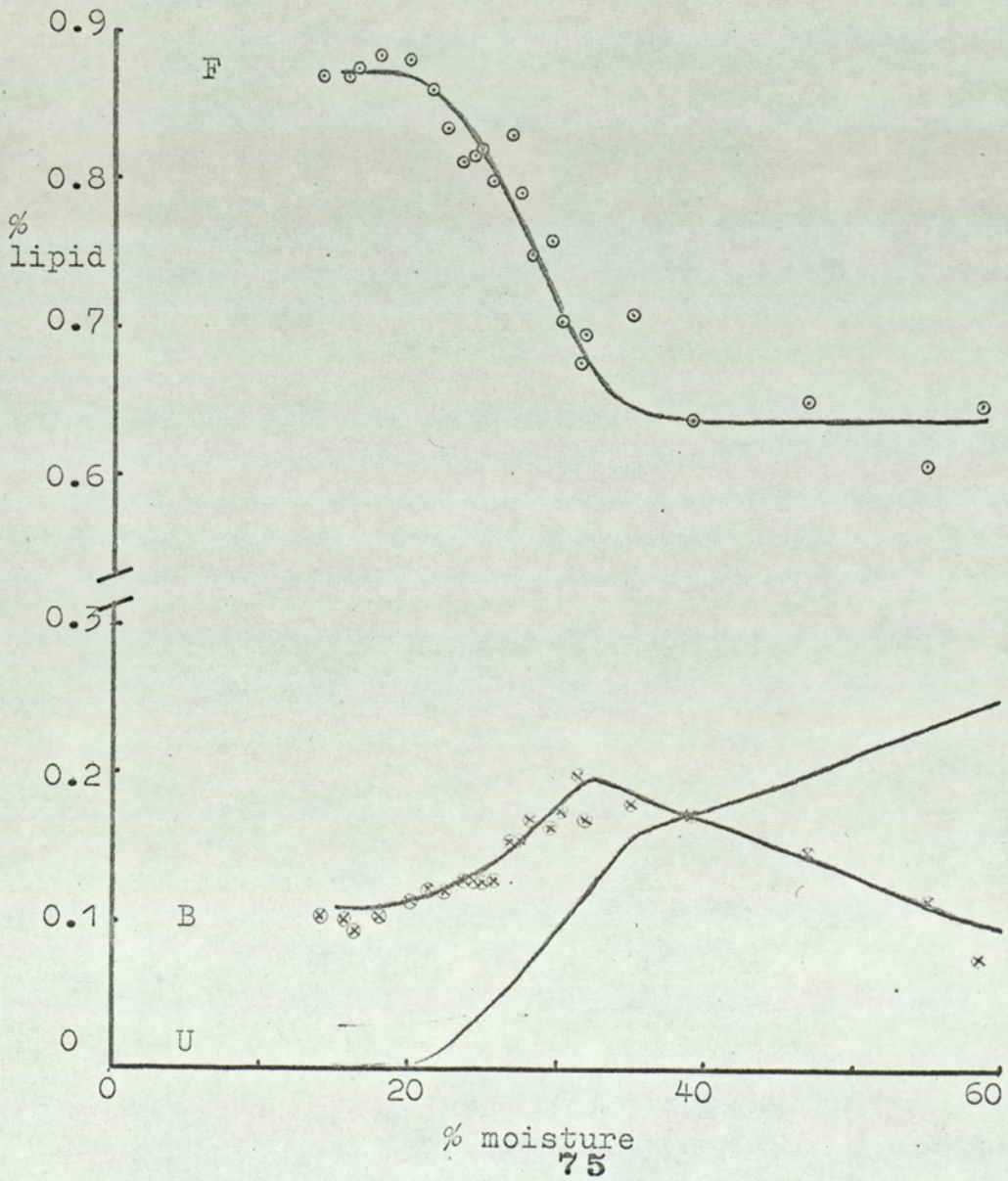
Figure 2:4

Effect of wetting on neutral lipid fractions of flour GOL 1. Results expressed as percentages of flour dry weight.

Standard Errors of Means

Free ± 0·0306

Bound ± 0·0376



The migrations of the lipid fractions that occur with increase of moisture are represented diagramatically in Figure 2:5. The presentation shows the different response of polar and neutral lipid fractions at different moisture levels.

Comparative study with a similar flour. GOL 2.

In order to assess the significance of the results reported above, a second flour, designated 'GOL 2', was subjected to a similar study in which lipid distributions were determined at key moisture levels. The second flour was chosen as being closely similar to GOL 1 in breadmaking potential. Full details of the flour composition are given in the Materials section.

Exactly the same procedures were used to study GOL 2, as were used for GOL 1. Flour samples were taken to moisture levels over the same range by the liquid nitrogen technique and the distribution of total free and bound lipids determined. The results are shown in Table 2:5 and plotted in Figure 2:6 together with the unextractable fraction obtained by difference.

Figure 2:5

Lipid fraction migration diagram. Effect of moisture level on flour GOL 1.

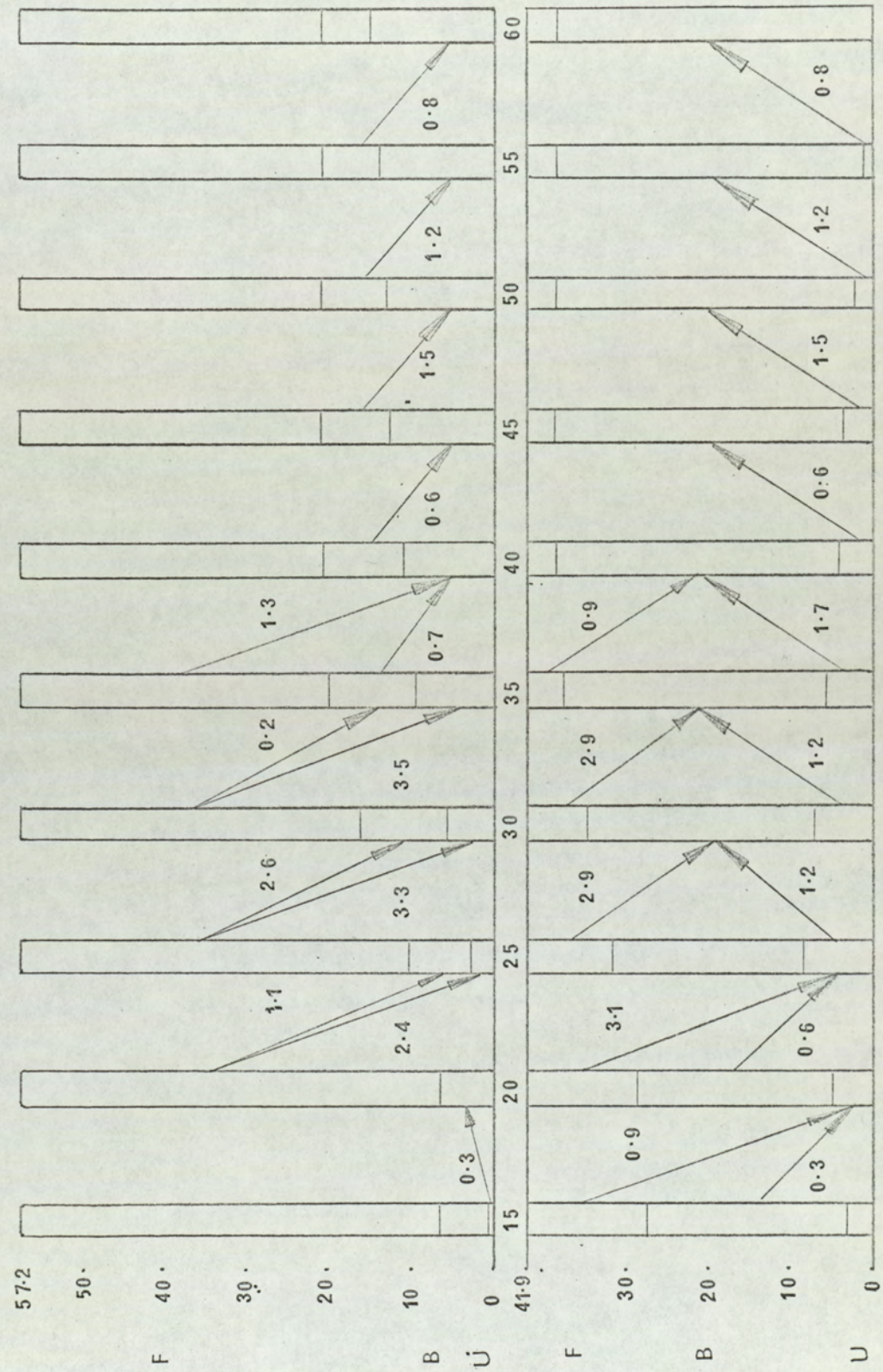


Table 2:5

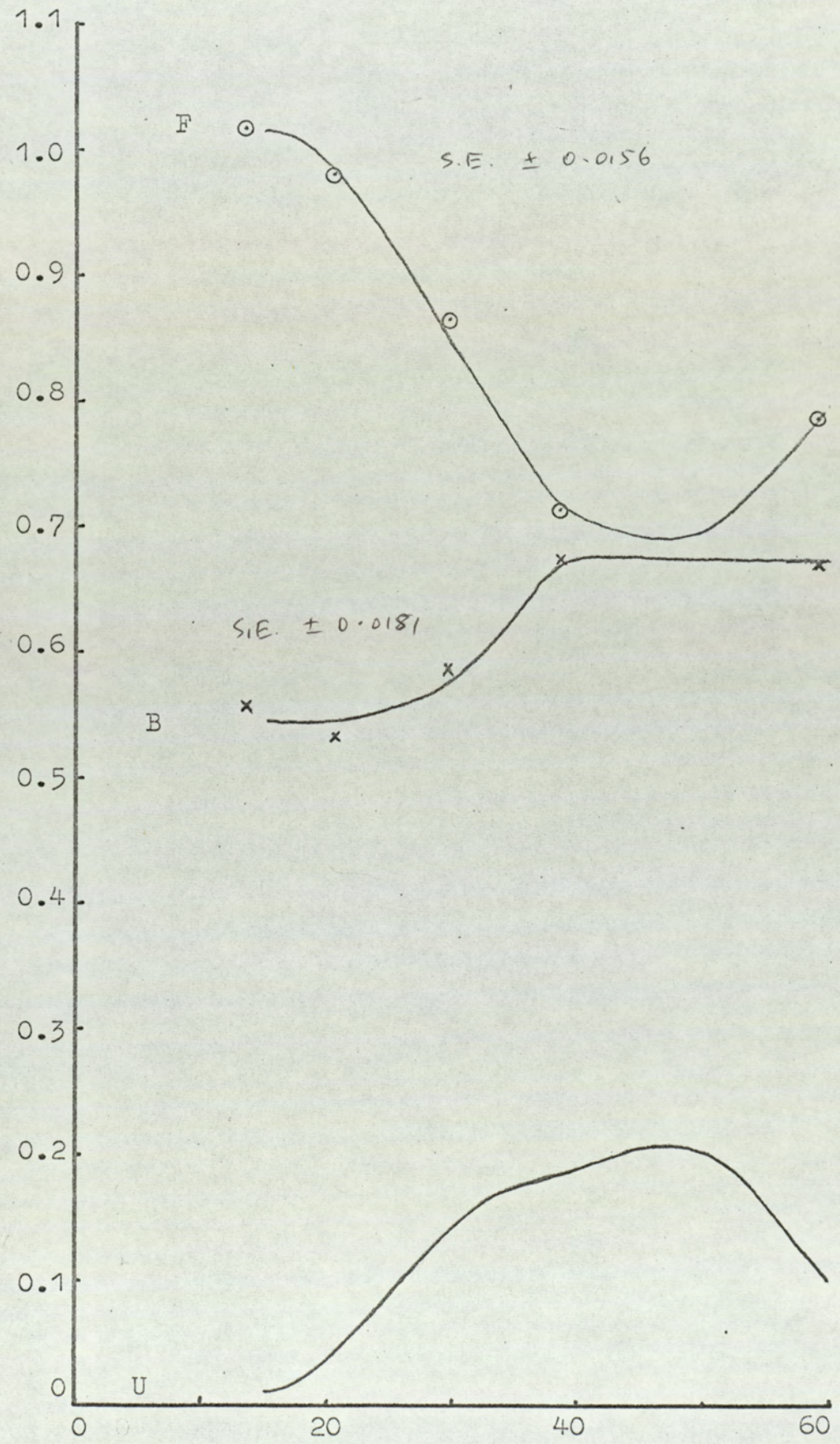
Effect of moisture level on lipid distribution. GOL 2

Expressed as % flour dry weight. Mean values.

Moisture level (%)	13.50	20.45	29.40	38.70	59.0	S.E of Means
Free	1.016	0.982	0.866	0.715	0.788	± 0.0156
Bound	0.556	0.532	0.586	0.673	0.670	± 0.0181
Free neutral	0.812	0.805	0.745	0.660	0.707	± 0.0372
Free polar	0.204	0.177	0.121	0.055	0.081	± 0.0279
Bound neutral	0.088	0.124	0.122	0.135	0.146	± 0.0397
Bound polar	0.468	0.408	0.466	0.538	0.524	± 0.0304
Total neutral	0.900	0.929	0.867	0.795	0.853	± 0.0216
Total polar	0.672	0.585	0.587	0.593	0.605	± 0.0123

Figure 2:6.

Effect of wetting on total lipid fractions of flour GOL 2. Results expressed as percentages of flour dry weight.



As expected the general pattern of response of GOL 2 was the same as was found for GOL 1. The decrease of free lipid again started at a lower moisture level than the increase of bound lipid and the total loss of free lipid between 15 and 45% was again considerably greater than the increase of bound lipid over the same moisture range. Above 40% moisture, there was no further increase of extractable bound lipid but it will be noted that, unlike the previous flour, the free lipid increased between 45 and 65% moisture. This result will be discussed later and for the moment, only the moisture range 15-50% will be considered.

Figure 2:7 shows the results of both flours for comparison. As can be seen the lipid distribution patterns of the two flours were broadly similar when the results are presented on a percentage flour dry weight basis. When presented on a percentage of total lipid basis as in Figure 2:8 the similarity of the lipid binding patterns of the two flours was even greater.

Neutral and polar lipid fractions.

The results of the separation of free and bound lipid into neutral and polar fractions are presented in Figures 2:9 and 2:10. These results show the effect of work free wetting on these flour fractions and are plotted as percentages of the total lipid of each flour.

To compare these results with those obtained from GOL 1, Figure 2:11 shows the free neutral, bound neutral and unextractable neutral lipid fractions of both flours, each expressed as percentages of their own total lipid.

Figure 2:7

Comparison of flours GOL 1 and GOL 2. Effect of wetting on total lipid fractions.

GOL 1 - - - - - - - -

GOL 2 ————————

Results expressed as % of flour dry weight.

See Figures 2:2 & 2:6 for Standard Errors of Means

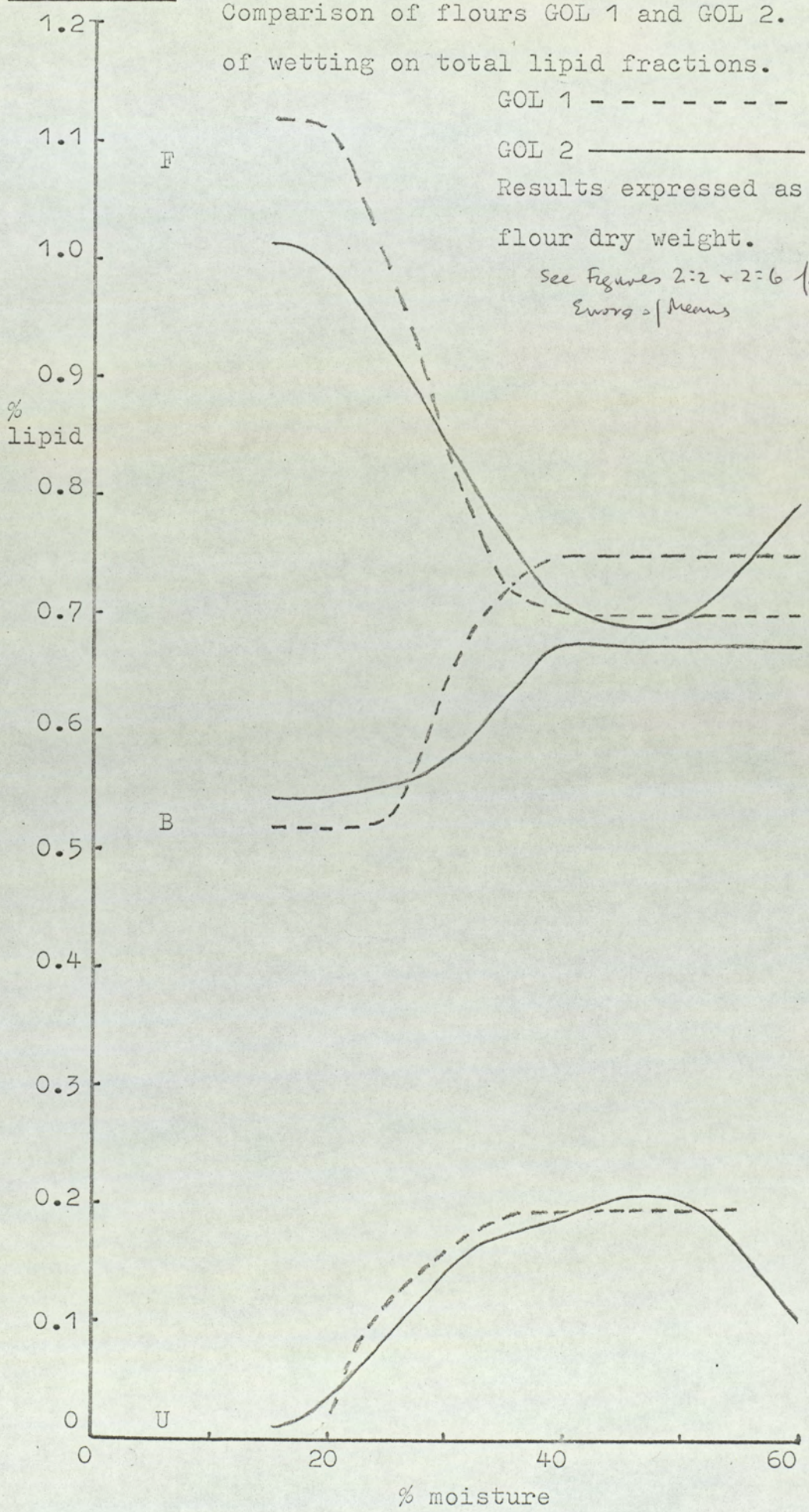
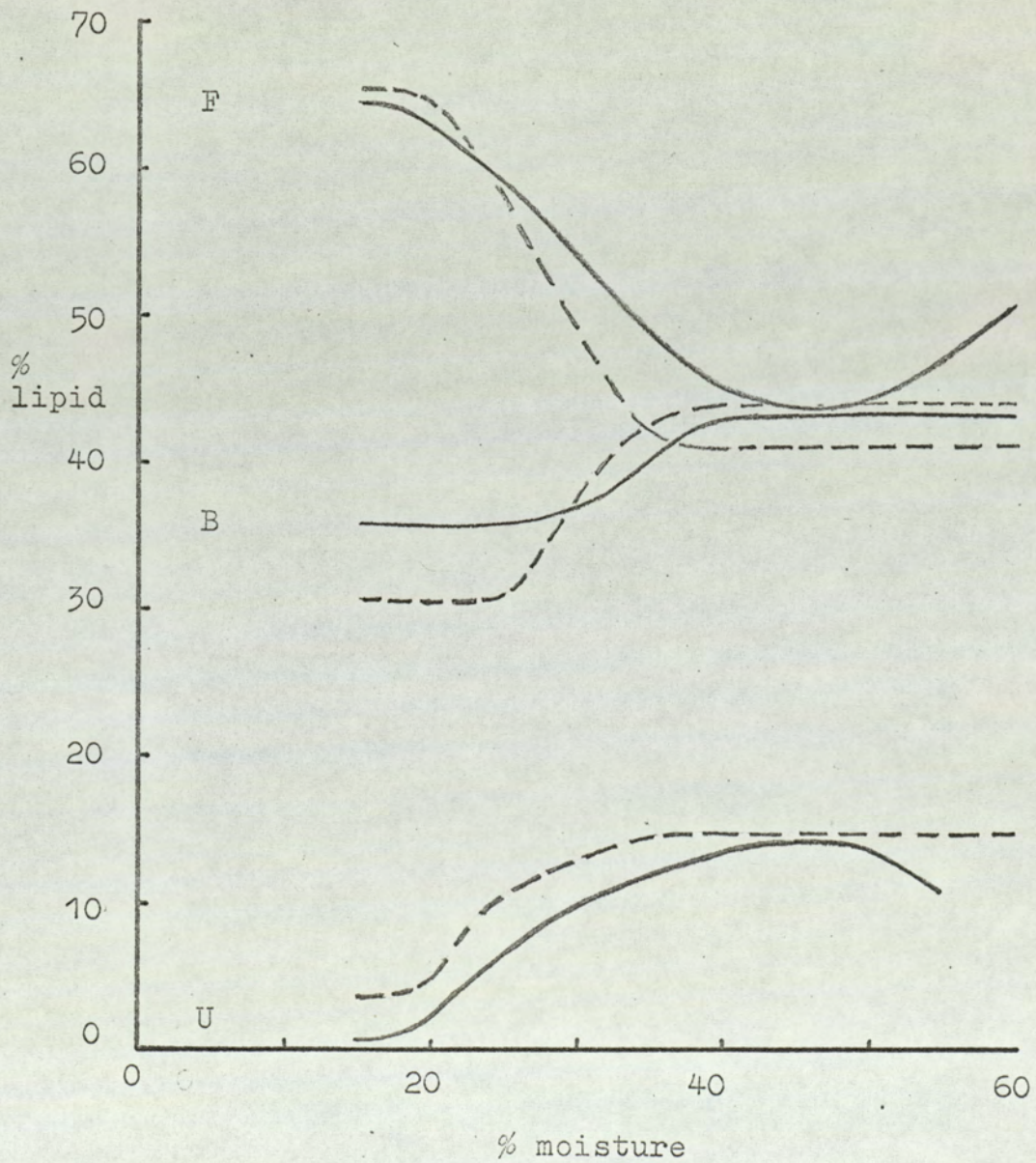


Figure 2:8.

Comparison of flours GOL 1 and GOL 2. Effect of wetting on total lipid fractions. Results expressed as percentage of total lipid present.

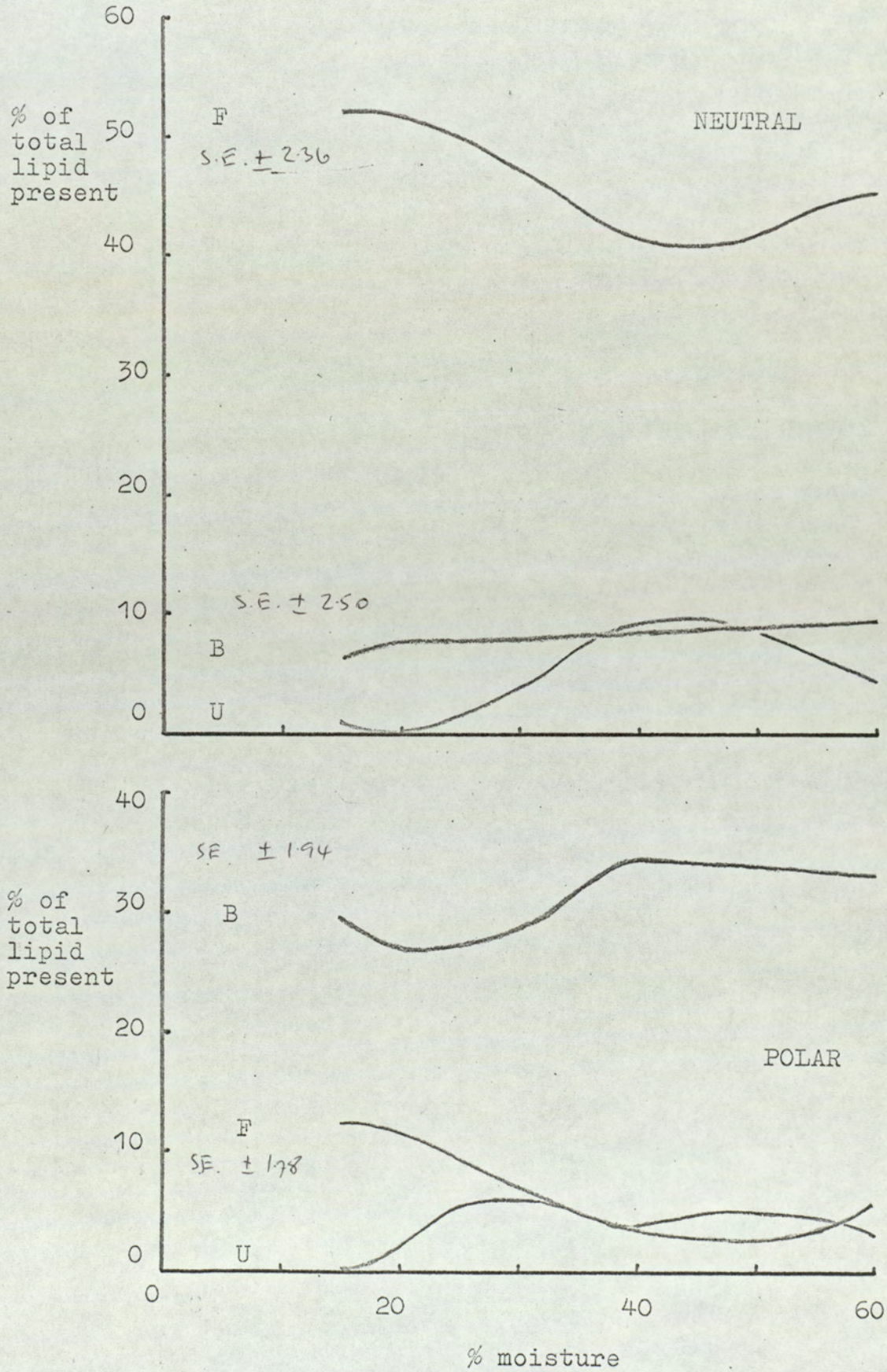
GOL 1 - - - - - - - - GOL 2

Standard Errors of Means	Free	Bound
GOL 1	± 0·540	± 0·970
GOL 2	± 0·995	± 1·150



Figures 2:9 and 2:10.

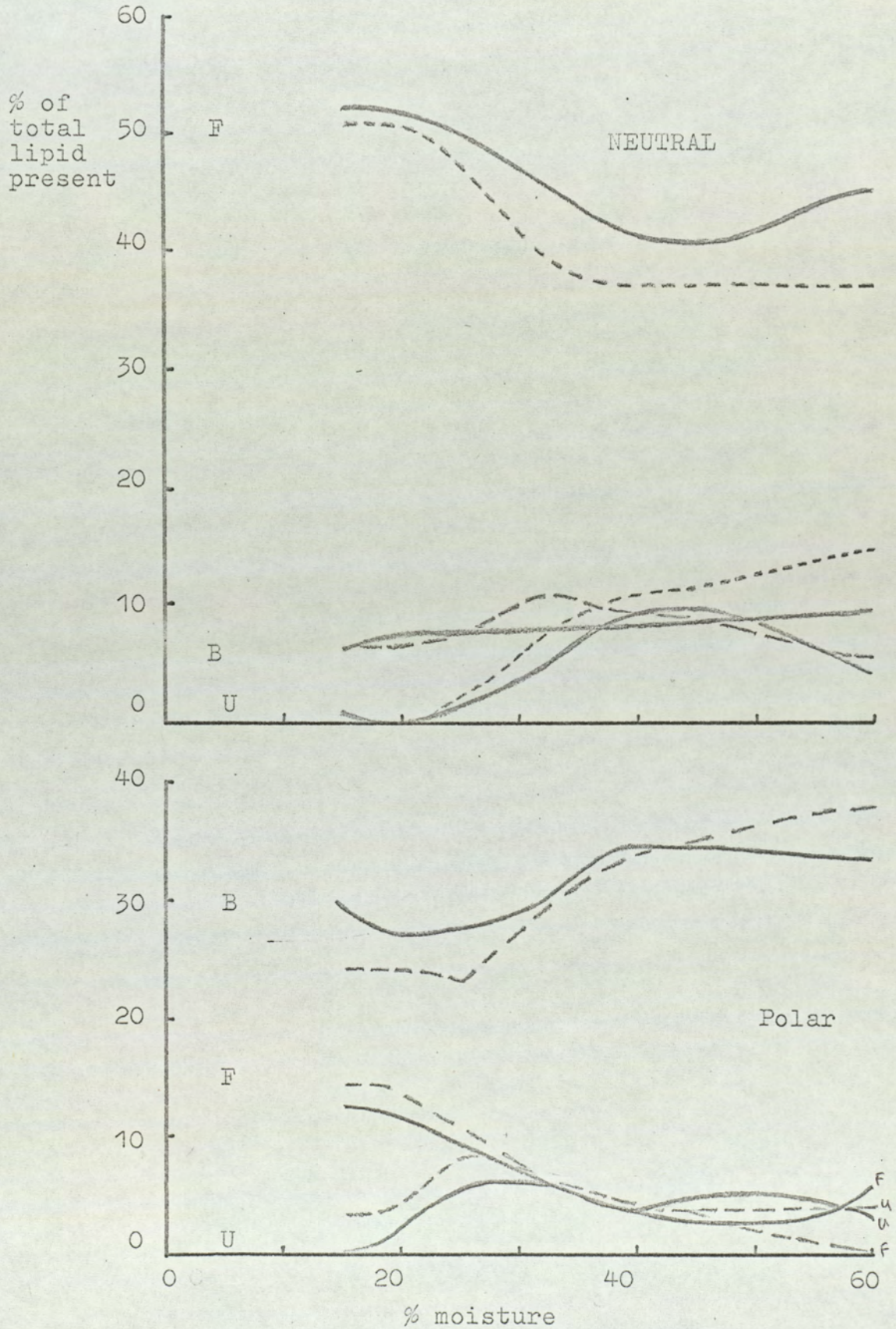
Effect of wetting on neutral and polar lipid fractions of GOL 2.



Figures 2:11 and 2:12.

Comparison of GOL 1 and GOL 2. Effect of wetting on neutral and polar lipid fractions. See pages 43 & 83 for Standard Errors of Means

GOL 1 - - - - - - GOL 2 ——————



While all these fractions showed closely similar results, the greatest differences lay in the larger, quicker loss of free neutral lipid of GOL 1 and the small maximum of bound neutral lipid of GOL 1 at 32% moisture. It is interesting to report that strong, unextractable binding of neutral lipid occured in both flours.

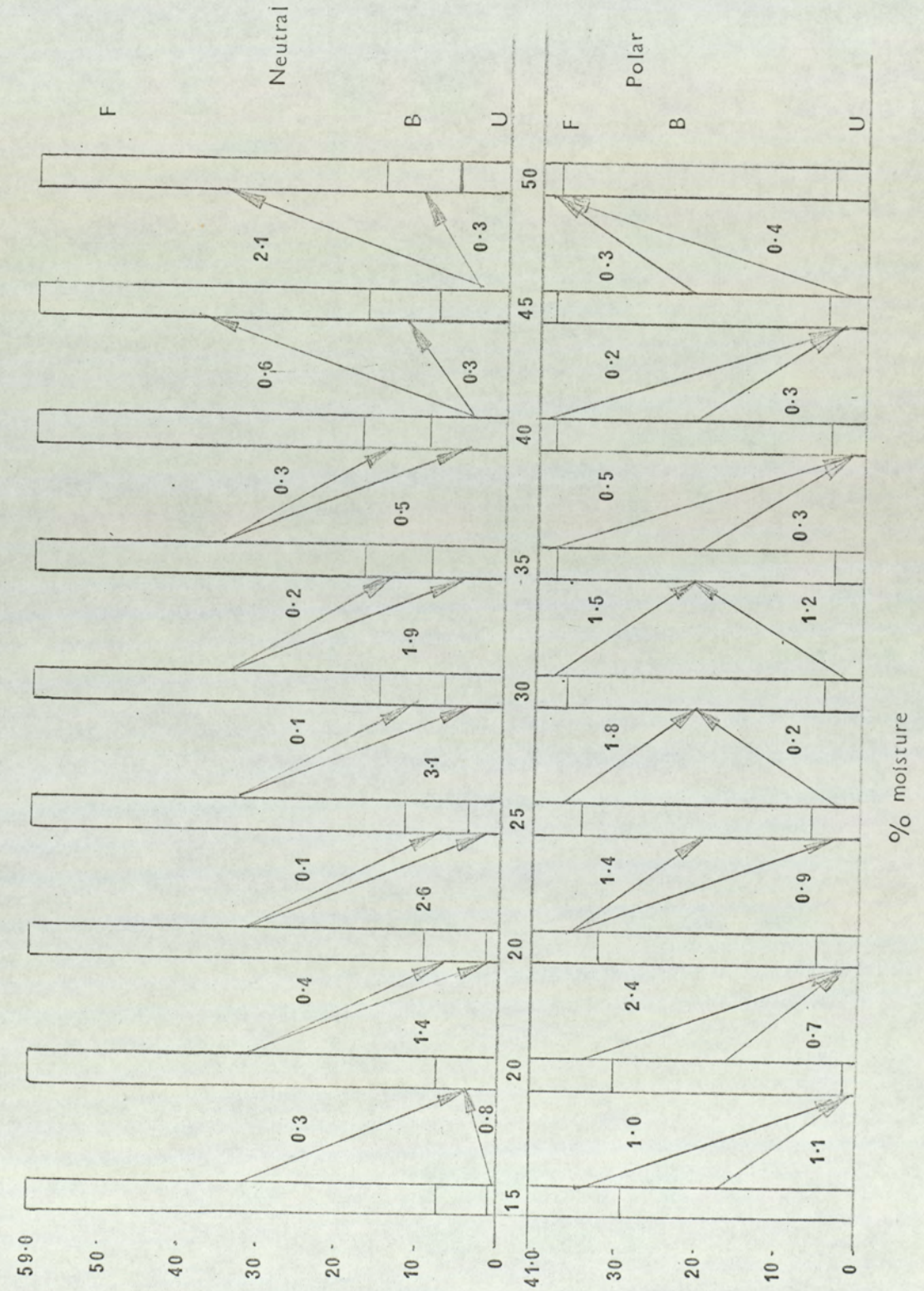
The polar lipid fractions of the two flours are compared in Figure 2:12 and are again shown to be broadly similar. The bound polar lipid of GOL 2 increased less and more slowly than GOL 1 although the free polar lipid of GOL 2 decreased similarly and by a similar amount. The greatest difference between the polar lipid fractions of the two flours lay in the unextractable fraction where at 50% moisture level, polar lipid was present in GOL 2 to the maximum for the moisture range, while in GOL 1 the level had returned to zero after a maximum value at 28% moisture.

In both flours, the effect of wetting was to bind most of the free polar lipid so that at 45% moisture the majority of the polar lipid was present in the extractably bound form. The effect of this wetting on the neutral lipid was a reduction of the free lipid by about a fifth (50-40%) in both flours. The bound and unextractable neutral fractions were present in similar amounts (10%) at 45% moisture, again in both flours, giving final proportions of 4:1:1 of the free, bound and unextractable neutral lipid.

The migrations of lipid fractions that result from work-free wetting of GOL 2 are shown in Figure 2:13. When compared with the result of GOL 1, Figure 2:5, the similarity between the response of the two flours is very striking.

Figure 2:13

Lipid fraction migration diagram. Effect of moisture level on flour GOL 2. Expressed as percentages of total lipid present.



In the two flours both neutral and polar fractions transfer between the same classes at similar moisture levels.

This information points to the conclusion that the effect of wetting is to produce a complex series of redistributions of the lipid fractions of flour. Furthermore the effect of this work free hydration is remarkably similar for two flours of the same breadmaking potential. No doubt lipid binding is only one manifestation of the many interactions that must occur between flour constituents as a result of hydration. Further evidence of this was shown by the considerable physical changes that were observed when the flour was wetted. These changes were considered worthy of further study and it was particularly hoped that they may be quantified. The intention therefore was to try to correlate the moisture levels at which changes in lipid distribution were found with moisture levels at which significant physical changes occurred.

SECTION THREE

Physical effects of work free hydration of flour.

Measurement of the physical effects of wetting flour. (GOL 1).

The results described in Section 2 drew attention to the effect of water on the physical nature of the flour, moistened in the absence of work. In this section three methods which were used to measure the physical effects of wetting flour are described and the subject of flour hydration discussed.

The first method was a simple measurement of volume loss to estimate the amount of shrinkage that was seen to occur when the ice-flour mixtures thawed in sealed beakers. The second method used the Instron Tensile Tester to detect physical differences between flours of different moisture contents. This apparatus is extremely sensitive and has been widely used for physical testing of materials including, more recently, wheat glutens and doughs. (Heaps et al. (1968)). The third approach measured the proportions of freezable and unfreezable water present in the flour at different moisture levels. Such measurements have been successfully carried out on mechanically developed doughs using a Differential Thermal Analyser as a Calorimeter (Davies and Webb (1969).

The results obtained using these methods will be considered in terms of moisture levels at which changes in physical properties of the flour occur. These moisture levels will then be discussed in terms of the interactions between lipids and other flour constituents as shown by the lipid binding study.

1. Volume shrinkage

The small open ended cylinders used to produce the photograph in Section 2 were also used in the measurement of shrinkage. Each cylinder was filled to the top with flour-ice mixture, taken from flour stocks with different ice contents prepared by the liquid nitrogen technique which had been stored at -20°C in a deep freeze cabinet. Each cylinder was covered with a metal plate and left for the contents to thaw for two hours at room temperature. The covers were then removed and rapeseed added to refill exactly each cylinder. The volume of rapeseed required to fill each cylinder is given in Table 3:1.

Table 3:1

Shrinkage of flours of different moisture content measured with rapeseed after 2 hours thawing.

Moisture %	Rapeseed volume ml.	Shrinkage %
23	4	9.8
30	5	12.2
34	9½	23.2
36	13	31.7
39	17½	42.6
45	25	61.0

To try to make these measurements more precise a repeat experiment was carried out in which dry flour was used to make up the shrinkage volume since the rapeseeds were too coarse to intimately fill the space left by shrinkage. The weight of flour involved in each case was noted and is given in Table 3:2.

Table 3:2

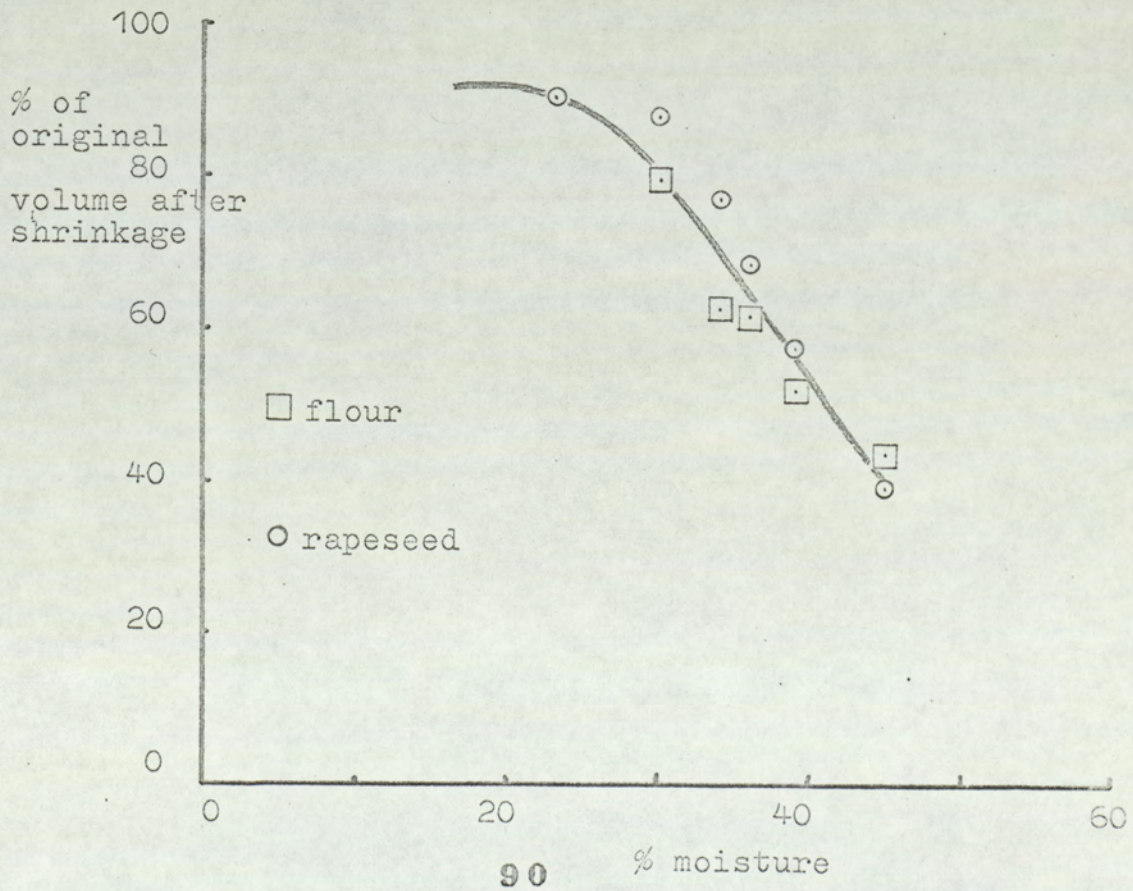
Shrinkage of flours of different moisture contents. Measured with flour after 2 hours thawing. Dry flour to fill empty cylinder: 23g.

Moisture	Flour	Shrinkage
%	g	%
30	4.80	20.9
34	8.70	37.9
36	8.93	38.8
39	11.19	48.7
45	13.06	56.8

The results of both experiments were plotted and are shown in Figure 3:1.

Figure 3:1.

Shrinkage of wetted flours.



These results show that shrinkage started between 25 and 30% moisture, probably at about 28% which is after the initial lipid binding effects have occurred. (see Fig. 2:2). The shrinking was then directly proportional to the increase of moisture level between 30 and 45% moisture. No further shrinkage occurred beyond 50% moisture. The moisture range at which the shrinkage occurred coincided with that at which polar lipid was transferred to the bound fraction, from either the free or unextractable fractions and neutral lipid was transferred to the unextractable fraction from either the free or bound fractions.

2. Instron Tensile Tester.

This apparatus has the advantage not shared by the majority of rheological dough testing methods that mechanical manipulation of the dough is not necessary before measurements are taken. This is an important advantage because the 'doughs' that are produced by the liquid nitrogen technique have received no mechanical work whatsoever in their preparation and as their properties alter as soon as the material is handled, conventional instruments were unsuitable for their examination.

When the flour contains more than about 30% moisture, the individual particles can still be distinguished although the material does appear to have a continuous structure. This structure however has no strength and would be called 'short' in dough terminology because it breaks without stretching.

The nature of the material can be likened to a dry crumbly cheese. As soon as the material is compressed or kneaded i.e. when mechanical work is introduced, a material more closely resembling dough is produced and it is this effect that the Instron apparatus examined.

Thawed flour-ice mixtures were subjected to a load of 180g by the apparatus and held at the constant deformation produced by this load to detect the effects of compression. The relaxation of the load was noted during the following minute at regular intervals of six seconds. Duplicate samples were examined over a range of moisture levels up to a limit of 51% since at this moisture a dough was produced that was too soft to accept the initial 180g load. The results are shown in Table 3:3.

Table 3:3.

Examination of work-free doughs with the Instron Tensile Tester. Relaxation of load with time. Means of duplicated values.

Load after time (seconds)	Moisture levels (%)				
	14.0	17.9	21.4	24.6	28.1
6	144	87½	86	93	86
12	139	74	70	77	71½
18	136½	66	62	68	63½
24	134	51½	57	63	58
30	132	56½	53	58	54
36	131	53½	49	55	52
42	130	51½	47	53	49½
48	129	49½	45	51	47
54	128½	47½	43	49	45½
60	128	44½	42	47	44

The values obtained after six and sixty seconds are plotted in Figure 3:2 and lines of best fit have been drawn through these values.

Figure 3:2.

Instron Tester. Load relaxation with time of wetted flours.

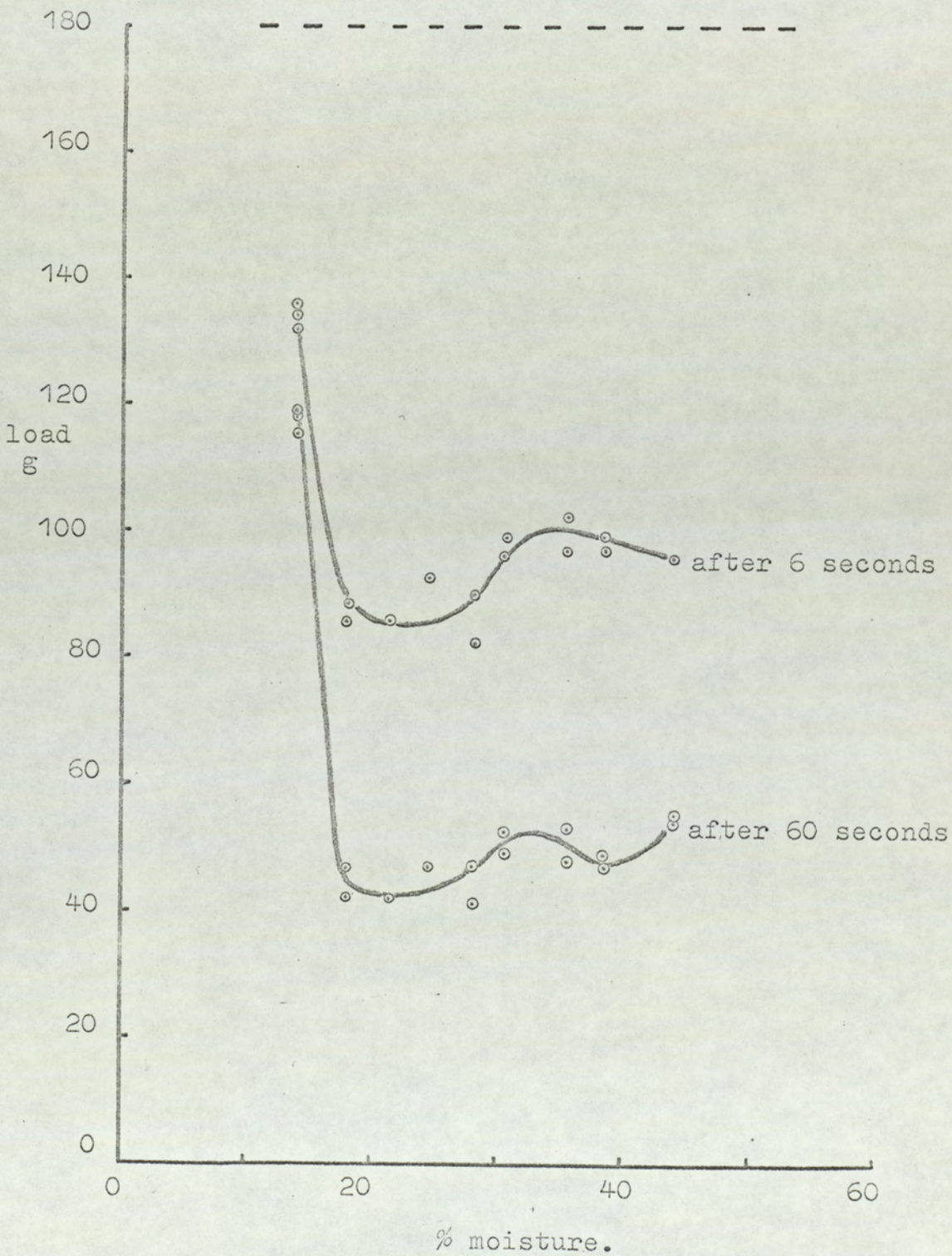


Table 3:3 continued load after time (seconds)	Moisture levels (%) 30.4	35.3	38.5	44.0
6	97½	100	98	96
12	82	83½	82	82
18	73½	74½	77½	74½
24	67½	68½	66½	69
30	63½	64	61½	65½
36	60½	60½	58	62½
42	56½	57½	54½	60
48	54½	54½	52	58
54	52½	52½	50	56
60	50	50½	48	54½

The apparatus shows the rate at which the material can relax stress produced by a deformation and the results shown in Figure 3:2 indicate that this property is affected by moisture level. The rate of relaxation depends upon how quickly the material can relax the stress produced by the initial load. This in turn depends on the number and strength of inter- and intramolecular forces within the material which are directly related to the extent and nature of dough constituent interactions.

Turning to the results in detail, the greatest effect on relaxation rate occurred between 14 and 18% moisture which coincided with the onset of unextractable binding of free lipid. With this small increase of moisture content of some 4%, the flour gained its maximum ability to relax the induced stress. This ability remained at a maximum for the moisture range 18-25%, the range at which unextractable binding occurred, but decreased between 25 and 35% moisture, now coinciding with the moisture range at which most of the extractable lipid binding was occurring.

Between 35 and 40% moisture the relaxation mechanism improved again and it was in this moisture range that both neutral and polar lipid was being extractably bound, some free neutral lipid was being unextractably bound and unextractably bound polar lipid was being transferred to the bound fraction.

Finally, between 40 and 45% moisture, the relaxation mechanism continued to improve immediately after deformation (6 seconds) but this improvement was not sustained and stress was less effectively relaxed after 60 seconds.

It was in this moisture range that there was an apparent exchange between bound and unextractable fractions of neutral and polar lipid, the former to the unextractable and the latter to the bound fraction.

Thus one can conclude that merely raising the moisture level of flour allows many interactions of importance to dough rheology to occur between the constituents among them the redistributions of lipid fractions. It is interesting and possibly significant that these redistributions occur over the same moisture range as the changes in physical properties measured by the Instron Tensile Tester.

3. Freezable and unfreezable water.

A differential thermal analyser has been successfully used as a calorimeter and shown to be a satisfactory method for determining freezable water in dough. An attempt was therefore made to use this method to examine freezable water in unworked moistened flours. However, difficulties were encountered due to the discontinuity of the moistened flours compared with the homogeneous nature of a mixed dough. Nevertheless when the results shown in Table 3:4 are plotted as in Figure 3:3 the amount of unfreezable water present in unworked doughs, 23%, was close to that found in mixed doughs prepared from the same flour. (24.85% Davies and Webb (1969)). This was further confirmed by the absence of freezable water in flour moistened to 23%.

One can conclude that when the moisture level is raised from 14 to 23%, all the added water is so intimately involved with the flour constituents as to be unfreezable at low temperatures.

Figure 3:3.

Proportion of freezable water in wetted flour of different total moisture contents. Determined by calorimetry. Line of unit slope drawn.

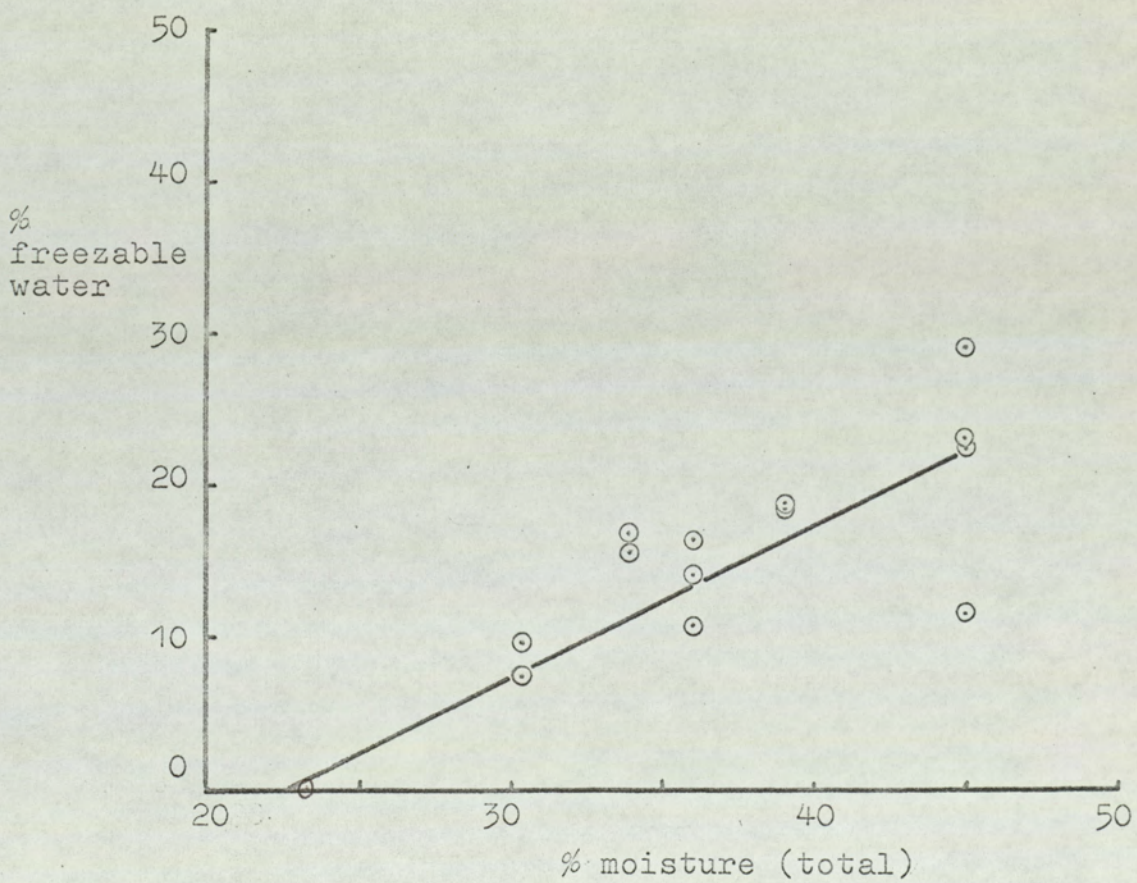


Table 3:4

Calorimetric determination of freezable water in wet flour.

Total moisture content (%)	Freezable water content (%) Mean values of three determinations.
30.2	7.50 ± 0.58
"	9.87 ± 0.30
33.8	16.90 ± 0.37
"	15.75 ± 2.30
36.0	16.60 ± 0.61
"	10.87 ± 0.11
"	14.05 ± 0.21
39.0	18.50 ± 0.49
"	18.95 ± 0.12
44.8	22.50 ± 1.99
"	11.55 ± 0.35
"	29.10 ± 0.71
"	23.20 ± 1.56

This is also the range of moisture levels over which strong unextractable binding of free lipid occurs as a result of work free wetting.

The results of this section have shown that work free wetting of flour produces both physical changes in the nature of the flour and redistribution of the lipid fractions. These effects are but two manifestations of the many interactions between flour constituents that result from hydration.

The consequences of wetting flour.

Although water is added to flour on every occasion that a dough is produced for breadmaking, wetting flour is not usually considered as a separate step. Invariably in dough mixing the combination of flour and water occurs in an mechanical mixer accompanied and probably aided by mechanical work. This explains why, although many people have speculated on the subject of flour hydration during dough mixing e.g. Hess (1955), Sandstedt (1955), Wall (1967) and Medcalf and Gilles (1968), there has been little interest shown in flour hydration in the absence of work. Since hydration is such an inevitable stage of dough production, the consequences of water addition, uncomplicated by the effect of mechanical mixing, are worth careful consideration. As has been seen from the results presented already, there is evidence of considerable flour constituent interaction.

The following survey was made with the intention of assembling together the factors involved in work free hydration of flour.

However, much of the published work on the subject of flour hydration has concerned mechanically mixed doughs and results are often considered from the breadmaking point of view. Nevertheless this information is still very relevant to an understanding of the processes involved in work free hydration of flour.

Hydration of flour.

Effective hydration of flour will only be achieved if the moisture is evenly distributed among and over the flour particles. Water distribution is one of the functions of dough mixing and a factor in flour hydration. A baker is interested in the maximum as well as the efficient incorporation of water in dough and the 'water absorption' of flour is considered responsible for this property. Flour 'water absorption' is the amount of water required to produce a dough of predetermined consistency in a Farinograph. Although this property cannot be attributed to a single constituent, an important factor is the amount of damaged starch present in flour. This damage occurs in the milling process particularly to hard wheats which by suffering greater starch damage produce flours with higher water absorption than soft wheats.

The hardness of wheat depends mainly on variety and to a lesser extent on the climatic conditions during maturation (Meredith (1969)). As hard wheat varieties also tend to be high protein varieties the properties are often considered to be related.

Different workers have come to different conclusions as to whether starch damage or protein content is the more significant factor in flour 'water absorption' as the gluten protein also makes a significant contribution to the hydration capacity of the flour. Moss (1961) considered protein content the more significant factor while Greer and Stewart (1959) thought starch damage showed the greater influence. Meredith (1966) found the two factors to be of almost equal importance.

Farrand's work has been useful in relating protein content and starch damage to maximum water absorption consistent with good bread quality (Farrand (1964), Farrand (1969)) but the importance of further factors were also revealed. For instance, water absorption is closely linked with alpha amylase activity in breadmaking (Tipples (1969)). Damaged starch is susceptible to alpha amylase attack which while necessary for gassing power can, in excess, overproduce dextrin. Alpha amylase can also effectively reduce the baking absorption by making the dough too slack during fermentation as enzyme degraded starch has a lower water capacity than damaged starch. On the other hand, in a mechanically developed dough where the period of fermentation is far shorter, more water than is indicated as sufficient by the Farinograph can be added without ill effects and give extra bread yield. However Lorenz and Johnson (1970) have found a limit of Damaged Starch Index of 4.65 for satisfactory continuous-mix bread production.

Although overall flour water capacity is of importance (Meredith (1969), other workers have wondered whether the rate of absorption is not more significant, particularly in brief dough processes. (Larsen (1964), Pratt (1964) and Bushuk (1966)). Particle size has been postulated as being responsible for the rate of absorption (Gracza (1960) Ponte et al. (1961), Pratt (1964) although Bushuk and Winkler (1957), Gur-Arieh et al. (1967) and Udani, Nelson and Steinberg (1968) have all shown this not to be the case. Muller and Hlynka (1964) reported that flour hydrates very rapidly while Gracza, Prigge and Manseth (1965) found that ten minutes were required to completely hydrate flour in a Farinograph. However one may question this latter conclusion as a dye of higher molecular size than water was used as an indicator of the completeness of hydration.

The factors that influence the water absorption of a flour are therefore largely concerned with flour hydration but also are related to the requirements of breadmaking. The flour constituents will now be considered individually but with two reservations. Firstly the behaviour of one individual constituent may be different in the presence of all the others and secondly, the method of isolation of a constituent may alter its behaviour.

Starch.

As already mentioned some of the starch in flour is damaged during the milling process, the proportion of damaged starch depending on the hardness of the wheat.

Farrand (1969) has shown that there is an optimum amount of damage for a particular flour if it is to be used for breadmaking. Several workers have shown that excessive starch damage causes bread failure and that it is not worth trying to induce additional starch damage. (Hampel (1954), Atkinson and Feuhrer (1960), Ponte et al. (1961), Schlesinger (1964), Schiller and Gillis (1964) and Tipples & Kilborn, (1966)).

Undamaged starch has a water capacity of 30% (Newton and Cook (1930)) to 35% (Sandstedt (1955)) while damaged starch will absorb about twice its own weight of water within half a second (Sandstedt and Schroeder (1960)). However the total cold water capacity of starch is less than is required for gelatinisation during baking. Additional water must therefore be transferred to the starch although damaged and undamaged starch both have the same water requirements for gelatinisation (Farrand (1964)).

Both Gracza (1960) and Meredith (1969) have speculated on the effect of lipids on capacity and rate of starch water absorption. Chlorination of flour, which particularly affects the lipid fraction, greatly increases absorption. Meredith (1969) agreed with Gracza (1960) that the effect on absorption is more likely to be due to a property change of the lipids associated with starch. If lipids do have an effect on starch absorption then one must query the results of Sandstedt (1955) and Larsen (1964) as both used solvents in their starch separations.

Protein.

Gluten protein has the main water-carrying function of flour and is considered to have a constant water requirement of 2.8 times its dry weight (Pratt (1964)). Larsen (1964) considered the gluten to have a slower rate of water absorption than starch and wondered whether protein might sometimes be incompletely hydrated in brief mixing processes. Milling is thought in some circumstances to denature part of the protein and reduce the water carrying ability (D'Appolonia and Gilles (1967)). The soluble protein is considered to have no water requirement (Pratt (1964)), however it will reduce the available free water.

To produce a satisfactory bread, the protein must be adequately hydrated and carry sufficient water to provide for the extra requirement of the starch at the gelatinisation stage in the oven. Flour protein hydration is described by Wall (1967) as the penetration of protein particles by water which then associates with polar sites to overcome the forces that cause the molecules to adhere.

Pentosans.

The pentosans are only present in flour in small amounts (about 2%) yet they must be considered significant in the context of hydration. Kulp (1968) reports that the soluble pentosans absorb 11 times and the insoluble pentosans 10 times their own weight of water. Therefore the pentosans absorb between a quarter and a third of the total water present in dough (Bushuk (1966), Kulp (1968)).

Hydrated pentosans are highly viscous and Neukom et al. (1967) suggest that they contribute to dough consistency. They are considered to form an intimate mixture with the gluten in which the starch granules are buried. If water insoluble pentosans are added to flour, they increase the water absorption, however they are considered to be deleterious to breadmaking properties (Sandstedt, Jolitz and Blish (1939) and Kulp and Bechtel (1963)). Whereas the water soluble pentosans are considered beneficial (Wrench (1965)). Udy (1957) suggests that there is an interaction between the gluten protein and water soluble polysaccharides of flour. Kulp (1968) considers that the different effects of water soluble and insoluble pentosans on breadmaking properties are due to these interactions. He suggests that water soluble pentosans interact with proteins not critically important to the protein network while the insoluble pentosan complex becomes associated with structure forming gluten proteins and interferes with the associative forces of gluten.

Lipids.

Although lipids have generally been considered not to have a hydration capacity of their own, the work of Chapman and coworkers suggests that this may not be so. In his studies of single phospholipid-water systems by differential scanning calorimetry (d.s.c.) he has shown that phospholipids bind about 20% water. (Chapman et al. (1967)). Furthermore, the water has an effect on the liquid crystallinity of the phospholipids. The presence of cholesterol in the phospholipid-water mixture also had an effect on the liquid crystallinity as was shown by d.s.c. (Ladbrooke et al. (1968)) and nuclear magnetic resonance spectroscopy (Chapman and Penkett (1966)).

These results not only suggest the possibility of the flour lipids having a water requirement but underline the possibility of interaction of lipid with other flour constituents. If the flour lipids are in a liquid crystalline form at temperatures below the 'capillary' melting point, and these temperatures are lowered even further by the presence of water and sterols, the possibility of the flour lipids being in liquid crystalline form at room temperatures is very real. This being so, hydrophobic interaction between lipid and other flour constituents seems likely.

Bushuk (1966) has considered the effect of lipids on the hydration of other flour constituents and thinks the non-polar lipids will have no effect on the overall distribution of water in the flour but may well effect the hydration capacity of hydrophilic constituents. He considered that polar lipids would affect the hydration capacity of gluten more than starch. Several workers have wondered about the influence of lipids on the redistribution of water in dough (Meredith (1969) Alcock and King (1950)). The presence of free lipid, concentrated on the starch granule surfaces with possible effects on the hydration properties as discussed by Gracza (1960) has already been mentioned and related to this is the profound effect of lipids on the pasting properties of starches (Osman and Dix (1960), Gray and Schoch (1962), Leach, McCowen and Schoch (1959), Medcalf, Youngs and Gilles (1968)). Therefore there is considerable evidence of the significance of the lipid fraction in flour hydration.

Salt and other inorganic materials.

The presence of soluble inorganic substances in the flour itself, in the added water and added to the flour as part of the breadmaking ingredients must also be considered in the hydration process. Traces of inorganic salts are naturally present in flour, the water used in breadmaking is not softened and common salt is always added to dough for breadmaking. Yeast foods, consisting of inorganic salts and certain oxidising agents are also regular ingredients.

Sodium chloride reduces the gluten water holding capacity by about 8% when added at the customary 2% level (Bushuk (1966)). The gluten structure is tightened by salt, as rheological studies show (Fisher, Aitken and Anderson (1949)) and less water can be held. The first benefits of adding salt are not realised until the fermentation stage when yeast activity is controlled. Fat coated (encapsulated) salt can be used in mechanically developed dough so that the salt is not released into solution until required (Fortmann et al. (1969)). The ionic concentration of the dough liquor effectively reduces the free water content of the dough and affects the ionic interactions between flour constituents (Bennett and Ewart (1965)). Mecham and Weinstein (1947), Wootton (1966) and Pomeranz et al. (1968) have shown a reduction in lipid binding when salt is present.

Metal ions also affect the oxidative gelation of pentosans (Kundig, Neukom and Deuel (1961) and the work of Fullington (1967), Dawson (1965) and Fullington and

Hendrickson (1966) suggests the involvement of metal ions in lipid -protein interactions which result in three way complexes of great stability. Acidity reduces the water holding capacity of gluten (Meredith (1969)) as do the added oxidants which affect the disulphide bonds and hence the mechanical properties of the gluten (Mecham (1968), Meredith (1969)).

Organic materials.

Flour contains some free sugar and in some bread-making processes sugar is added, which Bushuk (1966) considers to affect the rate but not the capacity of flour hydration. Like other soluble constituents of the flour, sugar reduces the available free water which will influence the rheological properties of the dough (Hlynka (1959)) and reduce the amount of hydrophobic bonding which Wehrli and Pomeranz (1969) suggest is dependent upon availability of free water and which Bushuk, Tsen and Hlynka (1968) consider an important feature of dough structure. Finally, the structure of water itself must also be considered as is shown by Tracey's work (1968) on the profound effect of drugs on dough properties by modification of the bound water.

Discussion.

Thus all the major flour constituents are influenced individually as well as collectively by water. Since our knowledge of hydration capacities of the protein, starch and pentosan fractions (Larsen (1964), Bushuk (1966)) shows that a breadmaking dough of 45% moisture has insufficient water for complete hydration of these three constituents (about 50% has been shown to be required (Bushuk (1966))

there will be competition for the available water. As this is limited in a dough, those constituents with faster rates of absorption will become hydrated first. It is suggested that this will be the starch, but as the gluten has greater capacity, there will probably be a redistribution of water with both time and mechanical development.

Using the information derived both from the literature and from the results of these first sections, the consequences of wetting flour without the introduction of mechanical work may be seen as follows.

When water is added in a limited amount, there will be competition for it from all the flour constituents and this will result in considerable changes in the individual components as well as the flour as a whole. The water will first be absorbed by the damaged starch, followed by the gluten, going into hydrophilic areas which probably also attract free polar lipids. There will be changes in conformation to accomodate the water and these changes, plus the presence of the water may permit movement to more stable arrangements. These movements, together with any release of energy will encourage interactions between constituents. New sites of interaction may be revealed, areas opened up and also closed in, releasing physically trapped material or entrapping fresh material.

Some interactions may result in the formation of chemical bonds if reactive sites become within range. If there is sufficient free water present redistribution between constituents may occur. Free water is essential for the existence of hydrophobic bonding and the character of the constituents may be completely changed by the presence of free water.

The fate of the lipids is probably governed by such an effect of water on the protein and the starch and one might imagine the dough structure at this stage to consist of hydrophobic areas within a hydrophilic mass. Hydrophobic bonding is an entropy effect and the exact arrangement would depend on that which gave greatest stability, as, without any mechanical work, the situation would be entirely governed by thermodynamic considerations.

However there will be a limit to all these effects as long as neither mechanical work nor other factors e.g. chemical action are introduced. One might therefore expect to reach a point at which further wetting causes no further interaction. Although such a limit of 45% moisture was seen in the lipid binding pattern of the first flour GOL 1, in the second flour, GOL 2, there appeared to be release of unextractable lipid to the free fraction at moisture levels above 45%. A probable explanation of the results, no doubt related to the inevitable differences that exist between flours from different grists, lies in the effect of this excessive amount of water which would tend to separate the free lipid from the substrate and even prevent as much binding as occurred at lower moisture levels.

The methods used to obtain the lipid binding results artificially divide the lipid into three fractions according to accessibility to solvent. Such an approach gave results which were repeatable and highly significant enabling changes in lipid distribution to be followed with confidence.

Moreover the experimental design ensured that the conditions of extraction were identical in each case since although the flour had experienced different amounts of water, the water was almost entirely removed by freeze-drying and the samples ground to powder of standard size before extraction.

Since neutral and polar lipids are not directly interconvertible in these circumstances these two lipid classes have been considered separately. It is possible however that mutual solubility effects may occur in a hydrophobic environment and will in certain circumstances lead to behaviour as a single lipid fraction.

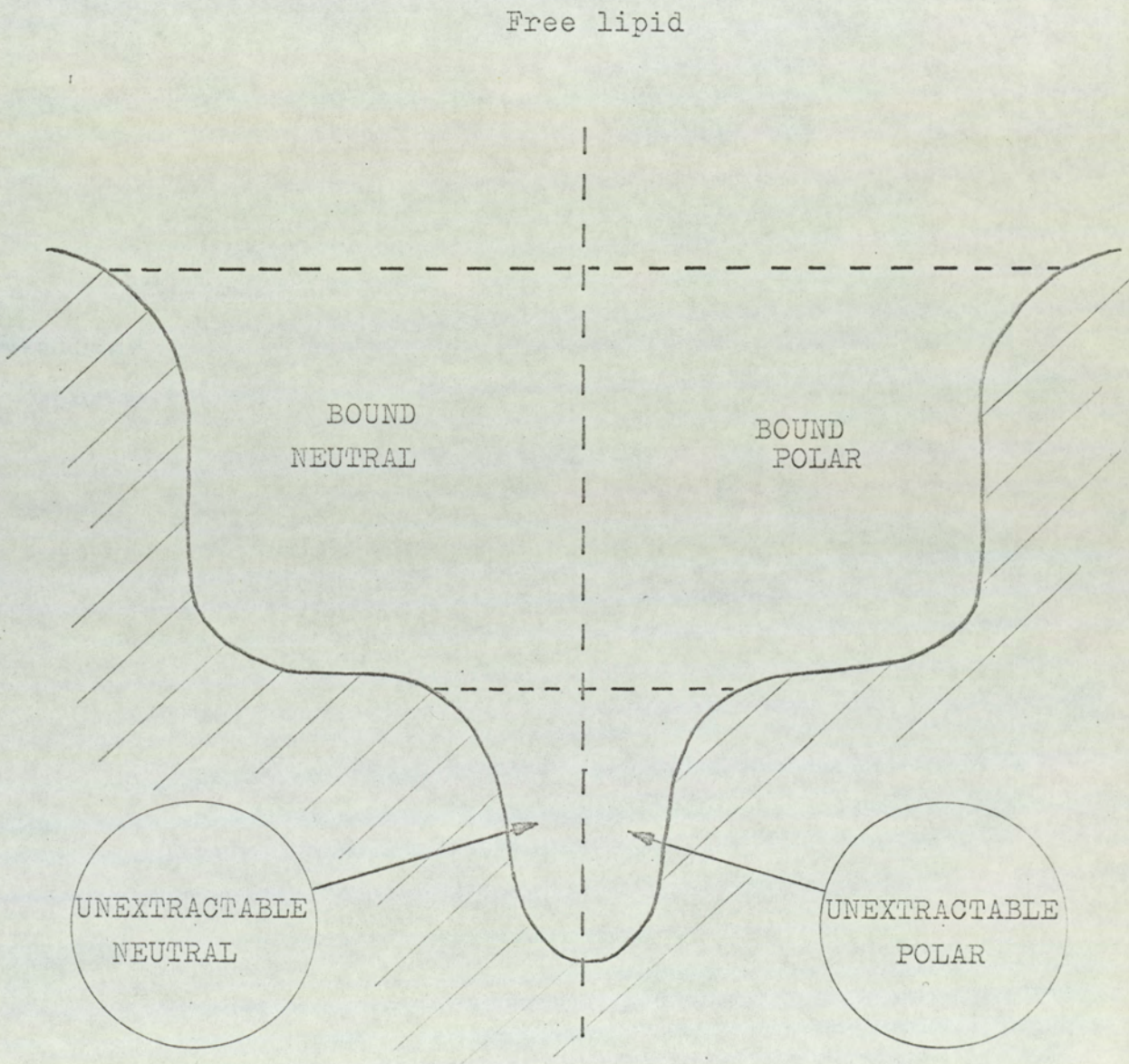
Lipid binding sites.

As an aid to clarity when discussing the nature of lipid binding it is proposed to consider that binding is occurring at a hypothetical site in the dough. Already a certain amount is known about such sites and later sections will describe studies directed towards learning more about their location and character. With this information it might be possible to resolve the nature of the various interactions responsible for lipid binding.

It is suggested that the sites be regarded as holding lipid at two levels, bound and unextractable and that the sites be considered to be divided into two halves, one for neutral and one for polar lipids. The sites are affected by moisture level such that there is movement in either half between the two levels and into the two levels from outside. They can be represented diagramatically as shown in Figure 3:4.

Figure 3:4.

A HYPOTHETICAL BINDING SITE.



Further study of the sites will follow these two lines.

1. The constituents of the flour (other than lipid) can be altered experimentally and the lipid binding response to work-free wetting examined. Such an approach will involve altering the sites and following subsequent changes in lipid binding.

2. The content and composition of the flour lipid can be altered in a flour with a known constitution and the response of the lipid binding to work-free wetting examined. In this case the sites will remain the same but the material available for binding will be altered and then the effect of work free wetting on these systems can be studied.

SECTION FOUR

Effect of wetting soft wheat flour.

A soft wheat flour.

This next section describes the effect of moisture level on the lipid distribution of a third flour, HIL. This flour was chosen as an example of a completely different type of flour to the two flours studied previously. The flour was milled from a soft wheat grist and 'air classified' to reduce the protein content. This produced a flour more suitable for making cake than bread. This flour had a significantly lower protein content, lower total lipid content and, by difference, a higher starch content than GOL 1 and GOL 2. (See Table 4:1).

Although many baked products made from soft wheat flour contain a high proportion of added fat relative to the amount of lipid naturally present, the natural lipid is essential to the baking properties. Baking failure will occur if the natural free lipid is removed from the soft flour by solvent extraction and only the return of the extracted lipid will restore the original baking properties to the flour. (Gilles and Shuey (1964)).

Compared with the two GOL flours discussed previously one would expect this flour to have a lower water absorption since it is known that less starch damage occurs during the milling of a soft wheat. The compositions of the three flours are compared in Table 4:1.

Table 4:1.

Composition of hard and soft wheat flours.

Protein, lipid and carbohydrate on a % flour dry weight basis.

	GOL 1	GOL 2	HIL
Moisture	14.05	12.50	12.60
Protein (N x 5.7)	14.5	15.0	6.9
Total extractable lipid	1.653	1.572	1.090
Carbohydrate by difference	83.8	83.4	92.0
Colour: Grade Value	2.5	1.4	3.0
Ash (Gross)	0.58	0.65	0.40

The effect of moisture level on the lipid distribution in this third flour, HIL, was studied to see whether the different proportions of protein and carbohydrate, showed any major effect. Such a study was undertaken to provide an indication of either the location or the availability of 'binding sites' when compared with the results already obtained for the two GOL flours.

Effect of moisture level on lipid distribution of HIL.

The examination of the effect of moisture level on the lipid distribution of this flour was carried out in exactly the same manner as used before on the previous two flours. When samples of the flour were raised to different moisture levels by the liquid nitrogen technique the unworked doughs thus produced were quite different from those from flour GOL 1. Whereas the hard wheat flour doughs had some structure above 30% moisture, those from the soft wheat flour had none at all and were more like slurries. The wetted flours were then freeze-dried and the distribution of free and bound, neutral and polar lipids determined as before. These results are shown in Tables 4:2, 4:3 and 4:4.

Table 4:2.

Effect of moisture level on free and bound lipid distribution. HIL.

Expressed as % of flour dry weight. (Mean values).

Moisture level %	Free	Bound	Total	Unextractable
12.6	0.680	0.410	1.090	-
29.0	0.644	0.384	1.028	0.067
33.7	0.588	0.425	1.013	0.082
38.3	0.565	0.462	1.027	0.068
48.4	0.517	0.504	1.021	0.074
53.0	0.533	0.492	1.025	0.070
Least significant difference. P=0.001	0.066	0.081		

Table 4:3.

Effect of moisture level on a soft wheat flour. HIL. Distribution of neutral and polar lipid. Mean values expressed as % of total extractable lipid.

Moisture level (%)	Free		Bound	
	% neutral	% polar	% neutral	% polar
12.6	52.0	10.4	9.7	27.9
29.0	51.0	8.0	10.5	24.7
33.7	47.2	6.8	12.0	27.0
38.3	45.6	6.2	13.1	29.2
48.4	42.6	4.7	12.4	33.8
53.0	44.1	4.8	10.8	34.3
Standard Errors of Means	± 3.4	± 2.4	± 3.6	± 2.8

Table 4:4.

Effect of moisture level on a soft wheat flour. HIL Neutral and polar distribution of free and bound lipid. Mean values expressed as % of flour dry weight.

Moisture level %	Free		Bound	
	neutral	polar	neutral	polar
12.6	0.567	0.113	0.106	0.304
29.0	0.556	0.088	0.115	0.269
33.7	0.513	0.075	0.131	0.294
38.3	0.497	0.068	0.144	0.318
48.4	0.464	0.053	0.135	0.369
53.0	0.482	0.051	0.118	0.374
Standard Errors of Means	±0.037	±0.028	±0.039	±0.030

The total values of free and bound lipid based on percentage of flour dry weight are plotted in Figure 4:1 and lines of best fit drawn. As before the unextractable fraction was obtained by difference.

When compared with the equivalent results of GOL 1 as shown in Figure 4:2 it is apparent that both the total lipid and the effect due to moisture are less in this soft wheat flour.

Qualitative comparison of hard and soft wheat flours.

As lipid contents of the flours were so different, a better comparison was obtained when the lipid fractions were compared as percentages of the total lipid of each individual flour. Figure 4:3 shows the total free and bound fractions of the two flours calculated on this basis and emphasizes the similarity of these two flours. A number of minor differences will be noted, the first being that moisture level change had less effect on the lipid fractions of HIL. Secondly, the initial decrease of HIL

Figure 4:1.

Effect of wetting on total lipid fractions of flour HIL.

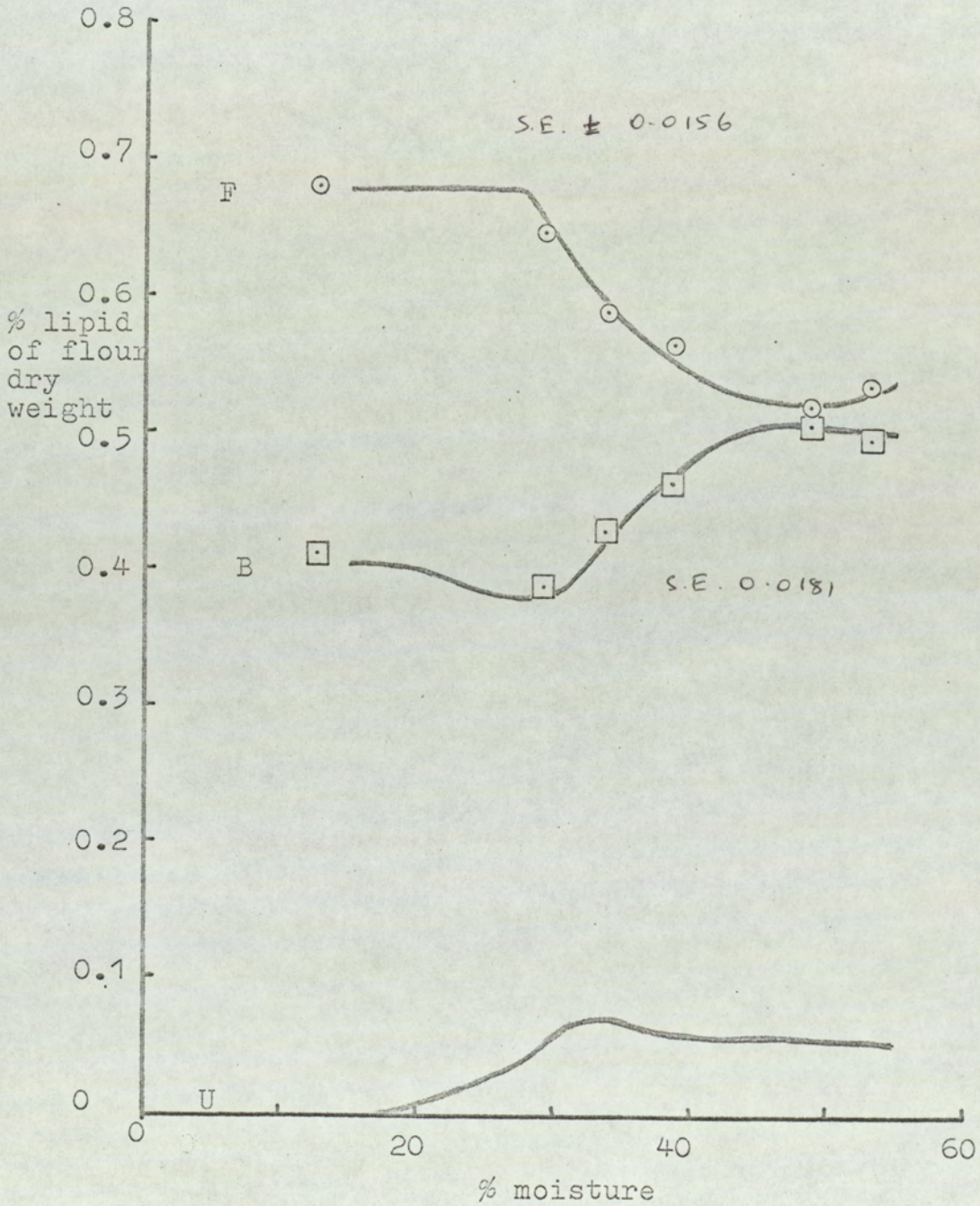


Figure 4:2.

Comparison of HIL and GOL 1. Effect of wetting on total lipid fractions. Results expressed as percentage of flour dry weight.

See Figures 2:2 & 4:1 for Standard Errors of Means.

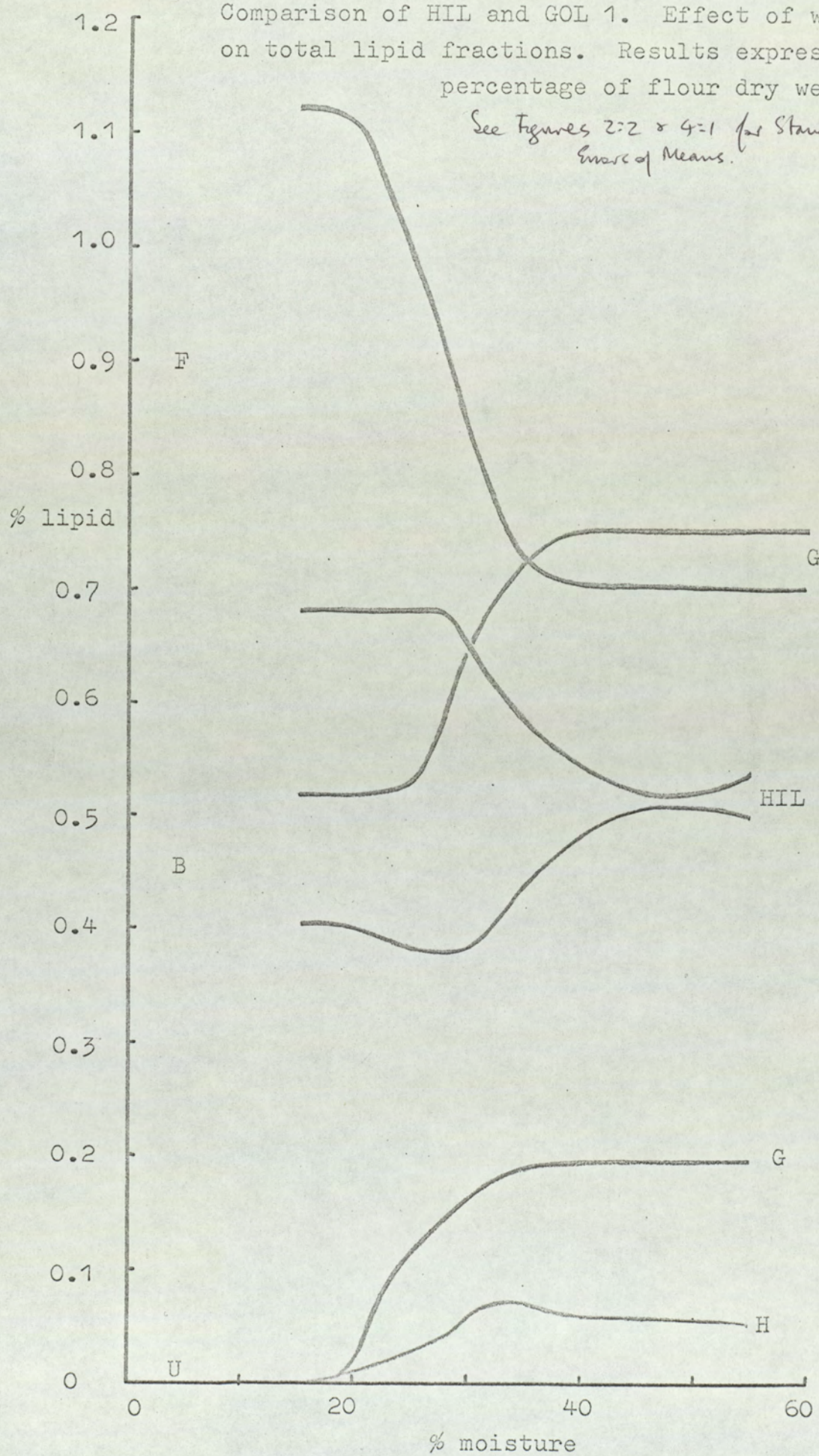
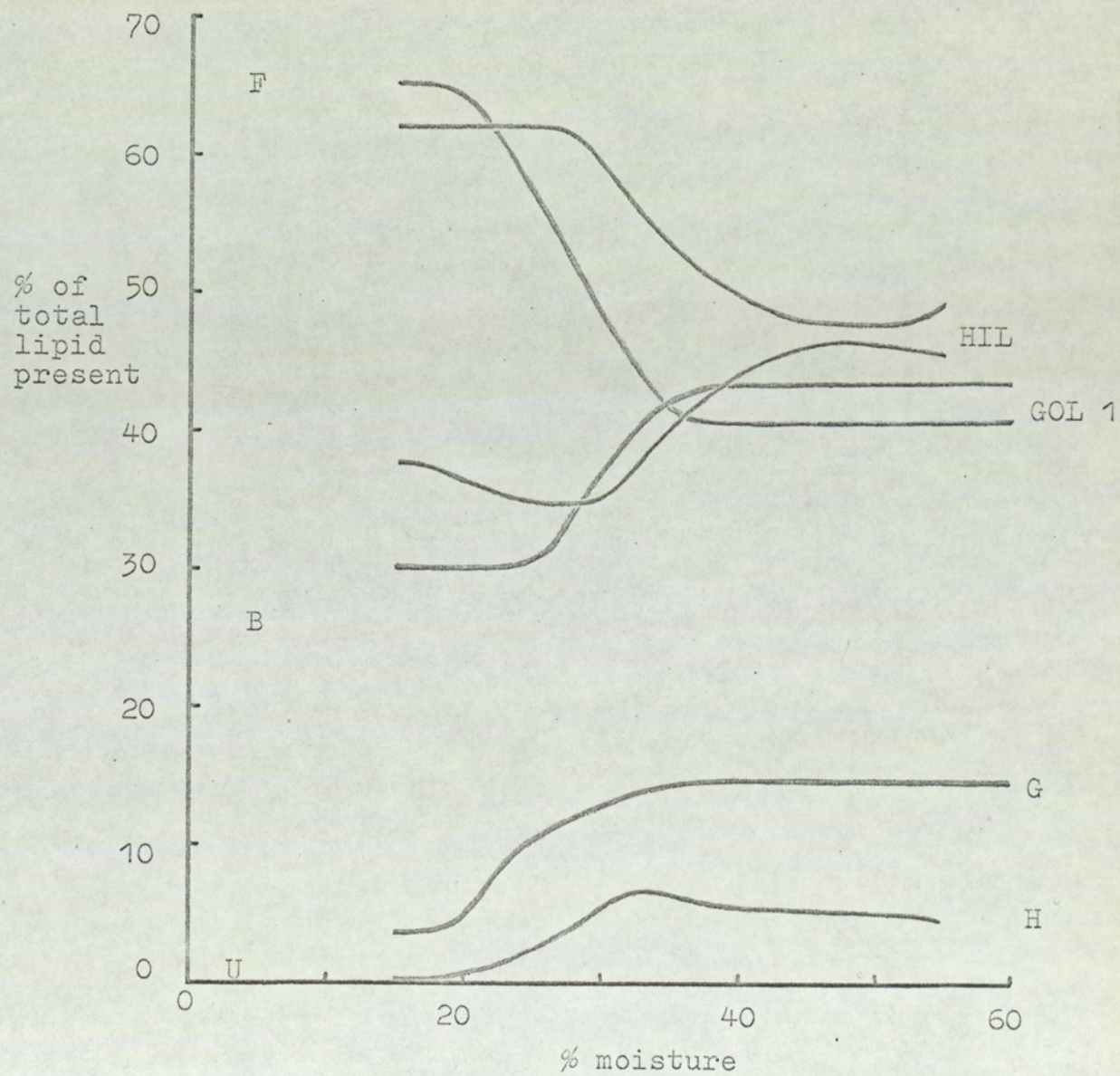


Figure 4:3.

Comparison of HIL and GOL 1. Effect of wetting on total lipid fractions.

Standard Errors of Means		Free		Bound
GOL	±	0.54	±	0.97
HIL	±	1.43	±	1.66



free lipid occurred at a moisture level nearly 10% higher than in the hard wheat flours. Thirdly, the HIL bound lipid decreased slightly during the first phase of wetting. This decrease suggested that the first effect of moisture level increase was the formation of an unextractable fraction, supplied in this soft flour by the bound fraction. But this unextractable fraction developed to only half the value shown by the previous two hard flours.

Polar lipid.

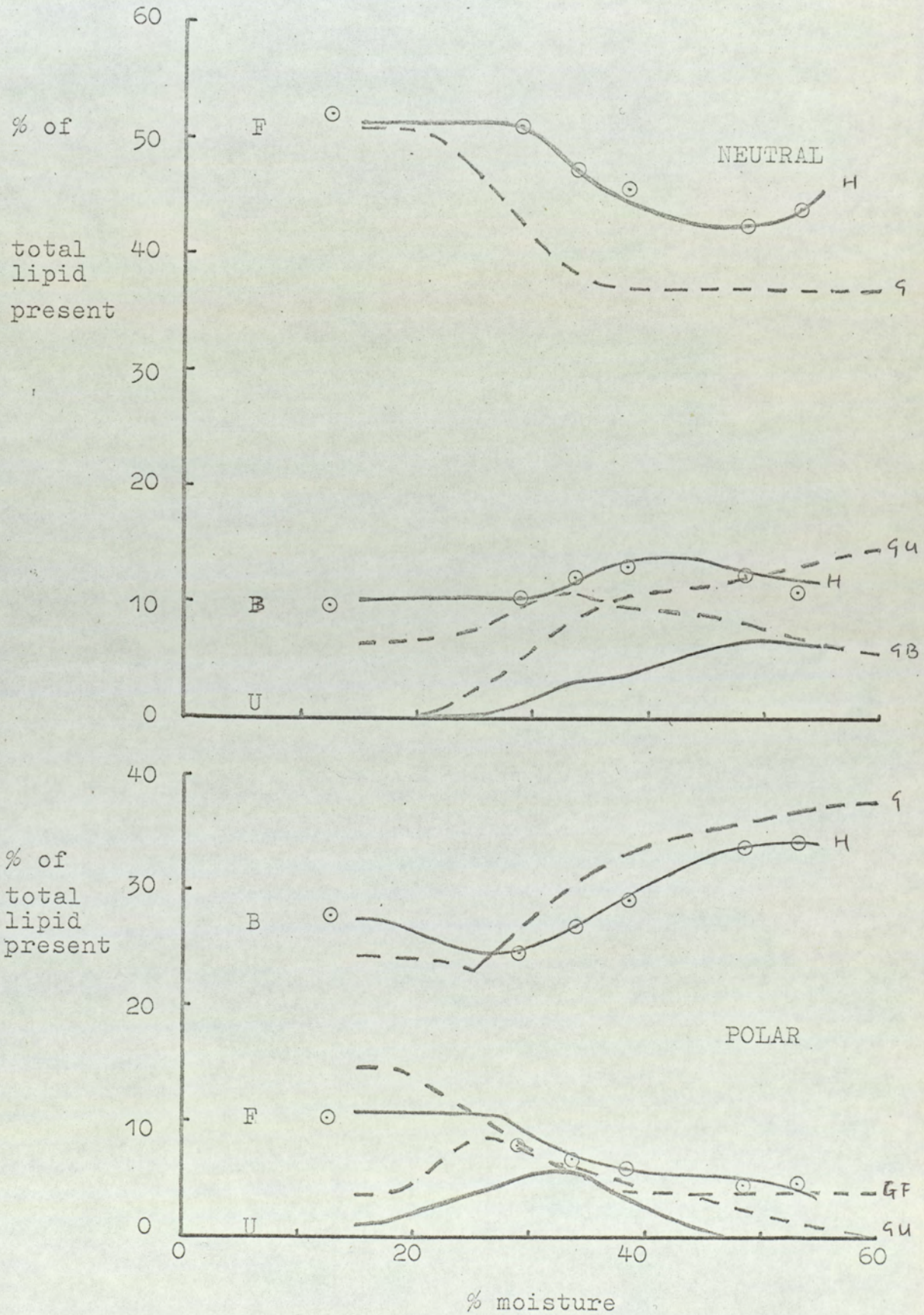
When the polar lipid fractions of the two flours are compared, as in Figure 4:4, it can be seen that they were for the most part similar. The HIL free lipid started to decrease at a higher moisture level and yet fell to a similar final value as that of GOL 1 at 50% moisture. The bound polar lipids increased by similar proportions and at similar rates although the increases did not start at exactly the same moisture levels. At 50% moisture the bound polar lipid was about 35% of the total in both cases. Although the unextractable fraction of HIL increased to the same level and at the same rate as GOL 1 it did decrease again at higher moisture levels.

Neutral lipid.

The neutral lipid fractions of HIL, shown and compared in Figure 4:5 with those of GOL 1, were mainly similar apart from the higher moisture level at which binding first occurred. Other minor differences were that although the free neutral lipids were of similar proportions at 14% moisture, those of HIL decreased less on wetting. Also the bound neutral lipids of HIL reached a maximum at 40% moisture before decreasing and the unextractable neutral fraction increased more slowly and rose to only half the value shown for GOL 1.

Figures 4:4 and 4:5.

Comparisons of neutral and polar lipid fractions of HIL and GOL 1 when wetted. See pages 43 & 116 for Standard Errors of Means



Qualitative conclusions.

In spite of its markedly lower protein content the general pattern of lipid response of HIL to moisture level increase showed a marked similarity to the two GOL flours. This must indicate that hydration produced the same sort of effects in both hard and soft wheat flours although on a slightly different scale. This may have been due in part to HIL having less lipid and protein but since the different lipid fractions did not all differ to the same degree, other qualitative factors must be involved.

When the differences in lipid distribution between the flours are considered, the most striking at first sight is the higher moisture level at which onset of loss of free lipid occurred. Since a soft wheat flour has a lower water absorption, a lower moisture level might have been anticipated. However, unextractable binding of the bound fraction was evident at about 20% moisture so that the actual difference between the lipid binding response of GOL 1 and HIL was the source of the unextractable fraction in the 20-25% moisture range.

In conclusion, the qualitative comparison of the flours has shown in each case that the same kind of lipid binding process was involved since a similar effect of work-free wetting on the lipid distribution occurred despite the different compositions and breadmaking potentials of the flours. When a critical, qualitative comparison of the three flours is made, as will be shown, the lipid system is almost identical in a number of respects and would suggest that at this hydration stage, before mechanical

dough development, the lipid response is not directly related to breadmaking quality.

Quantitative comparison of hard and soft wheat flour lipid distribution at 14% moisture.

Table 4:5 shows the lipid distribution at 14% moisture as percentages of the individual total lipids for the three flours and the proportions of some of these fractions.

Table 4:5.

Quantitative comparison of the lipid distribution of the three flours at 14% moisture. Lipid fractions expressed as % of total lipid. F= free, B=bound, T= total, N=neutral, P=polar and U=unextractable.

	GOL 1	GOL 2	HIL
Total lipid (% flour dry weight).	1.653	1.572	1.090
Free total	68.0	64.3	62.0
Bound total	31.5	35.7	37.5
Neutral total	59.3	59.2	61.4
Polar total	40.2	40.8	38.1
Free neutral	52.8	51.8	51.4
Free polar	15.2	12.5	10.6
Bound neutral	6.5	7.4	10.0
Bound polar	25.0	28.3	27.5
PT/NT	0.678	0.690	0.621
BT/FT	0.463	0.552	0.606
FN/NT %	89.2	87.7	83.8
BN/NT %	10.8	12.3	16.2
FP/PT %	37.9	30.6	27.9
BP/PT %	62.1	69.4	72.1
FN/FT %	77.7	80.5	83.0
FP/FT %	22.3	19.5	17.0
BN/BT %	20.5	20.7	26.7
BP/BT %	79.5	79.3	73.3

As can be seen the differences between some of the fractions of GOL 1 and GOL 2 are greater than the differences between HIL and GOL 1 or GOL 2. Further points which are worth noting are firstly the ratio of total polar to total neutral lipid which was the same for all three flours (2:3). Secondly the ratio of total free lipid to total bound lipid which was similar for the three flours (13:7). Thirdly the free to bound ratios of neutral and polar lipids were found to be similar for all three flours; neutral, 9:1, polar, 3:7. The neutral to polar ratios of free and bound lipids were also very similar but reversed; free, 4.2:1, bound 1:3.7.

Quantitative comparison of the effect of wetting on the lipid fractions of the soft and hard wheat flours.

After the flours had been wetted the different fractions were again similar in each flour as is shown in Table 4:6, and the ratios of these fractions were likewise similar for the three flours, although the proportions were changed as a result of wetting.

Table 4:6.

Quantitative comparison of the lipid distribution of the three flours at 45% moisture.

Lipid fractions expressed as % of total lipid.

	GOL 1	GOL 2	HIL
Free total	42.5	43.9	49.2
Bound total	45.5	42.9	45.8
Unextractable total	12.0	13.2	5.0
Extractable neutral total	47.5	50.6	57.5
Extractable polar total	40.5	36.2	37.5

		GOL 1	GOL 2	HIL
Free neutral		38.5	42.0	45.9
Free polar		4.0	1.9	3.3
Bound neutral		9.0	8.6	11.6
Bound polar		36.5	34.3	34.2
Unextractable neutral		11.8	9.5	5.0
Unextractable polar		0	3.7	0
PT/NT		0.853	0.716	0.741
FT/BT		0.934	1.025	1.072
FT/BT + UT		0.740	0.783	0.968
FN/NT %	N.F/B	81.1	83.0	80.0
BN/NT %		18.9	17.0	20.0
FP/PT %	P.F/B	9.8	5.2	8.8
BP/PT %		90.2	94.8	91.2
FN/FT %	F.N/P	90.7	95.7	93.3
FP/FT %		9.3	4.3	6.7
BN/BT %	B.N/P	19.7	20.0	25.4
BP/BT %		80.3	80.0	74.6
UN/UT %	U.N/P	100	72.0	100
UP/UT %		0	28.0	0
BN + UN/BT + UT %	B + U.N/P	36.1	32.3	32.6
BP + UP/BT + UT %		63.9	67.7	67.4
BN + UN/NT %		35.0	30.2	26.8
BP + UP/PT %		90.2	93.2	91.2

These constant proportionalities are particularly noteworthy in the light of the different natures and compositions of the three flours.

The differences between the soft and hard flours that were the result of wetting were principally in the unextractable fraction which in the case of the soft flour became only half the size of that of the hard flour and incorporated less neutral lipid. However HIL showed greater extractable binding of neutral lipid than the two hard flours.

The limiting factors of lipid binding due to work free wetting of flour.

The occurrence of no further binding beyond a moisture level of about 45% may be accounted for in two ways. The first possibility, prompted by the observation that equal amounts of free and bound lipid are present after wetting, could be the attainment of an equilibrium state. The second could be the attainment of a saturation situation in which all the binding sites available or accessible as a consequence of work free wetting are filled and no more sites can be revealed or reached without the introduction of mechanical work or some other form of energy.

To indicate which of these situations is the more likely the following were considered. For an equilibrium state one should expect the same proportions of free to bound in both neutral and polar lipids in each wetted flour, the free fraction being as significant as the bound. For a saturation state, the free fraction would be of less importance than the bound and unextractable fractions provided free lipid was present if there were binding sites available.

An equilibrium state

As shown in Table 4:7, not only are free to bound ratios of total and neutral fractions almost identical but the relative size of each lipid fraction is similar in each flour, particularly if the unextractable fraction is counted in with the bound. This evidence points to an equilibrium situation of the lipid distribution at 45% moisture.

Table 4:7.

Comparison of lipid fractions and proportions after wetting. (45%) Lipid fractions expressed as % of total lipid.

	GOL 1	GOL 2	HIL
Free total	42.5	43.9	49.2
Bound total	45.5	42.9	45.8
unextractable total	12.0	13.2	5.0
Free neutral	38.5	42.0	45.9
Free polar	4.0	1.9	3.3
Bound neutral	9.0	8.6	11.6
Bound polar	36.5	34.3	34.2
Unextractable neutral	11.8	9.5	5
Unextractable polar	0	3.7	0
BT + UT	57.5	56.1	50.8
BN + UN	20.8	18.1	16.6
BP + UP	36.5	38.0	34.2
FT/BT	0.934	1.025	1.072
FT/BT + UT	0.740	0.783	0.967
FN/BN	0.234	0.205	0.253

A saturation state.

If the lipid fractions are expressed as percentage of flour dry weight, the flours may be compared directly. As shown in Table 4:8 the two hard wheat flours were comparable in each fraction but the soft wheat flour could only be considered comparable with respect to the bound neutral and unextractable polar fractions. Otherwise the values of the other fractions were much lower.

Table 4:8.

Comparison of lipid fraction distribution after wetting.

Lipid fractions expressed as % of flour dry weight except HILP in which allowance has been made for the different protein content. Unextractable values are the maxima attained during wetting.

	GOL 1	GOL 2	HIL	HILP
BT	0.750	0.675	0.500	1.065
BN	0.148	0.136	0.128	0.273
BP	0.603	0.539	0.372	0.780
UT	0.203	0.205	0.068	0.145
UN	0.195	0.152	0.072	0.153
UP	0.066	0.080	0.060	0.128
UT + BT	0.953	0.880	0.568	1.210
UN + BN	0.343	0.288	0.200	0.426
UP + BP	0.669	0.619	0.432	0.920

If a correction is made to allow for the different protein contents of the flours (HILP) which assumes that lipid binding only involves the total protein and no other flour constituent, there is still no comparison between

the soft and hard flours. Unless a better basis on which to compare the flours can be found, e.g. the specific fraction involved in binding, and data from more flours is available, one must conclude that work-free wetting of flour results in a redistribution of lipid leading finally to an equilibrium situation. Furthermore, the observed results do not support a saturation state hypothesis.

Conclusions.

A study of three flours has shown that similarities and differences in breadmaking potential are not reflected in the lipid distribution since all three flours showed many more quantitative similarities than differences. The results showed that the lipids of these flours were distributed thus:-

	Free	to	Bound	at 14 % moisture
Total	65	:	35	
Neutral	90	:	10	
Polar	30	:	70	
	Neutral	to	Polar	
Free total	80	:	20	
Bound total	20	:	80	

The constant distribution of neutral and polar lipids in the bound fraction may reflect the ratio of neutral to polar lipid in the lipoprotein material of the original wheat grain. Flour consists of fragments of cellular material produced by breaking up ripe grain in a mill and some of the membraneous lipoprotein will probably be present unchanged in the flour as bound lipid.

It was shown that the effect on lipid distribution of wetting different types of flours, without mechanical mixing i.e. simple hydration was similar for all three flours.

The final proportions of lipid fractions in these flours were:-

	Free	to	Bound	to	Unextractable
Total	45	:	45	:	10
Neutral	70	:	15	:	15
Polar	10	:	90		

	Neutral	to	Polar
Free total	95	:	5
Bound total	20	:	80

The effect of wetting on the flour lipid fractions may be summarized as follows, the changes being expressed as percentages of the initial (14% moisture) value of the fraction.

Total Free - Decreases by 30%

Total Bound - Increases by 30%

Free polar - Almost entirely lost, initially to the unextractable fraction but finally to the bound fraction.

Free Neutral - Decreases by 20%, principally to the unextractable fraction, to some extent by way of the bound fraction.

Bound polar - Increases by 30%

Bound neutral - Increases slightly

Unextractable polar - Increases initially but decreases again at higher moisture levels.

Unextractable neutral - Increases throughout the range of moisture increase.

When 45% moisture has been reached an equilibrium has been established between free and bound, neutral and polar lipids in flour. This situation of lipid distribution is attained on each occasion that flour is wetted to produce a dough for bread or cake making.

Moreover, this is the situation at the moment that mechanical mixing commences and one can suggest that up to this point, there is little difference between flours of different breadmaking potential with particular respect to their lipid distributions.

The third flour was studied as a contrast to the first two to see if an alteration to the lipid binding substrate would produce a different response of the lipid distribution to hydration. Since no great difference was found between the flours, wetting apparently producing an equilibrium lipid distribution, it appeared that the pattern of lipid binding was unaffected by changes in binding site arrangement. The results suggest that in lipid binding the substrate is of less importance than the lipid.

Consequently this study will now concentrate on the effect of an alteration of the lipid fraction available for binding.

SECTION FIVE

Solvent extraction of flour and the effect of work-free wetting on such flours.

Solvent extraction of flour.

Investigations into the contribution of lipid to flour breadmaking properties have often been based on solvent extraction of flour since such extraction and subsequent lipid addition can have considerable effects on breadmaking properties. Different solvents have been used, usually non-polar in character and extracting about two thirds of the total lipid, since the polar solvent systems necessary to remove all the lipid render the flour useless for baking investigations, probably by denaturation of the gluten protein. Contradictory results of early work with defatted flours and results obtained in more recent and detailed studies have been reviewed in the introduction.

Although the same technique of fat extraction was used in this study, full attention was paid to the bound lipid remaining after extraction. The intention of the next stage was to remove free lipid to see how the new lipid distribution that resulted from extraction would be affected by work-free wetting.

Free lipid was extracted by percolation of petroleum spirit (b.pt. 40-60°C) through a tube packed with flour (GOL 1). This method was chosen to avoid the inevitable heating of the flour and extracted lipid that soxhlet extraction entails since the return of extracted lipid to the flour was intended at a later stage.

The fat-extracted flour, after air drying to ensure the removal of any remaining solvent, matched the graphic description of Cookson and Coppock (1956). The flour was more free flowing, whiter and apparently finer and lighter.

It dispersed easily in air and did not show the cakiness of the unextracted flour. One might therefore see the free lipid as holding the flour particles together as well as carrying the flour pigments.

The amount of lipid extracted matched the amount of free lipid previously found to be present and it was considered that this extraction procedure had eliminated free lipid. However, petrol is known to remove some non-lipid material e.g. purothionin, a petrol soluble flour lipoprotein which has been the subject of recent detailed studies. (Fisher, Redman and Elton. (1968) Nimmo, O'Sullivan and Bernardin. (1968)) and the precise effect of petrol extraction was investigated.

Results.

The yield of extracted lipid (1.10 % of flour dry weight) was slightly less than the total free lipid (1.12%) of GOL 1 and the proportion of neutral and polar lipid was found to be different. As shown in Table 5:1, the extracted lipid contained a lower proportion of polar lipid but was insufficiently different to be considered significant at this stage.

Table 5:1

Comparison of extracted lipid with free lipid of GOL 1. Expressed as % of flour dry weight.

	Extracted lipid	Free lipid
Total	1.100	1.120
Neutral	0.880	0.871
Polar	0.220	0.251

However, when the complete lipid distribution of the extracted flour was determined it became apparent that extraction of flour with non-polar solvent involved more than removal of free lipid. Table 5:2 shows the lipid distribution of GOL 1 before and after (GOL 1FE) extraction with petroleum spirit and the effect on each lipid fraction.

Table 5:2.

Lipid distribution of GOL 1 before and after extraction with petroleum spirit.

Expressed as % of flour dry weight.

Lipid fraction	GOL 1	GOL 1FE	Difference.	S.E. of Means
Free total	1.120	0.064	-1.056	± 0.009
Bound total	0.520	0.430	-0.090	± 0.016
Free neutral	0.871	0.046	-0.825	± 0.0306
Free polar	0.251	0.018	-0.233	± 0.0213
Bound neutral	0.107	0.100	-0.007	± 0.0376
Bound polar	0.413	0.330	-0.083	± 0.0283
Neutral total	0.980	0.146	-0.834	± 0.0216
Polar total	0.664	0.348	-0.316	± 0.0123

Effect of petrol extraction on flour neutral lipid.

Petrol extraction removed 0.825% from the free neutral lipid (95%) yet this was 0.055% less than found in the extract. Since only 0.007% was removed from the bound neutral fraction, some 0.048% must have been extracted from the unextractable fraction present in flour at 14% moisture.

Effect of petrol extraction on flour polar lipid.

Although extraction removed 0.233 % from the free

polar fraction, only 0.220 was collected. Bound polar lipid also lost 0.083% and one must conclude that 0.096% made up of 0.013% free and 0.083% bound polar lipid was unextractably bound as a result of the extraction process.

There was no preferential extraction of neutral or polar lipid from the free fraction since the proportion of neutral to polar lipid present in the unextracted free fraction was identical to the proportion of neutral to polar lipid lost as a result of extraction (78 N:22P).

Effect of petrol extraction on other flours.

When the other two flours, GOL 2 and HIL, had been extracted with petrol in the same way the distributions of lipids were determined and the results compared. Table 5:3 shows these results, GOL 2 was examined on two separate occasions and both sets of results are shown.

Table 5:3.

Lipid distributions of GOL 2 (twice) and HIL before and after extraction (GOL 2FE, HILFE) with petroleum spirit.

Expressed as % of flour dry weight.

Lipid fractions	GOL 2a	GOL 2FEa	GOL 2FEb	HIL	HILFE S.E. of Means
FT	1.021	0.048	0.084	0.676	0.035 ± 0.0156
BT	0.550	0.387	0.442	0.405	0.283 ± 0.0181
FN	0.833	0.036	0.065	0.560	0.027 ± 0.0372
FP	0.188	0.012	0.019	0.116	0.009 ± 0.0279
BN	0.077	0.091	0.090	0.105	0.070 ± 0.0247
BP	0.473	0.296	0.352	0.300	0.213 ± 0.0304
NT	0.910	0.127	0.155	0.665	0.097 ± 0.0216
PT	0.661	0.308	0.424	0.416	0.222 ± 0.0123

Extracted lipid	GOL 2 FEa	GOL 2FEb	HILFE
Total	1.106	1.040	0.766
Neutral	0.830	0.780	0.612
Polar	0.276	0.260	0.154

Tables 5:4 and 5:5 show comparisons of the effect on the three flours of petrol extraction.

Table 5:4

Comparison of effect of petrol extraction on GOL 1 GOL 2 (twice) and HIL. Neutral and polar lipids considered separately. Expressed as % of flour dry weight.

	GOL 1	GOL 2a	GOL 2b	HIL	S.E of Means
Neutral					
Extracted	+0.880	+0.830	+0.780	+0.612	
Free	-0.825	-0.797	-0.768	-0.533	± 0.0372
Bound	-0.007	+0.014	+0.013	-0.035	± 0.0397
Unextractable	-0.048	-0.047	-0.025	-0.044	
Polar					
Extracted	+0.220	+0.276	+0.260	+0.154	
Free	-0.233	-0.176	-0.169	-0.107	± 0.0279
Bound	-0.083	-0.177	-0.121	-0.087	± 0.0304
Unextractable	+0.096	+0.076	+0.030	+0.040	

Table 5:5

Comparison of effect of petrol extraction on GOL 1, GOL 2 (twice) and HIL. Neutral and polar lipids. Expressed as percentages of total lipids present.

Neutral	GOL 1	GOL 2a	GOL 2b	HIL
Extracted	+53.2	+52.9	+49.7	+56.0
Free	-50.0	-50.8	-48.9	-48.7
Bound	-0.3	+0.9	+0.8	-3.2
Unextractable	-2.9	-3.0	-1.6	-4.1
Polar				
Extracted	+13.3	+17.6	+16.5	+14.1
Free	-14.1	-11.2	-10.7	-9.8
Bound	-5.0	-11.3	-7.7	-8.0
Unextractable	+5.8	+4.9	+1.9	+3.7

Conclusions

Extraction of flour with petroleum spirit by percolation removed between 90 and 95% of free lipid with no preference for neutral or polar lipid. About a quarter of the bound lipid was also lost as a result of extraction. This principally polar fraction was partly extracted and partly unextractably bound. Extraction also removed a small amount of neutral lipid from the bound and unextractable fractions so that it might be considered that a substitution of neutral lipid by polar lipid occurred in the unextractable fraction.

Once again the three flours were remarkably similar to one another, particularly when compared on a percentage of total lipid basis. (Table 5:5)

Obviously fat extraction with petroleum spirit was not the straight-forward removal of free lipid that many workers considered it to be. These results will further help to explain the contradictory results obtained in early work with fat-extracted flour.

Effect of work-free wetting on the lipid distribution of fat-extracted flour. GOL 1.

In order to assess the effect of work-free wetting of fat extracted flour, samples were raised to moisture levels in the range 15-50% by the liquid nitrogen technique. The wetted, fat extracted flours were then freezedried, ground, sieved and the lipid distributions determined as before. The results obtained from GOL 1 are shown in Table 5:6.

Table 5:6

Effect of work-free wetting on the lipid distribution of fat-extracted GOL 1.

Mean values expressed as percentage of flour dry weight.

Moisture Level %	Free total	Bound total	Free neutral	Free polar	Bound neutral	Bound polar
14.1	0.064	0.430	0.046	0.018	0.100	0.330
20.9	0.071	0.389	0.056	0.015	0.091	0.298
31.5	0.037	0.433	0.028	0.009	0.104	0.329
41.1	0.004	0.435	0.003	0.001	0.124	0.311
S.E. of Means	± 0.009	± 0.016	± 0.0306	± 0.0213	± 0.0376	± 0.0283

The small amount of free lipid remaining after extraction virtually disappeared and there was no significant change in the amount of bound lipid obtained from the flour after wetting. The proportions of neutral and polar lipid in the bound fraction also remained unchanged. Evidently the binding of the small amount of remaining free lipid that did occur was unextractable.

Effect of work free wetting on the lipid distribution of fat-extracted soft flour. HIL.

The soft flour HIL was also wetted after extraction in exactly the same way as GOL 1. The results are presented in Table 5:7.

Table 5:7.

Effect of work-free wetting on the lipid distribution of fat-extracted HIL.

Mean values expressed as percentage of flour dry weight.

Moisture level %	Free total	Bound total	Bound neutral	Bound polar
11.6	0.035	0.283	0.070	0.213
23.1	0.059	0.314		
29.5	0.031	0.284		
35.6	0.022	0.311		
47.0	0.029	0.302	0.074	0.228
S.E. of Means	± 0.0156		± 0.0397	± 0.0304

Again there was no significant change in the amount of bound lipid or in the proportions of neutral and polar lipid in the bound fraction.

These experiments have shown that although petrol extraction did not have the anticipated effect of simple removal of free lipid, [illegible] effects on lipid distribution have been demonstrated and shown to be similar for the flours under examination. Also, work-free wetting of the extracted flours was shown to have no effect on the bound lipid fraction.

Thus the way was prepared for the addition of any lipid to extracted flour and the examination of the response to work-free wetting of a flour with a substituted free lipid fraction.

SECTION SIX

Reconstitution of fat-extracted flour and the effect of work-free wetting.

Reconstitution.

Flour reconstitution has always been looked upon as the return of lipid to solvent extracted flour to produce a flour for breadmaking studies that is comparable to the original. This is based on the assumption that fat extraction simply removes free lipid and reconstitution replaces it, there being no reason to suspect an overall change in lipid distribution from the breadmaking results.

This work has already shown that such an assumption cannot be made about fat extraction, therefore reconstitution must be considered in terms of addition of free lipid to flour from which not only has most of the free lipid been removed but has also suffered a lipid fraction redistribution. Thus the success of the reconstitution process cannot be judged in terms of the original flour but rather on the possible effects of this second process on lipid distribution.

Since a reconstituted flour is to be the subject of lipid distribution studies, the technique used to reconstitute the fat-extracted flour must fulfil a number of demanding requirements if there are to be a minimum of further effects on lipid distribution. The first major requirement of such a technique is a means of dispersing lipid evenly in the extracted flour and the second that the end product is a flour and not a dough. When the factors which are known to affect lipid binding are considered i.e. water, organic solvents, mechanical work with and without air present and heat, all the methods used hitherto must be considered suspect.

Although the introduction and dispersion of liquid fats was effectively achieved in a dough mixer (Smith and Andrews. (1957) Smith, Van Buren and Andrews. (1957)) a worked dough was produced. In order to disperse hard fat it was necessary for it to be pulverised or melted or dispersed in liquid oil and added to hot flour, again in a dough mixer (Pomeranz, Rubenthaler and Finney (1966) Elton and Fisher (1968)). The addition of lipid as a solution in petrol (Cole, Mecham and Pence (1960), Narayanan and Hlynka (1962) Tucker (1946) Olcott and Mecham (1947), Pomeranz, Rubenthaler and Finney (1965) and Cosgrove (1956)) butanol, carbon tetrachloride (Cole, Mecham and Pence (1960)) chloroform (Pomeranz, Rubenthaler and Finney (1965)) or buffer (Bloksma (1959)) produced good dispersion in flour or dough but with undoubted solvent effects on the breadmaking and lipid binding properties of the flour (Ponte (1968) Olcott and Mecham (1947)). While mixing lipid into a small part of the flour in a Stein mill and blending with the rest in a dough mixer was successfully used in breadmaking studies (Tao and Pomeranz (1968), Pomeranz et al.(1966), Pomeranz, Rubenthaler and Finney (1965), Pomeranz, Rubenthaler and Finney (1966), and Pomeranz et al. (1968)) this again could not be applied to these lipid binding studies.

In order to fulfil the particular requirements for a reconstitution method to produce flour suitable for such studies, the technique for adding water to flour was adapted to return lipid to extracted flour.

Reconstitution technique - Preliminary experiment.

Essentially, lipid was added to extracted flour as a fine powder suspended in liquid nitrogen. Lipid added directly to liquid nitrogen was sufficiently brittle to be ground and added as a suspension to extracted flour also suspended in liquid nitrogen. This technique dispersed lipid evenly in the flour by the boiling action of liquid nitrogen and permitted the return to the extracted flour of either the same amount of lipid as was removed by fat extraction or any other desired amount.

The reconstituted flour thus produced was visually indistinguishable from the original flour having the same creamy appearance and cakiness. When stirred vigourously in water it no longer produced a froth, a property peculiar to fat-extracted flours.

The feasibility of this method for producing reconstituted flours suitable for work-free wetting experiments was tested by reconstituting fat-extracted flour samples at different moisture levels. Lipid and water were added simultaneously to flour at the temperature of liquid nitrogen and after thawing, the work-free doughs thus produced were freezedried, ground, sieved and the free and bound lipids extracted. Table 6:1 and Figure 6:1 show the results obtained.

Table 6:1

Effect of reconstitution and wetting of fat-extracted GOL 1. Expressed as percentage of flour dry weight.

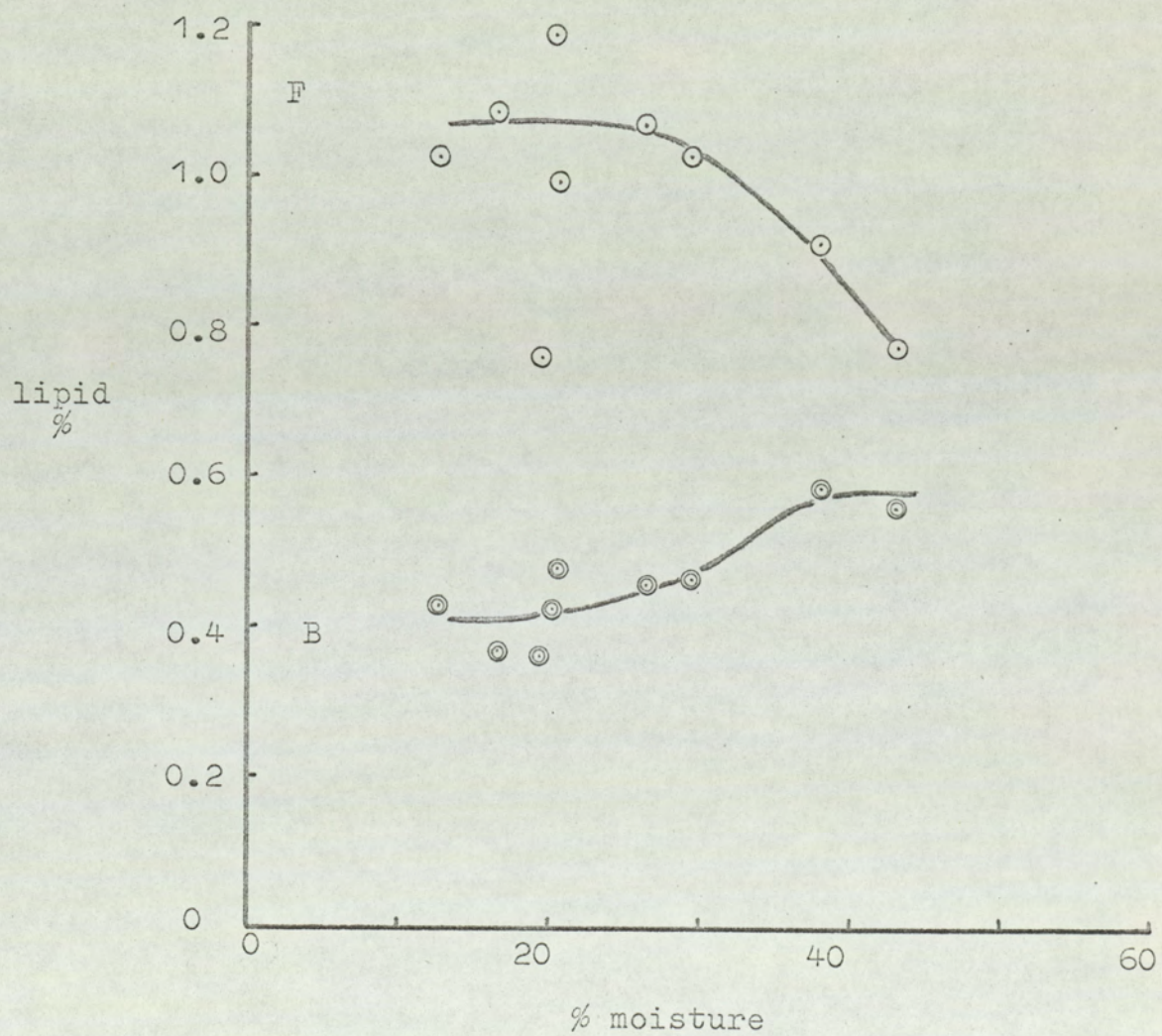
Mean values. 1.000 % lipid added.

Moisture level %	Free	Bound
12.7 fat-extracted	0.064	0.430
12.7	1.022	0.423
16.5	1.082	0.365
19.5	0.755	0.357
20.3	1.188	0.420
20.8	0.989	0.475
26.4	1.068	0.452
29.4	1.022	0.460
38.0	0.906	0.580
43.2	0.770	0.557
Standard Errors of Means	± 0.0156	± 0.0181

Figure 6:1.

Effect of wetting on lipid distribution of Reconstituted flour. GOL 1, first reconstitution. Free and bound lipid as percentage of flour dry weight.

Standard Errors of Means. Free, ± 0·0156. Bound ± 0·0181



Since a flour with the lipid well dispersed was produced and 96% of the added lipid was found to be free with no significant change in the amount of bound lipid this method of reconstitution was considered to be satisfactory. Lipid became bound as a consequence of wetting the reconstituted flour but the results showed a certain amount of scatter which was attributed to the simultaneous reconstitution and wetting of individual samples. In the second experiment, sufficient flour for the series was reconstituted in bulk to minimize lipid loss and provide a fixed level of added lipid for the whole series. Taking samples from this stock pile (stored at room temperature) for work-free wetting by the liquid nitrogen technique ensured that the effect of water on reconstituted flour was being studied and not the effect of lipid addition to wet flour.

Reconstitution of fat-extracted GOL 1 and the effect of work free wetting on the lipid distribution of this flour.

The liquid nitrogen technique was used to reconstitute 1030g of fat extracted flour by the addition of 9.9g extracted flour lipid (equivalent to 1.1% of flour dry weight). When thawed the reconstituted flour was stored in a sealed tin at room temperature. From the stock pile, 100g samples were individually wetted by the liquid nitrogen technique to selected moisture levels between 15 and 50%. After thawing and equilibration the samples were freezedried and the lipid distribution determined. The results that were obtained are shown in Table 6:2 and will be considered in two parts.

Firstly the individual and overall effects of fat extraction by percolation, reconstitution and wetting will be assessed and secondly the detailed effects of work free wetting on reconstituted flour will be discussed and compared with those of the original flour.

Table 6:2.

Effect of work-free wetting on lipid distribution of reconstituted flour GOL 1. Expressed as percentage of flour dry weight. Mean Values. Lipid added equivalent to 1.100%.

Moisture level %	Free			Bound		
	Total	Neutral	Polar	Total	Neutral	Polar
12.3	1.112	0.982	0.130	0.424	0.137	0.287
19.7	1.070	0.907	0.163	0.458	0.160	0.298
23.2	0.897	0.771	0.126	0.570	0.273	0.297
26.5	0.793	0.664	0.129	0.598	0.266	0.332
36.2	0.658	0.580	0.078	0.682	0.316	0.366
44.7	0.637	0.597	0.040	0.781	0.310	0.471
Standard Errors of Means		± 0.037	± 0.027		± 0.039	± 0.030

Table 6:3 shows the lipid distributions of the original flour, fat-extracted flour, anticipated reconstituted flour (fat-extracted flour and added lipid) and reconstituted flour.

Table 6:3.

Lipid distribution of flour GOL 1, before and after fat extraction and reconstitution. See Table 6:2 for Standard Errors of Means

Lipid fraction.	Original.	Fat-Extracted.	Reconstituted, Anticipated (A).	Found (F).	Difference between (A) and (F)
FT	1.122	0.064	1.164	1.112	-0.052
BT	0.520	0.430	0.430	0.424	-0.006
FN	0.871	0.046	0.926	0.982	+0.056
FP	0.251	0.018	0.238	0.130	-0.108
BN	0.107	0.100	0.100	0.137	+0.037
BP	0.413	0.330	0.330	0.287	-0.043
NT	0.987	0.146	1.026	1.119	+0.093
PT	0.664	0.348	0.568	0.417	-0.151

Effect of reconstitution on lipid distribution.

The initial impression of the effect of reconstitution was that most of the added lipid remained free and that there was no significant difference in the amount of extractably bound lipid. However, reconstitution was revealed as a more complicated process when the lipid fractions were considered separately.

Effect of reconstitution on neutral lipid.

As both free (0.056) and bound (0.037) fractions were greater than anticipated, unextractably bound neutral lipid must have been released (as had been found after fat extraction).

Effect of reconstitution on polar lipid.

Again, as found after fat extraction, loss of extractable free (0.108%) and bound (0.043%) lipid suggested that polar lipid (0.151%) became unextractably

bound, possibly displacing the 0.093% neutral lipid.

The net effect of fat extraction and reconstitution was a release of some 14.4% of the total neutral lipid from the unextractable fraction and unextractable binding of a considerable amount of polar lipid, 37.2% of the total polar lipid.

Effect of work-free wetting of reconstituted flour.

As shown in Table 6:4 there was a total loss of 26.7% free and an increase of 20.5% bound lipid as a result of work-free wetting, which suggested a net unextractable binding of 6.2%.

Table 6:4

Effect of wetting reconstituted flour to 50% moisture. Expressed as percentage of total lipid present.

Lipid fraction.	Standard Errors of means	Reconstituted flour - moisture level%. 15	50	Differences
FT	± 0.523	62.2	35.5	-26.7
BT	± 0.930	23.7	44.2	+20.5
FN	± 1.85	55.0	32.4	-22.6
FP	± 1.29	7.2	3.1	-4.1
BN	± 2.28	7.7	15.5	+7.8
BP	± 1.72	16.0	28.7	+12.7
NT	± 1.25	62.7	47.9	-14.8
PT	± 0.715	23.2	31.8	+8.6

The discrepancy between the 7.8% increase of bound and the 22.6% loss of free neutral lipid indicated that 14.8% neutral lipid was unextractably bound likewise 8.6% decrease of unextractable polar lipid would be necessary to account for the 12.7% increase of bound when only 4.1% free lipid was lost.

In Table 6:5, the original and reconstituted flours are compared after wetting. This demonstrates the effect of fat extraction and reconstitution at the wetted stage.

Table 6:5

Comparison of original and reconstituted flour GOL 1. after wetting to 50% moisture. Expressed as a percentage of total lipid.

Lipid fraction	Original	Reconstituted	Differences	S.E. of Means
FT	39.3	35.5	-3.8	± 0.523
BT	41.9	44.2	+2.3	± 0.930
FN	35.6	32.4	-3.2	± 1.85
FP	3.7	3.1	-0.6	± 1.29
BN	8.1	15.5	+7.4	± 2.28
BP	33.8	28.7	-5.1	± 1.72
NT	43.7	47.9	+4.2	± 1.25
PT	37.5	31.8	-5.7	± 0.715

The effects of fat extraction and percolation were almost reversed by wetting in that neutral lipid was unextractably bound and some unextractable polar lipid released. The greatest remaining difference was a much larger amount (almost double) of bound neutral lipid, 3.2% derived from the free and 4.2% from the unextractable fraction. The 5.1% reduction of bound polar lipid was principally due to unextractable binding.

Net effect of fat extraction, reconstitution and wetting on the lipid distribution of flour GOL 1.

The net effect is demonstrated in Table 6:6 where the original flour is compared with wetted reconstituted flour.

Table 6:6

Comparison of original and reconstituted wetted flour GOL 1. Expressed as percentage of total lipid present.

Lipid fraction	Original	Reconstituted flour at 50% moisture	Differences.	S.E. of Means
FT	62.8	35.5	-27.3	± 0.523
BT	29.1	44.2	+15.1	± 0.930
FN	48.7	32.4	-16.3	± 1.05
FP	14.1	3.1	-11.0	± 1.29
BN	6.0	15.5	+9.5	± 2.28
BP	23.1	28.7	+5.6	± 1.72
NT	54.7	47.9	-6.8	± 1.25
PT	37.2	31.8	-5.4	± 0.715

The most interesting result of this comparison is the large (9.5%) increase of bound neutral lipid and the amount of unextractably bound neutral (6.8%) and polar (5.4%) lipid.

Another way of considering the net effect is to compare the effect of wetting the original flour to the overall effect of fat extraction, reconstitution and wetting. Table 6:7 shows the differences between the lipid distributions of the original flour at 14 and 50% moisture and the differences shown in Table 6:6.

Table 6:7

Comparison of differences between lipid fractions after wetting and fat extracting, reconstituting and then wetting the original flour.

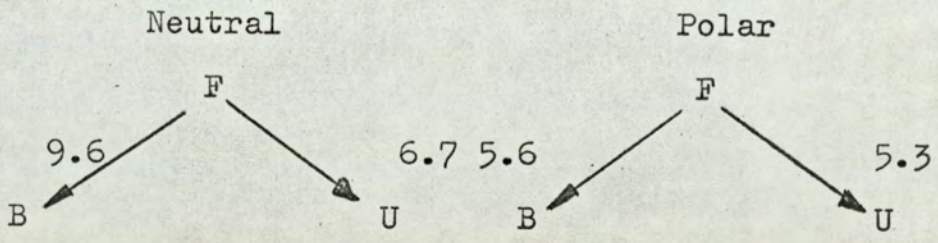
Lipid fraction	Effect of wetting original flour	Overall difference between the original flour at 14% and reconstituted flour at 50% moisture.	S.E. of Means
FT	-23.5	-27.3	± 0.523
BT	+12.8	+15.1	± 0.930
FN	-13.1	-16.3	± 1.85
FD	-10.4	-11.0	± 1.29
BN	+2.1	+9.5	± 2.28
BD	+10.7	+5.6	± 1.72
NT	-11.0	-6.8	± 1.25
PT	+0.3	-5.4	± 0.715

This comparison clearly shows the greater proportion of extractable binding that occurred in the reconstituted flour, particularly of neutral lipid of which less was unextractably bound. The reverse was found for polar lipid since less was extractably bound but more unextractably.

Diagram 6:1 shows a diagramatic representation of the net effect of fat extraction, reconstitution and wetting.

Diagram 6:1

Net effect of fat extraction, reconstitution and wetting. Values are expressed as percentage of total lipid present.



Diagrams 6:2, 6:3 and 6:4 show each stage separately.

Diagram 6:2.

Percolation extraction.

E = Extract

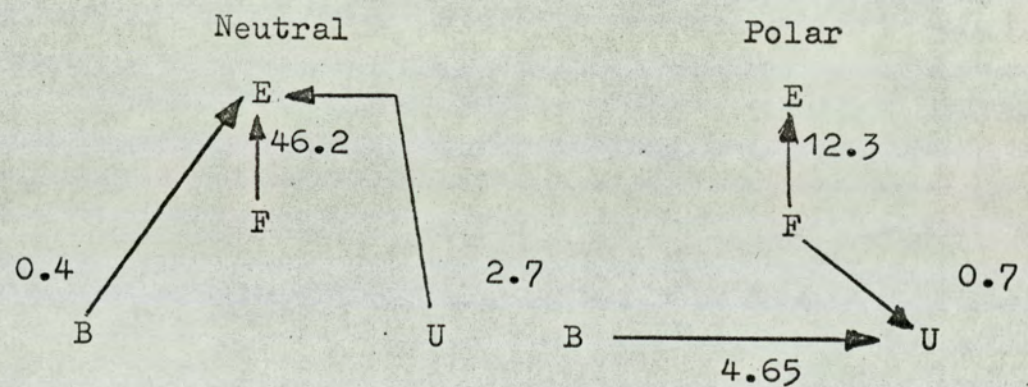


Diagram 6:3

Reconstitution.

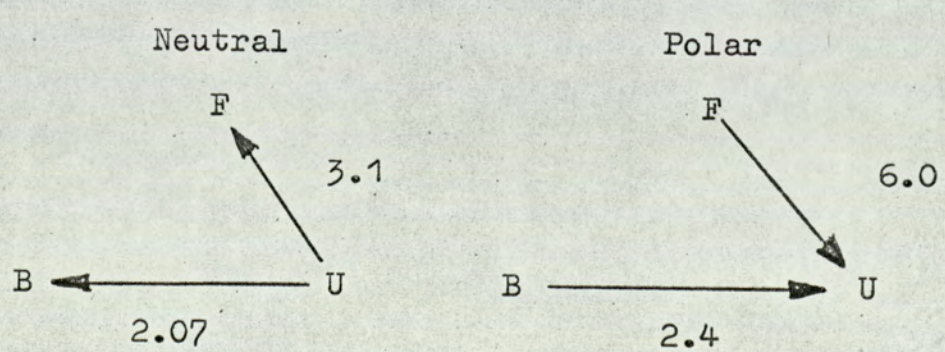
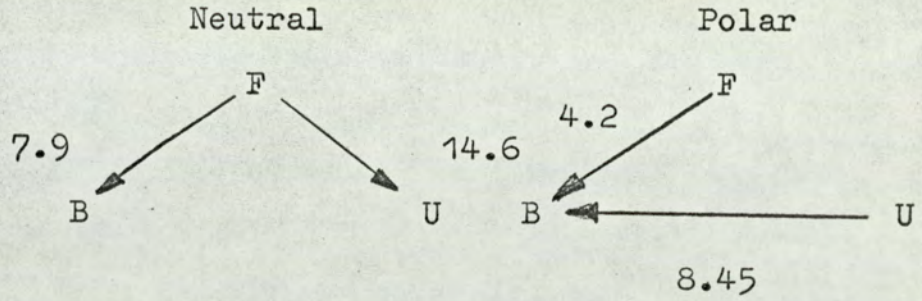


Diagram 6:4

Wetting reconstituted flour to 50% moisture.



These diagrams summarize the effects of fat extraction reconstitution and wetting on lipid distribution and make the point that both the first two stages, performed at about 13% moisture, involved release of unextractably bound neutral lipid and unextractable binding of polar lipid. The effect of wetting to 50% moisture was evidently to reverse this situation.

Effect of wetting reconstituted flour over the range 15-50% moisture.

The lipid distributions of reconstituted flour at different moisture levels were given in Table 6:2. These results have been plotted in Figures 6:2, 6:3 and 6:4. Also shown in these figures are the equivalent results obtained when the original flour was wetted. These figures should be considered in conjunction with Table 6:8 which shows a comparison of the effects of wetting the two flours. This direct comparison makes the overall similarity of the effect on the two flours immediately apparent.

Table 6:8

Comparison of the effects of wetting on the lipid distributions of original and reconstituted flour GOL 1. Differences between values expressed as percentage of total lipid present.

Lipid fractions	Original GOL 1	Reconstituted GOL 1R	S.E. of Means
FT	-23.5	-26.7	± 0.523
BT	+12.8	+20.5	± 0.930
FN	-13.1	-22.6	± 1.85
FP	-10.4	-4.1	± 1.29
BN	+2.1	+7.8	± 2.28
BP	+10.7	+12.7	± 1.72
NT	-11.0	-14.8	± 1.25
PT	+0.3	+8.6	± 0.715

Figure 6:2 compares the response of total, free, bound and unextractable fractions of the two flours to wetting. Generally the reconstituted flour, GOL 1R, showed an effect at a lower moisture level. More free lipid was lost more rapidly, the bound fraction increased by half as much again and there was a close similarity in the unextractable fractions although at a higher moisture level that of GOL 1R decreased.

Neutral lipid.

Figure 6:3 shows that while the two flours were similar, the major difference was the greater decrease of free neutral matched by a greater increase of bound neutral lipid in GOL 1R. GOL 1R also showed both a higher level and a greater loss of total extractable

Figure 6:2.

Comparison of Reconstituted with Original Flour GOL 1. Effect of wetting on total lipid fractions. Results expressed as percentage of flour dry weight.

Standard Errors of Means.	Free	Bound
GOL1	± 0.0090	± 0.0160
GOL1R	± 0.0156	± 0.0181

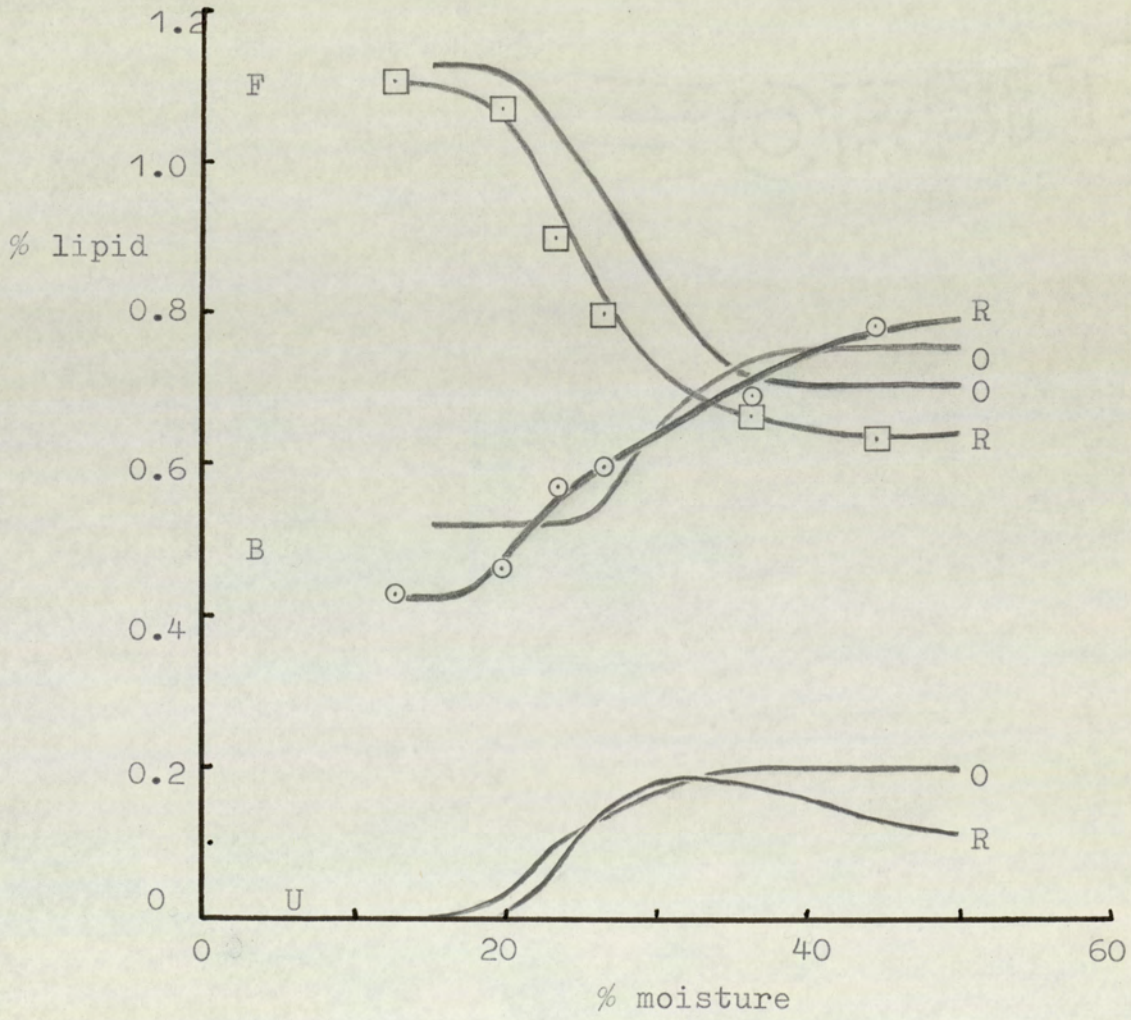
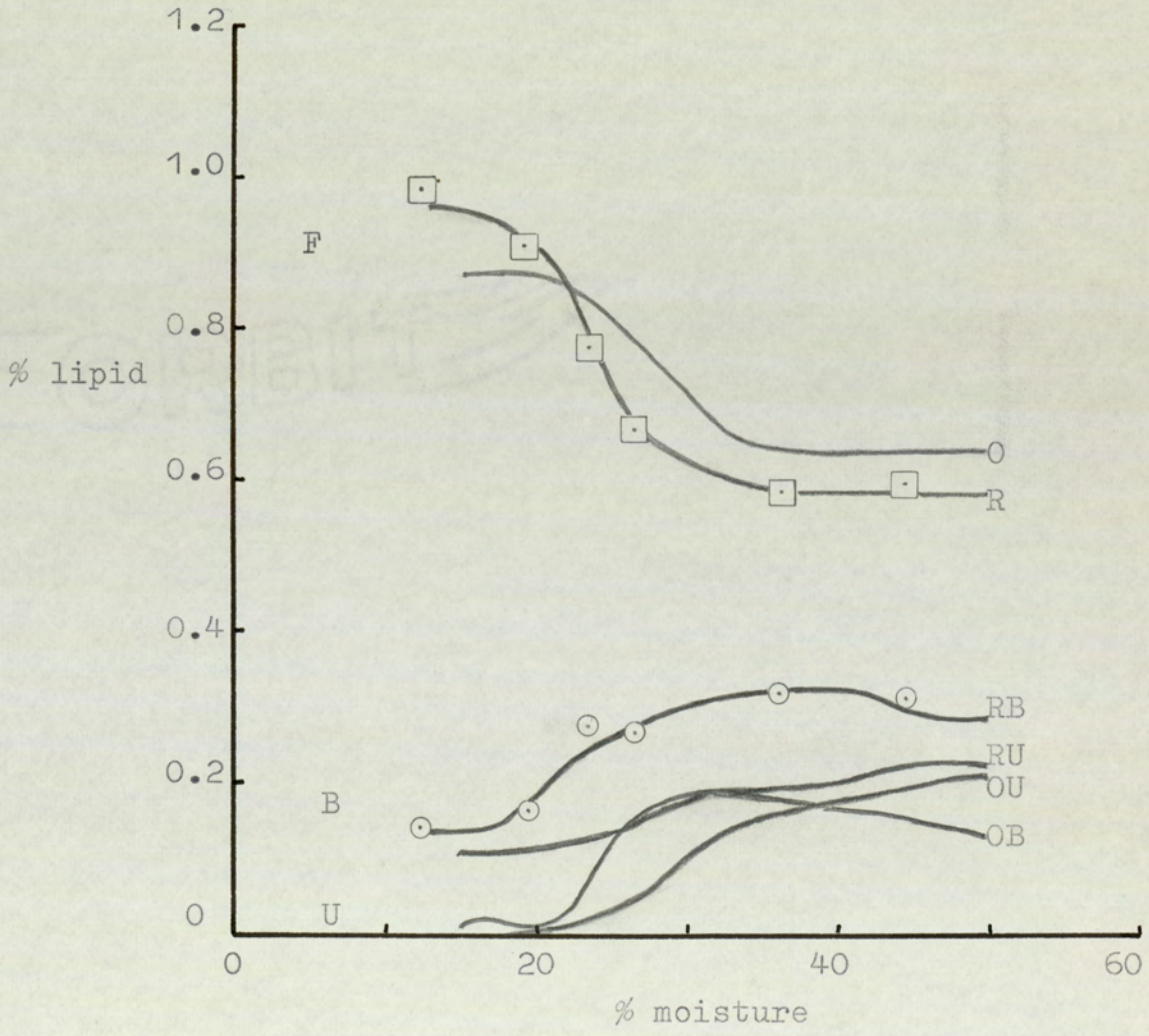


Figure 6:3

Comparison of Reconstituted and Original flours. Effect of wetting on neutral lipid fractions. Results expressed as percentages of flour dry weight.

Standard Errors of Means.	Free	Bound
Original	± 0·0306	± 0·0376
Reconstituted	± 0·0372	± 0·039



neutral lipid across the moisture range.

Polar lipid.

As shown in Figure 6:4, GOL 1R had less extractable free or bound polar lipid at 13% moisture than GOL 1. The free polar fractions fell to a similar value after wetting and the bound fractions of both flours increased by similar amounts although binding of GOL 1R started at a lower moisture level. The greatest difference between the two flours lay in the unextractable binding of polar lipids. In GOL 1R, polar lipid that had been unextractably bound at 13% moisture was released, while in GOL 1 polar lipid first became unextractably bound as the moisture level rose from 14 to 50% and was then released at higher moisture levels.

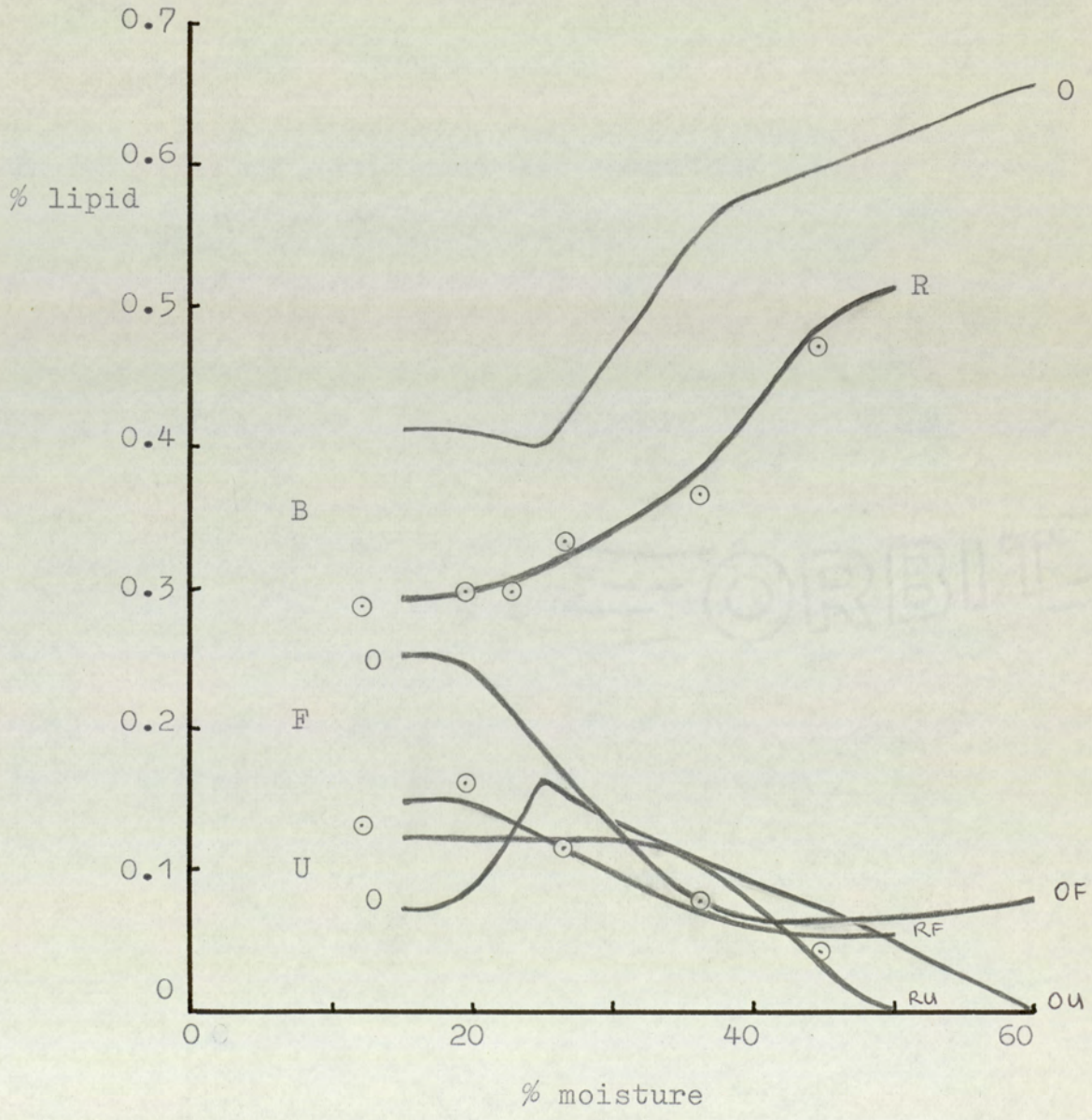
Conclusions.

The increased binding that occurred in fat extracted flours suggested that this procedure resulted in activation of more binding sites. The results of this study have indicated that such activation may be considered as removal of unextractable neutral lipid and unextractable binding of polar lipid. Reconstitution would appear to have been a continuation of this process since it resulted in further release of unextractable neutral lipid and more unextractable binding of polar lipid. In both processes, the binding sites were in an environment of limited moisture less favourable to hydrophobic bonding. This situation was completely reversed by work free wetting of the flour when unextractable polar lipid became extractable and neutral lipid unextractable.

Figure 6:4.

Comparison of Reconstituted and Original flours. Effect of wetting on polar lipid fractions. Results expressed as percentages of flour dry weight.

Standard Errors of Means.	Free	Bound
Original	± 0.0213	± 0.0283
Reconstituted	± 0.0279	± 0.0304



While fat extraction and reconstitution evidently affected lipid binding sites, the effects of lipid distribution will be given due consideration in the subsequent stages of this study, which will involve the reconstitution of fat extracted flour with different lipid fractions.

SECTION SEVEN

The effect of mechanical work and breadmaking ingredients on lipid binding.

Introduction

Although the main theme of this thesis is the work-free hydration of flour and its effect on the lipid-protein relationship, this section deals with other closely related subjects. An account is given of the effect of wetting on lipid distribution of flours with augmented lipid contents. From this work a study of the effect of mechanical work on lipid distribution developed. This section reports the results of these studies as well as subsequent investigations of the effect of other ingredients on lipid distribution and the distribution of water in dough.

Flours with augmented lipid contents.

In section Four it was concluded that flour free lipid was bound as a result of work-free wetting up to a moisture level of 40%. Beyond this moisture level no further binding occurred despite the continued presence of a substantial amount of free lipid and an equilibrium situation was suggested. However, Olcott and Mecham (1947) reported that when extra lipid was added to flour even more binding occurred. Eventually a binding capacity was reached of about three times the amount originally present in flour.

The liquid nitrogen technique of adding lipid to extracted flour without producing binding in the process was considered to be equally suitable for augmenting the lipid contents of flour. Such flours could then be wetted by the liquid nitrogen technique to see the effect of work free wetting on their lipid distributions. A comparison of the results with those of Olcott and Mecham would then

indicate whether their method of wetting was work free.

Effect of work-free wetting on flours with augmented lipid contents.

Measured amounts of lipid, extracted from flour GOL 1 with petrol, were added to unextracted samples of the same flour (GOL 1) by the liquid nitrogen technique. These flours showed a stronger colour and an increase in "cakiness" when compared with the original. These samples were then wetted to 50% moisture by the liquid nitrogen technique, freezedried after thawing, ground, sieved and the free and bound lipids extracted. The results are shown in Table 7:1.

Table 7:1

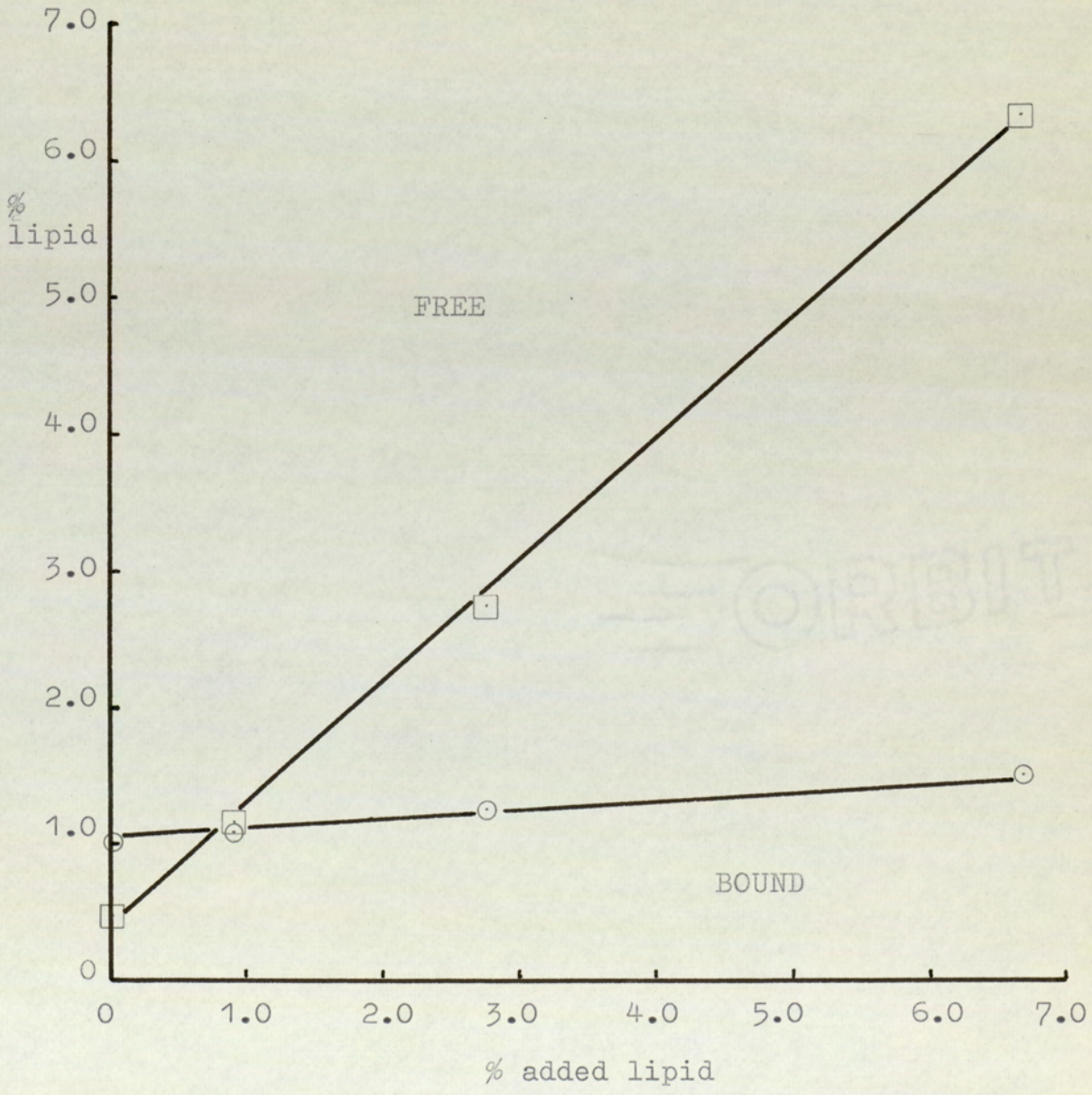
Effect of work-free wetting on the lipid distribution of flours with augmented lipid contents. Mean values, expressed as percentage of flour dry weight.

	Added lipid			
	0	0.8963	2.7569	6.6750
Total lipid known to be present before wetting	1.650	2.5463	4.4069	8.3250
Free	0.456	1.165	2.730	6.320
Bound	1.00	1.07	1.24	1.49
Unextractable	0.194	0.308	0.439	0.515
Bound by Olcott and Mecham's definition (B+U)	1.194	1.3813	1.6769	2.005

These results suggested that little extra binding had occurred despite the extra amount of lipid present. When these results are plotted as in Figure 7:1 it can

Figure 7:1

Effect of work-free wetting (to 50% moisture) on lipid distribution of flours with augmented lipid levels. Results expressed as percentages of flour dry weight.



be seen that the work-free wetting had not produced extra binding and the additional lipid remained free. Figure 7:2 compares these results with those obtained by Olcott and Mecham for the wetting of flours with augmented lipid contents by showing the amounts of free lipid obtained. Olcott and Mecham found that most of the lipid added to flour up to about 3.5% was bound. This is demonstrated in Figure 7:3 where a comparison is made between the results of the two studies. Lipid remaining in the flours after extraction with petrol is shown.

The considerable difference between the two sets of results suggested that the techniques employed for wetting the flours were having different effects. Accordingly, a similar series of flours with augmented lipid contents were prepared using the liquid nitrogen technique. These flours were then wetted in an imitation of the manner described by Olcott and Mecham. This involved adding equal amounts of flour and water to a blender bowl and mixing until a homogeneous dough was just formed. The distribution of lipids in these 'doughs' was determined after freezedrying, grinding and sieving and the results are shown in Table 7:2.

Figure 7:2.

Comparison of the effect of work-free wetting, ⊙, on free lipid levels with results reported by Olcott and Mecham (1947) ⊡ also on flours with extra lipid. Results expressed as percentages of flour dry weight.

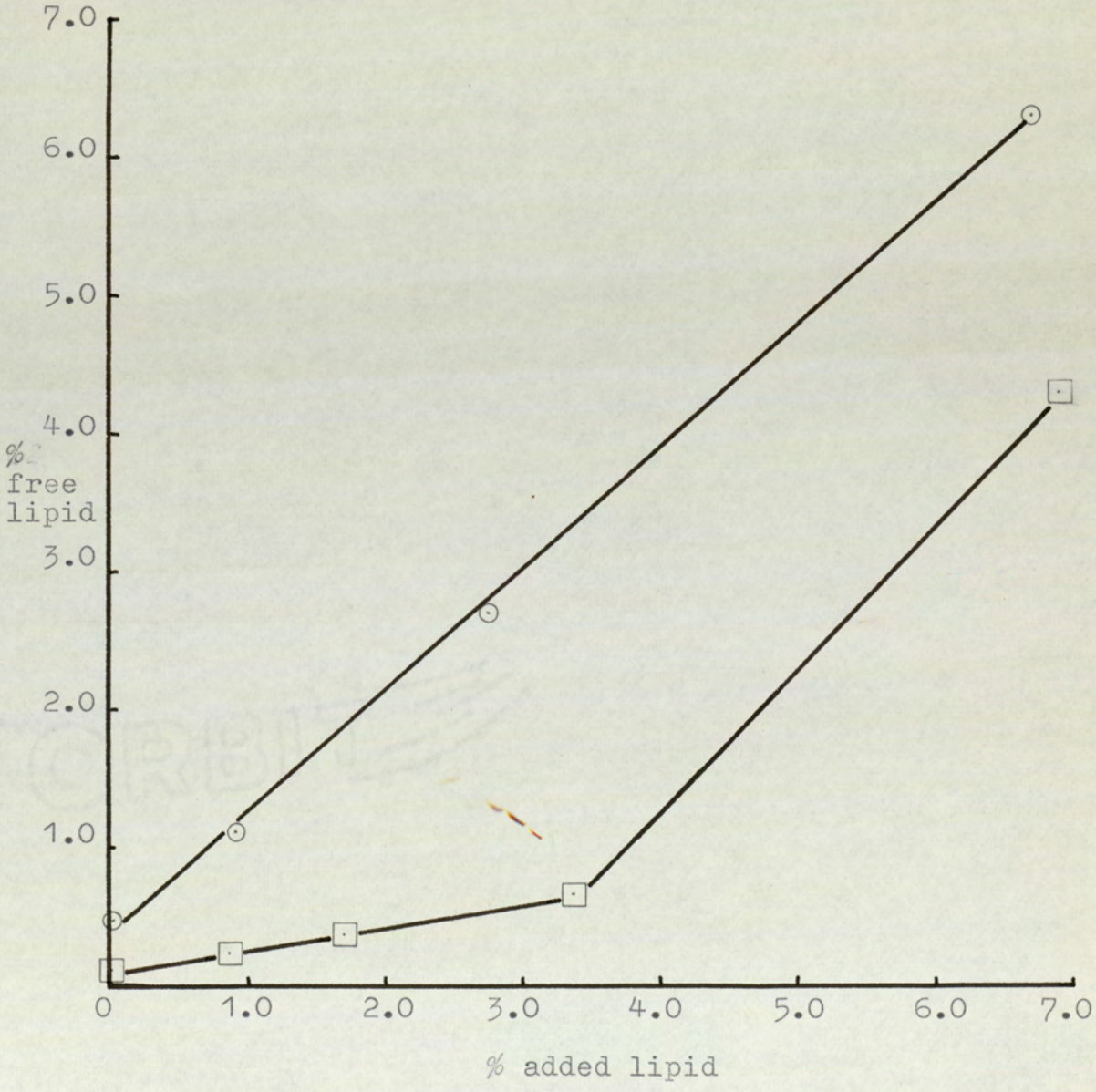


Figure 7:3.

Comparison of the effect of work-free wetting, on 'bound' lipid of flours having augmented lipid levels, ○, with results reported by Olcott and Mecham (1947), ⊡. Results expressed as percentages of flour dry weight. 'Bound' lipid from Olcott and Mecham's definition =B+U.

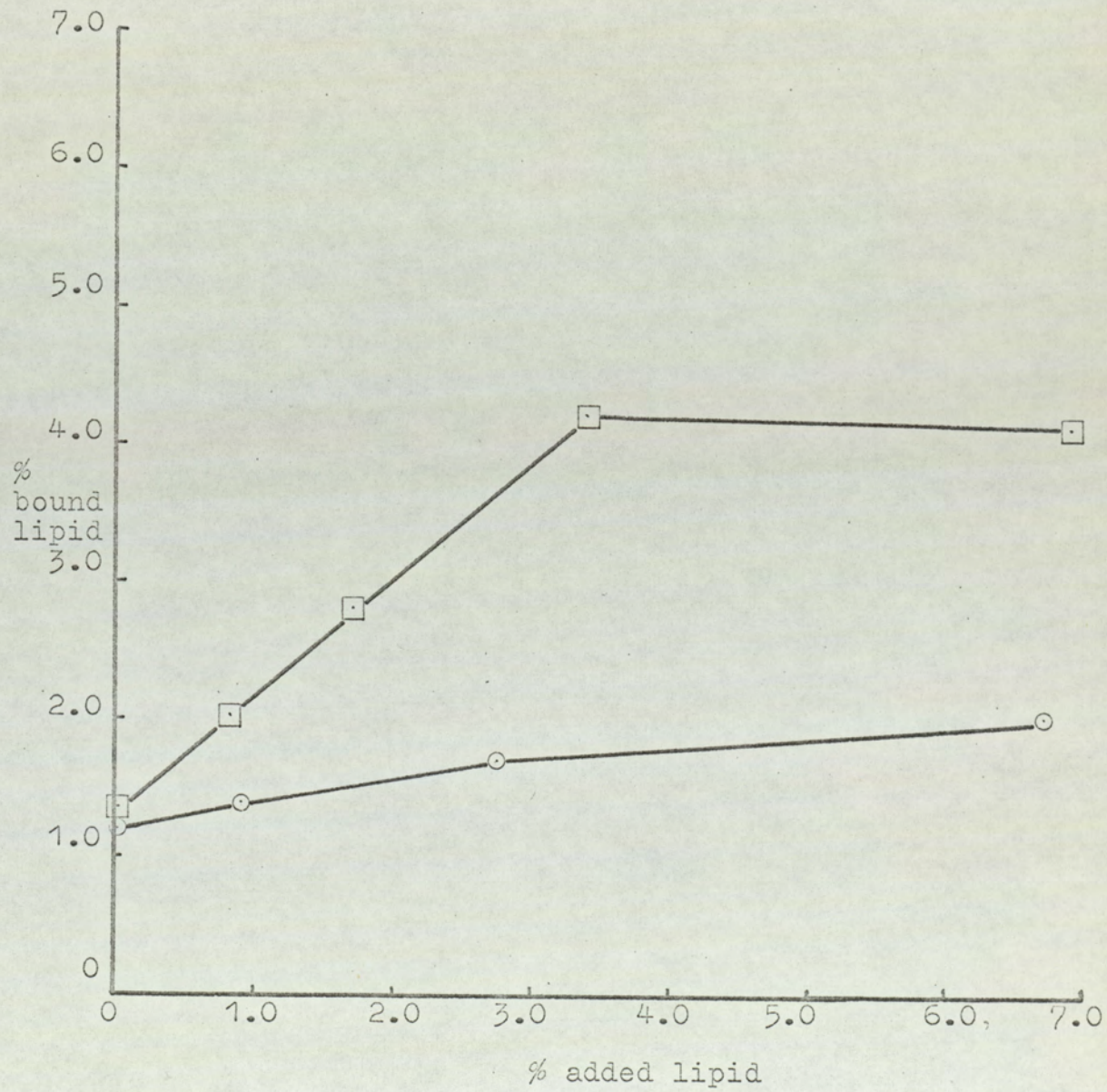


Table 7:2

Effect of wetting by blending on the lipid distributions of flours with augmented lipid contents. Mean values, expressed as percentages of flour dry weight.

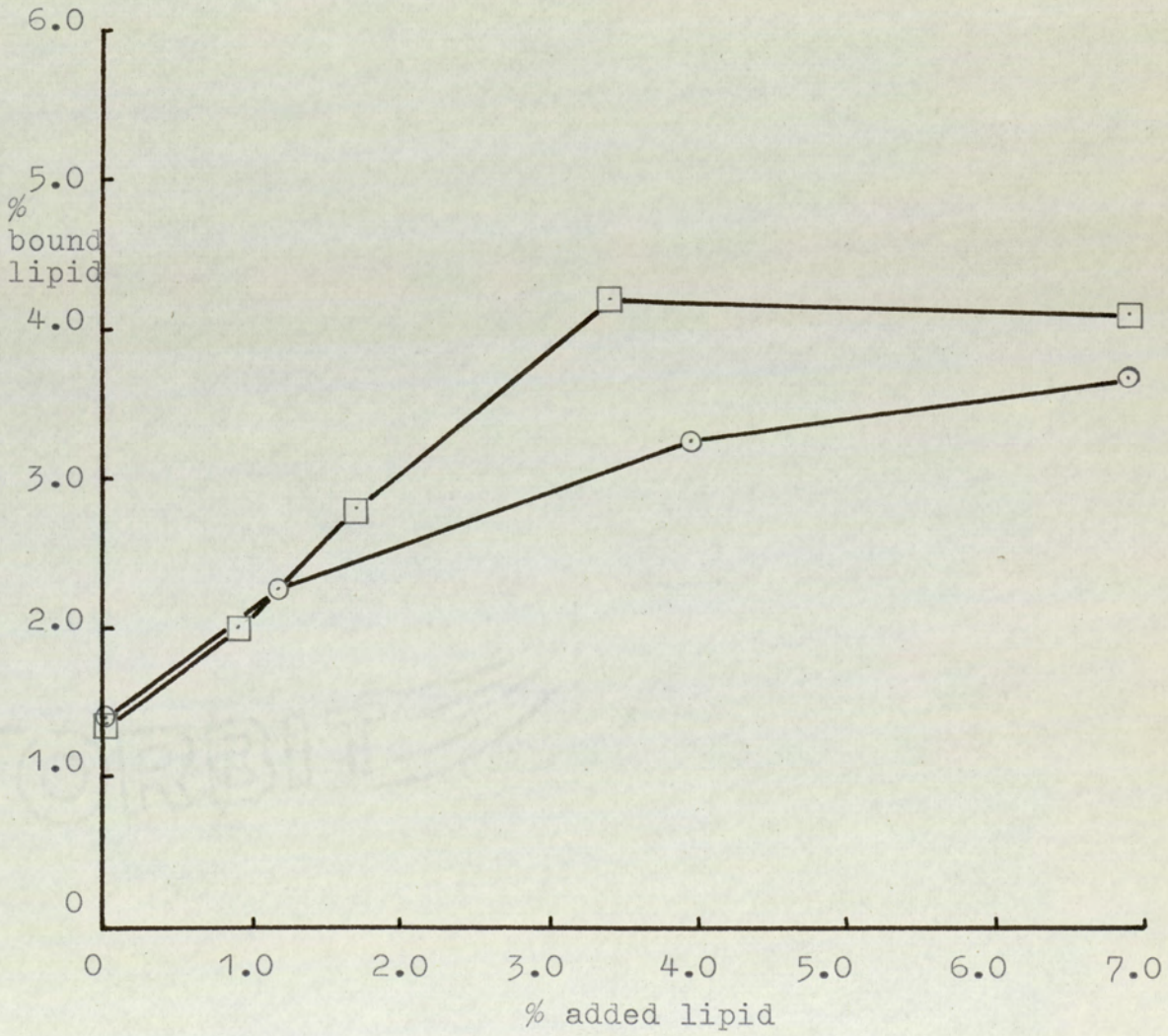
	Added lipid			
	0	1.175	3.960	6.900
Total lipid known to be present	1.650	2.825	5.61	8.55
Free	0.2513	0.572	2.353	4.870
Bound	1.16	1.90	2.69	3.41
Unextractable	0.239	0.353	0.567	0.270
Bound by Olcott and Mecham's definition(B+U)	1.3987	2.253	3.257	3.680

The use of Olcott and Mecham's wetting by blending technique produced much more binding of lipid. Figure 7:4 shows the increase of bound lipid at higher levels of added flour lipid found in both studies. This indicated that their wetting method had been successfully imitated. Furthermore, the difference between the liquid nitrogen technique and the 'blending' technique was obviously the introduction of mechanical work, albeit minimal and that this small amount of work was sufficient to cause the additional lipid binding.

It was considered that this result provided excellent justification for the use of the term 'work-free wetting' when describing wetting by the liquid nitrogen technique and emphasized the value of the technique. It was also interesting to note that when the colour of extracted

Figure 7:4.

Imitation of Olcott and Mecham's blending wetting technique. Effect on 'bound' lipid ⊙,(B+U) compared with Olcott and Mecham's results, ⊡.
Results expressed as percentages of flour dry weight.



lipids from doughs wetted by the two methods were compared, bleaching had occurred during the blending, probably by atmospheric oxygen in the open mixer (Hawthorn and Todd (1955)).

Effect of rewetting and freezedrying on lipid distribution

Freezedried, unworked dough samples, which had been prepared for the previous study were also used to examine the effect of rewetting unworked dough. These samples, freezedried and in fine powder form, were rewetted to 50% moisture using the Olcott and Mecham blending technique. Any further increase in binding would then be due to the work introduced by the blending technique and any effect of rewetting. Likewise any difference between these results and those obtained when the flour was first wetted by Olcott and Mecham's blending technique would indicate any freezedrying or rewetting effect. The lipid distributions of these rewetted freezedried 'dough' samples are shown in Table 7:3.

Table 7:3

Effect of freezedrying and rewetting of unworked doughs, the rewetting involving a minimal amount of work. Mean values expressed as percentage of flour dry weight.

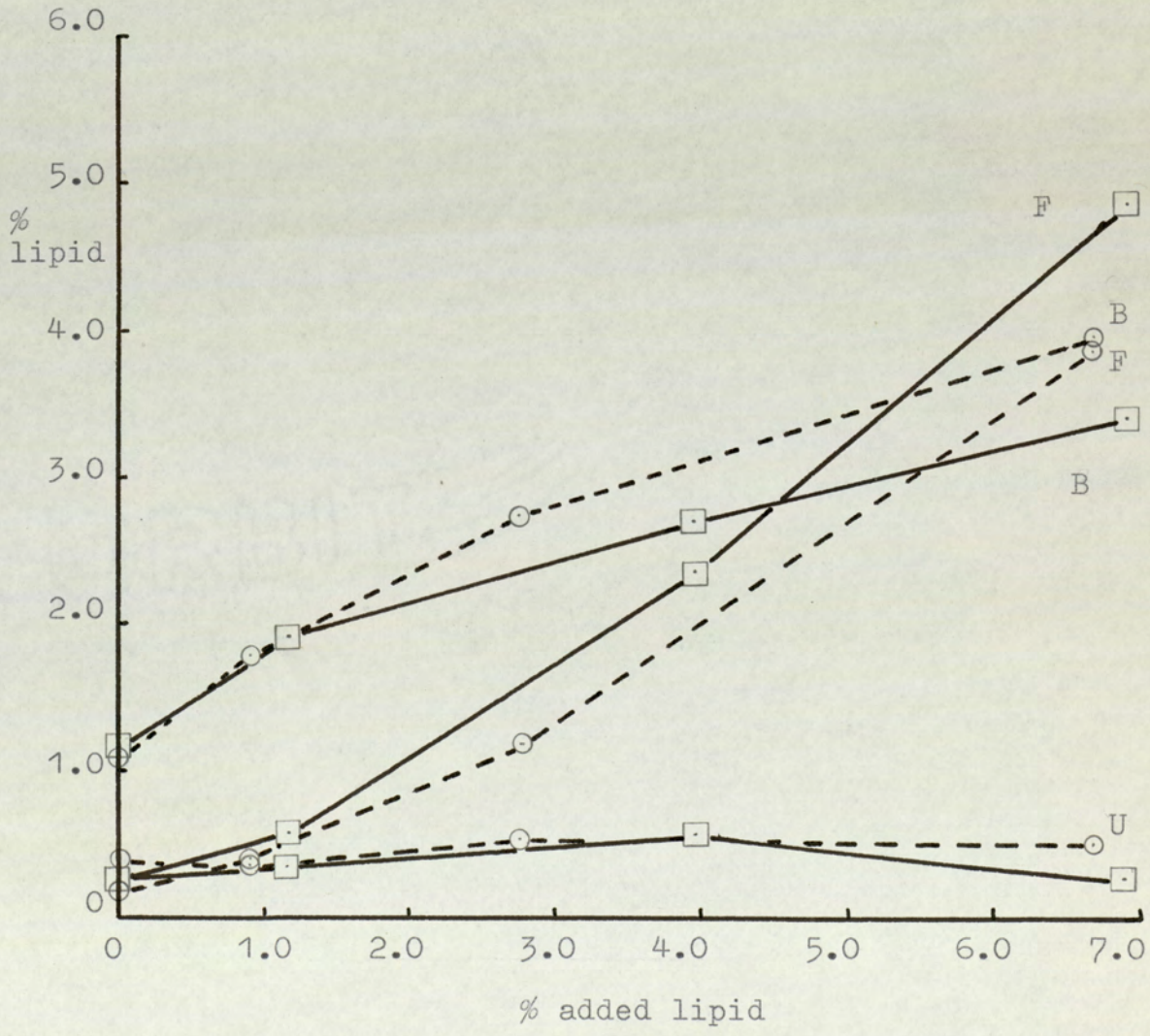
	Added lipid %			
	0	0.8963	2.7569	6.6750
After work-free wetting				
Free	0.456	1.165	2.73	6.32
Bound	1.00	1.073	1.24	1.49
After freeze-drying and 'blending' wetting				
Free	0.186	0.391	1.155	3.87
Bound	1.075	1.793	2.73	3.96
Unextract-able	0.389	0.3623	0.5219	0.495
Bound by Olcott & Mecham's definition (B+U)	1.464	2.1553	3.252	4.455

Again, the blending technique produced a considerable increase in lipid binding. Figure 7:5 compares the effect of blending wetting with blending-rewetting on lipid distribution.

The comparison suggests that the freezedrying and rewetting stage had produced no major effect, both sets of results were closely similar except for a small increase in binding in the rewetted dough.

Figure 7:5

Effect of freezedrying and rewetting wetted flours on lipid distribution of flours with augmented lipid levels. Comparison of the effect of blending wetting, □ , with blending wetting, for the second time, ○.
Results expressed as percentages of flour dry weight.



Effect of work-free wetting on fat-extracted flour reconstituted with extra amounts of lipid.

Samples of flour, extracted with petrol by percolation, were reconstituted using the liquid nitrogen technique to a range of lipid contents in excess of the amount originally present. These flours were then wetted to 50% moisture using the liquid nitrogen technique. After thawing and freezedrying, the distribution of free and bound lipids determined. The results are shown in Table 7:4.

Table 7:4

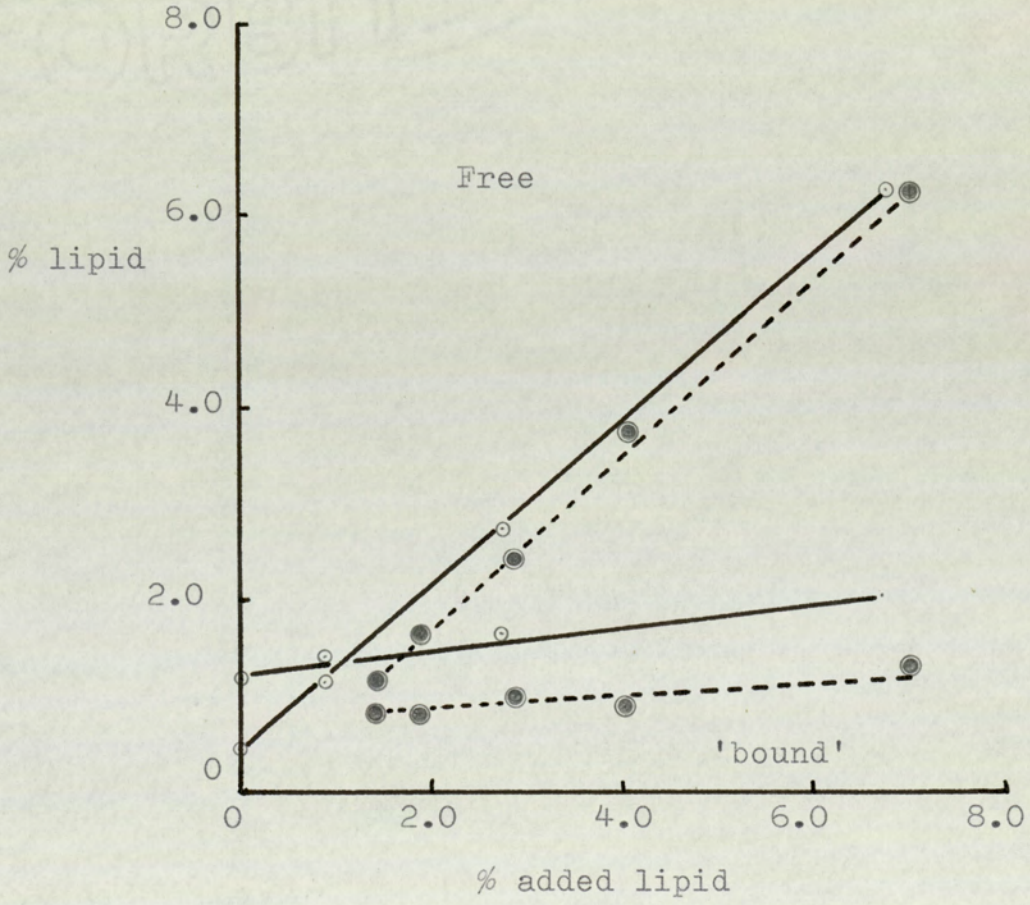
Effect of work-free wetting on the lipid distributions of fat-extracted flours, reconstituted to excessive levels. Mean values, expressed as percentage of flour dry weight.

Added lipid	Total lipid present	Free	Bound	Unextractable	'Bound' Olcott & Mecham (B+U)
1.423	1.951	1.16	0.588	0.203	0.791
1.890	2.418	1.63	0.605	0.183	0.788
2.880	3.408	2.41	0.643	0.355	0.998
4.090	4.618	3.74	0.596	0.282	0.878
7.050	7.578	6.25	1.088	0.240	1.328

These results suggested that little binding occurred as a result of the work-free wetting. If these results are compared, as in Figure 7:6, with those obtained for an unextracted flour, it can be seen that even less binding occurred in the extracted flour.

Figure 7:6.

Comparison of effect of work-free wetting on lipid distributions of unextracted, ⊙ , and fat-extracted, ◉ , flours with augmented lipid levels. Results expressed as percentages of flour dry weight, 'Bound' by Olcott and Mecham's definition.



Effect of heat on flours with augmented lipid contents.

A further set of results which were obtained from these flours with augmented lipid contents will be presented at this point. A series of unworked doughs produced from such flours were unintentionally heated during the final freezedrying stage. The experiment was repeated but with the omission of the heating, to demonstrate any effect on the lipid distributions due to heating. Table 7:5 shows the lipid distributions of the flours subjected to heat and in Figure 7:7 the comparison is made.

Table 7:5

Effect of heat on lipid distribution of wetted flour with augmented lipid contents.

Mean values expressed as percentage flour dry weight

	Added lipid %			
	0	0.8310	2.5284	4.5299
Total lipid present	1.650	2.4810	4.1784	6.1799
Free	0.322	0.709	1.580	3.038
Bound	0.905	0.985	1.190	1.239
Unextractable	0.423	0.787	1.4084	1.9029

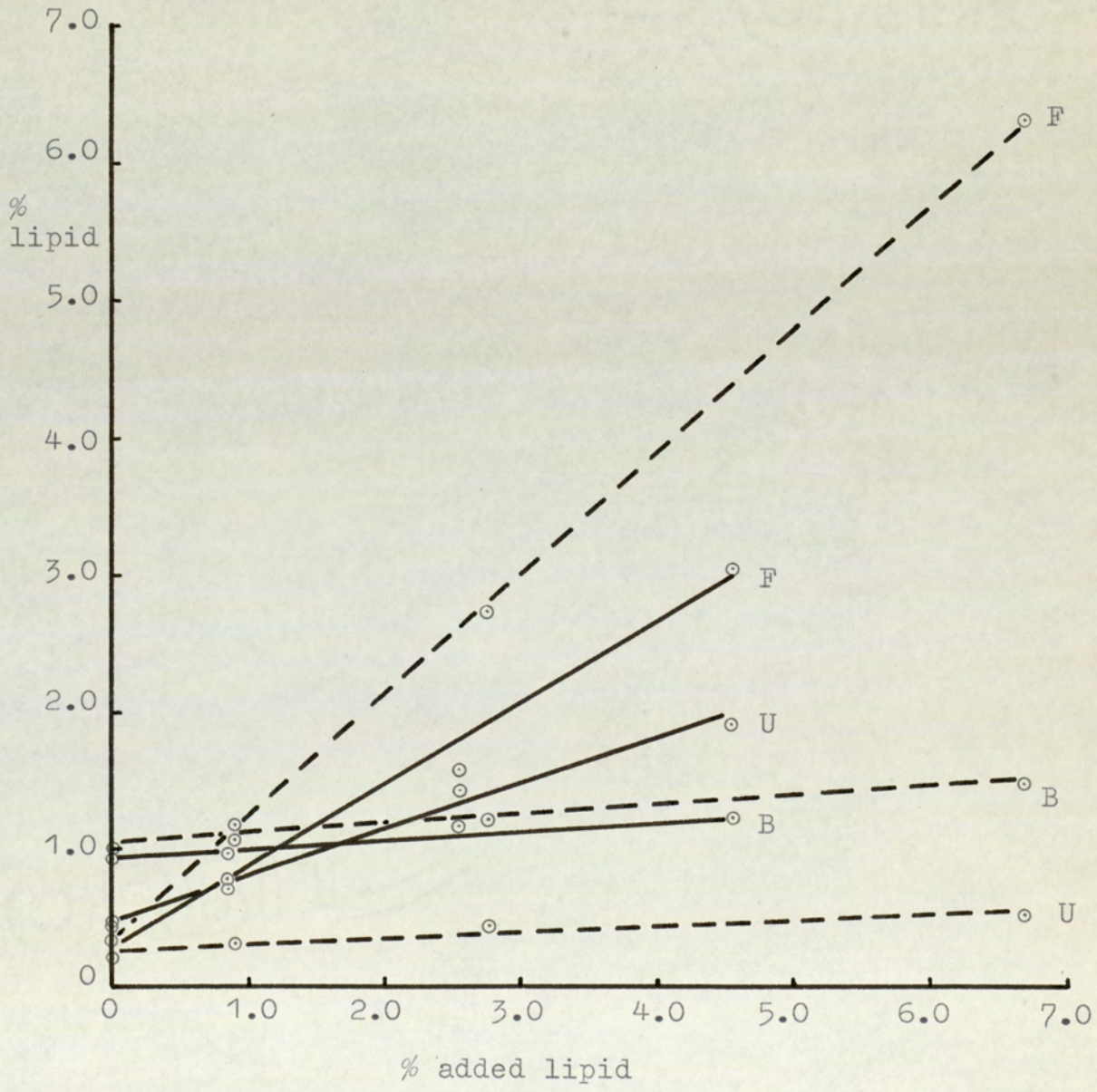
Clearly more lipid was bound in the flour with no added lipid as a result of heating, not [illegible] in the extractable bound form but [illegible] in the unextractably bound fraction. In flours with added lipid, the additional binding was mainly in the unextractable fraction. The binding due to heating may be considered therefore as being a strong type of binding but any further conclusions from

Figure 7:7.

Effect of work-free wetting on lipid distributions of flours with augmented lipid levels and the consequences of heating. Results expressed as percentages of flour dry weight.

With heat ——————

Without heat - - - - - - - - - -



these results must be drawn with caution since the amount of heat involved was not known.

However, these results substantiate the additional binding produced by baking reported by Baldwin et al. (1963) Daniels et al. (1966) and Chin and Pomeranz (1966). Future lipid binding studies may look more closely at this effect since controlled heating provides a means of introducing a specific amount of energy into a system.

Lipid binding capacities.

Olcott and Mecham found that an amount of lipid up to about three times that in the original flour could be bound and that this amount apparently approached the capacity of the flour. The total bound lipid present(extractably and unextractably bound) was then about 4.1% but this binding was probably due to the small amount of mechanical work introduced by the blending technique.

When their blending technique was imitated, total bound lipid (B+U) was found to be 3.65% and when the liquid nitrogen technique was used, 2.0%. This latter value was made up of 1.5% bound and 0.5% unextractable. The value for the worked doughs was made up of 3.35% bound and 0.30% unextractable. The small amount of work that the blending technique introduced was therefore responsible for the additional 1.85% extractable binding of lipid without increasing the capacity of the unextractable fraction. The effect of the presence of additional free lipid when flour was wetted without work was therefore to increase the amount of bound lipid by about 0.8% and unextractable by 0.3%. Presumably the equilibrium situation discussed in

Section Four was disturbed by the extra free lipid.

Although the main theme of this thesis is flour hydration in the absence of mechanical work the remarkably large effect that work has on lipid binding must be considered. Daniels et al. (1966) and Daniels et al. (1967) investigated the effect of full and overdevelopment of doughs of complete commercial breadmaking formulae on the lipid distribution and also found considerable lipid binding under certain conditions. The next piece of work links the two areas of study by investigating firstly the effect of mechanical development of simple flour and water doughs on lipid binding. Secondly the effect of atmosphere is examined since binding as well as bleaching was produced by wetting in a blender open to air, although according to the work of Daniels et al. (1967) an adverse effect on binding would have been anticipated under these conditions. Thirdly, the effect on lipid binding of other dough ingredients, particularly common salt (Mecham and Weinstein (1952), Wootton (1966) and Pomeranz et al.(1968)) will be examined. The fourth subject is the distribution of water in mechanically developed doughs.

Effect of mechanical work on lipid distributions of flour and water doughs.

Simple flour and water doughs were mixed at a constant rate ofwork input of 0.2hp.min./lb/min. to two total input levels of 0.4 hp.min/lb. and 4.0 hp.min./lb in a recording dough mixer. These two work levels were chosen because the first is a level introduced in a commercial breadmaking process (The Chorleywood Breadmaking Process) and the

second produces a grossly overdeveloped dough. The doughs were mixed with the lid of the mixing bowl in place but with no particular precautions to exclude air. The lipid distributions were determined after freezedrying, grinding and sieving and the results are shown in Table 7:6.

Table 7:6

Effect of mechanical work on dough lipid distributions. Mean values, expressed as percentage of total lipid present. (1.650%).

	Total values		
	Free	Bound	Unextractable.
14% moisture, no work	68.3	31.7	0
47% moisture, no work	42.5	45.5	12.0
47% moisture 0.4hp. min/lb.	12.5	83.7	3.8
47% moisture, 4.0 hp.min/lb.	20.5	72.3	7.2

Effect of mixing on lipid fractions.

Total lipid fractions.

As shown in Figure 7:8, when dough was mixed to 0.4 hp.min/lb. there was an even greater loss of free lipid (30%) than produced by wetting alone. Since the bound fraction increased by 38.2%, a release of 8.2% unextractable lipid was suggested. When mixed a further 3.6 hp.min./lb. to a total of 4.0 hp.min/lb. there was unextractable binding of 3.4% bound lipid and also a release of 8.0% to the free fraction.

Neutral lipid fractions.

Figure 7:9 and Table 7:7 show an increase of 38.8% of the bound fraction compared to a loss of 26.4% free when

Figure 7:8.

Effect of work-free wetting and mechanical mixing on total flour lipid distribution.

Results expressed as percentages of total lipid present.

Free, ○ , Bound, □ , Unextractable, ⬡ .

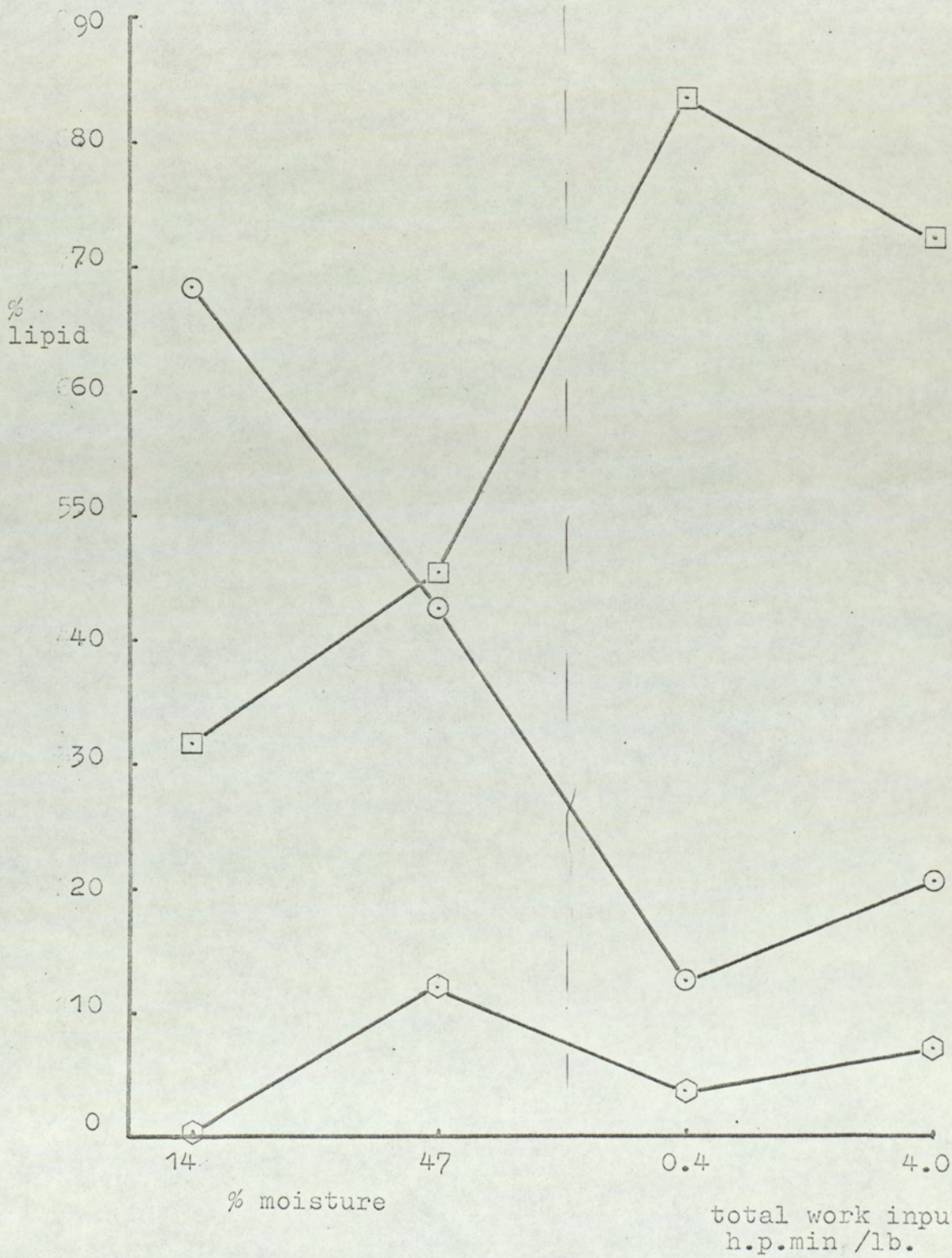
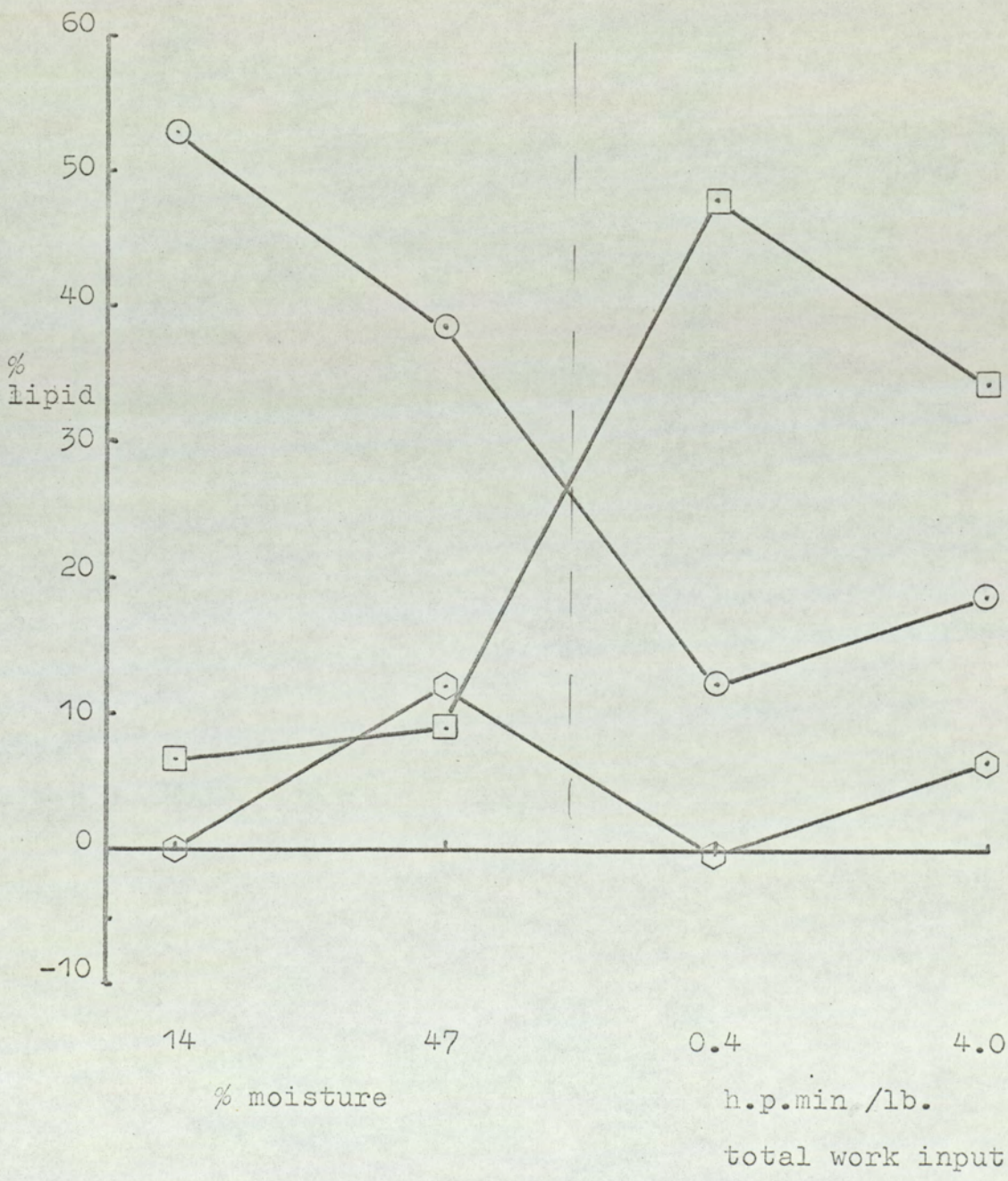


Figure 7:9.

Effect of work-free wetting and mechanical mixing on neutral lipid distributions. Results expressed as percentages of total lipid present.

Free, ○ , bound, □ , unextractable, ⬡ .



the dough was mixed to 0.4 hp.min./lb. which suggested that 12.4% unextractably bound neutral lipid became extractably bound. When overmixed, this was reversed to the extent that 7.0% of the extractably bound became unextractably bound and a further 6.5% of the bound became free.

Table 7:7

Effect of mechanical work on dough neutral lipid distributions. Mean values expressed as percentage of total lipid.

	Neutral lipid		
	Free	Bound	Unextractable.
14% moisture, no work	52.8	6.7	0
47% moisture, no work	38.5	9.0	12.0
47% moisture, 0.4 hp.min/lb.	12.1	47.8	-0.4
47% moisture, 4.0hp.min/lb.	18.6	34.3	6.6

Polar lipid fractions

Figure 7:10 and Table 7:8 show that only a small amount of polar lipid remained free after wetting and that after mixing to 0.4 hp.min/lb., 3.6% of this was unextractably bound and 0.6% extractably. Further mixing reversed this trend with release of 3.6% unextractable polar lipid, 1.5% to the free and 2.1% to the bound fraction.

Table 7:8

Effect of mechanical work on dough polar lipid distributions. Mean values expressed as percentage of total lipid.

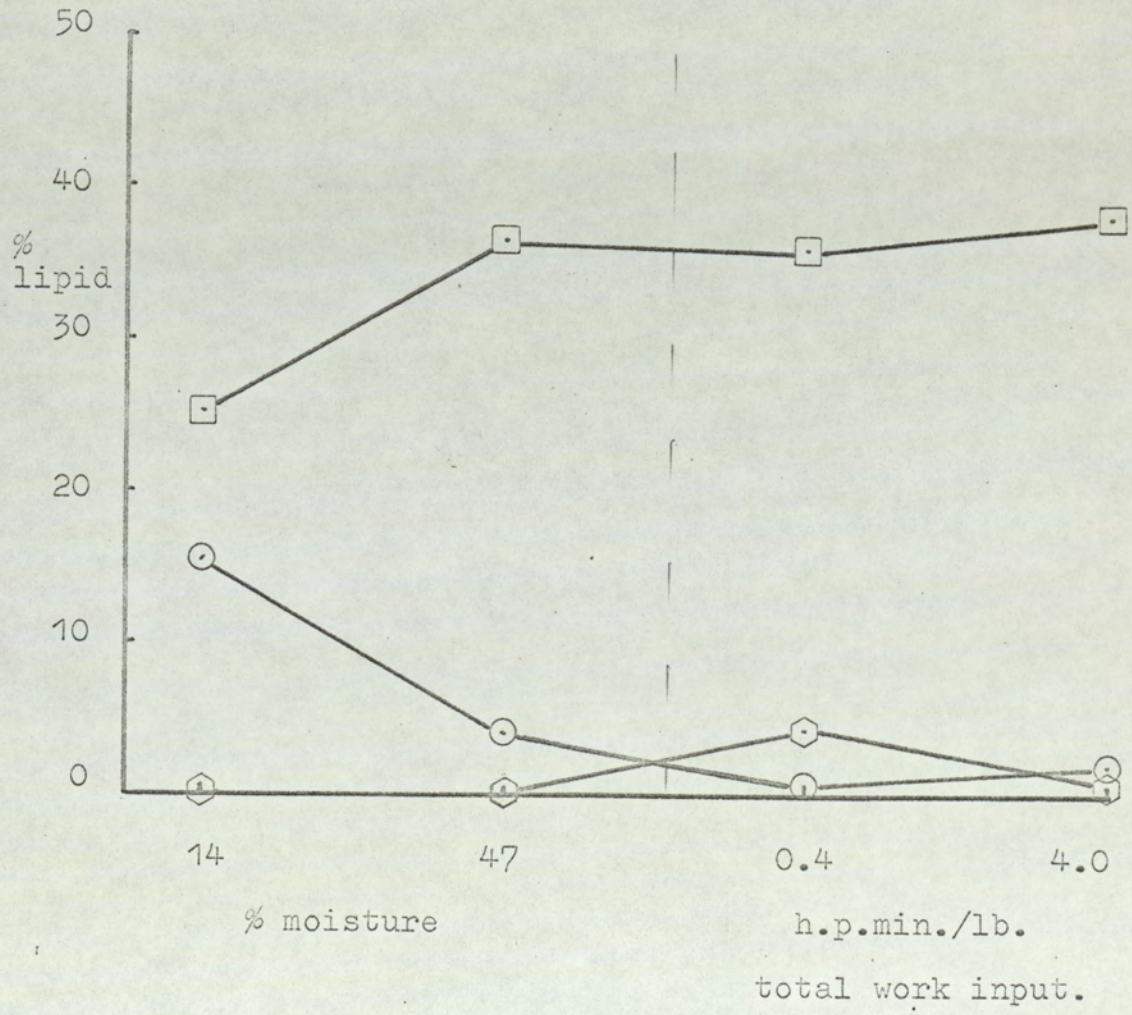
	Polar lipid		
	Free	Bound	Unextractable
14% moisture, no work	15.5	25.0	0
47% moisture, no work	4.0	36.5	0
47% moisture, 0.4hp.min /lb.	0.4	35.9	4.2
47% moisture, 4.0hp.min /lb	1.9	38.0	0.6

Figure 7:10.

Effect of work-free wetting and mechanical mixing on polar lipid distribution,

Results expressed as percentages of total lipid present.

Free, ○, bound, □, unextractable, ⬡ .



Work-free wetting of flour caused the unextractable binding of neutral free lipid and extractable binding of neutral and polar free lipid. The principle effect of the introduction of mechanical work in a dough mixer to a level of 0.4 hp.min /lb was to cause a further considerable increase of extractably bound neutral lipid. This came mainly from the free fraction but also from the unextractably bound fraction. The small effect on polar lipid was the unextractable binding of free and bound lipid. The main effect of overmixing was to produce a release of a small amount of bound neutral lipid.

Wetting flour in a blender was thought to introduce but little mechanical work yet the amount of lipid binding was not far short of that produced by 0.4 hp.min /lb. (Table 7:9)

Table 7:9

Comparison of lipid distribution of flour after wetting by blending and mixing to 0.4 hp.min /lb. Mean values expressed as percentage of total lipid.

	Free	Bound	Unextractable
Wetting in a blender	14.5	70.0	14.5
Work to 0.4 hp.min /lb.	12.5	83.7	3.8

It would therefore appear that little mechanical work is necessary for a considerable effect on lipid binding to be shown.

The increase of binding that was found in these air mixed doughs would not have been anticipated from the results obtained by Daniels et al. (1967) since they found mixing in air reduced lipid binding. However, their study

was on doughs of complete breadmaking formlae which included yeast, yeast food, chemical improvers, salt, shortening fat and enzyme active soya flour. It is thought these other ingredients, particularly the enzyme active soya (Daniels (1970)) were responsible for the contrast between these two sets of lipid binding results from doughs mixed in air.

Since common salt is added to all doughs intended for breadmaking and is known to affect lipid binding, a second similar pair of doughs were mixed, as before but with 2% added salt (on a flour basis) and the results shown in Tables 7:10, 7:11 and 7:12.

Effect of salt on dough lipid fractions.

Total lipid fractions

Table 7:10

Effect of salt on dough lipid distributions.
Mean values, expressed as percentage of total lipid present.

Total values.

47% moisture	Free	Bound	Unextractable
No work	42.5	45.5	12.0
0.4 hp.min /lb.without salt	12.5	83.7	3.8
with salt	17.8	70.6	11.6
4.0 hp.min /lb.without salt	20.5	72.3	7.2
4.0 hp.min /lb with salt	32.3	61.0	6.7

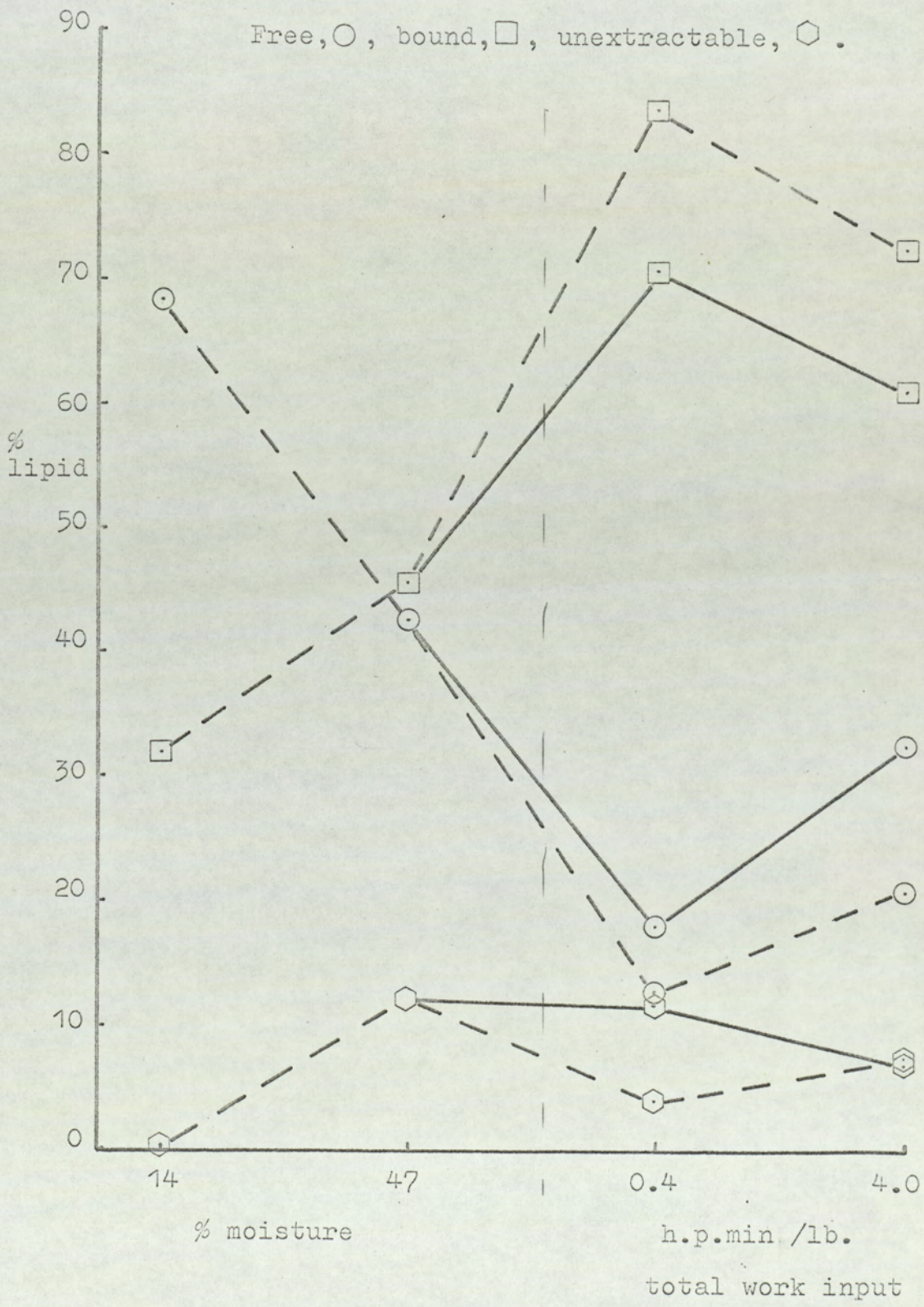
The effect of salt on dough lipid distributions is shown in Figure 7:11 where the results from doughs both with and without salt are shown.

When salt was present in dough mixed to 0.4 hp.min/lb.

Figure 7:11.

Effect of work-free wetting and mechanical mixing on total lipid distribution, with ———————— and without - - - - - - - - - salt present (2%). Results expressed as percentages of total lipid present.

Free, ○ , bound, □ , unextractable, ⬡ .



the unextractably bound fraction decreased less (by 13.1%) than when salt was absent. This resulted in less loss from the free and unextractable fractions. When the salted dough was overmixed, the bound fraction decreased by a little less (1.8%) but was still well below the unsalted level. Likewise the free fraction increased again by 6.5% more than was found in unsalted dough and finally reached a total that was 11.8% higher.

The presence of salt appeared to reduce binding and prevent release of unextractable lipid when dough was mixed to a level suitable for breadmaking. Overmixing reduced extractable binding further whether salt was present or not. However, when salt was present, the unextractably bound fraction was reduced by overmixing and not increased as was found in the absence of salt.

Neutral lipid fractions.

Table 7:11 and Figure 7:12 show the effect on neutral lipid both with and without salt present.

Table 7:11

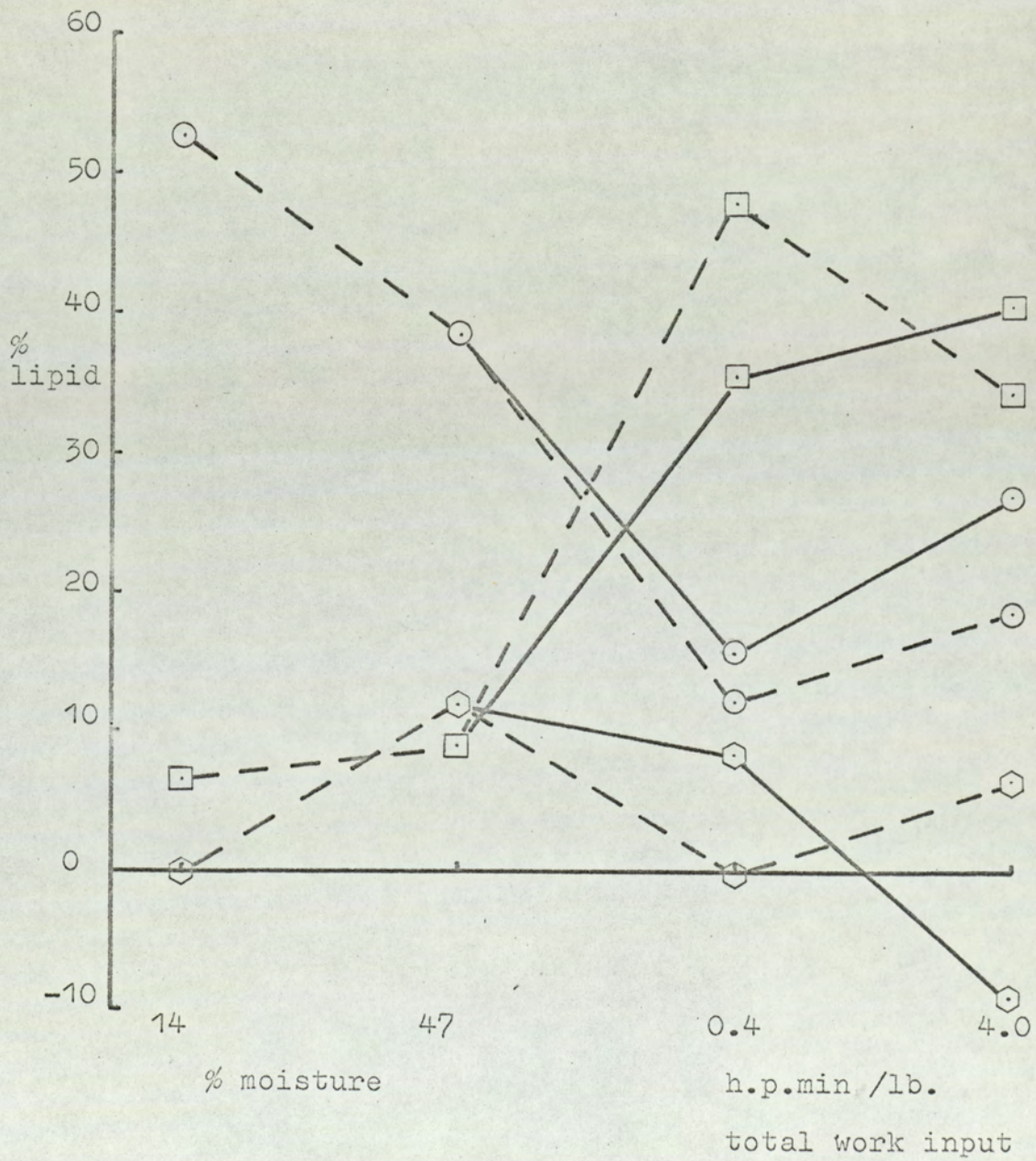
Effect of salt on dough neutral lipid distributions. Mean values expressed as percentage of total lipid present.

	Neutral lipid		
47% moisture	Free	Bound	Unextractable
No work	38.5	9.0	12.0
0.4 hp.min/lb.without salt	12.1	47.8	-0.4
0.4 hp.min/lb.with salt	15.7	35.5	8.3
4.0 hp.min/lb.without salt	18.6	34.3	6.6
4.0 hp.min/lb.with salt	27.0	40.6	-8.1

Figure 7:12.

Effect of work-free wetting and mechanical mixing on neutral lipid distribution, with ———— and without - - - - - - - - salt present (2%). Results expressed as percentages of total lipid present.

Free, ○ , bound, □ , unextractable, ⬡ .



When compared with Figure 7:13, it is apparent that mixing to 0.4 hp.min /lb. had a greater effect on neutral than polar lipid. The principle effect on neutral lipid was the smaller increase of extractably bound lipid. As this was not matched by a lower decrease of free it appeared that only a very small decrease of the unextractable fraction occurred. The unsalted dough lost all the unextractable neutral lipid during this mixing and the presence of salt appeared to prevent this happening.

Overmixing produced an increase of extractably bound neutral lipid and not a decrease. This gain was at the expense of the unextractably bound fraction which disappeared. This fraction increased in overworked unsalted dough.

Polar lipid fractions.

The minor effect of the presence of salt on polar lipid when mixing dough to 0.4 hp.min /lb. can be seen in Table 7:12 and Figure 7:13.

Table 7:12.

Effect of salt on dough polar lipid distributions. Mean values expressed as percentage of total lipid present.

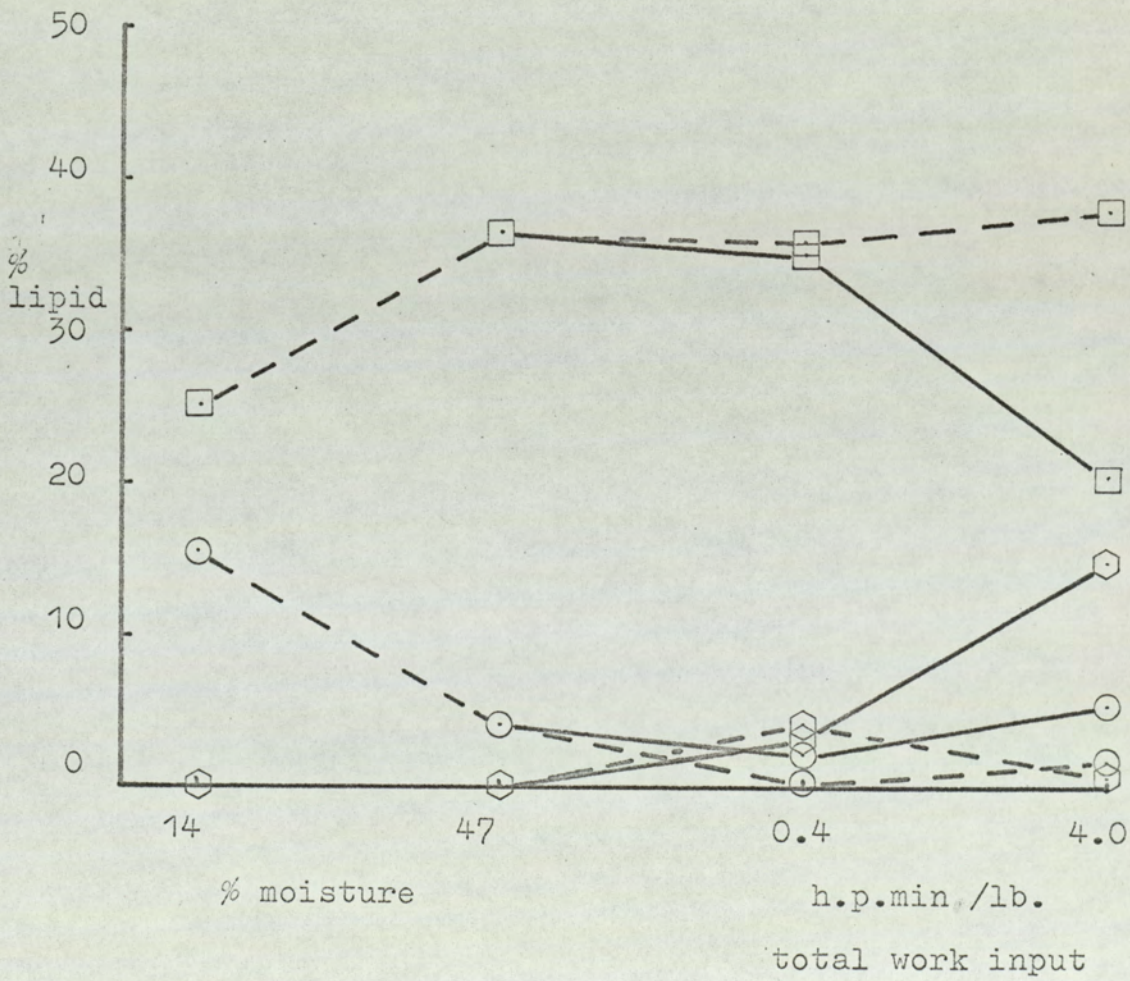
Polar lipid.

47% moisture	Free	Bound	Unextractable.
No work	4.0	36.5	0
0.4 hp.min/lb.without salt	0.4	35.9	4.2
0.4 hp.min/lb.with salt	2.1	35.1	3.3
4.0 hp.min/lb.without salt	1.9	38.0	0.6
4.0 hp.min/lb.with salt	5.3	20.4,	14.8

Figure 7:13

Effect of work-free wetting and mechanical mixing on polar lipid distribution, with ———————— and without - - - - - - - - salt present (2%). Results expressed as percentages of total lipid present.

Free, ○, bound, □, unextractable, ⬡ .



When the dough was overworked, the presence of salt drastically reduced the extractably bound fraction to the level present in flour at 14% moisture. This mostly became free although a small proportion became unextractably bound.

To summarize the effect of salt on worked doughs, the introduction of 0.4 hp.min./lb work to dough caused extractable binding of free neutral lipid, which was reduced by the presence of salt which also prevented the release of unextractably bound lipid. Overwork of unsalted doughs increased the unextractable neutral lipid at the expense of the bound neutral fraction and some neutral lipid became free. When salt was present both the extractably and unextractably bound fractions decreased. The former since more bound polar lipid became unextractably bound than unextractably bound neutral lipid became extractably bound. The latter since both unextractably bound neutral lipid and extractably bound polar lipid was released.

Other studies of the effect of salt on lipid binding in worked doughs have shown a reduction in total and phospholipid binding (Mecham and Weinstein (1952) and Wootton (1966)) in flour and water doughs. However, Pomeranz et al. (1968) found a reduction of total and neutral but not polar lipid in fat extracted flour as well as unextracted flour when salt was added which agreed with the results reported here. This would refute the suggestion (Mecham and Weinstein (1952) and Wootton (1966)) that added salt disrupts 'salt-like linkages' between lipid and

protein which have been postulated by Grosskreutz (1961), Lee and Wan (1963) and Pence et al. (1964), although it is necessary to qualify this since in this study polar lipid was found to be released when salted doughs were grossly overmixed.

The distribution of water in doughs.

The distinction between 'free' and 'bound' water in biological materials, especially foodstuffs, is often made and discussed but no complete agreement has been reached on their definition. While the concept is useful when considering the interaction of water with other components the definition rests entirely on the method of determination when any measurements are to be made.

A commonly used definition of 'free' water would appear to be that fraction of water which will freeze when the material is cooled to a sub-zero temperature. Numerous methods are described in the literature (Kuprianoff (1958) for the determination of freezable water, the proportion of unfreezable or 'bound' water then being inferred from the known total moisture level in the material. Among these methods is the calorimetric determination of this freezable water in which the energy change when the ice melts in the frozen material is measured. The Dupont 900 Differential Thermal Analyser was used as a calorimeter for this approach.

In this study, three series of doughs were mixed, Series A with GOL 1 with 2% added salt (flour weight basis) Series B, HIL with 2% added salt and Series C, GOL 1 without added salt. Various levels of added water were

used for each series of doughs, the range being limited by feasibility of mixing to the required work input and ease of handling.

A level of work of 0.6 hp.min /lb. was introduced to each dough at a constant rate of 0.2 hp.min /lb/min. At least four samples were taken from each dough, frozen and thawed in the Disc cell of the Differential Thermal Analyser and an endotherm of melting ice recorded as a peak. The area of this peak was measured and related to the weight of water involved in the transition by the use of a calibration coefficient for the machine. The amount of freezable water present was then calculated from the known amount of total water present in each sample.

Tables 7:13, 7:14 and 7:15 show the results obtained for Series A, B and C.

Table 7:13

Series A. Determination of freezable water in doughs of different moisture contents. Flour GOL 1 with salt. Mean values of 4 or more results.

Total water g/100g	Freezable water g/100g	
58.3	24.7	± 0.525
62.9	28.6	± 0.700
67.5	32.5	± 0.140
72.1	35.2	± 0.667
76.9	39.2	± 0.670
81.5	46.8	± 0.950
85.9	49.2	± 0.330
92.0	56.5	± 1.530

Table 7:14.

Series B. Determination of freezable water in doughs of different moisture contents. Flour HIL with salt. Mean values of 4 or more results.

Total water g/100g	Freezable water g./100g	
58.5	25.2	± 0.710
64.8	30.1	± 0.550
67.1	32.9	± 0.870
71.7	38.1	± 1.00
76.4	40.3	± 0.690
79.5	43.6	± 0.375

Table 7:15.

Series C. Determination of freezable water in doughs of different moisture contents. Flour GOL 1 without salt. Mean values of 4 or more results.

Total water g./100g	Freezable water g./100g.	
58.3	26.4	± 0.33
62.9	29.6	± 0.19
67.5	30.8	± 0.38
72.1	35.0	± 0.72
76.9	44.0	± 1.10
81.5	49.7	± 0.27
85.9	53.0	± 0.83
92.0	57.0	± 0.65

When freezable water was plotted against total water content in each series the two appeared to be directly proportional. Linear regression by the method of least

squares produced the values given for slope and x intercept in Table 7:16. The slopes in each series were each so close to unity that all the data were considered jointly. The reduction in scatter about the single fitted line compared to fitting three separate lines was not statistically significant.

Table 7:16.

Estimates of slope and x intercept by linear regression.

Slope

Series	Point estimate	95% confidence limit
A	0.976	0.874, 1.079.
B	0.881	0.754, 1.007.
C	0.998	0.815, 1.181.
All data	0.958	0.883, 1.033.

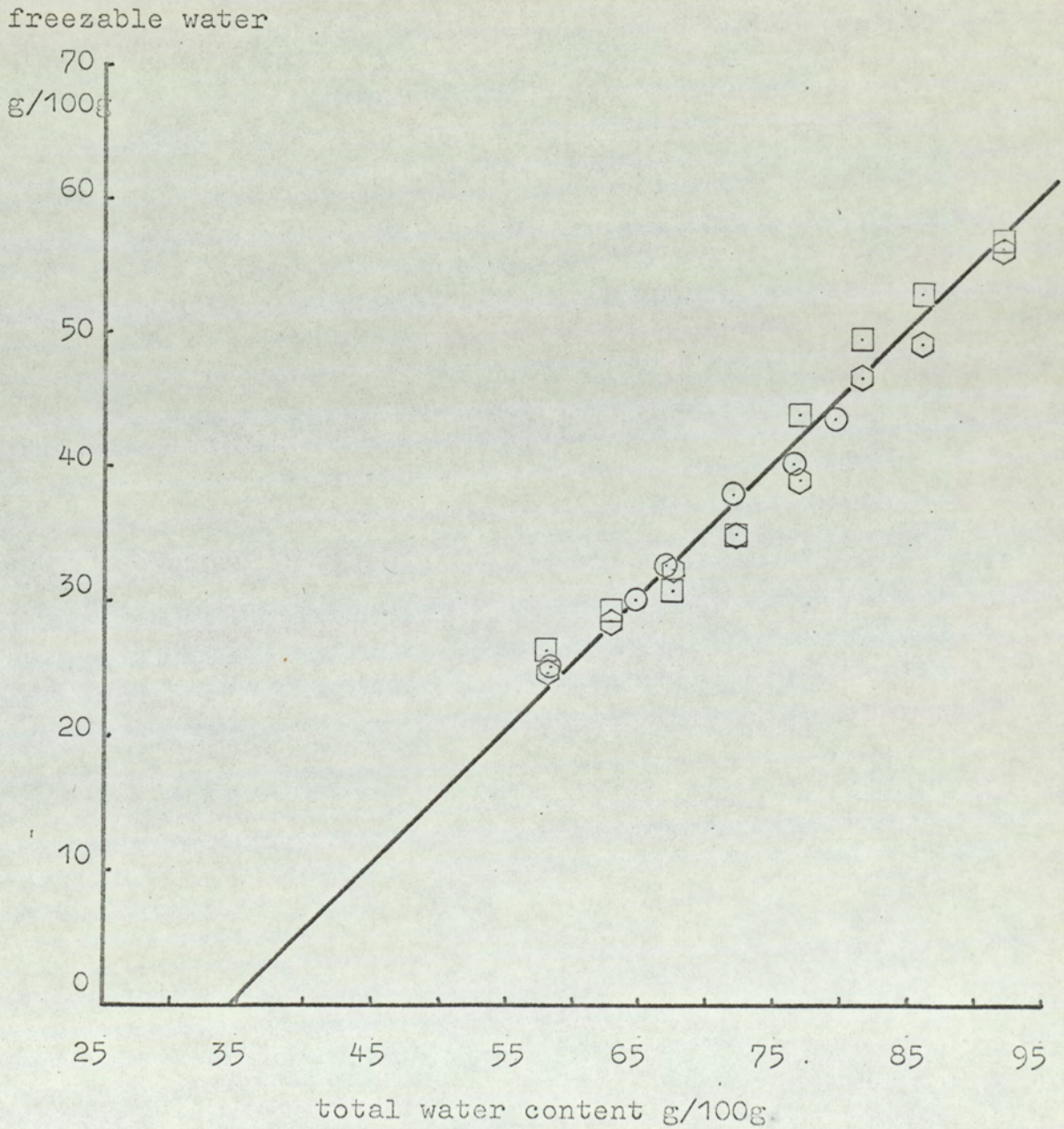
x intercept

Series	Point estimate	95% confidence limit
A	34.6	29.8, 38.5.
B	29.9	23.2, 35.0.
C	33.8	24.3, 40.4.
All data	33.0 $\pm$ 1.6	29.5, 36.0.

In figure 7:14 all the results have been plotted and a single line of unit gradient drawn. There was no significant difference between the results for hard wheat flour GOL 1 and the soft wheat flour HIL. There was also no significant difference between results when salt was present or absent. It was interesting that in the moisture range studied the proportions of freezable and unfreezable water were the same in these three dough systems, despite

Figure 7:14.

Freezable water contents of doughs compared with total water contents. A line of unit slope has been drawn. Results expressed as g.water/100$_g$ dry weight of flour.
GOL 1 with salt, ⊙, GOL 1 without salt, ⊡, HIL with salt, ⊙.



their different natures with respect to physical and baking properties.

The results suggested that above a certain moisture level, additional water was not sufficiently involved in the dough structure to prevent it freezing at about 0°C. In addition, within the range studied, the proportion of total water which was freezable at any particular moisture level was the same for each series of doughs and that each series had the same amount of unfreezable water (of mean value 33.0 ± 1.6 g/100g dry flour and equivalent to 24.85 % moisture). It would appear that the same moisture level had to be attained in each series before freezable water could be detected. Similar values have been found for wet flour by other workers, 29.0 ± 1.0 g /100g (Toledo, Steinberg and Nelson (1968)) and 28.6 g/100g (Vail and Bailey (1940)) using different methods.

The study of freezable water in flour wetted by the liquid nitrogen technique, using the Differential Thermal Analyser as a calorimeter and discussed in Section Three, supported the existence and magnitude of this threshold level of moisture. No freezable water could be detected in flour with moisture contents up to ~~0.30~~ 30.0 g/100g.

It is worth noting the coincidence at this moisture level of the first appearance of both freezable water and extractable binding of lipid due to wetting.

Salted doughs.

The absence of an endotherm at -22°C (the eutectic point of salt and water) in all thermographs suggested that added salt was not in solution in the freezable

fraction of the water. A salt eutectic endotherm was observed in salt solutions of a range of concentrations from 8% down to 0.1%. Figure 7:15 shows two typical thermographs, the first of a salt solution and the second of a dough.

The association of salt with the 'bound' water of dough has always been suspected since the addition of salt makes the dough protein less soluble and more compact. This has the effect of tightening the dough. The concentration of salt in the 'bound' water would in these circumstances be 4.97% in the case of GOL 1 and 5.9% for HIL.

Conclusions

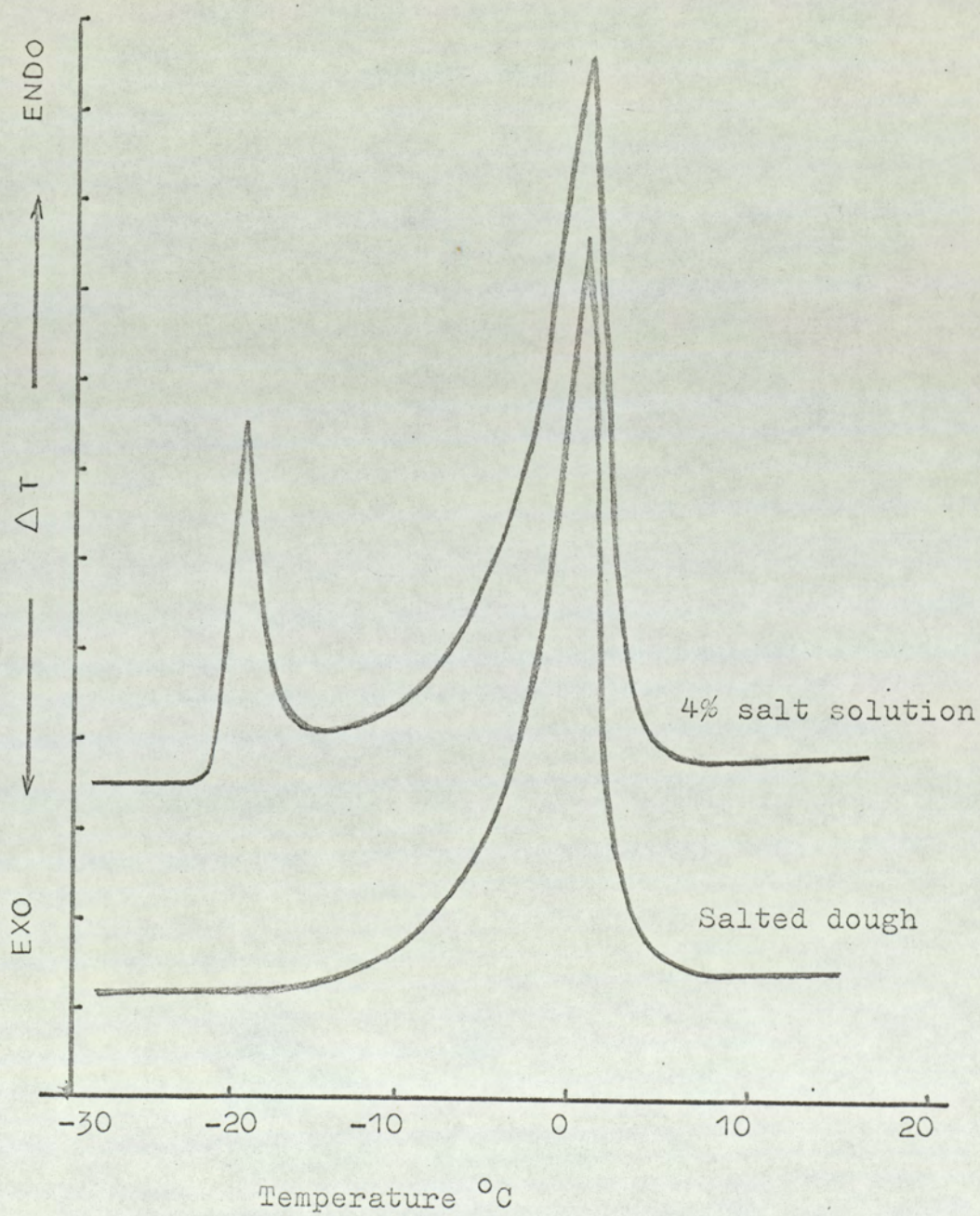
The results presented in this section are all relevant to the study of the lipid-protein relationship in flour and contribute information about dough structure and lipid binding sites.

The increase in lipid binding that resulted when only a small amount of mechanical work was introduced indicated that work exposed or activated more binding sites than hydration alone. Over-development and the presence of salt in the 'bound' water of dough was shown to reduce binding. However the presence of air in the dough mixer did not reduce binding but did bleach the flour pigment. Heat was found to increase unextractable binding as did overworking salted doughs in which a high proportion of polar lipid became unextractably bound.

There was a limit to the binding capacity of flour attainable by mechanical work even when an excess of free lipid was available. This gave an indication of the

Figure 7:15.

Thermographs of 4% salt solution and salted dough of 44% moisture content. Sample weights 5.0mg. Y axis sensitivity, 0.5°C/in. X axis sensitivity, 10°C/in. heating rate 10°C/min.



maximum number or capacity of binding sites available under these conditions.

Doughs were shown to contain the same amount of 'bound' water regardless of flour type or the presence of salt. The amount of bound water coincided with the amount necessary to cause extractable binding of lipid by hydration alone. Furthermore, when flour moisture was increased from 14 to 25%, the additional water was all 'bound' and simultaneously unextractable binding of neutral and polar lipid occurred.

The investigations reported in the next section are directly concerned with the main line of this thesis. The reconstitution of fat-extracted flours with neutral lipids and the response of such flours to work-free wetting will be examined.

SECTION EIGHT

Lipid binding in fat-extracted flours reconstituted with neutral lipids.

Introduction.

In this section a further manipulation of the wheat flour system is described whereby the effect of work-free wetting on fat-extracted flours reconstituted with neutral lipids only was studied. In Section Five, fat extraction was reported to bind polar lipid unextractably and release neutral lipid that was extractably and unextractably bound. In Section Six, reconstitution with total free lipid was reported to release even more neutral lipid and also to bind polar lipid. The wetting of flour reconstituted with total free lipid produced a reversal of the effects due to fat extraction and reconstitution. Bound polar lipid was released and free neutral lipid bound.

The work reported in this Section examined the effect of reconstitution of fat-extracted flours with only neutral lipids which left little free polar lipid available for binding induced by reconstitution. The effect of wetting on the lipid distribution of such flours was then examined in order to study the response of lipid binding sites to reconstitution and wetting when the availability of free polar lipid was strictly limited.

Reconstitution of fat extracted flour with neutral flour lipid.

The neutral lipid first chosen for reconstitution was the neutral fraction of flour free lipid removed from flour by percolation extraction. Separation of neutral from

polar fractions was achieved by adsorption of polar lipid onto silica gel from a chloroform solution. This technique was the same as that used for separation of neutral and polar fractions on a smaller scale in lipid distribution determinations. The lipid solution was shaken with two separate batches of activated silica gel to ensure complete removal of polar lipid. The proportion of neutral to polar lipid was exactly the same as obtained by separations on a small scale (4:1).

Fat-extracted flour (GOL 2) was reconstituted with 0.823% neutral lipid using the liquid nitrogen technique. This was the same level at which free neutral lipid was present in the original flour and involved adding 5.35g neutral lipid to 744g fat-extracted flour (dry weight 643g).

The reconstituted flour had a less creamy colour than the original unextracted flour, had regained its cakiness and lost its ability to produce a froth in water. Table 8:1 shows the lipid distributions of the original, fat-extracted and reconstituted flours.

Table 8:1

Effect on fat-extracted flour lipid distribution of reconstitution with neutral flour lipid. Mean values expressed as percentages of flour dry weight.

Lipid fraction	Standard Errors of Means	Flours: Original	Fat-extracted	Reconstituted	L.S.D P=0.001
FT	± 0.0156	1.021	0.048	0.834	0.066
BT	± 0.0181	0.550	0.387	0.417	0.081
FN	± 0.0372	0.833	0.037	0.794	
FP	± 0.0279	0.188	0.011	0.041	
BN	± 0.0397	0.077	0.091	0.085	
BP	± 0.0304	0.473	0.296	0.333	

The major part of the added neutral lipid remained free after reconstitution but a proportion of neutral lipid became unextractably bound (0.072%). This is shown in Table 8:2 where the lipid distribution of the reconstituted flour is compared with the anticipated lipid distribution if all the added lipid remained free after reconstitution.

Table 8:2

Comparison between anticipated and found lipid distributions of fat-extracted flour reconstituted with flour neutral lipid. Mean values expressed as percentages of flour dry weight.

Lipid fractions	Anticipated	Found	Differences. S.E. of Mean
FT	0.871	0.834	-0.037 ± 0.0156
BT	0.387	0.417	+0.030 ± 0.0181
FN	0.860	0.794	-0.066 ± 0.0372
FP	0.011	0.041	+0.030 ± 0.0279
BN	0.091	0.085	-0.006 ± 0.0397
BP	0.296	0.333	+0.037 ± 0.0304

Reconstitution also affected the polar lipid, 0.067% of the unextractably bound fraction was released, 0.037% became extractably bound and 0.030% free. This effect of reconstitution on polar lipid was the reverse of that found when fat-extracted flour was reconstituted with total free flour lipid. In the absence of added free polar lipid it was released from and not attracted to the binding sites.

Effect of work-free wetting on lipid distribution of flour reconstituted with neutral lipid.

Samples of the reconstituted flour were wetted by the liquid nitrogen technique over a range of moisture levels up to 55%. When the wetted flours thawed, the unworked doughs showed the same characteristic of shrinking at the same moisture levels as reported for the original flour in Section 2.

The lipid distributions were determined after freezedrying, grinding and sieving. The results are shown in Table 8:3.

Table 8:3

Effect of wetting on flour reconstituted with neutral lipid. Mean values expressed as percentages of flour dry weight. least significant differences. P=0.001. Free, 0.066. Bound, 0.081

Moisture contents %.	Totals Free	Bound	Unextractable
11.4	0.834	0.417	0.030
18.3	0.843	0.438	-
22.1	0.775	0.445	0.061
28.0	0.741	0.467	0.073
31.4	0.720	0.518	0.043
36.7	0.648	0.542	0.091
45.0	0.614	0.579	0.089
53.6	0.645	0.574	0.062

The unextractable fraction shown here was due to wetting only and no account has been taken of unextractable binding due to fat extraction.

The effect of wetting was to reduce free lipid and increase bound lipid. This is shown in Figure 8:1. Again there was no direct correlation between loss of free and increase of bound total lipids which suggested that lipid was unextractably bound and also released from the unextractable fraction.

Effect of wetting on neutral lipid distribution.

Table 8:4 shows the effect of work-free wetting on the distribution of neutral lipid in the reconstituted flour.

Table 8:4

Effect on neutral lipid distribution of work free wetting. Mean values expressed as percentages of total lipid (1.293%). Standard Errors of Means. Free, ±2.9. Bound, ±3.0.

Moisture content %	Neutral Free	Bound	Unextractable.
11.4	61.3	6.6	0.0
18.3	57.5	8.0	2.4
22.1	56.0	10.2	1.7
28.0	54.4	11.3	2.2
31.4	53.0	15.3	-0.4
36.7	47.7	15.0	5.3
45.0	46.2	18.1	3.6
53.6	48.8	15.0	4.0

The effect of wetting was to bind a quarter of the free neutral lipid, mainly extractably but also unextractably. Figure 8:2 shows this.

Figure 8:1.

Effect of work-free wetting on total lipid distribution of fat-extracted flour reconstituted with neutral flour lipid. Results expressed as percentages of flour dry weight.

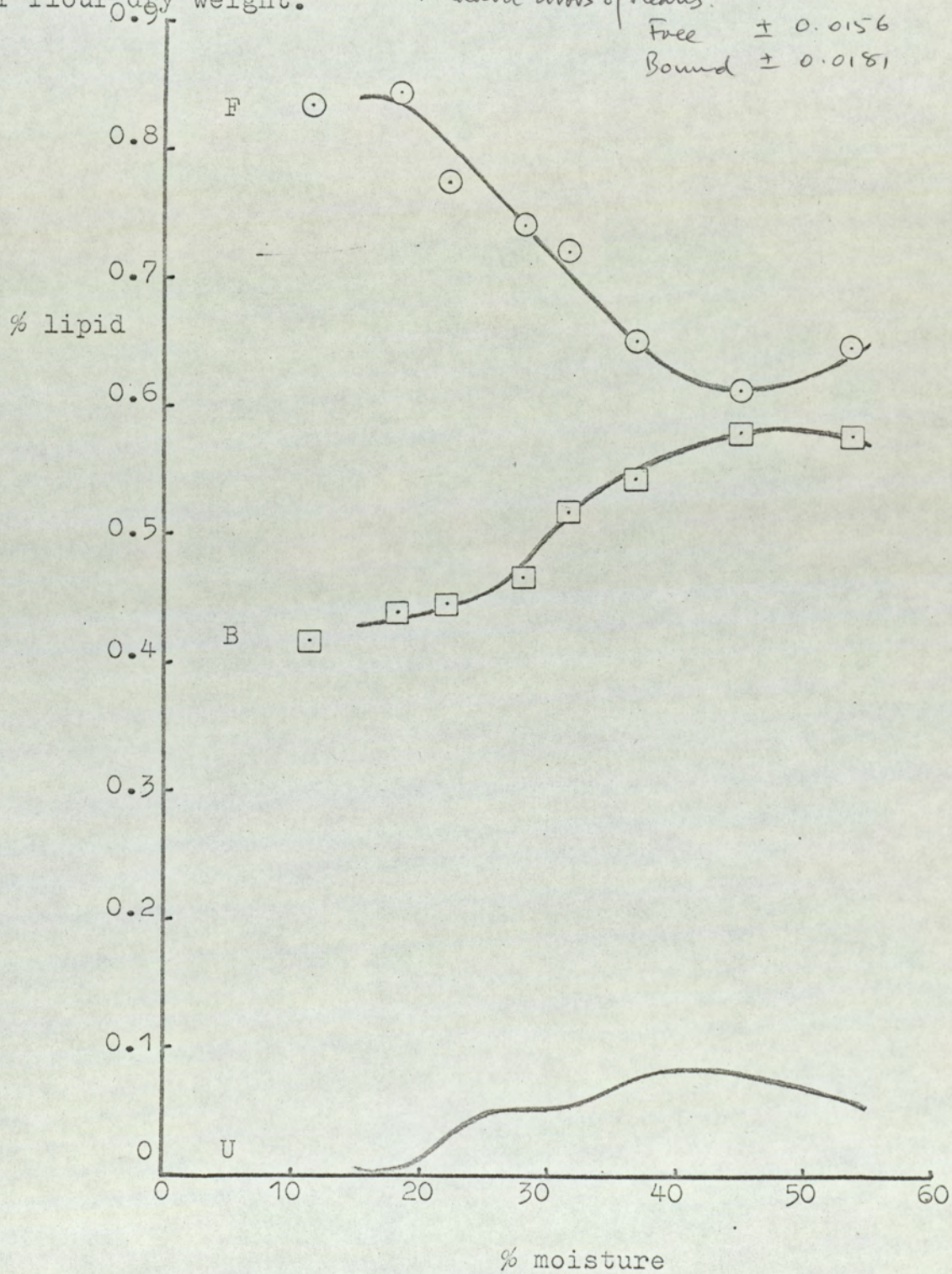


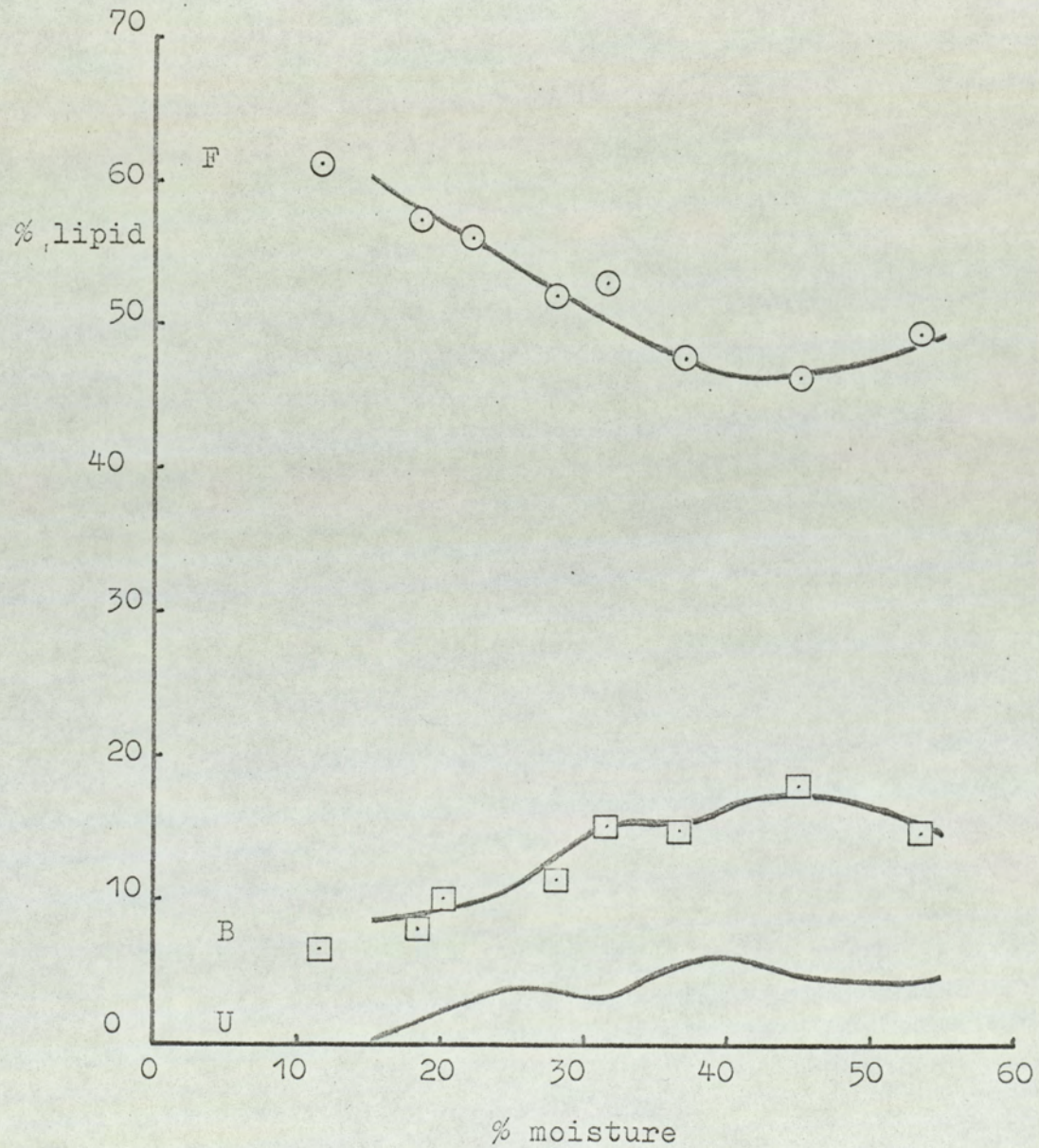
Figure 8:2.

Effect of work-free wetting on neutral lipid distribution of fat-extracted flour reconstituted with neutral flour lipid. Results expressed as percentages of total lipid present.

Standard Errors of Means

Free ± 2·9

Bound ± 3·0



Effect of wetting on polar lipid distribution.

The effect of work-free wetting on the distribution of polar lipid is shown in Table 8:5.

Table 8:5.

Effect on polar lipid distribution of work free wetting. Mean values expressed as percentages of total lipid (1.293%). Standard Errors of Means. Free ±2.08, Bound ±2.30.

Moisture content %	Polar Free	Bound	Unextractable
11.4	3.2	25.7	-
18.3	7.6	25.9	-4.6
22.1	4.0	24.3	0.6
28.0	2.9	24.7	1.3
31.4	2.6	24.8	0.7
36.7	2.5	26.9	-0.5
45.0	1.2	26.7	1.0
53.6	0.9	29.5	-1.5

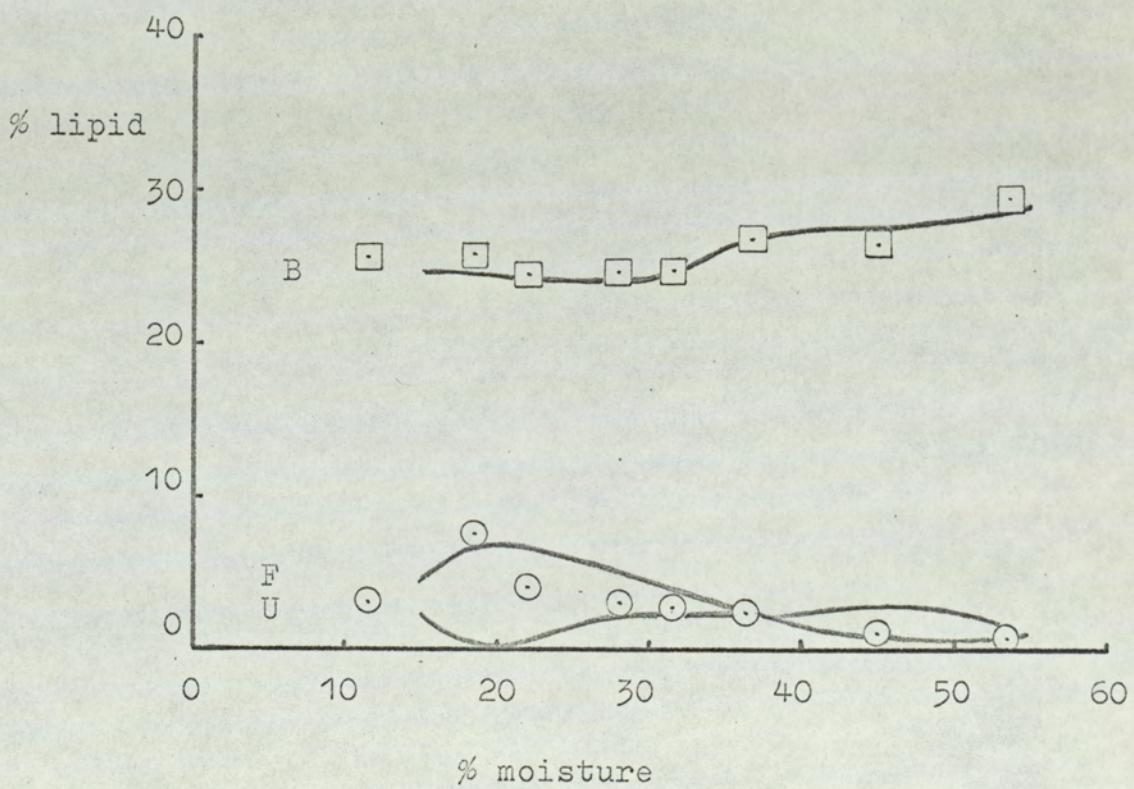
Wetting caused most of the free polar lipid that remained after fat extraction to be extractably bound. The first effect of wetting however was to release a small quantity (4.6%) of unextractably-bound polar lipid. These results are shown in Figure 8:3.

A similar proportion of free neutral lipid was bound in both the original flour (27%) and this reconstituted flour (25%) (with free neutral lipid). There was more binding of free neutral lipid however in the flour reconstituted with total free lipid (41%). Therefore it would appear that binding of free neutral lipid by

Figure 8:3

Effect of work-free wetting on polar lipid distribution of fat-extracted flour reconstituted with neutral lipid. Results expressed as percentages of total lipid present.

Standard Errors of Means.
Free ± 2·008
Bound ± 2·030



reconstitution of fat-extracted flour is encouraged by the return of free polar lipid.

Effect of reconstituting fat-extracted flour with a higher level of neutral lipid and wetting.

If an equilibrium between free and bound neutral lipid is produced by wetting, the amount of available free lipid may govern the amount of lipid that becomes bound. In order to examine this possibility, fat extracted flour was reconstituted with flour neutral lipid as before but to a much higher level. A level of 1.475% was obtained by adding 7.49g neutral lipid to 507g fat-extracted flour (dry weight) using the liquid nitrogen technique.

After 24 hours equilibration at room temperature, reconstituted flour samples were wetted to different moisture levels by the liquid nitrogen technique.

The distribution of lipids in the wetted flour samples were determined after thawing, freezedrying, grinding and sieving. Table 8:6 shows the distribution of the free and bound lipids at different moisture levels.

Table 8:6

Effect of work-free wetting on the lipid distribution of fat-extracted flour reconstituted with extra neutral lipid. Mean values expressed as percentages of flour dry weight.

Moisture content %	Free	Bound
13.3	1.535	0.379
19.3	1.540	0.419
22.2	1.519	0.388
26.3	1.455	0.460
28.0	1.470	0.473
30.8	1.400	0.513
37.8	1.358	0.519
44.8	1.230	0.690
55.0	1.148	0.709
Standard Errors of Means	± 0.0156	± 0.0181

The overall effect of work free wetting was to bind about 25% of the available free neutral lipid. Once again a small amount of unextractable material was released when the moisture level was raised. Figure 8:4 shows these results.

Effect of wetting on neutral lipid distribution.

The effect of wetting on neutral lipid distribution is shown in Table 8:7 and Figure 8:5.

When the moisture level was first increased (up to 20% moisture) unextractably bound neutral lipid was released. Thereafter free neutral lipid was bound both extractably and unextractably.

Figure 8:4.

Effect of work-free wetting on total lipid distribution of fat-extracted flour reconstituted with extra neutral lipid. Results expressed as percentages of flour dry weight.

Standard Errors of Means.
Free ± 0.0156
Bound ± 0.0181

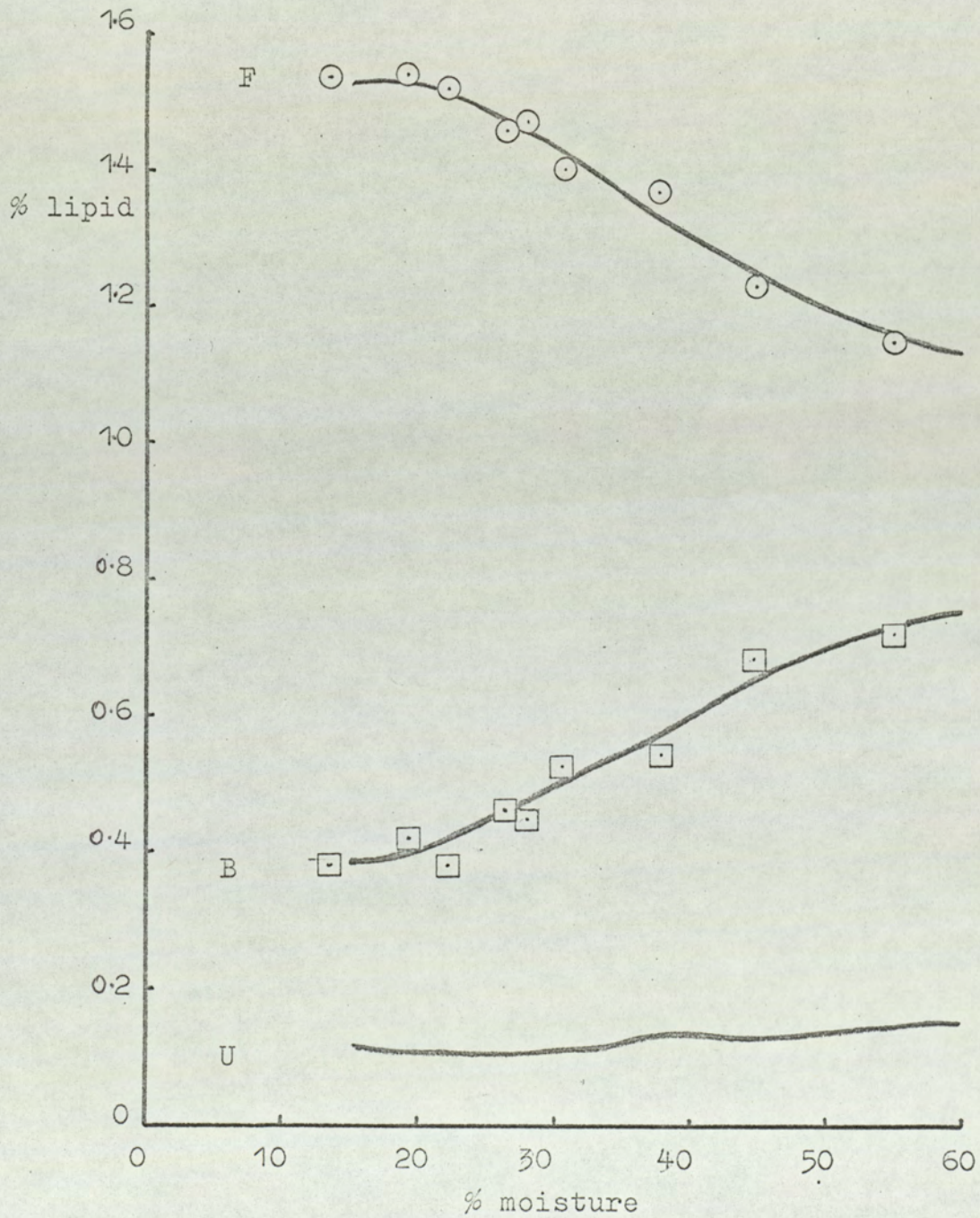


Figure 8:5.

Effect of work-free wetting on neutral lipid distribution of fat-extracted flour reconstituted with extra neutral lipid. Results expressed as percentages of flour dry weight.

Standard Errors of Means.
Free ± 0.0372
Bound ± 0.0397

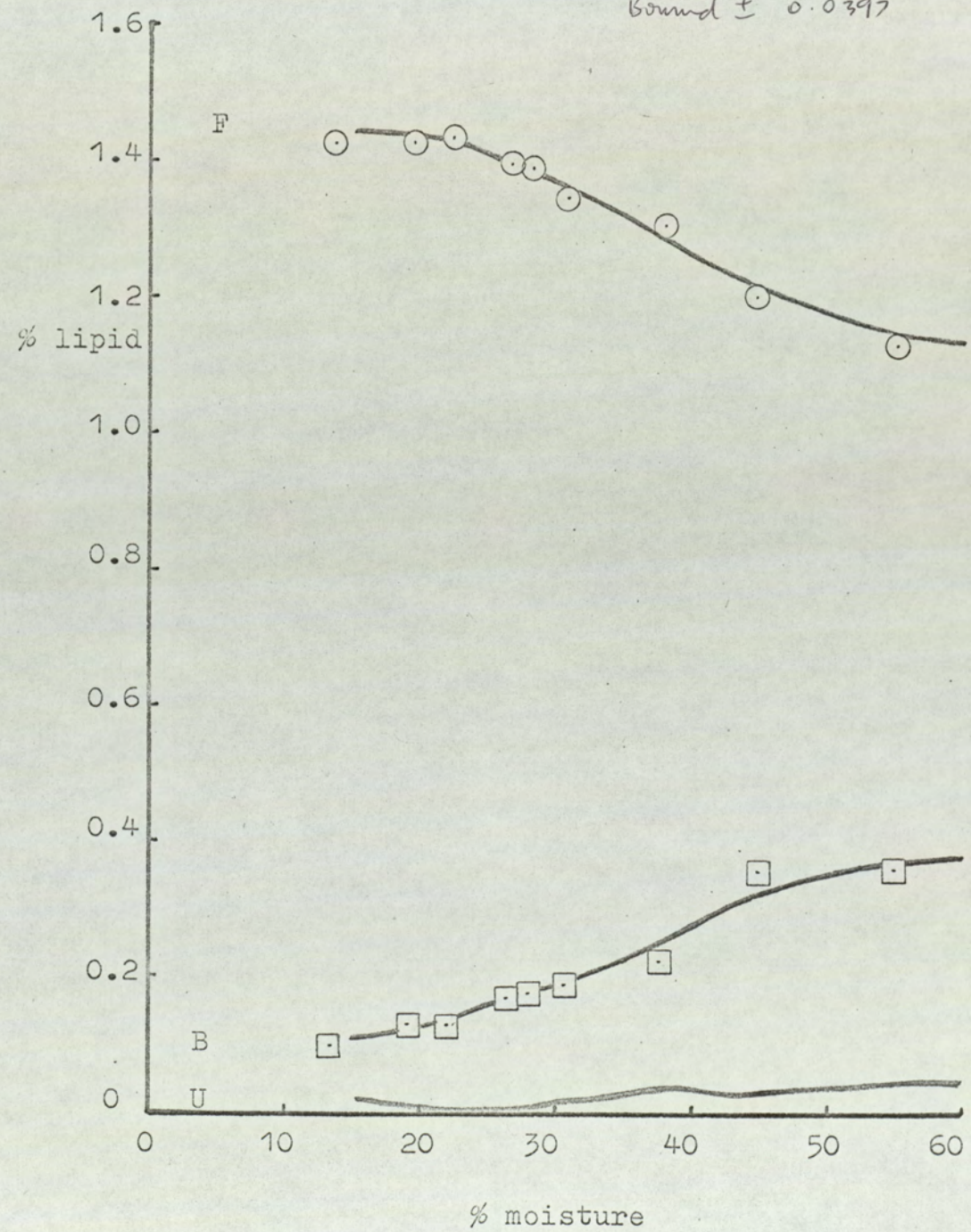


Table 8:7

Effect of wetting on neutral lipid distribution in flour reconstituted with extra neutral lipid. Mean values expressed as percentages of flour dry weight.

Moisture Content %	Neutral Free	Bound	Unextractable
13.3	1.427	0.098	-
19.3	1.428	0.122	-0.025
22.2	1.436	0.129	-0.040
26.3	1.395	0.166	-0.036
28.0	1.390	0.173	-0.038
30.8	1.342	0.187	-0.004
37.8	1.308	0.211	+0.006
44.8	1.200	0.353	-0.028
55.0	1.130	0.355	+0.040
S.E. of Means	± 0.0372	± 0.0397	

Effect of wetting on polar lipid distribution.

The effect of wetting on polar lipid distribution is shown in Table 8:8a and Figure 8:6.

Table 8:8a

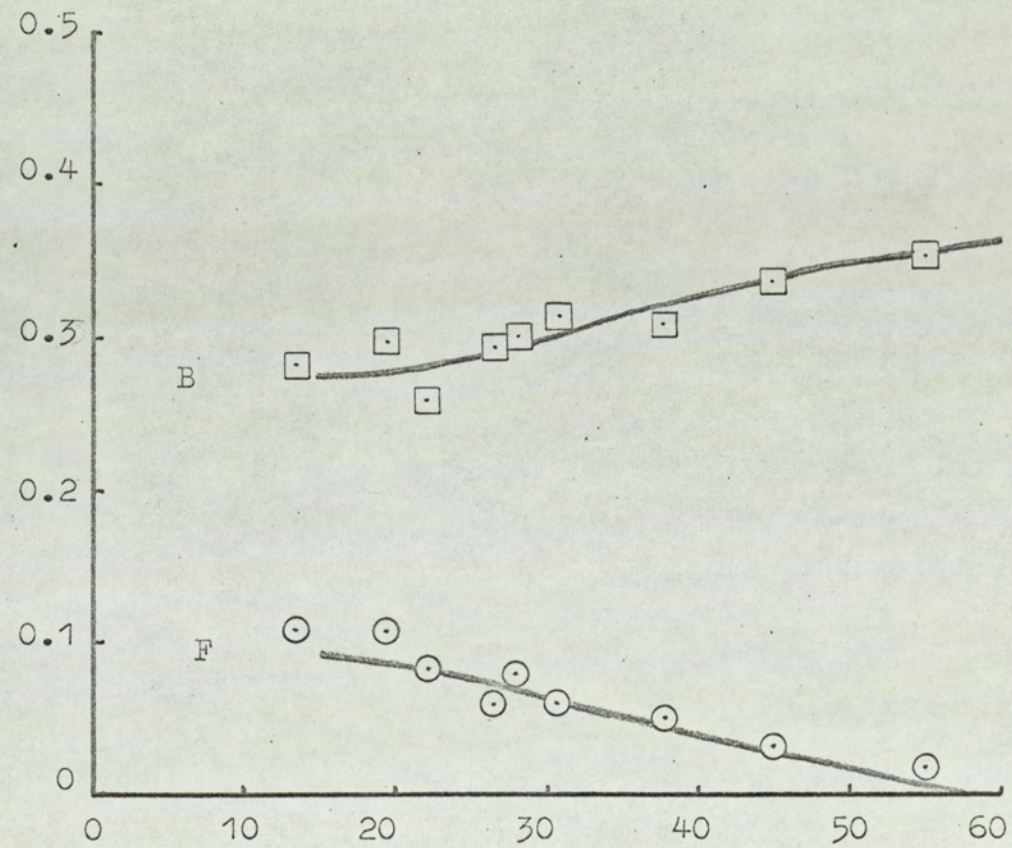
Effect of wetting on polar lipid distribution in flour reconstituted with extra neutral lipid. Mean values expressed as percentages of flour dry weight.

Moisture Content %	Polar Free	Bound	Unextractable
13.3	0.108	0.281	-
19.3	0.108	0.298	-0.017
22.2	0.083	0.259	0.047
26.3	0.060	0.294	0.035
28.0	0.080	0.300	0.009
30.8	0.058	0.315	0.016
37.8	0.050	0.309	0.030
44.8	0.030	0.337	0.022
55.0	0.018	0.354	0.017
S.E. of M.	± 0.0279	± 0.0304	

Figure 8:6

Effect of work-free wetting on polar lipid distribution of fat-extracted flour reconstituted with extra neutral lipid. Results expressed as percentages of flour dry weight.

Standard Errors of Means.
Bound ± 0.0304
Free ± 0.0279



A small proportion of unextractably bound polar lipid was also released when water was first added to the flour. The remaining free polar lipid was then bound as the moisture level was raised. There was the same final amount of bound polar as neutral lipid at 55% moisture.

The same proportion (a Quarter) of free neutral lipid was bound by wetting in both flours reconstituted with neutral lipid even though there was double the amount present in the second flour. Therefore a far higher level of neutral lipid binding occurred, due to wetting, when more neutral lipid was available. This level was higher than found in either the original flour or that reconstituted with free neutral lipid at the original level (0.355% compared to 0.146% and 0.225%) These results support the idea that an equilibrium situation between free and bound neutral lipid was produced by wetting. Firstly the amount of neutral lipid binding appeared to depend on the amount of free neutral lipid available. Although free neutral lipid remained available after wetting in the first flour, more binding occurred in the flour reconstituted with extra neutral lipid. Furthermore the ratio of free to bound neutral lipid was in each flour 3.2:1 after wetting. The presence of free polar lipid seemed less critical when such a large excess of free neutral lipid was available.

Reconstitution of fat-extracted flours with other neutral lipids.

Flour free neutral lipid was liquid at room temperature being largely unsaturated triglyceride fat. In order to establish which of this fat's characteristics are necessary for it to be bound, fat-extracted flours were reconstituted with other triglyceride fats. A solid, saturated triglyceride fat(commercial tripalmitin) and a liquid saturated triglyceride fat (coconut oil) were chosen, to demonstrate the requirement. If neither of the two fats were bound when the reconstituted flours were wetted, unsaturation would be indicated as a prerequisite for binding. If coconut oil was bound but not tripalmitin, the necessity for the fat to be liquid would be demonstrated. The fatty acid analysis of the two fats as well as flour free lipid is shown in Table 8:8b

Table 8:8b

Proportions of individual fatty acids present in tripalmitin, coconut oil and flour free neutral lipid. Expressed as percentages of total fatty acids present. Determined by gas-liquid chromatography as methyl esters.

Fatty acid	Tripalmitin	Coconut oil	Flour free neutral lipid.
10:0		8.0	
12:0		49.8	
14:0	1.5	19.1	
16:0	83.8	10.4	18.5
18:0	3.4	2.6	1.7
18:1	9.9	8.0	20.5
18:2	1.3	2.1	51.7
18:3			4.9
20:0			2.7

The tripalmitin sample contained a high proportion of palmitic acid and was solid at room temperature. Figure 8:7 shows a thermogram of tripalmitin which indicates the temperature range over which tripalmitin melted (40-60°c). Figure 8:8 shows that the high proportions of lauric and myristic acids in coconut oil are responsible for a melting point below room temperature. The shorter carbon chain length acids have lower melting points even though they are saturated.

Effect of wetting-fat extracted flour reconstituted with coconut oil.

Fat-extracted flour was reconstituted by the liquid nitrogen technique with coconut oil to a level of 1.16%. To obtain this level 9.09g. coconut oil were added to 783g. dry weight of fat-extracted flour. Reconstitution with this oil restored the cakiness to the flour and removed the tendency to produce a froth in water.

Samples of the reconstituted flour were wetted by the liquid nitrogen technique to a range of moisture levels up to 50%. The lipid distributions of these wetted flours were determined after thawing, freezedrying, grinding and sieving. The results are presented in Table 8:9 and Figure 8:9.

Table 8:9

Effect of wetting on lipid distributions of fat-extracted flour reconstituted with coconut oil. Mean values expressed as percentages of flour dry weight.

Moisture content %	Free	Bound	Unextractable.
13.0	1.230	0.425	-
24.8	1.192	0.463	0.008
35.1	1.107	0.504	0.044
48.1	0.941	0.665	0.049
L.S.D. P=0.001	0.066	0.081	

Figure 8:7

Melting thermogram of tripalmitin.
Sample weight 3.0mg, heating rate, 10°C/min. Y axis sensitivity 0.2°C/in. X axis sensitivity 20°C/in.

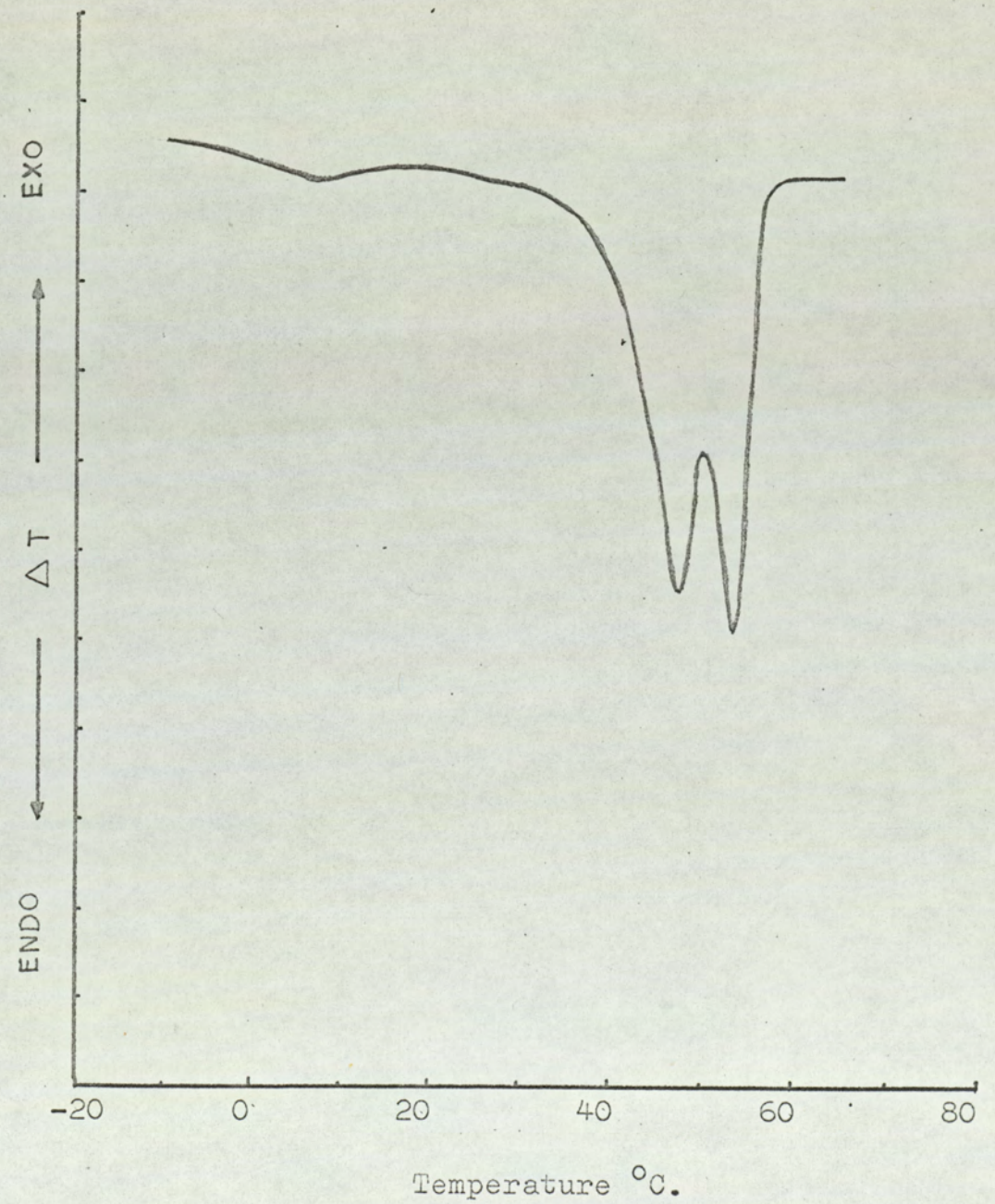


Figure 8:8.

Melting thermograms of coconut oil (4.8mg.) and flour neutral lipid (7.4mg.) Heating rates, 20°C/min Y axis sensitivity 0.2°C/in. X axis sensitivity 20°C/in.

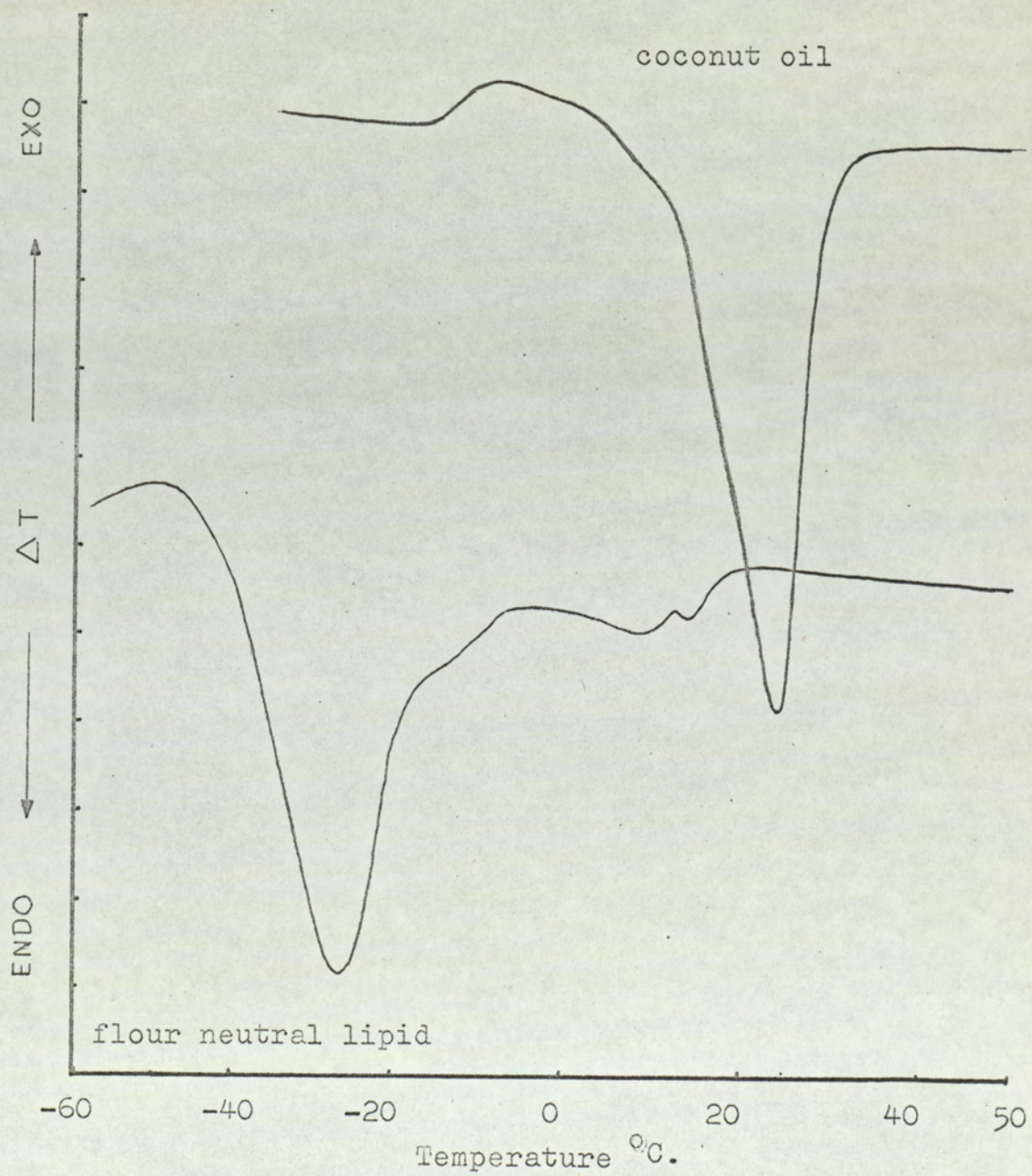
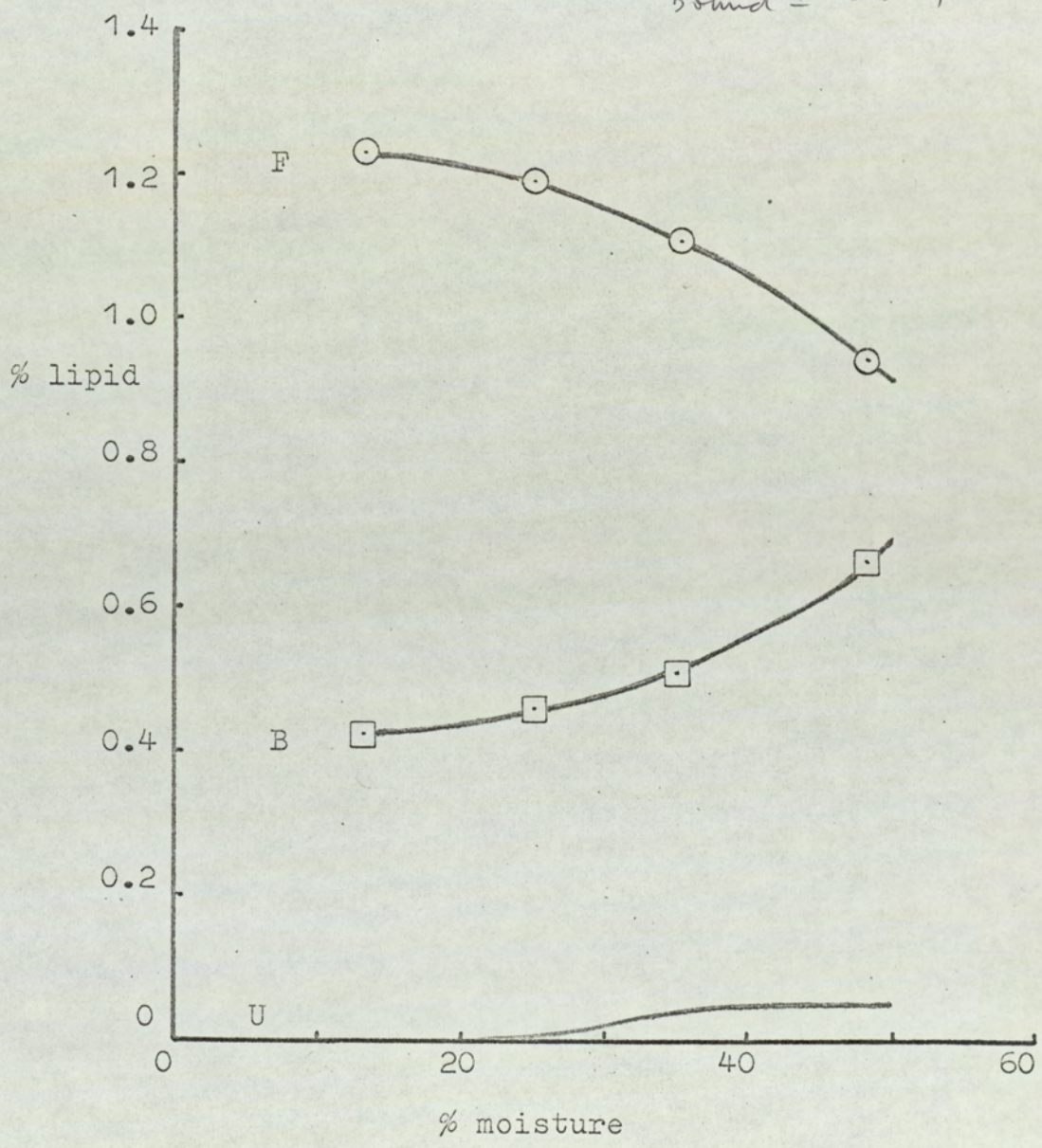


Figure 8:9

Effect of work-free wetting on the total lipid distribution of fat-extracted flour reconstituted with coconut oil. Results expressed as percentages of flour dry weight. Standard Errors of Means. Free ± 0.0156 Bound ± 0.0181



Wetting of flour reconstituted with coconut oil produced binding of this liquid, saturated triglyceride fat. At 50% moisture the bound neutral lipid was about 0.5% of the flour dry weight, an even higher level than found when flour was reconstituted with excess neutral flour lipid and wetted. Furthermore no free polar lipid was returned to this flour either. Since binding occurred one can conclude that binding was not restricted to unsaturated triglyceride fat.

The effect of wetting fat-extracted flour reconstituted with tripalmitin.

Fat-extracted flour was reconstituted with tripalmitin to a level of 1.095 % by adding 4.8g tripalmitin to 437g flour (dry weight) by the liquid nitrogen technique. Samples of the reconstituted flour were wetted by the liquid nitrogen technique to a range of moisture levels up to 50%. Lipid distributions of the wetted flours were determined after thawing, freezedrying, grinding and sieving. The results are shown in Table 8:10.

Table 8:10.

Effect of wetting on lipid distribution of flour reconstituted with tripalmitin. Mean values expressed as percentages of flour dry weight.

Moisture content %	Free	Bound
12.5	1.29	0.463
26.1	1.28	
36.0	1.24	
50.2	1.25	0.442
L.S.D. P=0.001	0.066	0.081

When this reconstituted flour was wetted there was no significant binding of tripalmitin. The slight loss of free lipid was probably the small amount of free polar lipid that remained after percolation extraction. Therefore a saturated triglyceride fat with a melting point above room temperature was not bound when flour was wetted.

Conclusions and Discussion.

The results in this Section have shown the effect of reconstituting fat-extracted flours with neutral lipids only and the effect of work-free wetting on such flours. The effect of reconstitution was to release unextractably bound polar lipid. This was the reverse of the effect of reconstitution with total free lipid.

When the reconstituted flours (with flour free neutral lipid) were wetted without mechanical work, a quarter of the free neutral lipid was bound in both flours. The increase of neutral lipid binding with increase of available neutral lipid coupled with a common final ratio of free to bound neutral lipid suggested that work-free wetting resulted in the establishment of an equilibrium situation between free and bound neutral lipid. Wetting flour reconstituted with saturated liquid triglycerides produced the greatest proportion of bound neutral lipid. Clearly the return of free polar lipid before wetting and the binding of free polar lipid by wetting was not necessary for neutral lipid to be bound.

The first effect of wetting was the release of a small proportion of unextractably bound polar lipid. The

overall effect of wetting on polar lipid was to bind all that which remained free after fat extraction. Free neutral flour lipid was bound because it was liquid at room temperature and not because it was unsaturated. Therefore the presence of unsaturation is not a prerequisite for a neutral lipid to be bound. The low melting point of flour neutral lipid is due to the predominantly unsaturated fatty acid components of the triglycerides.

These results give an insight into the factors which affect binding of neutral lipid. This is of particular interest as neutral lipid is added as an improver in breadmaking. Soft fat is adequate for fermented bread but there is a critical requirement for hard fat in modern breadmaking processes which involve mechanical dough development. These results show that while soft fat will be bound as a result of merely wetting fat-extracted flour and to an extent dependent on the amount added; hard fat will not be bound.

Elton and Fisher (1968) examined the effect of solid hydrocarbons on the quality of bread from mechanically developed doughs. They concluded from their results that for bread improvement there was a critical dependence on carbon chain length rather than melting point. Since the doughs were mechanically developed and baked and lipid distributions not determined these results cannot be directly related to the work reported here.

In order to gain further insight into the binding of

neutral lipid when flour is wetted, the next stage of the investigation examined fat-extracted flour reconstituted with a flour lipid fraction containing as low a proportion of neutral lipid as possible.

SECTION NINE

Reconstitution of fat-extracted flour with a neutral lipid deficient fraction of flour lipid.

Introduction.

In this Section reconstitution of fat-extracted flour with an acetone-insoluble fraction of flour free lipid is described. This fraction had a much lower proportion of neutral lipid than was present in total free lipid. The effect of reconstitution with this fraction was examined to see whether polar lipid was bound and neutral lipid released as occurred when fat-extracted flour was reconstituted with total free lipid. Alternatively the modified composition of the added lipid could have resulted in a different redistribution of lipid fractions. The effect of work-free wetting on the reconstituted flour was also examined, again to see whether the modified composition of the free lipid fraction also resulted in a different binding pattern.

Separation of an acetone-insoluble fraction.

The acetone-insoluble fraction of total free flour lipid was obtained by adding a concentrated petroleum solution of total free lipid to an excess of cold, freshly distilled acetone. A white bulky solid separated from a pale yellow solution. The acetone solution was removed by centrifugation followed by decantation and the solid washed with more cold acetone. Last traces of acetone were evaporated from the solid in a rotary evaporator. Nitrogen was bled into the evaporator and the sample held at 5°C under reduced pressure for two hours to completely remove acetone.

The product, a light, buff coloured solid, 14% of the

total free lipid, was stored under nitrogen at -20°C. This acetone-insoluble fraction contained 43% neutral and 57% polar lipid, a ratio of 0.75:1 compared to 4:1 (N:P) originally present in the total free fraction.

A low yield of a less stable material was obtained if reprecipitation was used to remove more neutral lipid. The presence of neutral lipid appeared to give added stability to the material since reprecipitated samples showed an even greater tendency to become intractable brown gums if warmed to above 10°C or exposed to air. For this reason the method of neutral and polar lipid separation involving silica gel (Morrison (1963)) was not used. In this separation methanol was necessary to strip polar lipid from silica gel but attempts to completely free the lipid from methanol also resulted in an intractable gum.

Effect of fat extraction.

Fat extraction by percolation removed 1.04% lipid from flour GOL 2, made up of 0.78% neutral and 0.26% polar lipid. Table 9:1 shows the lipid distribution of GOL 2 before and after fat extraction.

The effect of fat extraction on flour neutral lipid fractions was to remove 0.768% from the free neutral fraction and 0.012% from the unextractable fraction. The bound neutral fraction gained 0.013%, also from the unextractable fraction.

Fat extraction removed 0.169% from the free polar fraction and 0.091% from the bound polar fraction. A further 0.030% of the extractably bound polar fraction

became unextractably bound.

This release of unextractable neutral lipid and unextractable binding of polar lipid was exactly as found previously and reported in detail in Section 5.

Table 9:1.

Effect on lipid distribution of fat extraction by percolation of flour GOL 2. Mean values expressed as percentages of flour dry weight.

Lipid fractions	Original	Fat extracted	S.E. of Means
FT	1.021	0.084	± 0.0156
BT	0.550	0.442	± 0.0181
FN	0.833	0.065	± 0.0372
FP	0.188	0.019	± 0.0279
BN	0.077	0.090	± 0.0397
BP	0.473	0.352	± 0.0304

Reconstitution of fat-extracted flour with an acetone-insoluble fraction of total free flour lipid.

Fat-extracted flour was reconstituted with 0.38% of an acetone-insoluble fraction by the liquid nitrogen technique. If all added lipid remained free, the level of free polar lipid would have been slightly higher (0.236%) than originally present (0.188%) and free neutral lipid greatly reduced (0.833% to 0.228%) to give a neutral to polar lipid ratio of 1:1. The lipid distributions of the reconstituted flour as 'anticipated' if all added lipid remained free and as 'found' are compared in Table 9:2.

Table 9:2.

Lipid distributions of original and fat-extracted flour GOL 2, before reconstitution with an acetone-insoluble lipid fraction and after, both anticipated and found. Mean values expressed as percentages of flour dry weight.

	Total		
Flour	Free	Bound	Unextractable.
Original	1.021	0.550	
Fat-extracted	0.084	0.442	+0.005
Lipid added	0.380		
Reconstituted			
Anticipated	0.464	0.442	
Found	0.180	0.604	
Difference	-0.284 ± 0.0156 S.E of Means	+0.162 ±0.0181	+0.122

Considerable redistribution of lipid occurred when lipid containing a low proportion of neutral lipid was returned to fat extracted flour. Reconstitution resulted in a loss of 0.284% total free lipid (61%). Since the total extractable bound fraction only increased by 0.162%, 0.122% must have become unextractably bound. Although some binding was anticipated from previous results of reconstitution with total free lipid, binding on this scale without increase of moisture level was unexpected. When the effect on neutral lipid distribution was examined most of the loss of free neutral lipid was to the extractably bound fraction. Table 9:3 shows the neutral lipid distribution.

Table 9:3.

Neutral lipid distribution of original and fat-extracted flour, GOL 2, before and after reconstitution with an acetone-insoluble fraction. Mean values expressed as percentages of flour dry weight.

Flours	Neutral Free	Bound	Unextractable.
Original	0.833	0.077	
Fat-extracted	0.065	0.090	-0.025
Lipid added	0.163		
Reconstituted			
Anticipated	0.228	0.090	
Found	0.140	0.165	
Difference	-0.088	+0.075	+0.013
S.E. of Means	±0.0372	±0.039	

This result was the reverse of that reported in Section 6 when fat-extracted flour was reconstituted with total free lipid. When a lower proportion of free neutral lipid was added, as here, reconstitution resulted in binding. Previously, when a high proportion of free neutral lipid was added, more neutral lipid was released from the bound fractions. The effect of reconstitution on polar lipid distributions is shown in Table 9:4.

Table 9:4.

Polar lipid distributions of original and fat-extracted flour GOL 2 before and after reconstitution with an acetone-insoluble lipid fraction. Mean values expressed as percentages of flour dry weight.

Flour	Polar Free	Bound	Unextractable
original	0.188	0.473	
Fat-extracted	0.019	0.352	+0.030
Lipid added	0.217		
Reconstituted			
Anticipated	0.236	0.352	
Found	0.040	0.439	
Difference	-0.196	+0.087	+0.109
S.E. of Means	±0.0279	±0.0304	

A considerable proportion (83%) of free polar lipid became bound as a result of reconstitution, 0.087% extractably and 0.109% unextractably. Over a fifth (21.1%) of total polar lipid present was unextractably bound after reconstitution.

The unexpected binding of neutral lipid, in a similar amount to that of polar lipid, suggested that the response of neutral and polar lipids to reconstitution was not completely independent. Since the proportion of polar lipid was comparable to that of neutral present in the free fraction it may have exerted an influence on the reduced proportion of neutral lipid present. However, the considerable unextractable binding of polar lipid did not

extend to neutral lipid.

The remarkable effect of reconstitution of fat-extracted flour with a lipid fraction containing a low proportion of neutral lipid indicated that fat extraction appeared to expose more lipid binding sites, which were attractive to neutral and polar lipid, even at a moisture level of 11.4%.

Effect of work-free wetting on the lipid distribution of flour reconstituted with an acetone-insoluble fraction.

Such a high proportion of free lipid was bound as a result of reconstitution that wetting could be expected to have only a limited effect on lipid distribution. Samples of reconstituted flour were wetted by the liquid nitrogen technique over a range of moisture levels up to 50%. After thawing, freezedrying, grinding and sieving the lipid distributions were determined. The lipid distributions at the different moisture levels are shown in Table 9:5.

Table 9:5

Effect of wetting on the lipid distribution of flour reconstituted with an acetone-insoluble flour lipid fraction. Mean values expressed as percentages of flour dry weight.

Moisture contents%	Totals Free	Bound
11.4	0.181	0.604
18.6	0.212	0.585
24.2	0.178	0.617
29.7	0.152	0.603
34.7	0.155	0.632
39.0	0.123	0.648
44.8	0.145	0.645
49.2	0.120	0.600
S.E. of Means	± 0.0156	± 0.0181

After a small initial release of bound lipid, loss of free lipid was matched by an increase of extractably bound lipid up to a moisture level of 45%. Between 45 and 50% moisture a small proportion of bound lipid became unextractably bound. This amount was similar to the proportion of free lipid that had been bound due to wetting (0.050%). These results are shown in Figure 9:1.

The initial impression of the effect of wetting as insignificant was modified when the distributions of neutral and polar lipids were examined. These results, which are shown in Tables 9:6 and 9:7 and Figures 9:2 and 9:3, were more complicated and interesting than the distribution of total lipid fractions suggested.

Table 9:6.

Effect of wetting on neutral lipid distribution of flour reconstituted with an acetone-insoluble flour lipid fraction. Mean values expressed as percentages of flour dry weight.

Moisture content %	Neutral Free	Bound	Unextractable
11.4	0.140	0.166	0.057
18.6	0.151	0.098	0.114
24.2	0.111	0.170	0.082
29.7	0.103	0.221	0.039
34.7	0.123	0.240	0.000
39.0	0.103	0.169	0.091
44.8	0.120	0.186	0.057
49.2	0.102	0.132	0.129
S.E. of Means	± 0.0372	± 0.0397	

Figure 9:1.

Effect of work-free wetting on total lipid distribution of fat-extracted flour reconstituted with an acetone-insoluble fraction of flour free lipid. Results expressed as percentages of flour dry weight.

Standard Errors of Means.
Free ± 0.0156
Bound ± 0.0181

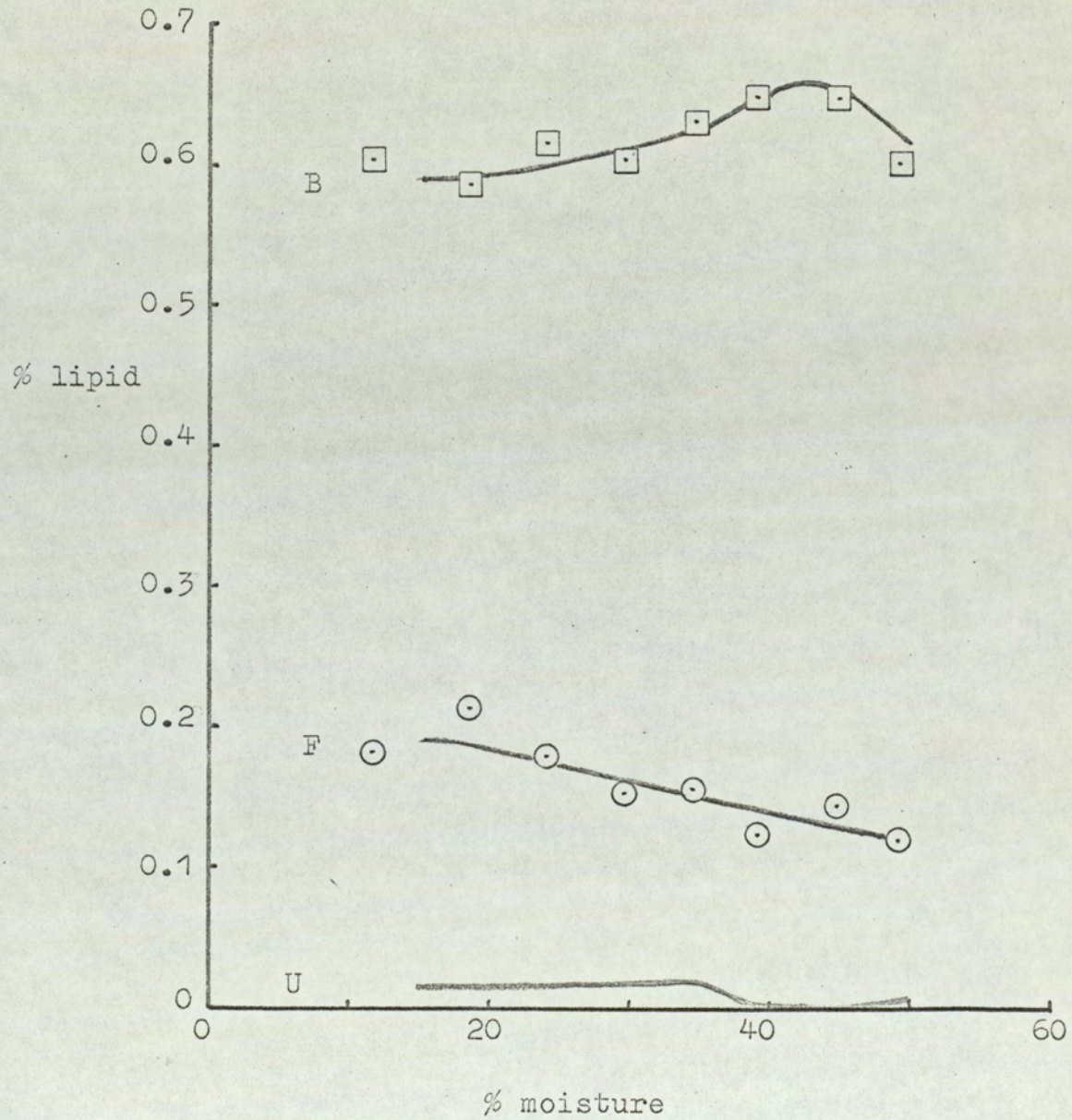


Figure 9:2

Effect of work-free wetting on neutral lipid distribution of fat-extracted flour reconstituted with an acetone-insoluble fraction of flour free lipid. Results expressed as percentages of flour dry weight.

Free, ○, Bound, □.

Standard Errors of Means ± 0.0372 ± 0.0397

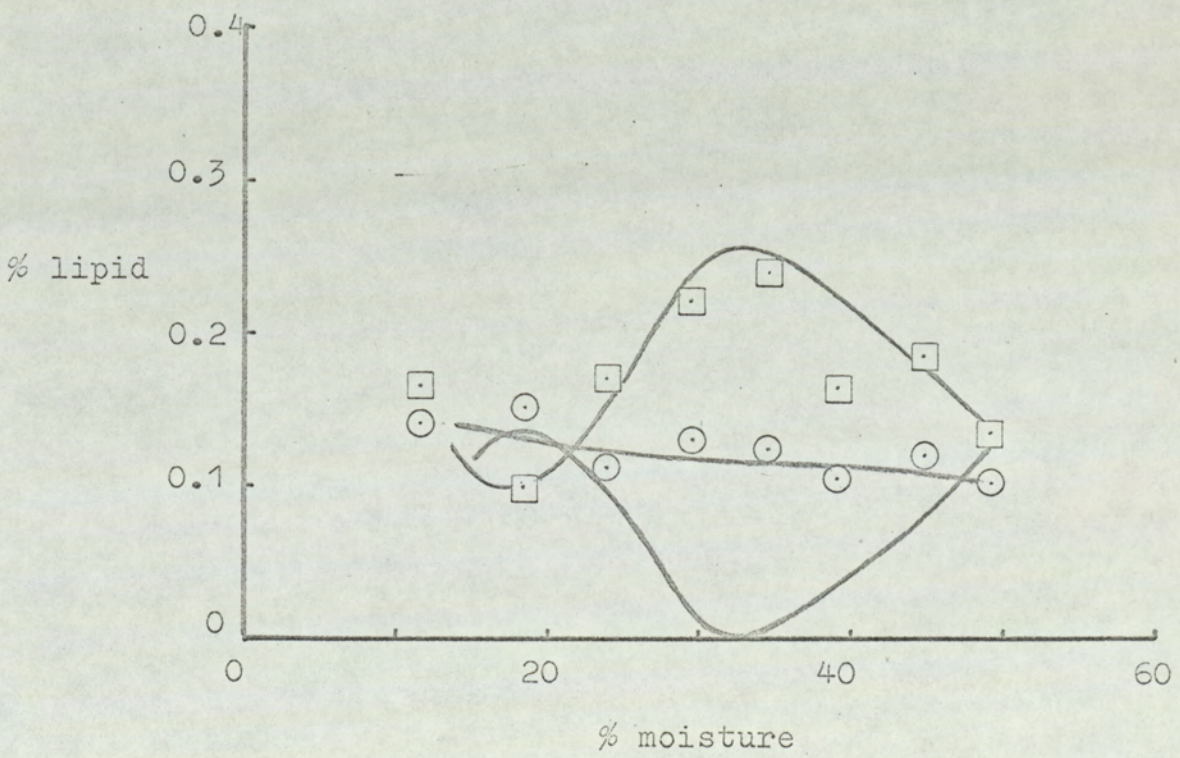


Figure 9:3

Effect of work-free wetting on polar lipid distribution of fat-extracted flour reconstituted with an acetone-insoluble fraction of flour free lipid. Results expressed as percentages of flour dry weight.

Standard Errors of Means Free ± 0.0279
Bound ± 0.0304

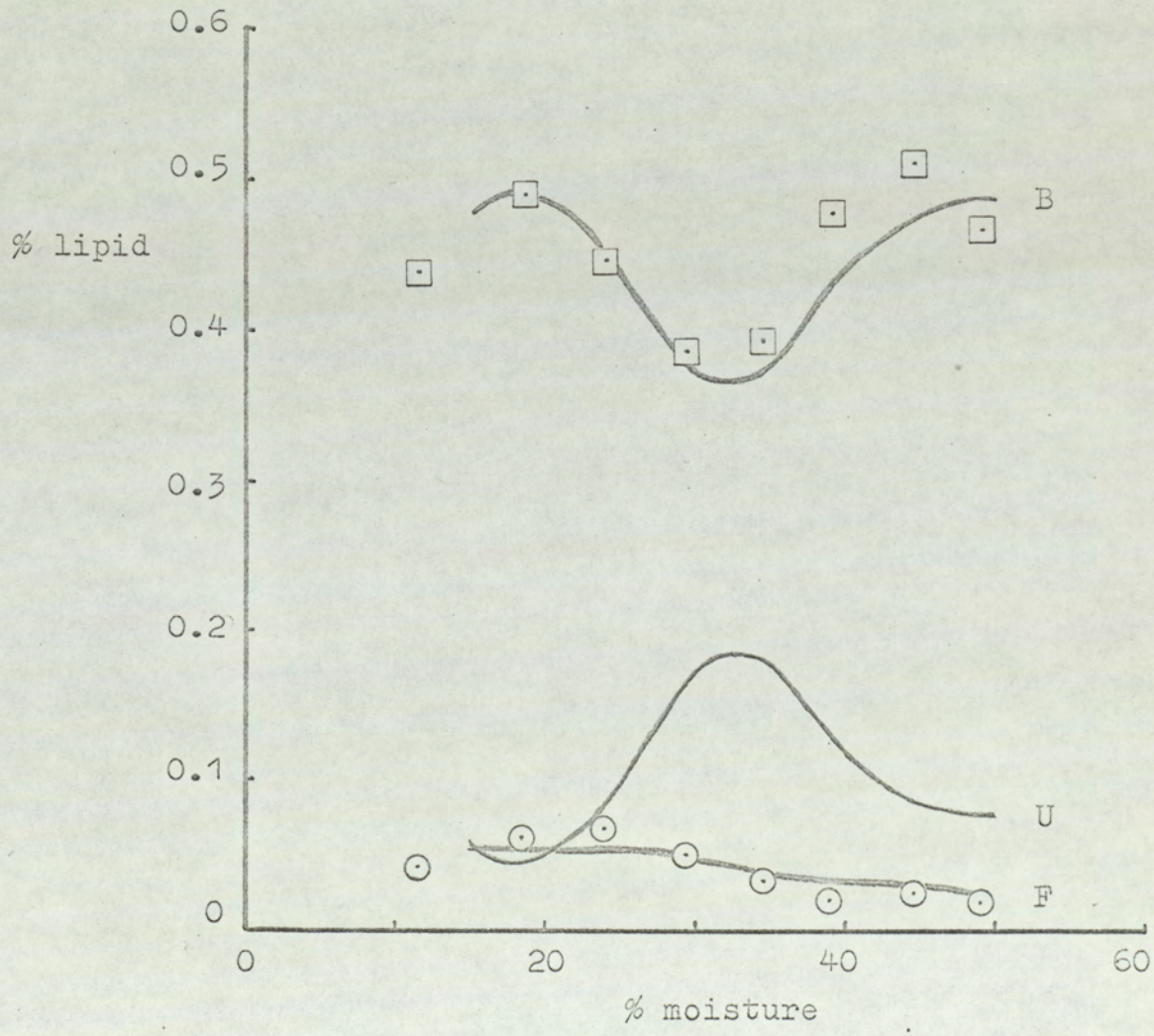


Table 9:7

Effect of wetting on polar lipid distribution of flour reconstituted with an acetone-insoluble flour lipid fraction. Mean values expressed as percentages of flour (dry weight).

Moisture content %	Polar Free	Bound	Unextractable.
11.4	0.041	0.439	0.108
18.6	0.062	0.487	0.039
24.2	0.067	0.447	0.074
29.7	0.050	0.387	0.151
34.7	0.032	0.392	0.164
39.0	0.020	0.479	0.089
44.8	0.025	0.512	0.051
49.2	0.019	0.467	0.102
S.E. of Means	± 0.0279	± 0.0304	

Both neutral and polar free lipid ~~was~~ were bound by wetting, at 50% moisture there was little polar lipid remaining free. A larger (x2½) amount of free neutral lipid was bound but the loss of free lipid was a small part of the effect of wetting.

The effect of wetting on the extractably and unextractably bound fractions, neutral and polar, could be considered together, over four moisture ranges. These ranges lay between three critical moisture levels which fell at 18%, 33% and 45%.

In the moisture range 11% to 18%, a proportion of extractably bound neutral lipid became unextractable and a similar proportion of unextractable polar lipid became extractable. This was a direct and equivalent exchange.

Between 18% and 33% moisture levels, the reverse occurred. Unextractable neutral lipid became extractable and vice-versa for polar lipid, in each case similar proportions were involved.

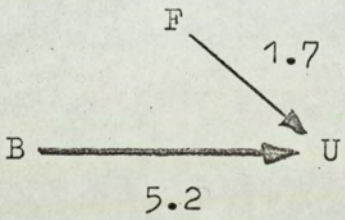
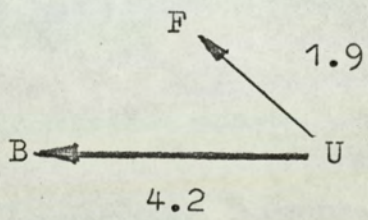
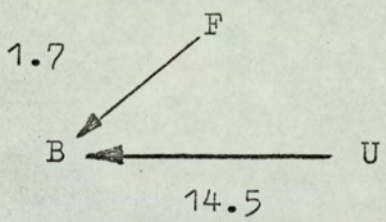
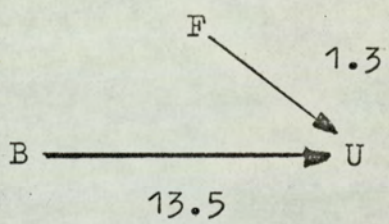
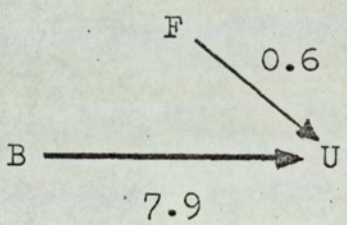
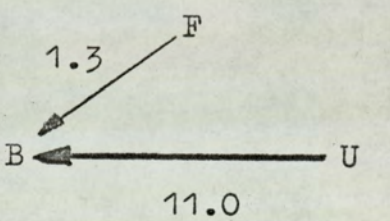
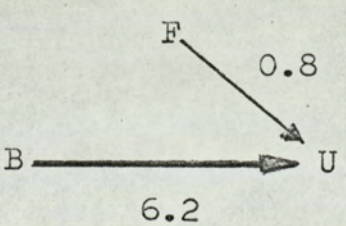
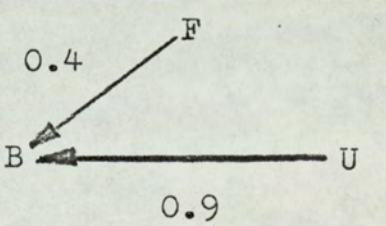
Between 33% and 45%, again there was a reversal, a proportion of extractable neutral lipid became unextractable and vice-versa for a similar proportion of polar lipid.

Between 45% and 50%. although unextractable binding of neutral lipid continued, this was not matched by release of unextractable polar lipid.

A summary of the results of wetting flour reconstituted with an acetone-insoluble fraction of flour lipid is shown in Figure 9:4.

Figure 9:4.

Changes in lipid distribution between each critical moisture level. Values expressed as percentages of total lipid present.

Moisture range %	Neutral	Polar
11-18		
18-33		
33-45		
45-50		

Conclusions.

Fat extraction and reconstitution with an acetone-insoluble fraction of flour lipid produced a flour in which the lipid distribution was weighted towards the bound fractions, both extractably and unextractably. The effect of wetting on this flour was to produce a clear pattern of behaviour involving rearrangement within the bound fractions

of both neutral and polar lipid. The reduction of available free lipid meant that these effects were not masked by a greater effect of binding of free lipid. Exactly the same patterns were found in studies of flours GOL 1, GOL 2 and HIL reported in Sections 2 and 4 although these effects were overshadowed by more extensive binding of free lipid.

The critical points in the moisture range were probably turning points in the hydration of flour. In terms of the lipid binding sites of flour they represented points at which the environment to bound lipid changed. Below a critical moisture level, for example 18%, the 'character' of the binding site favoured extractable binding of polar lipid and unextractable binding of neutral lipid. Above 18% moisture, the complete reverse was true, up to the next critical moisture level, 33%. At this next point a further reversal occurred.

The 'character' of binding sites would be determined by the degree of flour hydration. This would affect the protein conformation and the proportions and positions of hydrophilic and hydrophobic areas within the protein structure.

These results underlined the difference in behaviour between neutral and polar lipids yet indicated that the two classes were not independent.

From the results presented in Section 3, these critical moisture levels have already been shown to be significant points in flour hydration with respect to physical properties of the wetted flour.

Between 11% and 18% moisture, where unextractable neutral and extractably bound polar lipids were favoured, the relaxation properties of damp flour showed greatest change.

Between 18% and 33% moisture, where the greatest interchange of neutral and polar lipid was between bound and unextractable fractions, freezable water appeared at 25% moisture and flour lost its free flowing properties and first showed the shrinkage effect. Sufficient water to hydrate flour protein was present in this moisture range.

Above 33%. where unextractable binding of neutral lipid with corresponding release of unextractably bound polar lipid to the extractable fraction commenced, wetted flours were showing greatest shrinkage effect and relaxation properties improved again.

The demonstration of critical moisture levels, at which reversals in lipid distribution occurred, that this flour permitted was due to reconstitution with a fraction having a low neutral lipid content. This minimized the free lipid fraction by spontaneous binding of lipid when returned to fat extracted flour and prevented this fraction from overwhelming the reversal effects. This manipulation of the flour system has given further insight into the properties and functions of flour lipid binding sites and in conjunction with other results from this study will permit the construction of a more complete model for unworked dough structure.

DISCUSSION.

Discussion

"Water, water everywhere"

Coleridge.

While the importance of water in biological systems is ~~without question~~ not questioned, our knowledge of the role of water both in nature and in technology is still surprisingly incomplete. Recognised as the universal solvent, water nevertheless functions in nature in a more fundamental manner than as a mere medium in which biological processes may take place. It is this area of intimate interaction between water and the lipids, proteins and carbohydrates of the wheat complex that has formed the basis for research work reported in this thesis. It is therefore profitable to consider the consequences of altering the water balance within this complex system both from its biological and technological aspects.

As the wheat grain ripens in the ear, the dehydration of proteins occurs in an ordered, structured environment. If in the natural course of events the seed is dispersed into the soil and germinates, rehydration occurs. Many enzymes are then activated and controlled, ordered mechanisms directed towards the formation of a new plant are set in motion. However, at harvest, further changes in the grain are halted until, at milling, the ordered endosperm cell structure is grossly disrupted. When the biological potential of the flour system is released by rehydration, the consequences of this new chaotic situation will be both disordered and uncontrolled.

In these circumstances one can anticipate that a considerable variety of interactions will occur. For instance, Olcott and Mecham (1947) found that a proportion of flour free lipid became bound, solely as a result of raising the moisture content from 14 to 30%. It was this result that provided the stimulus for a detailed study of this otherwise neglected area.

A method of increasing flour moisture level, by small increments and without the introduction of mechanical work, was developed to examine in detail the consequences of flour hydration. As anticipated the resultant lipid redistribution that was found confirmed the complexity of the consequences of releasing this biological potential of flour. Some insight was also given into the processes leading to gluten formation and the part played by lipids in this phenomenon, the structure and properties of which account for the special breadmaking properties of wheat flour. Furthermore, this study has shown that work-free hydration provides an excellent means of examining a relatively simple lipid binding system.

A detailed picture of this fundamental process was given at any stage of hydration by wetting flour to any required level using the liquid nitrogen technique which avoided the introduction of mechanical work. Likewise, the distribution of water in unworked doughs as well as their physical properties could be determined over a wide range of moisture levels. Thus correlation could be made between changes of lipid distribution and changes in unworked dough structure at critical stages of hydration.

Every constituent of the endosperm fraction of wheat grain debris produced by milling wheat into flour is involved in hydration. As well as free starch granules there are free protein particles which are interstitial matter from the protein matrix(referred to as 'wedge' protein by Hess (1960)). Many clusters are also present, made up of fragments of protein matrix in which small starch granules are embedded (referred to as 'adhering' protein by Hess (1960)). The proportion of protein to starch in these 'clusters' decreases as the size of the cluster increases. During milling, free lipid is expressed from the germ and absorbed by the endosperm material (Stevens (1959)). This free lipid is distributed throughout the protein matrix and not, as Hess suggested (1954) concentrated in adherent protein. Most bound lipid is associated with protein as small inclusions throughout the protein matrix. These inclusions, 80% of which are polar lipids, are assumed to be remnants of cytoplasmic structures ocurring in endosperm cells at maturity (Seckinger and Wolf (1967)). A proportion of lipid with a high content of lysolecithin is bound to starch (Acker and Schmitz (1967), Wren and Merryfield (1970)). Moisture content is about 14% and entirely 'bound' i.e. intimately involved in the structure of flour constituents.

Having established a means of producing additional lipid binding in flour in a relatively simple manner the first study was extended. Initially the binding substrate was manipulated to see whether response of binding to work-

free wetting was related to either flour composition or breadmaking quality of flour involved. This was achieved by examining three flours, two with similar and one with completely different composition and breadmaking potential. The similar patterns of response to work-free wetting of lipid redistribution as well as identical water distribution suggested that lipid binding due to work-free hydration is a general flour phenomenon, largely unrelated to breadmaking potential. Pomeranz et al. (1966) and Daftary et al. (1968) also found that breadmaking potential of the source of a particular flour lipid fraction had no bearing on the effect of this lipid fraction on breadmaking quality.

Further manipulation of the unwetted flour system, for which a method was devised to replace the free lipid fraction with selected lipid fractions, demonstrated both unextractable binding of polar lipid and release of extractably and unextractably bound neutral lipid at low moisture levels solely due to petrol extraction and reconstitution. (See Figure D:3). Application of the work-free wetting technique to reconstituted flours and related studies of simple worked and salted doughs produced further information about the nature of lipid binding and its relationship with dough structure. The results of all these studies may be discussed profitably in terms of the hypothetical binding site proposed in Section 3. (See Figure D:1). While as yet it is impossible to give a full description of a real site of binding it can be described broadly in terms of the properties revealed by these studies.

Figure D:1.

A HYPOTHETICAL BINDING SITE.

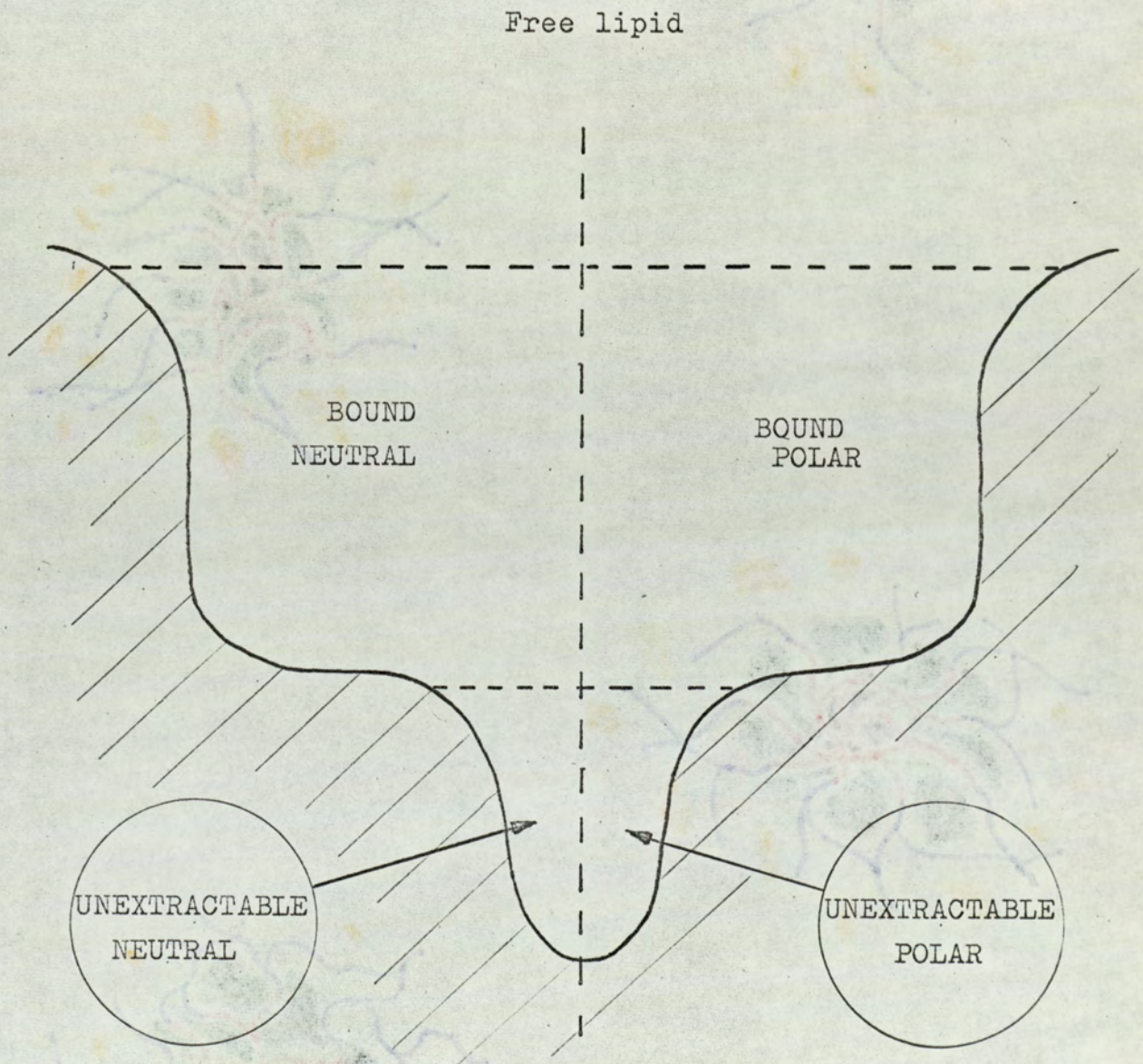
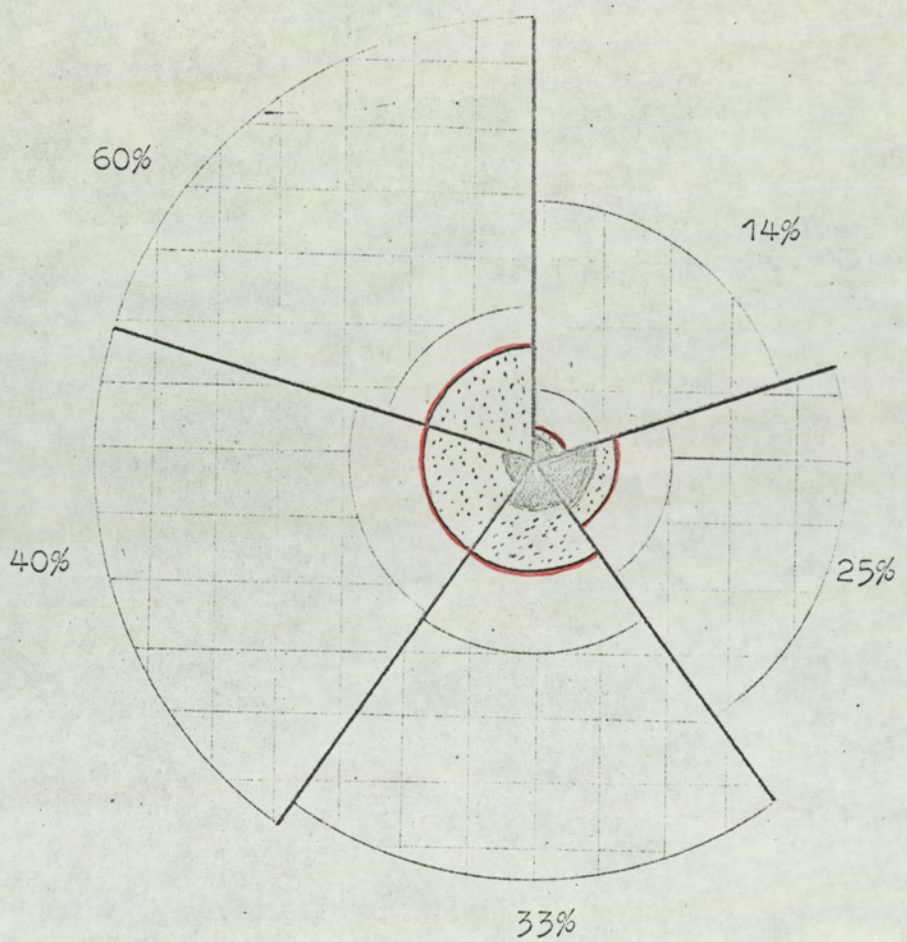


Figure D:2

Effect of moisture level on a diagrammatic binding site. Each sector represents the bound lipid at the indicated moisture level.



Boundary between bound and unextractable fractions

Bound polar

Bound neutral

Unextractable polar

Unextractable neutral

Figure D:3a.

Effect of fat extraction by percolation with petrol.

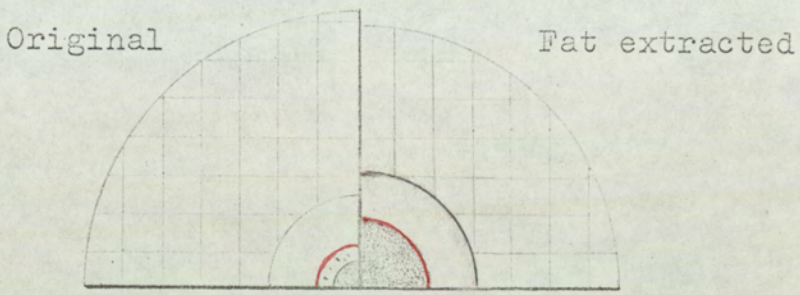
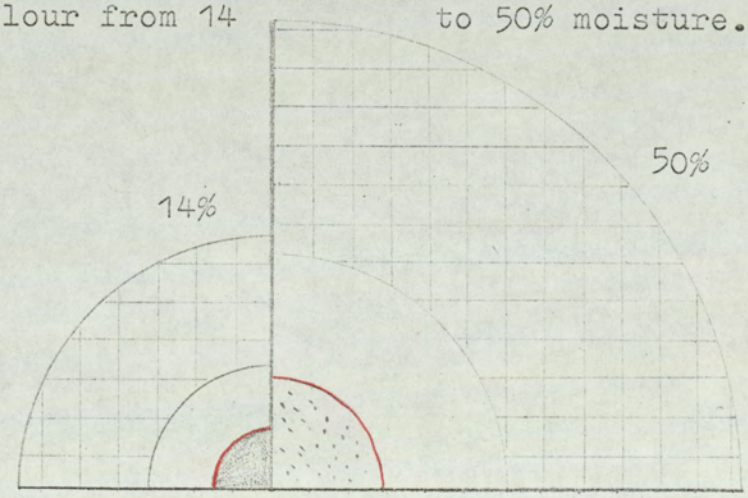


Figure D:3b.

Effect of work-free wetting on bound lipid of reconstituted flour from 14 to 50% moisture.



When flour leaves the mill, bound lipid is already present at the binding sites. (See Figure D:2). Both neutral and polar lipids are present in a 1:4 ratio at 14% moisture. Subsequent treatment of flour with solvent reveals the presence of a small proportion of unextractably bound neutral and polar lipid at this same moisture level. The difference between extractably and unextractably bound lipid is entirely arbitrary, being a measure of the proportion of lipid to which the Tsen solvent can gain access. This however is directly related to the structure of the material being extracted and therefore particularly relevant to this study.

Different response of neutral and polar lipid to work-free wetting and other manipulations of the flour system, particularly in the proportion of bound lipid which is unextractable might suggest that there are two different types of binding sites present, one for each of neutral and polar lipid. Since there is no quantitative correlation between the proportions of neutral and polar lipids migrating, either into the same fraction or interchanging between the same fraction, there is evidence of some independance of the two classes. Hoseney et al. (1970) found a separate association between neutral lipid and glutenin and between polar lipid and gliadin fractions of gluten. On the other hand, extractable and unextractable binding of free neutral and polar lipid occurs over the same moisture range i.e. at the same stage of hydration. When flour was reconstituted with neutral and polar lipid returned in

similar proportions(an acetone-insoluble fraction of flour lipid), both polar and neutral lipid became bound without any increase of flour moisture content. (See Figure D:5). Reconstitution with neutral lipid only or neutral lipid with only a low proportion of polar lipid e.g. return of free flour lipid to fat extracted flour, produced no such binding of neutral lipid without work free wetting. (See Figure D:4). Since more binding of free neutral lipid occurred on work-free wetting of fat extracted flour reconstituted with total free lipid than with neutral lipid only, the presence of free polar lipid appeared to encourage binding of free neutral lipid. This result contrasts with that of Daniels et al. (1969), Working with mechanically developed doughs they found less binding when free polar lipid was present in reconstituted flour. This points to a difference between binding produced by work-free wetting and by mechanical development. Hoseney et al. (1969), ~~has~~ have shown in studies with completely lipid-free flour that for free polar lipid to exert improving effects on flour breadmaking properties, either free neutral or bound neutral and polar lipid must also be present.

When examining lipid binding in mechanically developed doughs, Pomeranz et al. (1968) found that less binding of either extracted neutral or polar lipid occurred when both were returned together than when either were returned separately. In this case, each lipid class appeared to inhibit the binding of the other when mixed into a dough.

One can conclude that both neutral and polar lipid are present at the same site of binding, although in certain

Figure D:4a.

Effect of work-free wetting on flour reconstituted with flour neutral lipid at the original level.

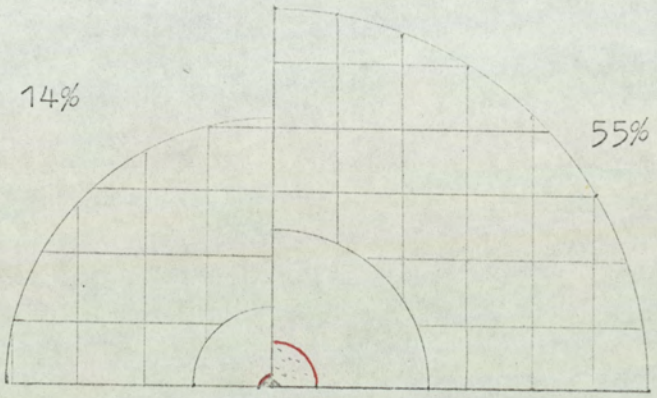


Figure D:4b.

Effect of work-free wetting on flour reconstituted with excess flour neutral lipid.

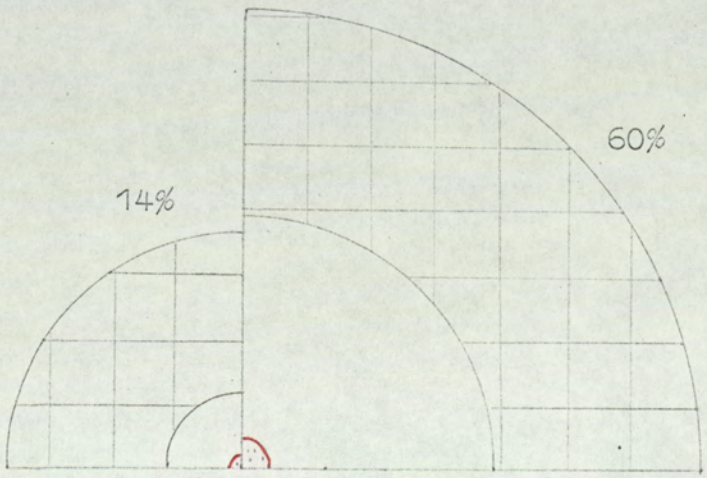
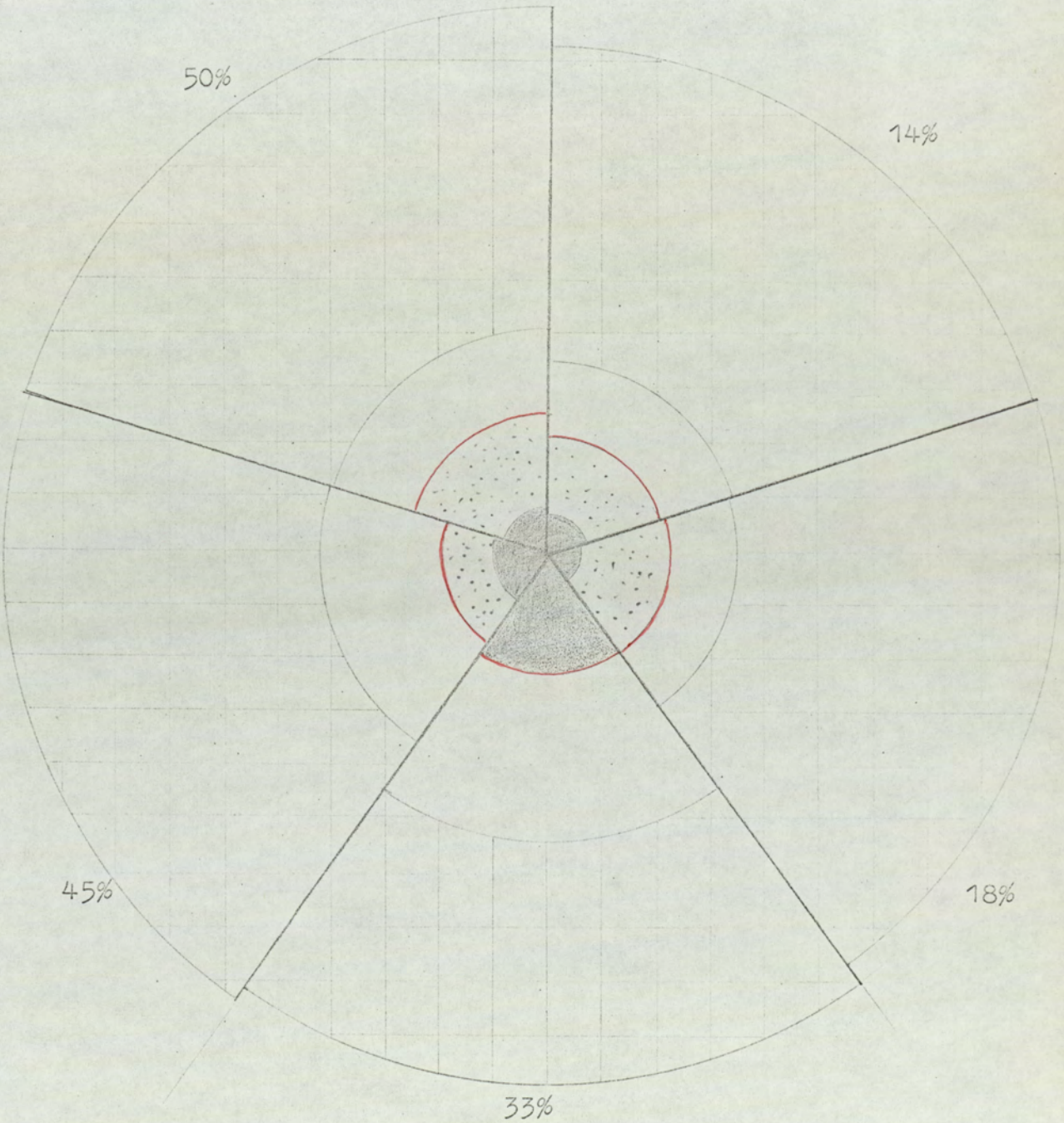


Figure D:5

Effect of work-free wetting of fat extracted flour reconstituted with an acetone-insoluble fraction. Moisture levels as indicated.



circumstances, when the conditions discriminate between the two classes (which are different both structurally and in terms of chemical activity), different responses are shown. An example of this is given when flour is extracted with petrol. Most of the free neutral and polar lipid is removed as well as a proportion of extractably and unextractably bound neutral lipid. At the same time, this treatment produces additional unextractable binding of polar lipid. (See Figure D:3). This same effect is seen when the extracted lipid is returned to the flour. (See Figure D:4). More unextractably bound neutral lipid became extractably bound or free and more free and bound polar lipid became unextractably bound.

These effects due to fat extraction and reconstitution have not been reported before because other workers have reconstituted flours by adding lipid during dough mixing and have not examined the lipid distribution of either the fat-extracted or unwetted reconstituted flour. Percolation of neutral solvent through flour must produce extraction gradients within sites such that the balance of neutral and polar lipid is left gravely disturbed. When different free lipid types are presented to extracted flour, the response varies with the lipid. For example, when free flour lipid was returned without the free polar fraction, the resulting lipid redistribution was the exact reverse of what happened when the polar fraction was present. (See Figure D:3 and D:4). Unextractably bound polar lipid was released and free neutral lipid unextractably bound. Extractable binding of neutral lipid also occurred at

reconstitution with an acetone-insoluble flour lipid fraction, in which neutral and polar lipid is present in similar proportions. (See Figure D:5).

The essential step in breadmaking is the proper formation and development of gluten from flour protein, either by mechanical dough mixing or by yeast fermentation. A dough thus made has the property of rising in proof as bubbles of carbon dioxide are produced by yeast fermentation. The dough must also be able to retain this gas until the open cell bread structure has been 'fixed' by starch gelatinisation, in the oven. Hydration of flour is a preliminary and inevitable stage in this process. By examining the results of work-free hydration the effects due to hydration alone are distinguished from and where possible can be related to those due to mechanical work. The results presented here clearly indicate that lipid is involved in both these vital stages of structure formation and substantiate the results of Baldwin et al.(1963,)Chiu et al. (1966) and Daniels et al. (1966) obtained from mechanically mixed doughs.

Work-free hydration itself proceeds in a number of distinct steps. (See Figure D:2). Each step is accompanied by different effects on lipid distribution within unworked doughs and changes in unworked dough physical properties resulting from structure formation and development. The original flour showed that during the course of hydration binding of neutral and polar lipid, extractably or unextractably, changes from one stage to the next. This

same effect, preferential unextractable binding of first one then the other lipid type was most clearly shown in flour reconstituted with a lipid fraction with a low proportion of neutral lipid. (See Figure D:5). Since there was so little free neutral lipid in this flour available for extractable binding, the subtle changes in proportions of extractably and unextractably bound lipid were not masked.

During the first stages of flour hydration, all added water is 'bound' i.e. immediately absorbed into the structures of flour constituents. Probably no relationships between separate flour particles are produced yet but unextractable binding of both neutral and polar lipid occurs. (See Figure D:2). This additional binding of lipid is probably located at the sites of binding already present.

With the appearance of free water, above 25% total moisture content, the first major signs of structure formation e.g. shrinkage, are noticeable, indicating interactions between separate flour particles. Since during this stage free neutral lipid and unextractably bound polar lipid becomes extractably bound, further involvement of lipid in development of dough structure is indicated.

In the final stage, a limit is reached to the degree of structure formation due solely to hydration and unextractable binding of neutral lipid is the distinctive feature of lipid redistribution.

Binding sites are evidently sensitive indicators of the

hydration process. The distribution of lipid within a binding site is an indication of the conformation of the local protein which in turn is related to the spatial arrangement of hydrophilic and hydrophobic parts of the protein structure (Chapman (1969)).

Binding of polar lipid can be envisaged at the interface between hydrophilic and hydrophobic areas with neutral lipid confined to the latter (c.f. the associations of neutral and polar lipids with different gluten fractions of developed dough proposed by Hoseney et al. (1970)). As gluten structure is formed and developed from flour protein, firstly by hydration and secondly by mechanical development, the capacity or number of binding sites is altered and so is their 'character'. This change of 'character' is demonstrated by the different lipid binding responses to work-free wetting between critical moisture levels.

Obviously no single type of polar lipid interaction is occurring in such a complex system as wetted flour with the wide rage of lipid types classified as polar in this study. While some electrostatic interactions are possible (Grosskreutz (1961), Lee and Wan (1963), Pence et al. (1964) and Zentner (1964)), a combination of interactions of different types, e.g. hydrogen bonding and London- van der Waal's dispersion forces must account for much binding of polar lipid (Chapman (1969)).

Hydrophobic bonding of neutral lipid, stabilized by the considerable entropy gain of non-polar material leaving an aqueous environment appeared to be confirmed by the appearance of considerable unextractable binding of neutral

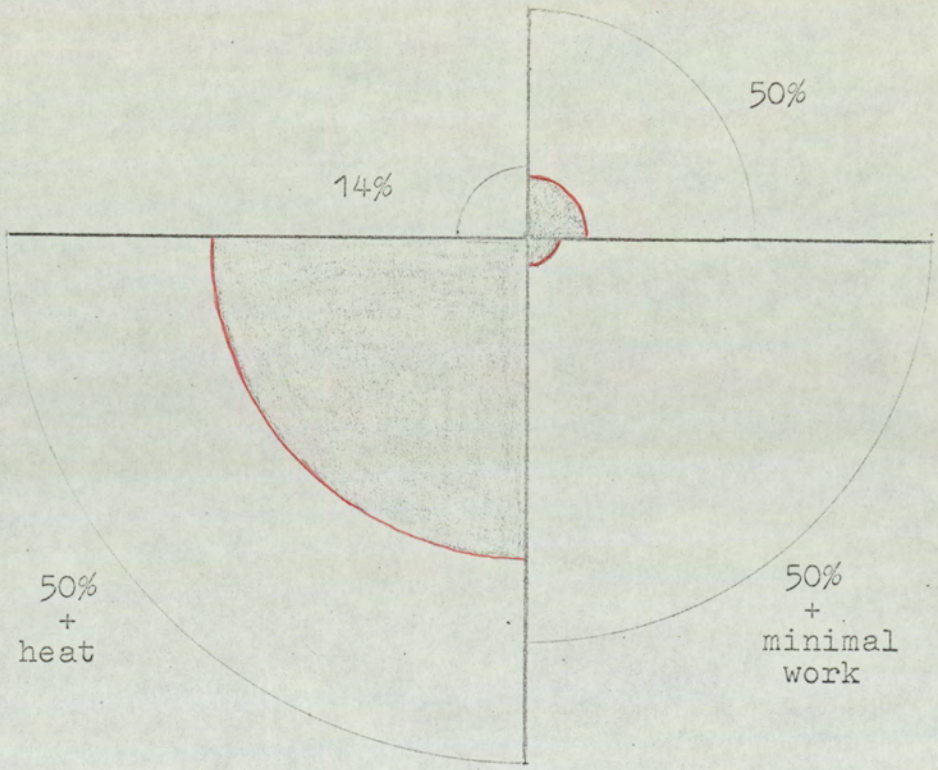
lipid on heating. (See Figure D:6). An increase in neutral lipid binding when bread dough is baked (Baldwin et al. (1963) Chiu et al. (1966) and Daniels et al. (1966)) also supports the likelihood of hydrophobic binding of neutral lipid since the stability of only this particular interaction increases with temperature (Werhli and Pomeranz (1969)).

The well known improving effect of added triglyceride fat to dough for production of bread by panary fermentation and the importance of a high melting component in the essential added fat ingredient of mechanically developed dough indicates a special role for neutral lipid in breadmaking. This role must however be qualified since a proportion of neutral lipid must be 'free' when the dough enters the oven. This has been shown by the poor loaves produced by returning only neutral lipid to mechanically developed doughs made from fat-extracted flour. (Daniels et al. (1969)). Additional neutral lipid binding was found in the doughs from which these poor loaves were produced. The deleterious effect of high levels of neutral lipid binding on loaf quality was also shown by production of both effects by mixing under a reduced pressure (below 15in./Hg.) (Daniels et al. (1969a) Chamberlain et al. (1970)).

During fermentation in traditional bread production little additional binding (Chiu et al (1966)) occurs. This may explain part of the beneficial effect of added fat to this type of bread in addition to the advantages of crumb softening and staling retardation. Results reported in this thesis showed that it was only necessary for neutral lipid to be in the liquid phase for some binding to occur during

Figure D:6

Flour with excess free lipid (a) at 14% moisture (b) at 50% moisture (c) at 50% moisture after blending-wetting (d) as (b) but heated during freezedrying. Total bound and unextractable fractions shown.



work-free wetting.

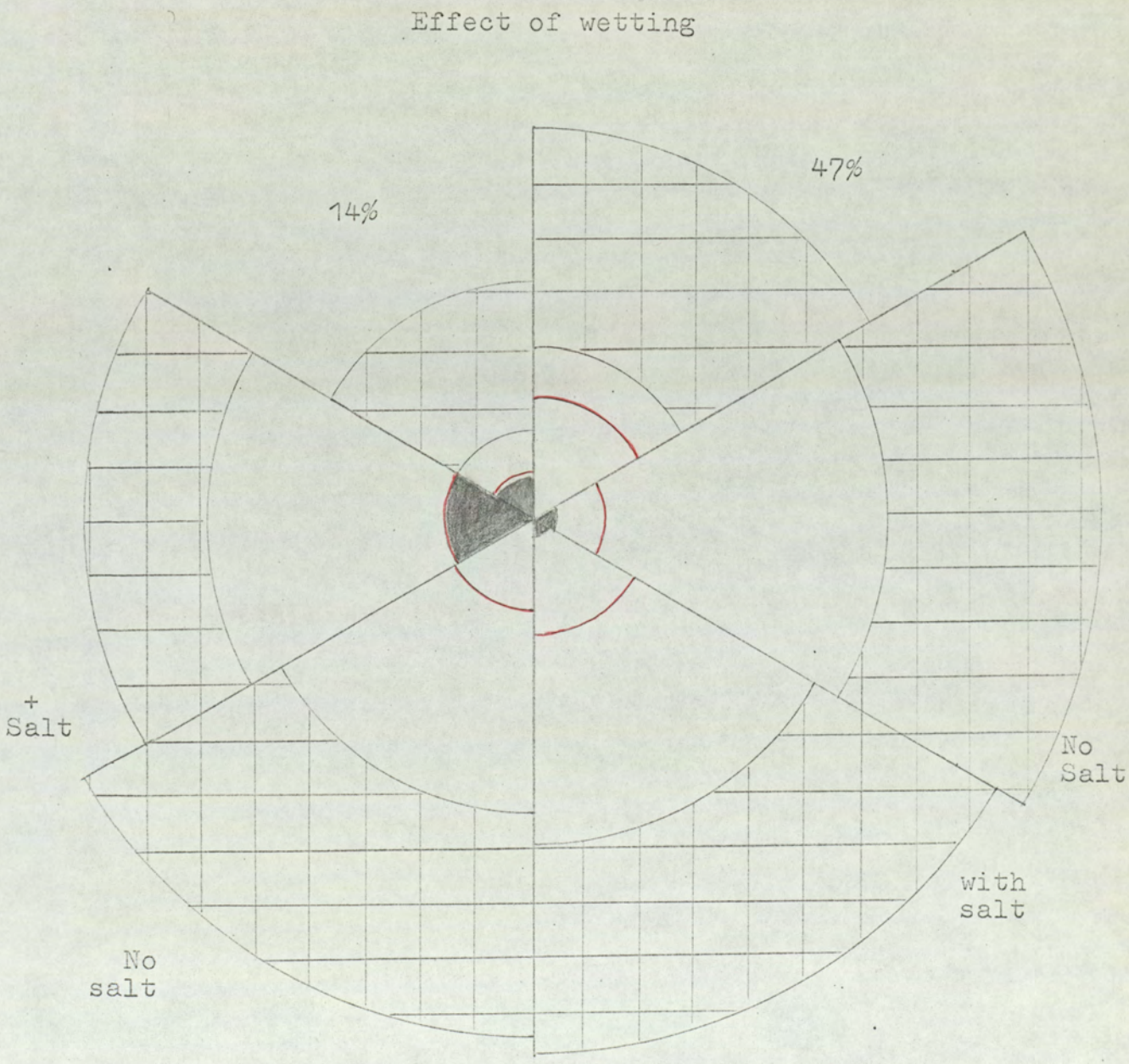
Free flour lipid, including any added fat, acts as a single blend (Baker and Mize (1942)), the value of added hard fat may be related to the overall reductions in binding that results, although other possible roles for the hard fat fraction have been discussed by Elton and Fisher (1968). The critical requirement for hard fat (Baldwin et al. (1963) (1965) Pomeranz, Rubenthaler and Finney (1966) Pomeranz and Hayes (1968)) was shown to be limited to fat melting between 61 and 85°C, mineral wax serving equally well to give good bread volume.

The presence of common salt in the dough formula not only has organoleptic advantages and fermentation moderating properties but it also slightly reduces the binding of neutral lipid (See Figure D:7) as shown in the study reported here and that of Pomeranz et al. (1968). This may be as a result of the tightening of gluten structure reported by Bennett and Ewart (1965) with consequent effects on the binding sites, probably by the presence of sodium and chloride ions in the structural 'bound' water of gluten (Davies and Webb (1969)).

Neutral lipid is not the only fraction of flour lipid of importance in breadmaking. Daftary et al. (1968) singled out glycolipids as being essential to the production of acceptable bread in the presence of free neutral lipid. Similarly, Hoseney et al. (1969) found that both glycolipid and free neutral or total bound lipid ~~was~~ were required for the production of satisfactory bread, starting from completely lipid free flour.

Figure D:7.

Effect of mechanical work to 0.4 and 4.0 h.p.min./lb., with and without added salt, on lipid distribution.



The most important conclusion to be drawn from the work reported in this study is the extent of the development of gluten structure produced solely by hydration and the consequent effect on lipid distribution. When water has been added to flour to raise the moisture content to 45% without any mixing, already phenomena of significance to breadmaking have occurred. The formation of gluten structure has been started, a third of free neutral lipid and most (90%) free polar lipid has been bound.

While water for rehydration and germination of wheat grain is unquestionably vital to the farmer in the natural course of events, it is also essential to the baker. The effect of rehydration of the apparently chaotic wheat flour system is turned to advantage by the baker to form a dough from which bread can be produced. The work reported in this thesis has shown that the water the baker adds to flour is more than a diluent and lubricant and solvent but an initiator and an essential structural component of dough.

There still remains much to be learnt from a simple flour and water system and two closely related areas are suggested by this work for future examination. Firstly the use of heat to produce lipid binding in flour could be studied quantitatively. Such an approach should yield information about the energies involved in binding and the nature of the interaction. Differential thermal analysis may provide an ideal means of examining this area. Secondly the amount of mechanical work that produced considerable lipid binding when introduced to the flour-water system was so small that a study of low levels of work input should also produce much information.

The overall objective of such studies would be further elucidation of the nature of lipid binding and dough structure and the clarification of the relationship between lipid binding and breadmaking properties. Eventually it is hoped that an understanding of these factors will aid in the more efficient production of high quality bread from an increasingly wide range of flour types.

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J. Fd Technol. (1969) 4, 117–123.

An improved method of adjusting flour moisture in studies on lipid binding

R. J. DAVIES,* N. W. R. DANIELS* AND R. N. GREENSHIELDS†

Summary. A method is described for the controlled adjustment of flour moisture level over the range 14–60%. Water was added as powdered ice to flour dispersed in liquid nitrogen thereby avoiding the introduction of mechanical work. The effect of moisture on the distribution of free and bound lipids in a hard wheat flour was investigated using this technique. Free lipid decreased above 20% moisture, while extractable bound lipid increased only above 25% moisture. These different critical moisture levels are considered with reference to the distribution of neutral and polar lipid fractions in the moistened flour.

Introduction

Changes in the relative proportions of free and bound lipids occur during the mixing of wheat flour doughs (Baldwin *et al.*, 1963; Ponte *et al.*, 1964, 1966; Chien-Mei & Pomeranz, 1966; Wootton, 1966) and have been shown to be influenced by the rate of dough mixing, by the total mechanical work input and by the atmosphere in the dough mixing chamber, (Daniels *et al.*, 1966, 1967).

The simple addition of water to flour has been shown to bring about the binding of flour lipids even in the absence of mechanical work (Olcott & Mecham, 1947). It was found that while increasing the flour moisture from 10% to 20% was without effect, ether extractable lipid fell from 70% of the total lipid to 39% at 30% moisture. Further addition of water caused no change in lipid binding provided dough development was avoided.

The object of the present investigation was to obtain a method that would allow a more precise control of flour moisture adjustment in the absence of mechanical work over a wider range than that used previously and to investigate lipid binding in detail in the critical area between 20% and 30% moisture.

Materials and development of method

The flour used in these experiments was an untreated unbleached commercial bread flour containing 14·0% moisture and, on a dry basis, 12·45% protein (nitrogen ×

* Authors' address: Spillers Limited, Technological Research Station, Station Road, Cambridge.

† Author's address: Department of Biological Sciences, The University of Aston in Birmingham, Gosta Green, Birmingham 4.

5·7), 0·5% ash and 1·65% total lipid. Light petroleum boiling between 40° and 60°C was redistilled before use. All other solvents were of analytical reagent quality and were used without further purification. Silicic acid, 100 mesh (Mallinckrodt Chemical Works, St Louis, U.S.A.), was freed of fines by sedimentation in methanol and dried at 105°C in a vacuum oven before use in the fractionation of neutral and polar lipid.

Attempts to raise the flour moisture content by equilibration over water in a closed vessel failed owing to mould growth on the damp flour. The addition of mould inhibitors (e.g. toluene, as used by Olcott & Mecham, 1947) was avoided in view of possible effects on lipid distribution. Moisture was more effectively raised by allowing the flour to fall repeatedly through a column of dense fog generated by passing steam over blocks of solid carbon dioxide. However, above 20% moisture the method failed owing to the caking of moist flour particles which prevented their even access to the fog. The addition of a measured amount of water in the form of a fine spray to a thin layer of flour also produced an unsatisfactory distribution of moisture. The greatest disadvantage of these methods was the lack of fine control over the moisture level that could be obtained. The unpredictable nature of the results of these techniques also produced a time lag since a moisture determination was necessary before proceeding further.

The addition of powdered ice to flour at sub-zero temperatures (Olcott & Mecham, 1947) appeared to offer a more precise control of moisture content but had the disadvantage that a long period of storage (1 month at −9·4°C) was required to allow the ice to sublime into the flour. Trial experiments showed that it was difficult to obtain a powdered ice fine enough for intimate dispersion in flour by grinding ice in a mortar cooled to −20°C. Invariably, even after prolonged storage below freezing point, when the mixture was brought to room temperature the moisture was found to be unevenly distributed in the flour, giving rise to damp clumps in surrounding dry flour.

It was noticed that when ice was ground at −20°C, the ice surface melted under the pressure of the pestle preventing the required breakdown to a fine powder. A considerable improvement in the fineness of grinding was obtained when ice was ground below the surface of liquid nitrogen (−196°C) added to the mortar. Moreover, the addition of the required weight of flour to the finely divided ice suspended in boiling liquid nitrogen produced a mixture in which the ice was intimately mixed with the flour before storage and equilibration at −20°C. The procedure finally used in this work for moisture adjustments in the range 14–60% was to measure the required amount of water into liquid nitrogen held in an iron mortar (a ceramic mortar was unable to withstand the extremely low temperatures used) and grind quickly to a finely divided powder. This was added to flour (100 g) similarly suspended in boiling liquid nitrogen in a beaker and the slurry transferred to a deep-freeze cabinet at −20°C to allow the nitrogen to boil off and the ice–flour mixture to reach equilibration by sublimation. It was found that even with the higher moisture levels an equilibrium was reached rapidly and the mixture was ready for use within 24 hr.

The effect of moisture level on lipid binding was studied by allowing the frozen

moisture to thaw at room temperature for at least 30 min before taking a sample for moisture determination by the A.A.C.C. vacuum oven method (Anon, 1962). The remaining damp flour was dried in the frozen state and the free and bound lipids quantitatively extracted.

The procedures followed were those adopted by Daniels *et al.* (1966). Free lipid was defined as that lipid removed by a 7-hr soxhlet extraction with light petroleum (b.p. 40–60°C). Bound lipid was defined as that remaining after the extraction of free lipid and removed by the solvent system recommended by Tsen, Levi & Hlynka (1962). The lipids obtained by these extractions were then quantitatively separated into neutral and polar fractions using a method based on that described by Parkes & Hummel (1965). The lipid material (100 mg) was dissolved in chloroform (50 ml) and shaken for 10 min with silica gel (2 g), filtered on a sintered glass funnel (Pyrex POR 4) and washed with chloroform (4×25 ml). The neutral lipid was that present in the solution. Polar lipid was then removed by washing the silica gel with methanol (5×25 ml). Solvents were removed by rotary evaporation and the proportions of neutral and polar lipid determined from the dry weight present in the two solutions. Thin layer chromatography was used to ensure that the method classified the lipid classes consistently. Plates were developed with either a solvent comprised of light petroleum (b.p. 40–60°C), ethyl ether and glacial acetic acid 60 : 40 : 1 v/v to separate the neutral lipids or chloroform methanol and water 80 : 25 : 2 v/v to separate the polar lipids.

Results and discussion

The method of moisture adjustment described proved most effective since a particular moisture level could be obtained to within 1% of that required with an even moisture distribution throughout the flour. This was verified both by repeated moisture determinations on the same sample and by the absence of small dough particles in the dry bulk of the flour which were seen when spray moistening of the flour was tried. The conditions of the technique eliminated completely any question of mechanical development of the wetted flour at all moisture levels and also avoided the possibility of mould contamination. The possibility that treatment with liquid nitrogen or freeze drying could have influenced the extractability of lipids was examined by subjecting a sample of flour to the routine described but with the omission of thawing before freeze drying. There was no change in free and bound distribution even though sufficient ice had been added to raise the moisture to 40% indicating that any change in free and bound lipid distribution was due to the increase in moisture level of the flour alone.

The effect of moisture on extractability of lipids was studied over the range 14–60%. Samples were prepared at intervals of less than 5% moisture throughout the range and at smaller intervals (about 1%) in the area of particular interest (18–35% moisture). The results of this study are shown in Fig. 1. Expressing all extracted lipid as a percentage of the total lipid extractable from flour at 14% moisture, the amount of free lipid

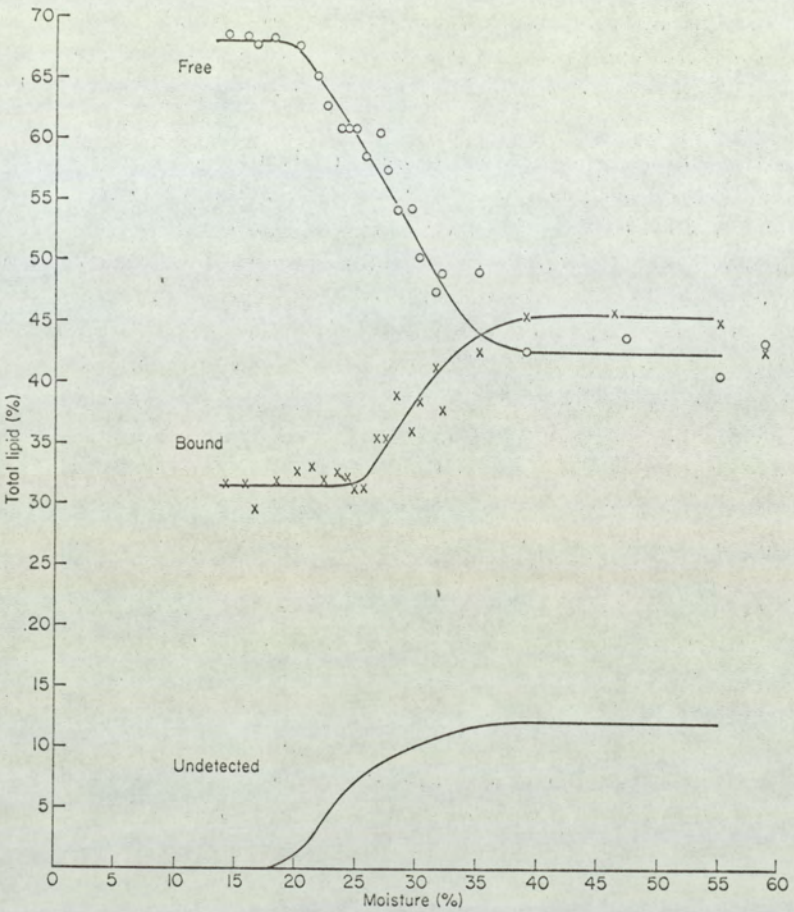

FIG. 1. Variation of lipid distribution with moisture level. ○, Free lipid; ×, Bound lipid; undetected calculated by difference. Standard errors of the plotted means; free ± 0·60%; bound ± 0·97%.

remained constant at 68% as the flour moisture was raised to 20%. Above 20% and up to 40% moisture the free lipid decreased steadily to give a constant value of 42·5% above 40% moisture. However, the amount of bound lipid at 14% flour moisture, 31·5%, remained constant until the moisture level rose to 25%. At this point bound lipid started to increase until it reached a maximum value of 45·5% at a moisture level of 40% and remained constant thereafter. These results were in agreement with those of Olcott & Mecham (1947) and further indicated that the increase in binding was a gradual process.

An interesting feature of the lipid binding results was the discrepancy between the loss of free lipid and the corresponding gain in bound lipid in the 20–25% moisture

range. It would appear that between 20 and 25% moisture a proportion of lipid material became undetectable even though subjected to the very efficient Tsen solvent extraction. A possible explanation may be that this lipid was bound in a very strong manner in a form which was resistant to solvent attack. Although this change commenced when 20% moisture was attained, further weaker binding, which increased the (extractable lipid) fraction referred to as 'bound', did not occur until 25% moisture was reached.

The fractionation of the free and bound lipids into neutral and polar gave a better insight into these changes, not only in this critical range but over the whole range. For instance it was evident that the initial strong binding in the undetected form involved polar lipid since the polar free lipid fell from 15·2 to 11·0% between 15 and 25% moisture without any increase in the extractable polar bound lipid. Unextractable lipid, some 12% of the total lipid at its maximum, remained fairly constant above 30% moisture although the proportion of neutral to polar lipid increased with increasing moisture. These results were in agreement with the findings of Olcott & Mecham who observed that loss of free lipid was matched by a loss of ether extractable phosphorus. They concluded that binding of phospholipids preferential to other constituents of flour lipids occurred during hydration of the flour although the initial stages of hydration were not studied in detail.

It is interesting that these quite significant changes in the lipid distribution pattern occurred only because the moisture level had increased. The possibility that these changes coincided with the presence of free water in the flour is suggested from the results of Toledo, Steinberg & Nelson (1968) which confirmed an earlier result from Vail & Bailey (1940). These workers, the former using nuclear magnetic resonance and the latter a freezing point depression technique, found that only above 25% moisture could liquid (free) water be detected in moistened flour. The amount of non-liquid (bound) water remained constant and independent of total moisture content of the flour above moisture levels of about 25%.

Across the range of moisture levels, the wet flour or dough that resulted when the flour–ice mixture was allowed to thaw showed interesting changes of physical form. Between 14 and 28% the flour changed from a free flowing to a sticky powder. The onset of this change occurred at a moisture level of about 20% and coincided with the onset of changes in lipid distribution (Fig. 1). Above 28%, towards the end of the changes in lipid distribution the moist flour exhibited for the first time a tendency towards a continuous structure. The material could hardly be called a dough yet over a period of some 2 hr the volume significantly decreased. This shrinking effect became more pronounced as the moisture level was further raised to the 50% level. At 50% moisture the dough appeared to have a wet surface after 1 hr at room temperature and had the consistency of a stiff batter after 2 hr.

Fig. 2 shows the appearance of the moistened flours over the range of moisture levels discussed above. The flour–ice mixtures were placed in open ended cylinders

(3·5 cm × 4·5 cm) and allowed to thaw for 2 hr at room temperature after which the cylinders were lifted off. The flour at 18% moisture collapsed. Between 20 and 28% the cylindrical form remained but did not shrink. Above 28% the volume decreased with increasing moisture so that at 45% moisture the volume was reduced to less than half the original.

Other workers in these laboratories (Webb *et al.*, 1969) concluded that a minimum moisture level of some 35% was required to hydrate an unworked flour of similar composition. This would suggest that a redistribution of lipids during the increase of moisture from 14 to 35% was necessary before dough formation was possible. The formation of a structure that contracted at this same moisture level (Fig. 2) was also

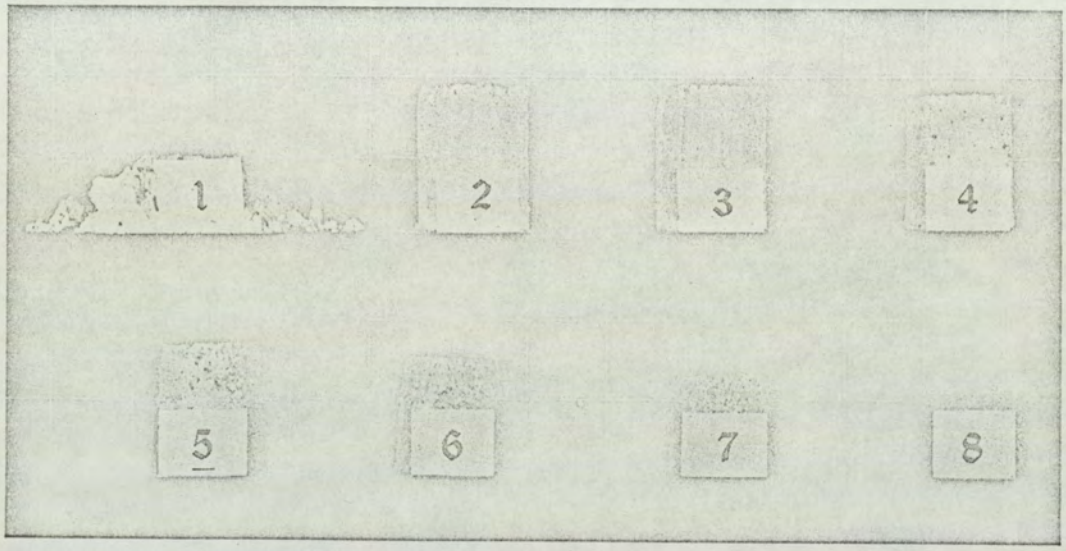

FIG. 2. Effect of moisture on the shrinkage of flour cylinders. Moisture levels; (1) 18%, (2) 23%, (3) 28%, (4) 30%, (5) 34%, (6) 36%, (7) 39%, (8) 45%

considered significant, particularly as Webb *et al.* (1969) found that a dough could be formed by the introduction of mechanical work to a flour at 35% moisture.

The intimate dispersion of finely divided ice in flour suspended in liquid nitrogen permitted the controlled adjustment of moisture level in flour without the introduction of mechanical work. This method further allowed the examination of changes in lipid distribution resulting from the effect of moisture at initial stages of dough formation. It was apparent that some interactions between flour components occurred at moisture levels at least 20% below those normally employed in breadmaking. Further work is in hand to elucidate the full significance of the pattern of events which occurs when water is added to flour both with and without the introduction of mechanical work and also to correlate these events with the presence of free water. It is proposed to ex-

tend this work to include soft wheat flour and so obtain further information regarding the nature of the interactions between different components of flour during dough formation.

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REPRINTED FROM CHEMISTRY AND INDUSTRY, 1969, pp. 1138—1139

Calorimetric determination of freezable water in dough

by R. J. Davies and T. Webb*

Spillers Limited, Technological Research Station, Station Road, Cambridge

** External research student of the Department of Biological Sciences, University of Aston in Birmingham*

Water is not only the major component of most foodstuffs, but is intimately involved in the physical and chemical properties of these materials. It is likely that water is associated with the other components of the foodstuff to different degrees and an understanding of this relationship is of consequence in food technology. The distinction between 'free' and 'bound' water has often been made and discussed but no complete agreement has been reached on their definition. However, the concept is a useful one when hypotheses regarding the interaction of water with other components present are proposed, but when any measurements are made the definition rests entirely on the method of determination. A commonly used definition of 'free' water would appear to be that fraction of the water which will freeze when the material is cooled to a sub-zero temperature. Numerous methods are described in the literature[1] for the determination of freezable water, the proportion of unfreezable or bound water then being assumed from the known total moisture level in the material. Among these methods is the calorimetric determination of this freezable water in which the energy change is measured when the ice in the frozen material melts. This paper reports the application of this approach to wheat flour doughs using a Differential Scanning Calorimeter (d.s.c.).

Method

Three series of doughs were mixed using two different flours. Series I involved flour A, an untreated, unbleached hard wheat flour suitable for breadmaking, with 2 per cent added salt (flour weight basis); series II, flour B, an untreated, unbleached soft wheat flour not suitable for breadmaking, with 2 per cent added salt and series III, flour A again but without the addition of salt.

Various levels of added water were used for each series of doughs, the range being limited by the feasibility of mixing to the required work input and ease of handling.

The doughs were mixed on a constant dough weight basis (470g) at 30 $\pm$ 1 °C in a stainless steel-clad farinograph bowl attached to a Brabender do-corder. A level of work of 0·6 h.p. min/lb was introduced into each dough at a constant rate of 0·2 h.p. min/lb/min.

After mixing, the doughs were immediately sealed in aluminium foil to prevent loss of moisture until samples were taken for analysis. Four samples (c. 5 mg) were cut from the centre of each dough and enclosed in a tared, lidded aluminium cup. Each cup was then weighed and placed on what is normally the reference thermocouple of the d.s.c. cell of a differential thermal analyser (Dupont 900 d.t.a.). An empty lidded cup was used as reference. The cell was cooled to −50 °C and then heated at a rate of 10 °C/min. to a temperature of +50 °C. This allowed sufficient time for the heating to stabilise at the set rate and the sample and reference platforms to equilibrate before and after the ice/water transition at about 0 °C. Each sample was frozen and thawed only once, the endotherm being presented as a peak (see Fig. 1). The areas under the peaks were measured by planimeter and a calibration factor was determined to relate the area of the endotherm peak to the weight of water involved in the transition. This factor included the latent heat of fusion of ice and a calibration coefficient for the machine for water at 0 °C. The total water present in the samples was known and the amount of freezable water in each dough was calculated from the endotherm peak. Both quantities were then presented as a percentage of the dry flour present in the dough.

Results

When the results were plotted as freezable water versus total water (Fig. 2) it was apparent that over the range of moisture levels examined:

(a) the two were directly proportional giving a line of unit gradient.

(b) there was no significant difference between the three series.

In view of the different natures of the three systems, particularly in regard to their known physical and baking properties, the observed similarity was interesting.

The results suggested that above a certain moisture level extra water was not sufficiently bound to prevent it freezing at about 0 °C. In addition, within the range studied, the proportion of the total water which was freezable at any particular moisture level was the same for each series of doughs and that each series had the same amount of unfreezable water of mean value 0·33 $\pm$ 0·016 g/g dry flour (24·85 per cent moisture), *i.e.* that the same moisture level must be attained in each series before freezable water could be detected. Similar values have been found for wet flour

J. Fd Technol. (1970) 5, 149–156.

A method for reconstituting fat extracted flour

R. J. DAVIES, N. W. R. DANIELS AND
R. N. GREENSHIELDS*

Summary

A low temperature technique is described for the reconstitution of fat extracted wheat flour which allows the return of lipid material with the minimum alteration of flour properties. A study of the effect of moisture level on lipid binding in reconstituted flour is compared with a similar study of the original flour. Flours with augmented lipid contents were produced and confirmation that the method avoided the introduction of mechanical work obtained.

Introduction

Investigations of the role of lipid in breadmaking using the technique of extracting flour with solvent and subsequently reconstituting have produced many contradictory results as reviews of the subject show (Cookson & Coppock, 1956; Fisher, 1962; Daniels, 1963). Apart from the choice of a suitable method of lipid extraction, reconstitution also presents problems if changes in flour properties are to be avoided. A successful method of reconstitution would have to satisfy the following requirements if subsequent study of lipid binding was intended. Firstly it is essential that the lipid be completely and evenly dispersed throughout the flour; secondly, the use of solvent (including water) must be avoided; thirdly, mechanical work must be excluded and finally adequate precautions must be taken against the risk of lipid oxidation. Such precautions are necessary since these factors have been reported to affect lipid binding or breadmaking properties of flour (Daniels *et al.*, 1966; Daniels *et al.*, 1969; Davies, Daniels & Greenshields, 1969; Pomeranz, Shogren & Finney, 1968; Ponte *et al.*, 1964). To try to fulfill these requirements, a technique for adding water to flour (Davies *et al.*, 1969) was adapted to return lipid to extracted flour. The essential features of the technique are the grinding of the material to be added to a fine powder in liquid nitrogen and the addition of this fine powder to flour suspended in liquid nitrogen. This allows the return of lipid material to flour without the introduction of mechanical work or other factors which might influence lipid binding.

Authors' address: Spillers Limited, Technological Research Station, Station Road, Cambridge.
* Present address: Department of Biological Sciences, The University of Aston in Birmingham, Gosta Green, Birmingham 4.

Materials and methods

An unbleached, untreated flour of a commercial breadmaking grist was used which contained 14% moisture and, on a dry basis, 14·5% protein (nitrogen × 5·7), 0·58% ash and 1·65% total lipid, 1·13% of which was free and 0·52% bound. All solvents were of analytical reagent quality and used without further purification except for light petroleum (b.p. 40–60°C) which was redistilled. Fat extracted flour was obtained by percolation of light petroleum through flour (5 l/Kg) contained in a glass cylinder (130 × 8) cm for 24 hr, followed by air drying. This removed free lipid as efficiently as Soxhlet extraction yet without the use of heat. Free lipid was stored at −20°C under nitrogen after the solvent had been removed also under nitrogen on a rotary flash evaporator.

To reconstitute, the extracted flour was added to liquid nitrogen contained in a beaker and the level of boiling liquid nitrogen maintained to keep the suspension as a mobile slurry. The appropriate weight of flour lipid was then added to liquid nitrogen in a metal mortar. The lipid was sufficiently brittle at this temperature to be ground down to a fine powder with a pestle. This powder, suspended in liquid nitrogen, was then transferred to the beaker containing the suspension of flour in liquid nitrogen. The liquid nitrogen boiled off and produced an excellent dispersion of the lipid throughout the flour.

When required for studies on lipid binding, portions of the reconstituted flour were raised to required moisture levels by the technique previously described (Davies *et al.*, 1969), and the distribution of free, bound and unextractable lipids determined. Flour was extracted for 7 hr in a Soxhlet extractor using light petroleum (b.p. 40–60°C). The extracted lipid was called free lipid. The dried extracted flour was then re-extracted by the method of Tsen, Levi & Hlynka (1962) which used chloroform, methanol and water. The lipid thus extracted was called bound lipid. The remaining lipid, derived by difference, was called unextractable.

Flours with augmented lipid levels were obtained using the same liquid nitrogen technique but with greater proportions of added lipid. These flours (Series A) were wetted to 50% moisture by the liquid nitrogen technique, thawed, freeze-dried and then finely ground for lipid distribution determinations. Flours of series A.W. were flours of series A at the final stage, i.e. freeze-dried and ground (see diagram), which were remoistened to 14% by the liquid nitrogen technique. This minimized any effects due to the heat evolved during hydration of the freeze-dried material. This material was then wetted to 50% moisture in a mixer by the method described by Olcott & Mecham (1947).

Results and discussion

Reconstitution of flour

Flour that had been fat extracted and reconstituted by the liquid nitrogen technique

was compared visually and physically with unextracted control flour and fat extracted flour. As graphically described by Cookson & Coppock (1956) fat extraction of a flour produces a whiter, fine or light material which has not the cakiness of the control flour but is easily dispersed in air when disturbed. It would seem that as well as bearing the fat soluble pigments the free lipid tends to hold the flour particles together. Furthermore, when a suspension of 20% fat extracted flour in water was beaten, a frothiness was obtained which was not found in the original flour. The reconstituted flour retained none of these differences and was indistinguishable from the original flour. Reconstitution by the liquid nitrogen technique had successfully restored the original appearance and handling properties to the fat extracted flour.

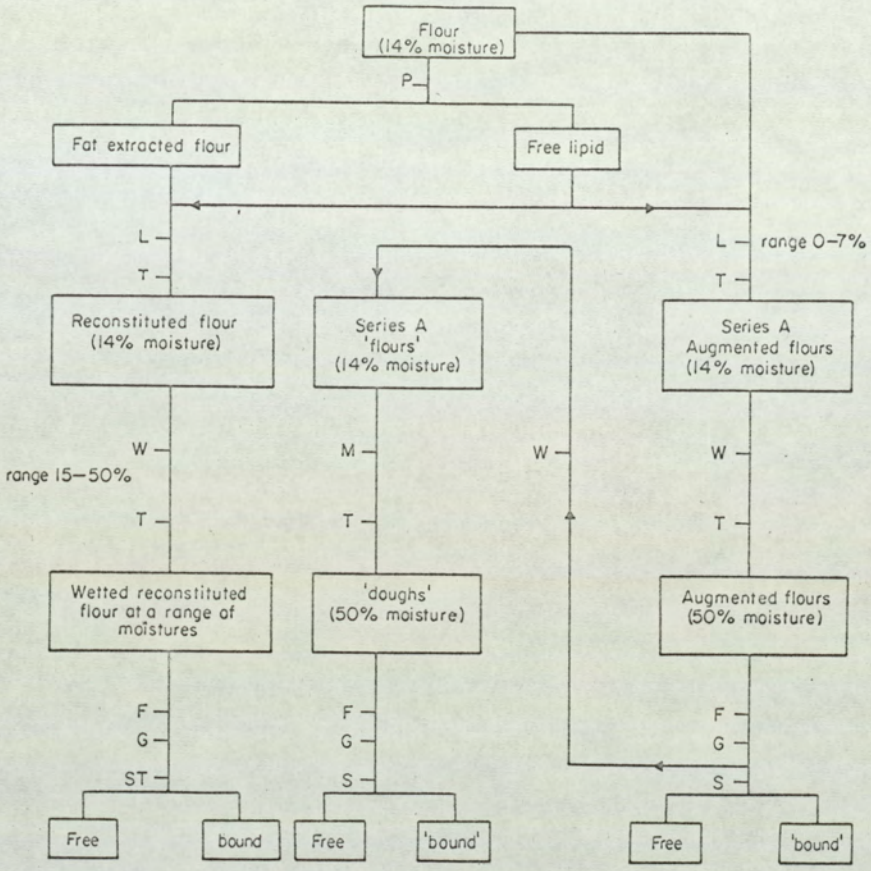
In order to assess the effect of reconstitution on lipid distribution, free and bound lipids were determined and the results compared with those obtained from the original flour (Davies *et al.*, 1969) and from the original flour after fat extraction. The results are presented in Table 1. Reconstitution by the liquid nitrogen technique restored the

TABLE 1. Lipid distribution of original fat extracted and reconstituted flours at 14% moisture: expressed as % of flour dry weight in each case (% of total lipid of original flour in parentheses).

Flours	Free	Bound	Total	
Original	1·130 (68·5)	0·520 (31·5)	1·650 (100·0)	1·10 (66·7) removed by fat extraction
Fat extracted	0·064 (3·9)	0·422 (25·6)	0·486 (29·5)	1·095 (66·25) flour lipid returned
Reconstituted	1·107 (67·0)	0·424 (25·7)	1·531 (92·8)	

free lipid close to the original level without increasing lipid binding. The bound lipid found in the reconstituted flour was 0·424% compared with 0·422% in the extracted flour. It should be noted that percolation with light petroleum reduced the recoverable bound lipid from 0·520% to 0·422%; this loss will be discussed elsewhere. The new technique permits the restoration of free lipid without further effect on lipid binding.

Judged by the criteria of reproducing the appearance, handling and lipid distribution of the original flour it was thought that the liquid nitrogen technique was a satisfactory method of producing a reconstituted flour. A particular advantage of the method was that a flour was produced and not a dough. Furthermore factors such as heat, water, solvent and mechanical work, which influence lipid binding, were avoided during the production of the flour.



KEY

F Freeze-drying
G Grinding to a fine powder
L Addition of lipid using the liquid nitrogen technique
M Addition of water using the technique described by Olcott and Mecham (1947)
P Petrol extraction by percolation
S Petrol extraction in a Soxhlet for 7 hr
ST Petrol extraction in a Soxhlet for 7 hr followed by extraction with Tsen solvent
T Thawing
W Addition of water using the liquid nitrogen technique

Effect of water on reconstituted flour

When the moisture level of the original flour was increased, a distinct pattern of lipid binding occurred (Davies *et al.*, 1969). As a further test of reconstitution, the effect of moisture level on the lipid distribution of the reconstituted flour was investigated to see the effects of reconstitution on lipid binding properties. As with the original flour the moisture level was adjusted using the liquid nitrogen technique (Davies *et al.*, 1969). The distribution of free, bound and unextractable lipid was determined over the same moisture level range (14–50%) as the original flour. The effect of moisture level on the free lipid of the original and reconstituted flours is shown in Fig. 1. A similar loss of free lipid was found for both flours.

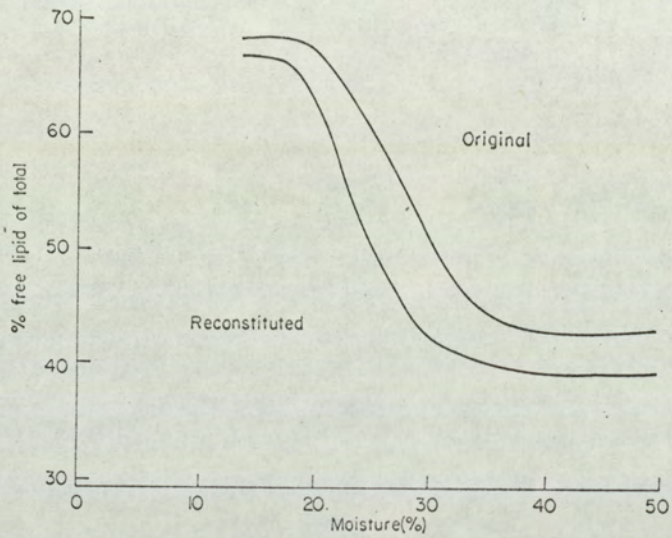


FIG. 1. Effect of moisture level on free lipid. Comparison of reconstituted with original flour. Total lipid 1·65% of flour dry weight in each case.

As shown in Fig. 2, although a small loss of extractable lipid followed reconstitution, increasing the moisture content led to a comparable increase of bound and unextractable lipid in both flours. Furthermore the same changes of physical form across the range of moistures were noted, e.g. when the flour had a moisture level greater than 28% a continuous structure was apparent and shrinkage occurred with time. It was concluded that reconstitution appeared to have little significant effect on the lipid binding properties of the flour.

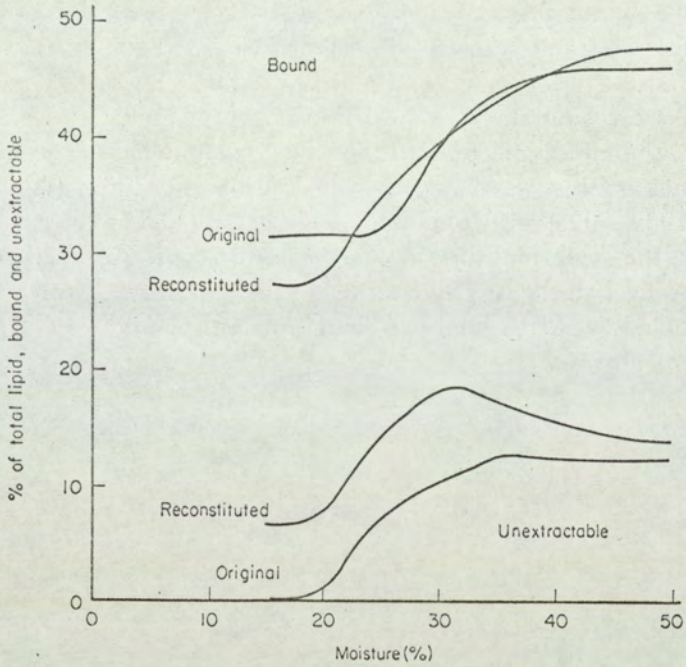


FIG. 2. Effect of moisture level on bound and unextractable lipid. Comparison of reconstituted with original flour. Total lipid 1·65% of flour dry weight in each case.

Flours with augmented lipid levels

The liquid nitrogen method of lipid addition proved to be an excellent means of producing flours with augmented lipid levels. The required lipid levels were predetermined and the only observable change in the flour was a stronger colour and an increase in 'cakiness'. The work of Olcott & Mecham (1947) suggested that if such flours were wetted to 50% moisture much of this excess free lipid would become bound. However, we have found (Davies *et al.*, 1969) that when a control flour was wetted without the introduction of mechanical work, only a proportion of the available free lipid became bound. It was possible therefore that either our results represented an equilibrium at 50% moisture which would be altered by the presence of excess lipid or that the method of water and excess lipid addition used by Olcott & Mecham (1947) introduced mechanical work or some other lipid binding factor. The flours with augmented lipid levels were wetted to 50% moisture by the liquid nitrogen technique to see whether any additional binding occurred above that found for the control flour. Fig. 3 shows that in the absence of work, only an insignificant amount of additional lipid binding occurred when the moisture was raised by the liquid nitrogen technique.

To investigate the possibility that the lipid binding reported by Olcott & Mecham

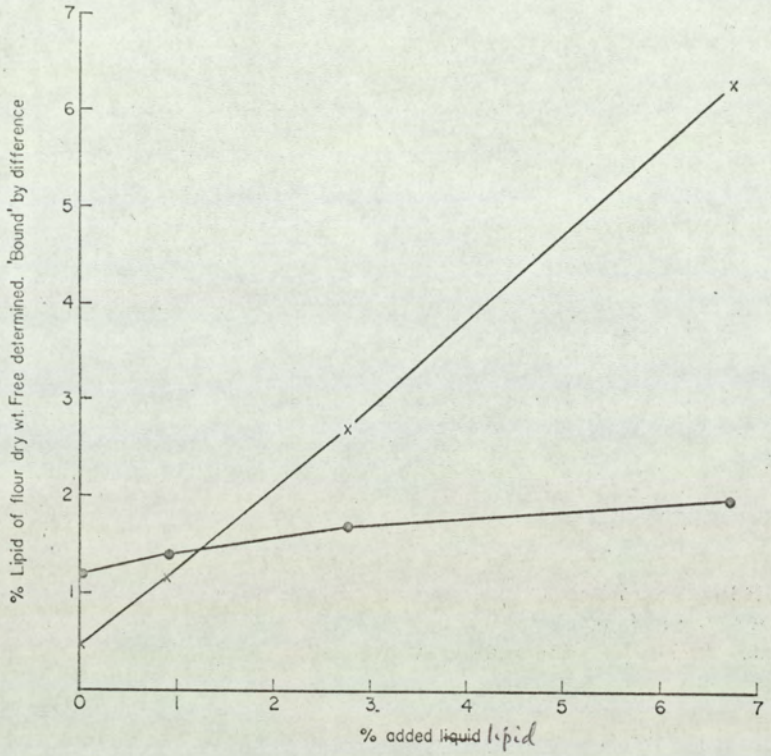


FIG. 3. Effect of wetting to 50% moisture by the liquid nitrogen technique of flours with augmented lipid levels: series A, × ; free lipid, ●; Lipid remaining after petrol extraction: (bound + unextractable).

(1947) was caused by their method, their wetting technique was imitated. The flours (series AW) were added to the amount of water required to raise the moisture to 50% in an Atomix mixer. The speed of the mixer was kept to a minimum using a Variac transformer and mixing stopped when the addition and blending was completed. The distribution of lipids in these 'doughs' was then determined. The results (Fig. 4) matched those of Olcott & Mecham (1947) very closely and indicated that the binding of much of the available free lipid was probably produced by the minimum mechanical work introduced by the mixer. Apart from confirming the value of the liquid nitrogen technique for avoiding the introduction of work, this experiment confirmed that the flour had a limited capacity for binding in the presence of excess free lipid under these experimental conditions. A comparison of the colour of the extracted free lipid of the two series showed that bleaching had occurred during the mixing of the AW series, probably by atmospheric oxygen in the open mixer (Hawthorn & Todd, 1955).

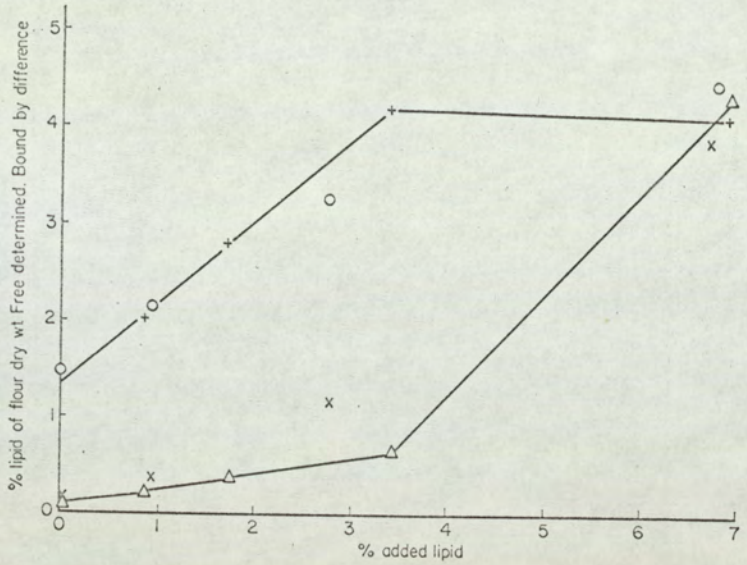


FIG. 4. Effect of wetting to 50% moisture in a mixer on flours with augmented lipid levels. Series AW, ×; free lipid, ○; bound lipid (by difference). Results reported by Olcott & Meecham (1947) with a similar flour, △; free lipid, +; bound lipid.

This method fulfills the requirements for producing a reconstituted flour suitable for lipid binding studies. A satisfactory flour was produced without the introduction of mechanical work which matched the appearance and lipid binding properties of the original flour. It is proposed to use this technique to prepare synthetic flours containing specific free lipids for further studies of lipid binding.

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