EXTRACTION OF FLOUR BY MIXTURES OF BUTANOL-1 AND WATER

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ABSTRACT

Flour, when suspended in aqueous butanol-1, reversibly absorbs or loses water. When the solvent contains 17.5% water (by weight), the equilibrium water content of the flour is 48% (d.b.) and rises very rapidly with increasing water content of the solvent. A flour was extracted stepwise with various butanol-1-water mixtures. With increasing water content, the extracted lipids increased from 1.16 to 1.37%. Likewise, the extracted nonlipids increased from 0.06 to 0.27% and larger amounts were extracted by percolation. Gluten development was retarded when the extract contained more than 10% water; further increases in water content resulted in greater retardation. When gluten development was retarded, gluten yield could be increased by increasing the mixing time or the rest period between mixing and washing. After 15 min. of mixing and 60 min. of rest, gluten yields were normal in all cases. The retardation of gluten development and the loss of baking quality after extraction with butanol-1 containing more than 10% water may be due to the extraction of carbohydrates, more particularly water-soluble pentosans, rather than lipid constituents. This suggestion is supported by the decrease of water absorption of the flour with increasing water content of the solvent.

Water-saturated butanol-1 was proposed as solvent for the extraction of wheat and flour pigments by Binnington, Sibbitt, and Geddes in 1938 (1). In 1955 Mecham and Mohammad introduced it as solvent for the extraction of lipids from wheat products (2). This solvent has since then been used extensively for isolation of wheat lipids (among others 3–6), for preparation of lipid-"free" flour (among others 7–9) or proteins (among others 10–15). Extractions of flour with dry butanol and with water-saturated butanol result in doughs with very different properties. We had no knowledge, however, of the effect of extractions by mixtures with intermediate water contents. Further, we observed that flour can absorb much water from a butanol-water mixture. Consequently, the water content of the solvent phase during extraction can differ greatly from that of the added solvent. Therefore we sought for answers to the following questions: How is the water distributed between the flour and the solvent phase? What is the effect of water content on the amount of lipids and nonlipids extracted? How does the extraction affect flour and dough properties?

[1]Manuscript received December 3, 1965. Contribution from Institute for Cereals, Flour and Bread TNO, Wageningen, The Netherlands.

We performed a series of stepwise extractions with butanol-water mixtures with various butanol:water ratios. In the extracts lipids, non-lipids, and water were determined. We studied the properties of extracted flours mainly by means of gluten washing after various mixing times; we had applied this technique successfully in previous work on the extraction with acetone-water mixtures (16). It was modified by the use of a GRL mixer (17), which permits elimination of oxidation of doughs by atmospheric oxygen. Variations in the time interval between mixing and gluten washing were another modification. Farinograph measurements and baking tests gave additional information.

Materials and Methods

Unless stated otherwise, analytical results, dough compositions, and solvent:flour ratios refer to flour with 14.0% moisture. Quantities of solvents or extracts are always given in g. or ml. per g. of flour: "2.5 g./g. of solvent" means 2.5 g. of solvent per g. of flour

Flour. A commercial, unbleached, improver-free bread flour with 1.76% of Kjeldahl N and a dry gluten yield of 8.9% was used. Other analytical data are given in Table IV, flour A; Fig. 4 shows its farinogram.

Stepwise Extractions with Butanol-Water Mixtures. The main series of extractions (B to I inclusive) was performed with 2.5-kg. portions of flour. Each extraction consisted of four steps: extraction with 1) butanol with higher water content, 2) butanol with water, 3) dry butanol, and 4) dry diethyl ether. The water content of the solvent added for step 1 was chosen higher than for step 2 (see Table I) so that the water contents of the first two extracts would not differ too widely. Step 3 with dry butanol was included to avoid phase separation upon addition of diethyl ether to butanol with too high a water content. Diethyl ether was used to facilitate drying of the extracted flour.

Table I gives amounts of extract, and water contents of solvents and extracts. We began each step by slow agitation of a suspension of flour in 2.5 g./g. of solvent, at room temperature for at least 6 hr. in step 1 and at least 4 hr. in steps 2 to 4. In steps 1 to 3, after sedimentation, we replaced the supernatant by the same weight of solvent for the next step. In step 4, after agitation, we filtered the suspension by suction and washed the filter cake with another 1.0 ml./g. of diethyl ether.

Extracts with less than about 9% of water were cloudy (1). After centrifugation, we added the sediments to the bulk of the flour.

We dried the extracted flour first for 9 hr. at 35°C. in the vacuum of a water aspirator. Residual butanol was removed by spraying water

TABLE I

AMOUNTS OF EXTRACTS OBTAINED AND THEIR WATER CONTENTS

EXTRACTION	AMOUNT OF EXTRACT REMOVED				WATER CONTENTS OF SOLVENTS ADDED AND OF EXTRACTS				
					Step 1		Step 2		Step 3[b] Extract
	Step 1	Step 2	Step 3	Step 4[a]	Added[c]	Extract	Added	Extract	
	g./g. flour	*g./g. flour*	*g./g. flour*	*g./g. flour*	*% by wt.*	*% by wt.*	*% by wt.*	*% by wt.*	*% by wt.*
B	1.43	1.56	1.59	2.94	0.2	2.0	0.0	1.5	1.2
C	1.06	0.97	0.95	2.84	11.2	9.4	10.0	9.6	6.7
D	1.31	1.19	1.49	2.72	14.5	11.6	13.0	12.2	8.4
E	1.15	1.22	1.29	2.72	17.0	13.2	15.0	13.8	9.3
F	1.24	1.16	1.10	2.66	19.2	14.4	17.0	15.3	10.8
G	1.29	1.22	1.13	2.77	21.0[d]	15.5	18.0	16.4	11.4
H	1.36	1.31	1.23	2.70	23.0[d]	16.3	19.0	17.1	12.1
I	1.38	1.37	1.25	2.69	25.0[d]	17.0	20.0	17.9	12.9
J	2.23		2.29	3.94	10.3			9.0	4.8
K	2.28		2.17	3.80	20.0			15.6	8.5

[a] Unlike those with extracts from steps 1 to 3, which were actually weighed, the weights in this column were calculated as the product of volume and specific gravity.
[b] The solvent added was dry butanol in all extractions.
[c] Moisture in the flour in excess of 14.0% was considered part of the solvent.
[d] Two-phase mixture.

(2) on the flour until average moisture content was about 20% (as-is basis). After equilibration for 60 hr. in a closed container at room temperature, we dried the flour for 48 hr. at 35°C. in a rotary oil pump vacuum. Lumps were reduced with a percussion grinder. We left the dried flour in the atmosphere until its moisture content was more than 10%.

Two supplementary extractions (J and K), the products of which were mainly used for baking tests, were performed in essentially the same way. The only deviations are that (a) 2.0-kg. portions of flour were used with (b) a higher solvent:flour ratio, 3.5 g./g. Moreover, (c) we combined steps 1 and 2 into one step. Details can be read from Table I.

We assumed that the moisture determination in flour measures total volatile material, including both water and residual butanol. Contrary to this, the Karl Fischer titration measures only water. Therefore we attempted to measure residual butanol in extracted flours as the difference between their moisture content and the results of Karl Fischer titrations. Results obtained led to the conclusion that the amounts of residual butanol cannot have been much larger than 0.2%. We could not obtain more precise results.

Percolations with Butanol-Water Mixtures. We performed these percolations with 250-g. portions of flour in 4 × 50-cm. columns. To minimize swelling of the flour in the column, we agitated a suspension

of flour in 2.5 g./g. of a butanol-water mixture with higher water content for 4 hr., poured the slurry into a column, and percolated it at room temperature with a butanol-water mixture to an amount and with a water content given in the table below. Moisture in the flour in excess of 14.0% was considered part of the solvent.

Water content of solvents, % by wt.		
Added for making slurry	12.6	20.3
Solvent phase of slurry (estimated from data in Table I)	10.3	15.0
Solvent for percolation	10.0	15.0
Amount of butanol-water mixture added for percolation, ml./g.	10.0	6.0
Total extract, ml./g.	15.6	11.9

These percolations took several days. After this, we percolated the columns with 2 ml./g. of dry butanol, washed their contents in a beaker with 2 ml./g. of diethyl ether, and then filtered them by suction. We first collected fractions of 2 ml./g., four and three times, respectively. The remainder of the extracts was collected in one portion; see Fig. 3. We analyzed fractions of the extracts for lipid and nonlipid materials; the extracted flour was not used for further experiments.

Percolation with Light Petroleum. We percolated 4 kg. of flour in a 7 × 140-cm. column with light petroleum (b.p. 40°–60°C.) at room temperature and under carbon dioxide. We collected in total 2.82 ml./g. of extract, containing dry material to the amount of 0.94% of flour weight. Similar experiments had shown that, after 2.5 ml./g. is collected, the extraction can be considered exhaustive; the extracted material contains only a negligible amount, about 0.01% of flour weight, of nonlipids. We dried the extracted flour at 35°C., finally in a rotary oil pump vacuum; it was mainly used for baking tests and in the description it is indicated by the code P.

Lipids and Nonlipids in Extracts. We weighed 25- or 50-ml. portions of extracts and then evaporated them in vacuum on a water bath. We dried the residues overnight at 85°C. in vacuum, and finally at 102°–105°C. at atmospheric pressure. Usually this final step did not further reduce weight. Only if large amounts of nonlipid material were present was it difficult to attain constant weight. The weight after this procedure measures the sum of lipid and nonlipid material.

Nonlipids were removed from this residue by the procedure of Folch *et al.* (18), modified so as to increase the volume of the upper phase. We added to the residue 6.43 ml. of chloroform, 4.16 ml. of methanol, 3.41 ml. of water, and 12 μmoles of calcium chloride. After equilibration and phase separation, we removed the top layer. We washed the bottom layer twice more with 4 ml. of "pure solvent upper

phase" containing calcium chloride (18). Finally it was evaporated, dried, and weighed as described above. The weight thus obtained is reported as lipids. Heating at 102°–105°C. before the separation did not materially affect the results.

Nonlipids were obtained by difference. As a consequence, negative values are occasionally reported.

Farinograms. These were recorded at 30°C. with 50.0 g. of (extracted) flour (14% m.b.) and 1% of sodium chloride at a consistency of 550 B.U. maximum, or after 10 min. if the maximum came later. We chose this consistency because at higher water contents doughs spread over the mixer wall in the GRL mixer even after moderate mixing times.

Gluten. We mixed doughs from 100 g. of flour, and the composition used for farinograms, in a GRL mixer (17) at 41 r.p.m. for various times at 30°C. in either nitrogen or oxygen. We washed gluten by hand from dough samples of about 15 g. with 0.007*M* phosphate buffer (pH 6.8). Resistance and extensibility were estimated manually. Before weighing, we removed excess of water by pressing the wet gluten ball against the skin of the hand. After weighing the wet gluten, we spread it over filter paper and dried it for 90 min. at 130°C.; then we weighed the dry gluten.

Between mixing and gluten washing we allowed the dough samples various rest periods at 30°C. We washed either three dough samples after rest periods of 45, 60, and 75 min. or seven samples after 15, 30, 45, 60, 75, 105, and 150 min.; we used results of the latter series to study the effect of rest period on gluten yield.

Gluten Phosphorus. We washed gluten from 50 g. of dough after mixing for 10 min. in nitrogen with a 0.1*M* sodium chloride solution; in the last stage of washing, distilled water was used. For purification we dispersed the gluten in 0.0025*M* acetic acid, centrifuged the dispersion for 10 min. at 800 × *g*, and precipitated the gluten from the supernatant by neutralization with sodium hydroxide (19). Phosphorus in the purified dry gluten was determined colorimetrically as molybdenum blue (20).

Baking Tests. These were performed with four samples: the original flour (A), two samples that had been extracted with butanol-water mixtures (J and K), and a sample after extraction with light petroleum (P). To make sure that differences between mixing times were due solely to mechanical action and not to an improver effect of oxygen, we prepared all doughs in nitrogen.

The composition of doughs was as for farinograms; in addition they contained 2% of yeast. The water addition to sample P was 57.4%,

corresponding to a maximum consistency of 550 B.U. in the farinograph. The schedule of the baking tests was as follows:

Mixing: 5, 10, or 15 min.; GRL mixer at 41 r.p.m. Dough temperature after mixing and during fermentation, 28°–30°C.
First fermentation: 40 min., after a mixing time of 5 min.; 35 min., after a mixing time of 10 or 15 min.
Intermediate proof: 25 min.
Tin proof: Duration was adjusted so as to have 250 ml. of carbon dioxide per loaf produced with all samples: samples A and P 60 min.; sample J, 67 min.; sample K, 72 min.
Baking: 20 min. at 230°C.

After mixing, we weighed portions of dough corresponding to 71 g. of flour. Loaf volumes were measured by means of rapeseed displacement.

The slowing down of the rate of carbon dioxide production in samples J and K by 10 and 20%, respectively, cannot be explained by residual butanol. Addition of 0.2% of butanol to flour slowed down the gas production in the second hour (as measured by a S.J.A. fermentation recorder) by only 2%; for 0.4% of butanol this reduction was about 8%. The observed reduction of yeast activity may be due to removal of fermentable carbohydrates by the extraction.

Water in Extracts. We determined water by Karl Fischer titration of a weighed amount of extract after dilution with methanol; the end point was established amperometrically (dead-stop method).

The concentration of lipids in the extracts is so low that addition of iodine to unsaturated compounds during the titrations, if it occurs, cannot cause an error in the water contents of more than 0.05%.

Fat in Flour. We determined fat after acid hydrolysis by Van de Kamer's method as described by Bloksma (9), as well as according to Weibull (21).

Moisture, Nitrogen, and Ash in Flour. Moisture in flour or extracted flour was determined as the loss of weight upon heating for 90 min. at 130°C. in an oven with free ventilation. It is reported on as-is basis.

Nitrogen was determined according to a Kjeldahl procedure.

The residue after ashing for 4 hr. at 600°C. is reported as ash.

General. All solvents used for extractions were freshly redistilled. During extractions and filtrations we prevented access of atmospheric oxygen as well as possible. We used analytical grade reagents throughout.

Results

Water in Extracts. The right-hand part of Table I gives water contents of extracts. Usually an extract contains less water than does the

solvent added; the flour absorbs water from the solvent mixture. Only to dry butanol does the flour lose water.

This looks like an equilibrium between solvent water and (absorbed) flour water. From the amounts of added solvents and extracts removed, and their water contents, water contents of the flour after each extraction step are obtained by elementary arithmetic. In Fig. 1 these flour water contents are plotted against the water content of the corresponding extract. In this graph the "water content of flour" is the water absorbed in addition to possible absorption of butanol and water in the ratio in which they are present in the solvent phase. Points obtained after the water content was increased (open circles) are described by the same line as points obtained after the water content was decreased (solid circles); this supports the idea of an absorption equilibrium, the isotherm (for room temperature) of which is drawn in Fig. 1. Small-scale experiments with other flours of different types gave results that approached very closely the results in Fig. 1. The isotherm in Fig. 1 is probably typical for a variety of flours. Apart from the absence of hysteresis, it resembles the isotherm describing adsorption of water from the gas phase (22).

The water content of the flour increases steeply if the water content of the solvent approaches 18%; this is still 2% lower than the water concentration at saturation, which is 20.1%. In spite of the addition of two-phase mixtures with up to 25% of water in extractions G, H, and I, 17.9% is the highest water content recorded for an extract. If water is added to such a solvent-flour system with a high water content, most of the addition is absorbed by the flour.

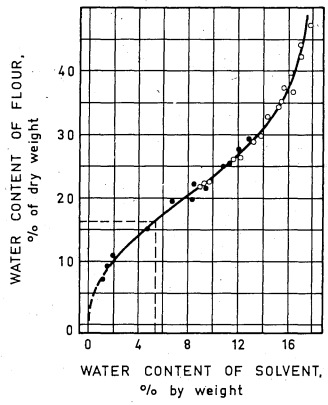

Fig. 1. Absorption isotherm derived from water contents in Table I. Open circles were derived from measurements after transfer of water from solvent to flour, solid circles after transfer from flour to solvent.

Lipids and Nonlipids in Extracts. Table II gives amounts of lipids and nonlipids in extracts and the total amounts removed in a complete stepwise extraction. (In Fig. 9 these total amounts are plotted against the water contents of the extracts.) Lipids extracted increase regularly with increasing water content; there is a relatively sharp increase in nonlipids when the water content of the extract passes beyond 10%.

Relative amounts of nonlipids in the first three steps of extractions B to I are plotted against water contents in Fig. 2. For a constant

TABLE II
AMOUNT OF EXTRACTED LIPID AND NONLIPID MATERIAL

Extraction	Extracted Material								Extracted Material [a]	
	Lipids				Nonlipids				Lipids	Nonlipids
	Step 1	Step 2	Step 3	Step 4	Step 1	Step 2	Step 3	Step 4		
	g./10⁵ g. extract	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*g./10⁵ g. extract*	*% of flour*	*% of flour*
B	452	204	72	29	24	11	4	−1	1.16	0.06
C	520	302	176	92	22	24	10	2	1.27	0.06
D	519	250	128	42	34	39	19	8	1.28	0.14
E	551	293	135	51	45	44	24	7	1.30	0.15
F	586	292	146	65	36	56	31	3	1.40	0.15
G	559	267	113	49	53	62	36	5	1.31	0.20
H	556	248	103	42	60	64	38	6	1.32	0.23
I	578	262	88	38	65	63	57	8	1.37	0.27
J	372		130	43	14		−2	−5	1.29	0.01 [b]
K	400		129	43	68		29	5	1.35	0.24

[a] Total of all four (or three) steps.
[b] If negative values from steps three and four are neglected, 0.03.

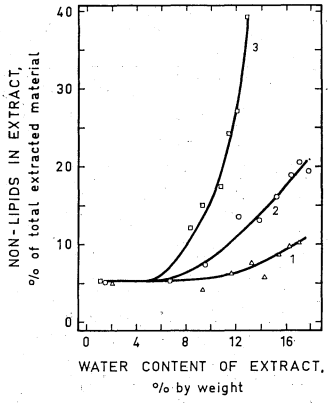

Fig. 2. Relative amounts of nonlipids in extracts of extractions B to I inclusive against water content of extracts. The numbers 1 to 3 refer to the steps within each extraction.

water content, the relative amount of nonlipids increases from step 1 to step 3. The two percolations gave similar results, which are presented in Fig. 3; the relative amount of nonlipids increases when the percolation is continued (Table III). These results correspond to an amount between 1.0 and 1.5% of rapidly extracted lipids in the flour, and a large stock of nonlipids that are slowly extracted by butanol-water mixtures.

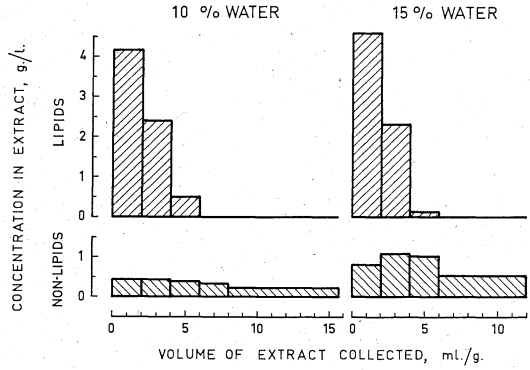

Fig. 3. Lipids and nonlipids extracted by percolation. The integrated amounts are given in Table III as a percentage of flour. The volume of extract is expressed in ml. per g. of flour.

TABLE III

COMPARISON OF YIELDS OF LIPID AND NONLIPID MATERIALS IN A STEPWISE EXTRACTION AND BY PERCOLATION

Water Content of Solvent	Type of Extraction	Solvent: Flour Ratio	Extracted Material [a]		Relative Amount of Nonlipids
			Lipids	Non-lipids	
% by wt.		*ml./g.*	*% of flour*	*% of flour*	*%*
10	Stepwise	7.3	1.26	0.06	5
	Percolation:				
	first 1,000 ml.	4.0	1.31	0.18	12
	total extract	15.6	1.33	0.51	28
15	Stepwise	7.6	1.33	0.17	11
	Percolation:				
	first 1,000 ml.	4.0	1.38	0.38	22
	total extract	11.9	1.41	0.91	39

[a] For the stepwise extraction, smoothed values are read from Fig. 9.

Residual Fat in Extracted Flours. The extraction of lipids from flour is reflected by a decrease in fat content found after acid hydrolysis. This is shown by columns 3 and 4 of Table IV. None of the extractions has completely removed flour lipids. The decrease in fat

content after acid hydrolysis does not quantitatively agree with the amount of lipids found in the extracts, the latter being greater. Mean values of the differences are 0.37% for Van de Kamer's procedure, and 0.25% for Weibull's method; standard deviations of these differences are 0.04 and 0.06%, respectively. Experimental evidence is not sufficient to conclude that results of Weibull's method agree better with the lipid contents of extracts than do figures obtained by Van de Kamer's procedure. The loss of water-soluble hydrolysis products of phospho- and glycolipids partly explains why the acid hydrolysis methods yield decreases that are smaller than the amounts of lipids in the extracts.

The difference between columns 2 and 3 of Table IV roughly corresponds to triglycerides. The results confirm that triglycerides are more easily extracted than are free fatty acids and constituents that yield fatty acids upon acid hydrolysis, such as phosphatides (4,23).

Nitrogen and Ash. In agreement with literature data (11,15,24,25), column 5 of Table IV shows that only a small part of the extracted nonlipid material consists of amino acids and their derivatives, such as peptides, and proteins. The low nitrogen contents of the extracts is confirmed by the nitrogen content of the extracted flours, for which values between 1.74 and 1.77% were found (originally 1.76% N). Even if one assumes a protein factor of 6, not more than 0.07% of amino

TABLE IV

ANALYTICAL DATA ON ORIGINAL AND EXTRACTED FLOURS, AND ON EXTRACTS

FLOUR[a]	FAT AFTER ACID HYDROLYSIS			N IN EXTRACTS[c]	ASH	WATER ADDITION FOR FARINOGRAM	GLUTEN-P[d]
	Van de Kamer		Weibull: Total				
	Fatty Acids[b]	Total					
	% of flour	*% of flour*	*% of flour*	*% of flour*	*% of flour*	*ml./100 g. flour*	*p.p.m.*
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*
A	0.78	1.30	1.35	....	0.44	54.2	810
B	0.40	0.46	0.32	0.005	0.45	56.6	530
C	0.36	0.39	0.33	0.006	0.45	55.6	460
D	0.34	0.37	0.29	0.009	0.42	53.0	440
E	0.32	0.35	0.29	0.010	0.42	52.8	400
F	0.30	0.36	0.29	0.010	0.41	52.9	360
G	0.30	0.35	0.26	0.009	0.41	52.4	360
H	0.30	0.33	0.26	0.011	0.41	51.7	380
I	0.29	0.33	0.28	0.012	0.40	51.7	340
J	0.36	0.40	0.30	....	...	54.6	...
K	0.32	0.34	0.29	....	...	52.6	...

[a] The original flour is A. Extracted flours are denoted by the same letter as the extraction from which they result.
[b] Free fatty acids and fatty acids from compounds, such as phosphatides, that are hydrolyzed by hot acids.
[c] Determined in a proportional mixture of all four extracts.
[d] μg. per g. gluten (dry basis).

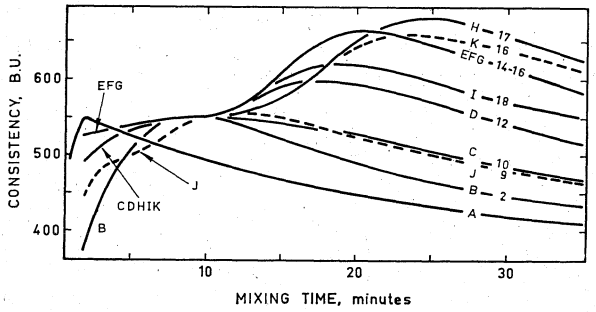

Fig. 4. Farinograms of the original (A) and extracted (B to K) flours. Numbers indicate approximate water contents of extracts.

acids and their derivatives is removed in extraction I.

Figures for ash in column 6 shows decreases of mineral matter up to 0.04%.

In extraction I, amino acids, etc., and mineral matter do not account for more than 0.11% of the flour, whereas in total 0.27% nonlipids are removed. About 60% of the nonlipids cannot be explained as originating from these classes of compounds.

Farinograms. Extractions with the two lowest water contents of the solvent result in increased water absorption (column 7 of Table IV). Further, the water absorption of the extracted flour decreases with increasing water content of the solvent, ending up well below the original water absorption. Farinograms in Fig. 4, except those of samples A to C inclusive and J, show a flat part at about 10 min. followed by a new increase in consistency leading to a maximum after 20 min. or later. Similar shapes of farinograms have been observed after extraction with acetone-water mixtures (16). This peculiar shape was most pronounced with flours H and K, although in extraction I the water content was still higher than in extraction H.

Effect of Rest Period on Gluten Yield. Figure 5 shows two examples of the increase in dry gluten yield with longer rest period. We observed this effect of rest period in all cases that gluten yield was below normal. It was not found with flours A to C; with the other flours the effect gradually disappears with longer mixing time.

Effect of Mixing Time on Gluten Yield. For a study of the effect of mixing time on gluten yield, the rest period must be kept constant. Gluten yields after 60 min. of rest were obtained either as the height at 60 min. of a smooth curve through experimental points as in Fig. 5, or as the mean value of gluten yields after 45, 60, and 75 min. of rest.

Figure 6 shows dry gluten yields as dependent upon mixing time after various extractions. The retardation of gluten development re-

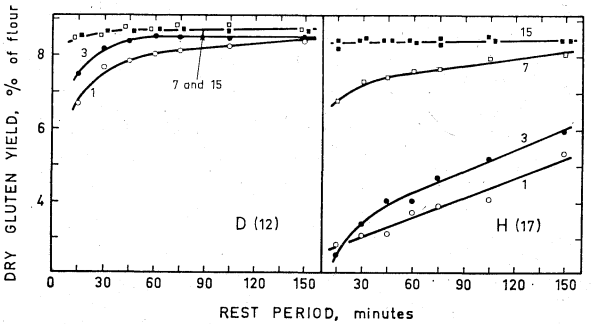

Fig. 5. Dry gluten yield from extracted flours D and H (12 and 17% of water in extracts, respectively) as a function of rest period (between mixing and gluten washing). Labels indicate mixing times in min.

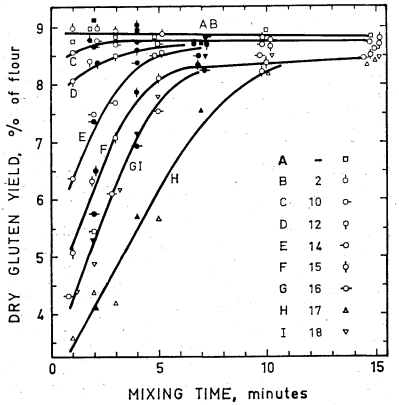

Fig. 6. Dry gluten yield as a function of mixing time. Rest period was 60 min. Approximate water contents of extracts are tabulated with the signs and code letters. Open signs refer to mixing in nitrogen, solid signs in oxygen.

ported earlier (16) is confirmed, although the solvent and mixer are different. This retardation is more marked the higher the water content of the solvent; the only exception from this rule is, as with the farinograms, the order of flours H and I. All samples attain a normal or nearly normal gluten yield after 15 min. of mixing.

Table V gives gluten yields after longer mixing times than in Fig. 6. They remain at the normal level of 8.5 to 8.9% even after 40 min. of mixing, provided that it is done in nitrogen. In oxygen, however, gluten yields fall sooner or later. This dependence of gluten breakdown upon the mixing atmosphere is contrary to the insensitivity of gluten development to it; in Fig. 6 no difference can be detected between nitrogen and oxygen points. That gluten breakdown requires oxygen confirms the earlier hypothesis that it is due to an oxidation process (16); the second part of that hypothesis – that thiol groups in

TABLE V

DRY GLUTEN YIELD AS DEPENDENT ON MIXING ATMOSPHERE AND MIXING TIME[a]

MIXING ATMOSPHERE	MIXING TIME	FLOUR[b]								
		A	B	C	D	E	F	G	H	I
	min.									
Nitrogen	25	8.8	8.6[c]	8.5[c]	8.7	8.6	8.6	8.5	8.6	8.6
	40	8.8				8.9	8.5	8.6	8.5	8.6[c]
	45				8.6[c]					
Oxygen	16				8.8	8.9				8.5
	20	8.6	8.6	8.6	4.6		8.6	8.6	8.1	4.2
	21					4.3				
	24								3.0	
	26			7.6			3.6	3.0		
	35	5.2[c]					1.2[c]	0.0	0.0	0.0

[a] Time interval between mixing and gluten washing, 60 min.
[b] See footnote a in Table IV.
[c] Dough spread over mixer wall.

doughs from defatted flour are oxidized more rapidly than in normal doughs – has since been disproved (9,25). Gluten yields after long mixing times in oxygen are not sufficiently regular for conclusions to be drawn as to the effect of the water content of the solvent during extraction.

Gluten Properties. The ratio wet gluten yield to dry gluten yield is a measure of its water-binding capacity. For normal flours this ratio is about 3. During development, mixing time and rest period generally affected the water-binding capacity in a way similar to that of gluten yield. The wet:dry ratio was higher with longer rest period as well as with longer mixing time. The latter effect is shown in Fig. 7. Only flours A and B show a maximum after short mixing times. The effect

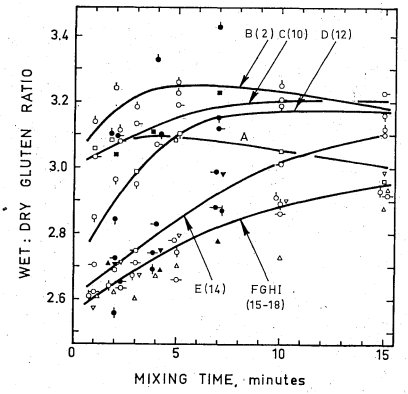

Fig. 7. Water-binding capacity of gluten as a function of mixing time. Rest period was 60 min. Numbers in parentheses indicate approximate water contents of extracts. The signs have the same meaning as in Fig. 6.

of the water content during extraction upon the water-binding capacity of the gluten is generally parallel to the effect on water absorption of the flour as measured in the farinograph (see column 7 of Table IV); however, no differences between flours F through I can be detected.

After long mixing times in nitrogen, all flours gave normal values, between 2.8 and 3.1, for the wet:dry gluten ratio. After long mixing times in oxygen, this ratio increased up to values between 3.7 and 4.7 before the gluten structure broke down.

Gluten from extracted flours had a granular appearance, except after the longest mixing times. The extractions decreased both resistance and extensibility of the gluten. This effect was slight with flours B to D and was marked with F to I, which group originates from extractions with a high water content. Flour E had an intermediate position: after mixing times of 5 min. or more it behaved as if belonging to the first group; after shorter mixing times it had the characteristics of the second group. A longer rest period led to increases in resistance and extensibility. After very long mixing times the resistance decreased, irrespective of the mixing atmosphere; under these conditions the extensibilities varied widely.

We determined gluten prosphorus to detect changes in the amount of phosphatides bound to the protein. Column 8 of Table IV shows a regular decrease of the phosphorus content with increasing water content of the extracting solvent. If the decrease of gluten P is a measure of the extracted lipid P, then one can calculate the P content of the lipids removed in the various extractions; for comparison, the P content of lecithin is 4.0%. The P content of the extracted lipids, calculated on the basis of this assumption, increases from 0.21% for extraction B to 0.30% P in the lipids removed in extraction I. One can also estimate the "differential" P content of the lipid fraction that was extracted by procedure I but was not removed by extraction B; for this we calculated 0.8%. Apparently, only a small part even of this latter fraction consists of phosphatides that are bound to gluten proteins or would be bound to them upon mixing.

Baking Tests. These were undertaken to obtain confirmation of the results obtained by gluten washing. The extracted flours, J and K, which were used in test baking gave dry gluten yields of 8.5 and 6.2%, respectively, after 3 min. of mixing and 60 min. of rest. These yields, together with the water contents of the extracts (Table I), the amounts of extracted lipids and nonlipids (Table II), and the water absorptions (Table IV) justify the conclusion that these flours are considered similar to flours C and G, respectively. (This conclusion is also illus-

trated in Fig. 9, in which some of these quantities are plotted against the water content of extracts.)

Results of baking tests are shown in Fig. 8. The most obvious con-

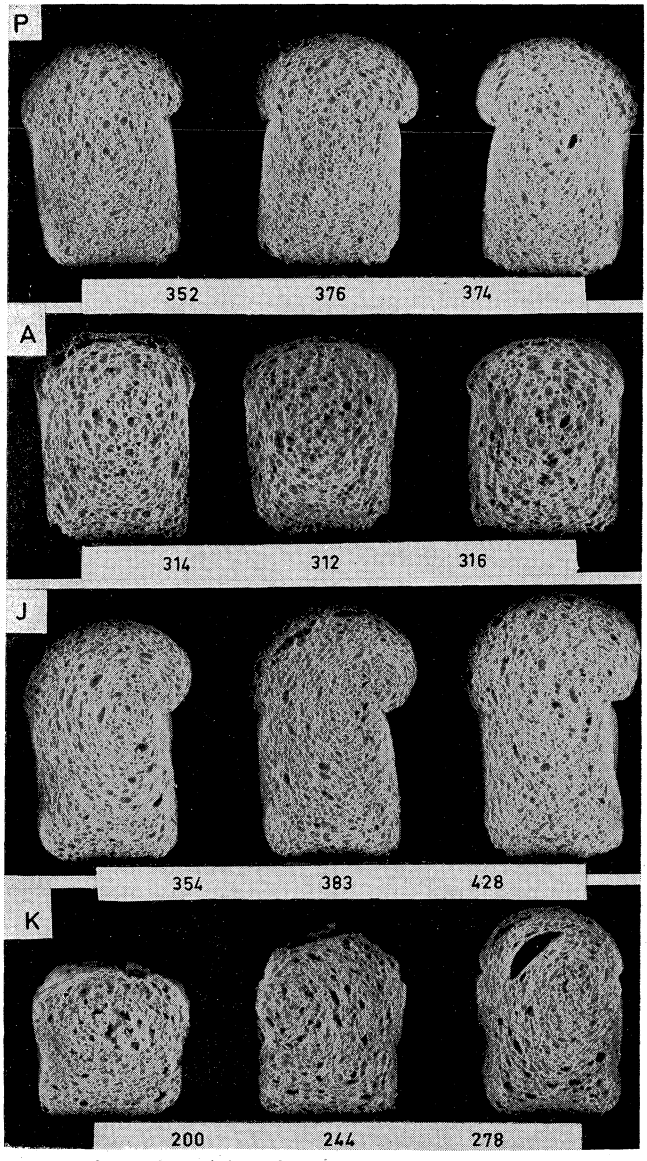

Fig. 8. Loaves from the original flour (A), the same flour after extraction with butanol-water mixtures with low (J) and high (K) water content, and after extraction with light petroleum (P). Loaf volume in ml. is given under each cross-section. Mixing time (left to right): 5, 10, and 15 min.

clusion is that, whereas extraction by butanol with a high water content (flour K) affects baking quality adversely, extraction with a moderate water content in the solvent (flour J) is beneficial. This latter extraction has results comparable to those of extraction by light petroleum (flour P). In addition to higher volumes, flours P and J produced loaves with much finer crumb structure than does the original flour A.

Loaf volumes from flour A are, like gluten yields, only slightly dependent upon mixing time. Both flours J and K show a considerable increase in loaf volume with increasing mixing time. For flour J this was not quite expected because of gluten yields from flour C; for flour K this increase completely agrees with gluten yields from flour G. In previous (unpublished) experiments it was found that, after extraction with light petroleum, gluten yield is not more dependent upon mixing time than with the original flour; this is confirmed by the rather small effect of mixing time on the loaf volumes from flour P.

Discussion

Water Content during Extraction, Extracted Lipids and Nonlipids, and Gluten Development. The central theme of this study comprises the relationship among the water content during extraction, the amounts of lipids and nonlipids extracted, and gluten yield after various mixing times. For purposes of discussion, some of the latter quantities are plotted against the water content in Fig. 9; the water content of the extract that contained most water (first extract of B, J, and K, and second extract of C to I) was used to characterize a complete extraction. Gluten yields after 3 min. of mixing and 60 min. of rest are smoothed values from Fig. 6.

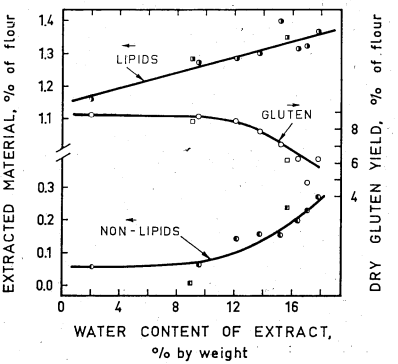

Fig. 9. Amounts of extracted lipids and nonlipids and dry gluten yield against the water content of that extract of each extraction that contained most water. Circles: extractions B to I; squares: extractions J and K. For details see text.

The plots of extracted lipids and nonlipids against the water content during extraction demonstrate that the "cleanness" of the extraction rapidly deteriorates if the water content of the extracts surpasses 10%. By increasing this water content one can obtain a slightly higher lipid yield, though at the expense of a much heavier contamination by nonlipids if the water content exceeds 10%. The data of Fig. 2 suggest that for a clean extraction of lipids the number of steps should be limited. This conclusion is corroborated by the results obtained by percolation, which may be considered as a very large number of infinitesimal steps; large amounts of nonlipids are extracted by prolonged percolation (see Fig. 3). A comparison of stepwise extractions and percolations with equal water contents is made in Table III. These considerations provide a basis for selecting the extraction procedure and water content that best fit a particular purpose.

The water content during extraction also affects gluten development. A plot of dry gluten yield after 3 min. of mixing and 60 min. of rest against the water content shows a marked decline of gluten yield at a water content of 10%. It coincides with the increase in extracted nonlipids. There is no corresponding change in the amount of extracted lipids or gluten-bound phosphatides. This raises the question whether the retardation of gluten development after extraction by butanol-water mixtures with more than 10% of water might be due to the removal of nonlipid constituents rather than of lipids.

Nature of Extracted Nonlipids. The nature of these nonlipid constituents has not been studied. From nitrogen and ash determinations we concluded that only less than 40% of the extracted nonlipids could consist of amino acids and their derivatives, and of mineral matter. This directs our attention to the carbohydrate fraction. Glass found "surprisingly large amounts" of mono- and disaccharides in extracts obtained with "water-saturated butanol-1" (27). In our opinion, changes in dough properties can better be explained by the hypothesis that water-soluble pentosans are removed by solvent mixtures with high water content.

The changes in dough properties referred to are the observed reduction of water-binding capacity of both flour and gluten and increase in peak time of the farinogram. Water-soluble pentosans, though not as important for the loaf volume as is the protein fraction, increase the water absorption and speed dough development (28–31). Moreover, pentosanases reduce the volume of loaves obtained from natural (32) or reconstituted (33) flours.

Effect of Mixing Time and Rest Period. The effect of mixing time on gluten yield from extracted flours had been found in previous

work (16). Then the rest period was not varied; consequently, its effect was not observed.

From the slopes of graphs of gluten yields and wet:dry weight ratios against mixing times and rest periods, one can estimate how long a rest period is equivalent to mixing for a unit of time. For both properties, values in the order of 30 units are found. The physical changes concerned proceed 30 times as fast during mixing, as in a resting dough. This ratio agrees fairly with the values of 30 to 60 found earlier for chemical reactions in the same mixer at a higher speed (34).

The author cannot explain why the physical processes contributing to gluten development would be retarded by extraction of lipids. If lipid layers covering proteins are removed, one would expect an increase in the rate of swelling of the protein. An explanation based on removal of nonstarch polysaccharides is more promising. In a normal dough, these constituents may initially absorb most of the water (35), which can be transferred to the protein as the gluten network develops. If so, doughs without them would during the initial stage of mixing contain too much water for the protein, and run short of water when gluten development approaches completion. The shape of the farinograms is in agreement with this hypothesis.

The increase in water-binding capacity of gluten before its complete breakdown after prolonged mixing in oxygen might be interpreted as follows. When placed in a suitable solvent, a cross-linked polymer swells. The extent of swelling increases with a decreasing number of cross-links (36). If we replace the cross-linked polymer with the gluten proteins and substitute water for the solvent, the increased water-binding capacity suggests a fission of cross-links between protein molecules. Such a fission could be the beginning of a further breakdown of the cross-linked structure, eventually leading to reduction of gluten yield. We have considered the possibility that the fission of cross-links is an oxidative cleavage of disulfide bonds; stress during mixing could make these bonds more reactive (37). However, exploratory disulfide titrations in doughs from flour I after mixing in oxygen for 30 min. did not give any indication of a decrease of the number of disulfide bonds during mixing.

Gluten Washing and Baking Tests. There is some parallel between results of gluten washing and test baking: after extraction with butanol-water mixtures, loaf volumes increase considerably with longer mixing time, whereas with the original flour and the flour after extraction with light petroleum, mixing time has no effect, or only a small effect. Nevertheless, there are also deviations. Flour C shows only slight retardation of gluten development, whereas in baking

tests with the corresponding flour J the effect of mixing time is obvious. After 15 min. of mixing, differences between gluten yields have become negligible; differences in loaf volumes are still large. Possibly these deviations could be explained if baking demands more from the gluten properties than does gluten washing. Then the increased loaf volumes of extracted samples J and P could be explained by an improvement in gluten properties, which is not reflected by the results of washing.

In any case, it is remarkable that high loaf volume is obtained after an extraction with water content under the critical amount of 10%, whereas with water content of more than 10%, baking quality apparently is destroyed by the extraction. Though this destruction cannot be explained completely in terms of gluten development, it is most probably due to the same process as is the retardation of this development.

Water Content of Extracts. As a result of absorption of water by flour from butanol-water mixtures, the solvent phase during extraction will generally be poorer in water than the added solvent. Only if the solvent contains less water than is in equilibrium with the flour-water content will the solvent extract water from the flour. The dashed lines in Fig. 1 show that for a flour with 14.0% moisture (as-is basis; that is, 16.3% on dry basis) this will occur with solvent mixtures containing less than 5.5% water. The actual water content of the solvent during extraction with "water-saturated butanol-1," as described in the literature, has always been lower than the content at saturation, that is, 20.1%. The difference depends upon the solvent:flour ratio. The water content of the extract can be calculated from this ratio and the absorption isotherm in Fig. 1. Results of such calculations are plotted in Fig. 10. Curve 1 of this graph shows the water content of a first extract if the flour originally contained 14.0% moisture. Curves 2 to 4 describe the water content of subsequent extracts obtained by replacing the preceding one with an equal weight of fresh solvent. In the calculations it is assumed that at each replacement 1.2 g. of solvent is retained per g. of flour (with 14% moisture). As a consequence, the lines for all subsequent extracts converge at point Q, the abscissa of which is 1.2.

In most extractions with "water-saturated butanol-1," described in the literature, the solvent:flour ratio was between 1.7 and 2.5 g./g.; occasionally the extraction was repeated up to six times with equal or smaller quantities of solvent. Figure 10 shows that water contents of these extracts generally have been between 14 and 17%: they have been well above the critical water content of 10% discussed above. Therefore, the properties of the extracted flour or gluten samples are

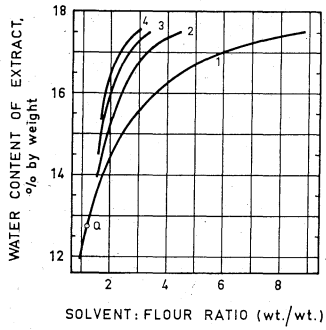

Fig. 10. Water content of extracts as a function of the solvent:flour ratio, as derived from the isotherm in Fig. 1. Lines 1 to 4 refer to subsequent extracts obtained by replacing the supernatant with fresh solvent. Details are given in the text.

expected to be similar to those of the present samples D to I and K. In a few cases extractions with dry butanol are reported (38,39); then properties of the extracted flour will be like those of the present sample B.

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