Relationship Between Lipid Content and Composition and Loaf Volume of Twenty-Six Common Spring Wheats[1]

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ABSTRACT

Flours of 26 common spring wheats grown at three locations in western Canada and whole grain meal of the samples from one location were subjected to various lipid analyses in an attempt to identify a constituent(s) that can be used to predict breadmaking quality. Free lipids were extracted with *n*-hexane and bound lipids with water-saturated *n*-butanol after the *n*-hexane extraction. Free lipids were fractionated by silicic acid column chromatography into nonpolar (NL), glyco (GL) and phospho (PhL) lipids. The sum of GL and PhL was defined as polar lipids (PL). Highly significant correlations were obtained between loaf volume and various parameters derived from lipid content and composition. Linear regression equations based on these relationships and multiple linear regression equations in which loaf volume was expressed as a function of protein content, lipid content, and lipid composition parameters were developed for estimating loaf volume. The best estimation was obtained from the equation with protein content, NL/PL, and NL/GL as independent variables. For screening of plant breeders' populations, a good estimation of loaf volume could be obtained from either NL/PL or NL/GL determined on whole grain meal.

Besides the proteins, flour lipids also contribute significantly to breadmaking quality (Chung et al 1978; Chung and Pomeranz 1981; Daftary et al 1968; MacRitchie 1977, 1981). Several researchers have attempted to find a quantitative relationship between lipid data and loaf volume (Berger 1982; Fisher et al 1964, 1966; Pomeranz et al 1966). Chung et al (1980) developed procedures for lipid extraction and fractionation that produced data ranking hard red winter wheat (HRW) flours in order of their breadmaking potential. Later, Chung et al (1982) reported correlations between loaf volume and content of several lipid classes for flours of U.S. hard red winter wheat varieties.

Whereas many investigations have been carried out on the relationship between results of various technological tests used to measure breadmaking potential and flour constituents for bread wheats grown in Canada (Baker and Kosmolak 1977; Bushuk et al 1969; De LaRoche and Fowler 1975; Fowler and De LaRoche 1975a,b; Orth et al 1972), none of these studies included flour lipids.

In this study, lipid data for flours of 26 common spring wheats grown in three locations in western Canada were obtained and analyzed statistically to investigate possible relationships with loaf volume. Lipid data were also obtained for whole grain meals of these varieties grown at one of the three locations; these data were compared with flour data and statistically correlated with loaf volume.

MATERIALS

The wheat samples used were from the 1981 Uniform Quality Nursery grown in western Canada at Swift Current, Regina, and Lethbridge by A. B. Campbell of the Agriculture Canada Winnipeg Research Station. Relevant technological data (Table I) indicate that the samples covered a broad range of breadmaking quality (loaf volumes ranged from 515 to 1,055 cm^3).

All chemicals used were of analytical or reagent grade.

METHODS

Preparation of Flour and Grain Meal

Wheat grain was stored at 4° C until milled into flour or ground into meal. Flour was milled on a Buhler experimental mill, type MLU-202 after 24 hr of tempering to 15.5% moisture. The wheats were ground into whole grain meal on a U/D cylone sample mill to pass a screen with 0.5-mm openings.

Analytical Methods

Moisture, protein (N × 5.7), and ash contents were determined by AACC method 44-15A, 46-12, and 08-01, respectively (AACC 1983). Loaf volumes (RLV) were determined by rapeseed displacement of loaves baked by the GRL remix baking test (Kilborn and Tipples 1981). The baking test was replicated three times; means of replicates are reported.

The variation in RLV with location for each variety (caused in part by natural variation in protein content resulting from variations in environment and soil fertility among the three locations) can be reduced by normalizing the volumes to a protein content equal to the average for all samples. For each sample, normalized loaf volume (RLVc) was obtained by multiplying RLV by the ratio of measured protein content divided by 13.2, the average flour protein content. This calculation assumes that RLV varies directly with protein content for the range of protein contents covered by the samples.

Extraction and Fractionation of Lipids

n-Hexane-soluble (free, FL) lipids and water-saturated *n*-butanol soluble (bound, BL) lipids after extraction of FL were extracted from 5 g (db) of flour or whole grain meal using the procedure of Bekes et al (1983a). The sum of FL and BL was defined as total lipids (TL). FL was fractionated by silicic acid (Florisil) column chromatography into nonpolar lipids (NL), glycolipids (GL), and phospholipids (PhL) by sequential elution with chloroform, acetone, and methanol. The dimensions of the column were 4 × 120 mm. Florisil (100–120 mesh) was from Fisher Scientific Co. (Toronto, Canada). Polar lipid (PL) was calculated as the sum of GL and PhL.

Lipid extractions and fractionations were replicated three times and the values were averaged. Average total recovery of lipids from the Florisil column was 98% (94.7–98.8%). All lipid results are reported in milligrams per 100 g, dry basis. Coefficients of variation for determination of lipid contents were in the range of 5–9%; GL had the highest C.V. and FL had the lowest.

Statistical Analyses

Analyses (correlation matrix, multivariate analysis, stepwise multiple linear regression) were done using the SAS statistical program package (SAS Institute 1982) on the University of Manitoba mainframe Amdahl computer.

RESULTS AND DISCUSSION

Results of Breadmaking Quality Tests

Protein contents of the samples varied from 11.0 to 16.9% for

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TABLE I
Wheat Samples and Technological Data[a]

Sample	Kernel Type[c]	Swift Current				Regina				Lethbridge			
		Flour Yield (%)	Protein		RLV[b] (cm^3)	Flour Yield (%)	Protein		RLV (cm^3)	Flour Yield (%)	Protein		RLV (cm^3)
			Grain (%)	Flour (%)			Grain (%)	Flour (%)			Grain (%)	Flour (%)	
Benito	HRS	73.0	14.8	14.4	895	72.4	14.7	14.0	855	73.7	14.0	13.1	745
Bulbul	HWS	...	...	...	...	67.1	11.9	10.8	690	72.4	11.0	10.3	595
Chester	HRS	73.6	15.0	14.5	760	72.8	14.4	13.5	875	74.6	13.6	12.7	850
Columbus	HRS	73.8	15.1	14.6	800	70.1	14.6	14.0	780	75.5	14.5	13.8	835
Cook	HWS	75.3	14.4	13.8	955	70.1	13.4	12.5	870	76.3	12.9	12.0	825
Glenlea	HRS	73.7	14.5	14.0	525	69.3	14.3	13.3	540	73.5	12.8	12.2	595
H-Ra^{2}F$_2$	SWS	69.4	14.9	14.2	570	64.0	14.2	12.8	660	68.7	12.6	11.7	530
James	HRS	76.6	14.6	14.3	775	73.1	14.8	14.0	935	76.4	14.0	13.0	625
JIT-35-2L	HRS	73.9	14.1	13.5	720	69.6	16.9	15.7	1,000	73.5	14.0	13.3	780
Kenya 321	SWS	67.1	14.2	12.5	570	68.0	13.0	11.8	655	69.1	13.2	11.7	640
Len	HRS	75.1	14.9	14.1	825	68.2	14.7	13.5	655	73.5	14.4	13.0	750
Manitou	HRS	74.3	14.3	13.8	820	73.6	14.4	13.7	850	74.8	13.7	12.9	730
McKay	HRS	74.4	13.6	12.8	585	70.4	12.8	11.5	555	75.1	12.1	11.1	685
M.J. INTA	HRS	73.1	15.1	14.4	530	65.3	14.3	12.9	585	71.8	14.4	13.0	610
Mn 70170	HRS	75.5	14.5	14.0	760	71.5	13.2	12.4	790	76.1	12.8	12.0	675
NAPB NHS 183-74	HRS	75.1	13.6	13.2	870	72.0	13.5	13.0	860	76.4	12.1	11.5	750
NAPB NHS 1001-75	HRS	75.3	13.9	13.3	770	69.6	12.3	11.4	775	75.6	12.5	11.4	660
ND 560	HRS	75.0	14.8	14.4	685	70.2	15.1	14.0	745	74.8	14.4	13.3	720
ND 563	HRS	71.6	15.5	15.0	515	68.6	15.0	13.9	635	70.5	14.7	13.8	605
Neepawa	HRS	72.1	15.4	14.7	860	72.0	14.3	13.6	840	72.5	14.0	13.2	750
Pavon sib.	HWS	72.5	15.4	14.0	925	66.8	13.1	12.0	830	72.4	13.1	12.1	775
RL2520//Tc*6/KF	HRS	74.2	14.4	13.9	715	70.4	13.9	13.3	730	76.1	13.9	13.2	800
Saric 70/Np	HRS	73.5	16.0	15.7	965	70.5	16.7	15.8	1,055	73.8	15.8	15.4	800
SD 2355	HRS	73.5	14.5	14.3	940	69.8	14.6	13.6	890	73.3	15.1	13.7	735
Tesopaco sib.	SRS	70.3	13.1	12.5	535	66.6	12.6	11.8	650	70.5	11.3	10.4	665
Tobary/Romany	SRS	70.8	13.4	12.6	750	67.6	12.5	11.4	715	72.0	12.0	11.1	660

[a] All data reported on 14% mb.
[b] RLV = Remix loaf volume.
[c] HRS = hard red spring, HWS = hard white spring, SWS = soft white spring, and SRS = soft red spring.

TABLE II
Lipid Content (mg/100 g, db) of Flours from Wheats Grown at Three Locations

Sample	Kernel Type[b]	Swift Current					Regina					Lethbridge				
		Lipids		Free Lipids[a]			Lipids		Free Lipids			Lipids		Free Lipids		
		Free	Bound	NL	GL	PhL	Free	Bound	NL	GL	PhL	Free	Bound	NL	GL	PhL
Benito	HRS	1,268	745	1,087	89	92	1,096	705	945	80	71	1,160	638	1,010	69	81
Bulbul	HWS	...	...	...	...	...	753	735	640	65	48	678	717	588	52	38
Chester	HRS	1,034	690	913	60	61	986	635	837	87	62	924	700	781	83	60
Columbus	HRS	940	694	814	64	62	1,092	655	956	78	58	1,162	630	1,014	78	70
Cook	HWS	1,106	645	906	102	98	996	675	827	100	69	1,103	717	923	112	68
Glenlea	HRS	1,063	750	965	50	48	920	680	832	39	49	870	647	780	49	49
H-Ra^{2}F$_2$	SWS	807	617	731	34	42	811	600	722	44	45	769	589	690	38	41
James	HRS	1,018	720	893	61	64	998	740	836	87	75	1,060	730	941	55	64
JIT-35-2L	HRS	1,034	600	911	65	58	1,152	714	984	91	77	989	705	858	74	57
Kenya 321	SWS	1,070	714	956	50	65	1,044	745	918	63	63	820	700	724	50	46
Len	HRS	1,319	735	1,146	79	94	1,256	725	1,116	65	75	931	685	819	67	45
Manitou	HRS	1,182	720	1,021	74	87	1,393	782	1,215	110	68	1,110	745	934	72	64
McKay	HRS	848	548	756	45	47	884	579	791	44	49	822	741	705	53	64
M.J. INTA	HRS	800	714	726	33	41	841	720	754	41	46	967	756	865	48	54
Mn 70170	HRS	1,290	700	1,134	74	82	1,048	798	899	76	73	930	645	812	61	57
NAPB NHS 183-74	HRS	1,335	685	1,153	85	97	1,172	695	990	96	86	1,089	654	925	97	67
NAPB NHS 1001-75	HRS	1,056	735	919	70	67	882	745	730	78	74	977	700	844	66	67
ND 560	HRS	1,224	710	1,088	65	71	1,175	735	1,035	74	66	1,058	700	938	65	55
ND 563	HRS	1,031	689	945	42	44	982	650	879	48	55	851	714	766	40	45
Neepawa	HRS	1,265	739	1,100	83	82	1,108	718	946	83	79	1,144	725	1,000	72	72
Pavon sib.	HWS	925	784	769	94	62	634	617	519	60	55	1,287	617	1,100	109	78
RL2520//Tc*6/KF	HRS	1,138	745	1,011	65	62	1,150	750	990	82	78	1,207	780	1,044	87	76
Saric 70/Np	HRS	1,184	738	1,021	87	76	1,305	728	1,099	109	97	1,440	710	1,278	76	86
SD 2355	HRS	1,385	760	1,176	104	105	1,334	794	1,126	113	95	1,298	748	1,140	76	80
Tesopaco sib.	SRS	686	696	621	33	32	875	650	772	54	49	837	531	726	63	48
Tobary/Romany	SRS	1,016	750	881	79	56	975	753	835	67	73	958	714	834	59	65

[a] NL = nonpolar lipids, GL = glycolipids, and PhL = phospholipids.
[b] HRS = hard red spring, HWS = hard white spring, SWS = soft white spring, and SRS = soft red spring.

grain and 10.3 to 15.8% for flour. As expected, the protein content of flour was significantly correlated to that of grain ($r = 0.967$, 0.01 level). Protein content varied markedly with location. Milling yields ranged from 64.0 to 76.6% (average, 73.4%). Flour ash contents (data not shown) varied from 0.35 to 0.56% (average, 0.41%).

RLV varied from 515 cm^3 to 1,055 cm^3. RLV was positively correlated with protein content ($r = 0.425$). Normalization of the loaf volume results to the average protein content narrowed the range of values (RLVc = 452–916 cm^3). However, multivariate analysis of the RLVc data indicated that normalization to average protein content did not eliminate entirely the effect of location on loaf volume. Obviously other factors that depend on location contribute to loaf volume.

Grain and Flour Lipid Results

Comparison of lipid contents of whole grain and flour for the Swift Current samples (Tables II and III) showed that the TL content of the grain (average, 3,006; reported in mg/100 g, db) was much higher than that of flour (average, 1,786). Most of the difference in TL results from the much higher content of FL in grain. FL for grain varied from 1,317 to 2,800 (average, 2,084) and for flour from 686 to 1,385 (average, 1,081).

BL varied from 616 to 1,222 (average, 921) for grain and 548 to 784 (average, 705) for flour. Also, the FL/BL ratios for grain (average, 2.32) were substantially higher than those for flour (average, 1.53).

Regarding the subfractions of free lipids, NL for grain (average, 1,922) was much higher than for flour (average, 946). The average contents of PL and PhL were somewhat higher in grain than in flour, whereas free GL was higher in flour than in grain. Comparative values were 162 and 136 for PL, 60 and 69 for GL, and 98 and 68 for PhL.

Because of the large difference between average grain and flour values for NL, the average NL/PL and NL/GL ratios differed by a factor of approximately two. Average values for grain were 12.58 and 34.78, and for flour were 7.36 and 14.84. Similar variability was obtained by Chung et al (1982) for the HRW varieties that they analyzed. They explained their results on the basis of different lipid contents and compositions of endosperm, germ, and aleurone parts of the wheat grain. Our average results for FL, NL, and NL/PL are systematically higher than those of Chung et al (1982). HRW wheat flours contained substantially higher free PL than our spring wheat

TABLE III
Lipid Content (mg/100 g, db) of Whole Grain Meals of Wheat Grown at Swift Current

Sample	Kernel Type[b]	Lipids		Free Lipids[a]		
		Free	Bound	NL	GL	PhL
Benito	HRS	2,514	749	2,298	84	132
Chester	HRS	2,117	848	1,976	51	90
Columbus	HRS	1,883	1,020	1,735	71	77
Cook	HWS	1,917	1,050	1,686	89	141
Glenlea	HRS	2,200	837	2,085	43	72
H-Ra^{2}F$_2$	SWS	1,317	1,010	1,230	30	57
James	HRS	1,748	948	1,592	51	103
JIT-35-2L	HRS	2,038	900	1,890	65	83
Kenya 321	SWS	1,986	937	1,846	42	98
Len	HRS	2,538	974	2,337	74	127
Manitou	HRS	2,408	914	2,216	67	125
McKay	HRS	1,636	967	1,531	41	65
M.J. INTA	HRS	1,700	741	1,607	28	65
Mn 70170	HRS	2,800	835	2,606	62	132
NAPB NHS 183-74	HRS	2,356	616	2,154	87	115
NAPB NHS 1001-75	HRS	1,900	867	1,742	59	76
ND 560	HRS	2,434	1,222	2,275	56	102
ND 563	HRS	1,896	796	1,794	38	64
Neepawa	HRS	2,287	1,018	2,091	70	126
Pavon sib.	HWS	1,847	710	1,663	78	106
RL2520//Tc*6/KF	HRS	1,985	1,100	1,827	56	102
Saric 70/Np	HRS	2,268	940	2,065	75	128
SD 2355	HRS	2,750	1,050	2,539	71	140
Tesopaco sib.	SRS	1,474	848	1,404	27	43
Tobary/Romany	SRS	2,043	1,142	1,881	76	86

[a] NL = nonpolar lipids, GL = glycolipids, and PhL = phospholipids.
[b] HRS = hard red spring, HWS = hard white spring, SWS = soft white spring, and SRS = soft red spring.

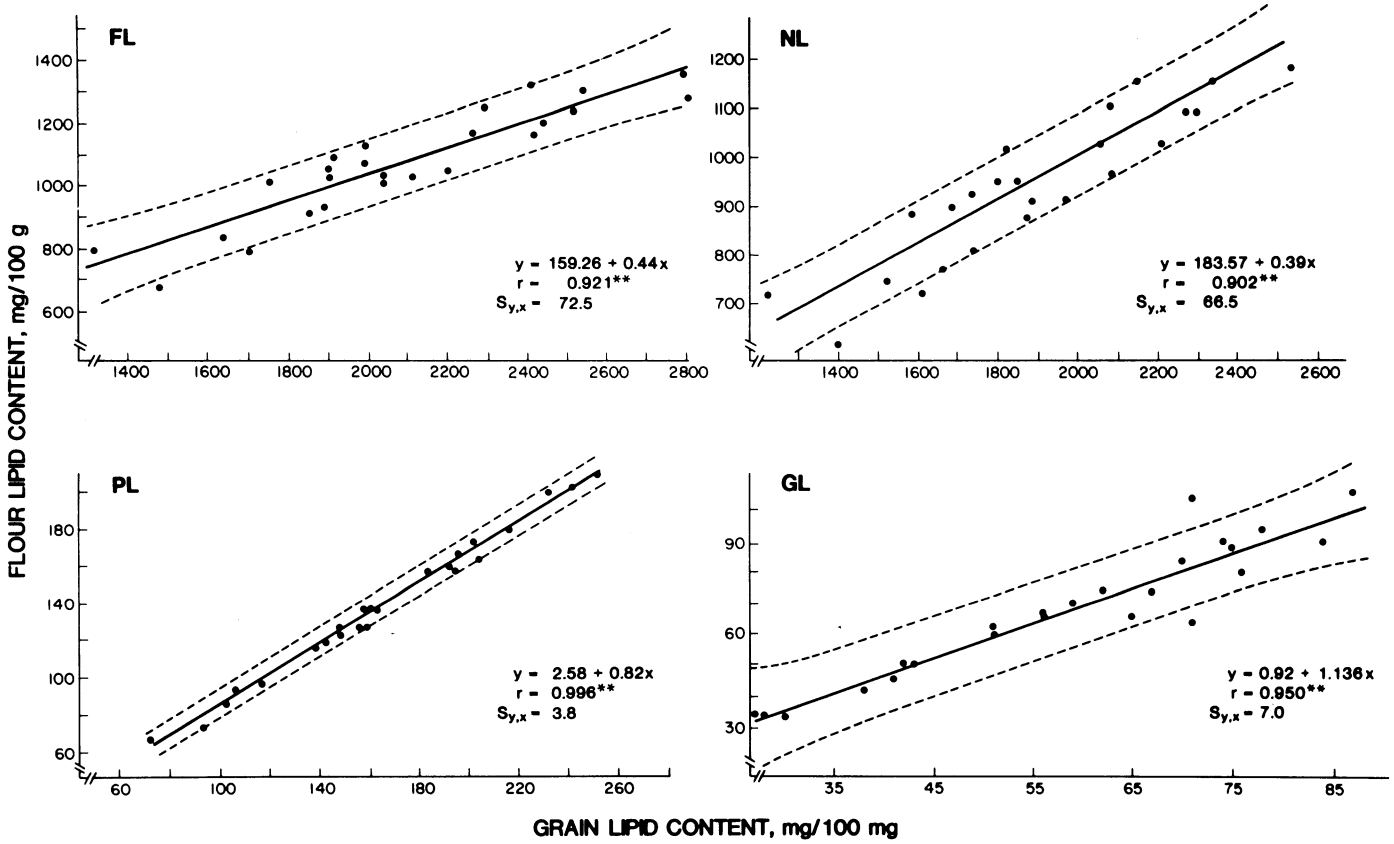

Fig. 1. Relationships between flour and grain lipid contents. **FL**, free lipids; **NL**, nonpolar lipids; **PL**, polar lipids; and **GL**, glycolipids. Dashed curves indicate 95% confidence limits.

flours. It is not clear if the differences obtained in the two studies result from differences in samples, environments, or analytical procedures.

Positive correlations were obtained for contents of analogous lipid fractions in grain and flour. The *r* values were: TL, 0.869; FL, 0.918; BL, 0.056; NL, 0.902; PL, 0.975; GL, 0.938, and PhL, 0.933. All correlations except that for BL were significant at the 1% probability level. The positive correlations for different free lipid subfractions of wheat and flour were previously shown by Tweeten et al (1981) and Chung et al (1982). Our *r* value for free NL is much higher than that obtained by Chung et al (1982) for winter wheats (0.48). The two studies gave similar *r* values for PL.

Because of the highly significant correlations between grain and flour values for FL, NL, PL, GL, and PhL, the values for flour can be estimated with a reasonable degree of accuracy from the values for grain. Linear regression equations for the flour subfractions of FL are given in Figure 1.

TABLE IV
Linear Correlation Coefficients for Loaf Volume (RLV) and Loaf Volume Adjusted for Protein Content (RLVc) with Flour and Grain Lipids[a]

	Flour[b]				Grain
Lipid	SC (n = 25)	R (n = 26)	L (n = 26)	All (n = 77)	SC (n = 25)
RLV					
FL[c]	0.618**[d]	0.473*	0.671**	0.556**	0.531**
NL	0.505**	0.359	0.616**	0.455**	0.456*
PL	0.906**	0.853**	0.839**	0.871**	0.905**
GL	0.928**	0.869**	0.835**	0.874**	0.908**
PhL	0.820**	0.738**	0.654**	0.758**	0.829**
BL	0.341	0.302	0.135	0.286*	0.051
TL	0.627**	0.471*	0.646**	0.558**	0.511**
NL/PL	−0.944**	−0.791**	−0.637**	...[e]	−0.895**
NL/GL	−0.924**	−0.816**	−0.619**	...	−0.827**
RLVc					
NL/PL	−0.984**	−0.973**	−0.961**	−0.976**	−0.910**
NL/GL	−0.968**	−0.972**	−0.951**	−0.968**	−0.863**

[a] Correlation coefficients (*r* values) were calculated from average values of triplicates of RLV and RLVc and lipid contents (mg/100g sample, db).
[b] SC = Swift Current, R = Regina, and L = Lethbridge.
[c] FL = hexane soluble (free) lipids, NL = nonpolar lipids, PL = polar lipids, GL = glycolipids, PhL = phospholipids, BL = water-saturated butanol extracted (bound) lipids after FL extraction, and TL = total lipids.
[d] *,** Significant at the 5% and 1% levels of probability, respectively.
[e] These calculated *r* values are invalid because the three station values are significantly different.

TABLE V
Regression Equation Constants and Correlation Coefficients for Estimation of Remix Loaf Volume (RLV) by Linear Regression

No. of Variables	x_i	a_o	a_i	r
RLV with flour—free lipids and protein				
1	GL[b]	371.212	5.24	0.874**[c]
2	Protein	75.65	24.21	
	GL		4.92	0.904**
3	Protein	513.29	56.68	
	NL/PL		−49.59	
	NL/GL		−12.62	0.981**
RLV with grain—free lipids and protein				
1	GL	316.41	7.18	0.831**
2	PL	634.02	1.91	
	NL/GL		−5.69	0.944**
3	Protein	183.46	32.33	
	NL/PL		1.82	
	NL/GL		−5.88	0.959**

[a] Linear regression analysis determine the best prediction equation of the form $RLV = a_o + \sum_{i=1}^{n} a_i x_i$ by increasing the number of independent variables.
[b] GL = glycolipids, NL = nonpolar lipids, and PL = polar lipids.
[c] ** Significant at the 1% level of probability.

Results of Statistical Analyses

Correlation coefficients for RLV with various lipid parameters were calculated for each set of samples from one location and for the combined data for the three locations (Table IV).

The correlations between RLV and lipid fractions were all positive for both flour and grain. The linear correlation coefficients were significant (at the 1 or 5% level of probability) for all fractions except for BL. For each lipid fraction, the three location *r* values were not significantly different according to the significance test (Sokal and Rohlf 1969). In such instances, it is statistically valid to combine the data for the three locations to calculate the *r* value for the combined sample.

Significant (at the 1% level of probability) negative correlation coefficients were obtained for RLV with the ratios NL/PL and NL/GL. However, for both parameters the *r* values for the three locations were significantly different, and therefore the data could not be combined to give a valid *r* value for all the samples. Again, the *r* values for grain were similar to the values for flour.

Correlation coefficients for RLVc (loaf volume normalized to average protein content) with NL/PL and NL/GL were not significantly different for the three locations. Accordingly the data for the three locations were combined to give an *r* value for the entire population. The values obtained were significant at the 1% probability level. This result is consistent with the assumption, made above, that most of the interlocation variability in loaf volume results from the variation in protein content.

Results for grain for the Swift Current location showed the same trends as the results for flour. For each lipid fraction and for the NL/PL and NL/GL ratios, the *r* value for grain was equal to or slightly lower than that for flour. However, in all cases except BL, the coefficients of correlation were significant at the 1 or 5% levels of probability. When the RLVc values were used instead of RLV in the calculation of the *r* values, slightly higher values were obtained.

Results presented herein show that RLV can be estimated from either flour or whole grain values for PL, GL, NL/PL, or NL/GL.

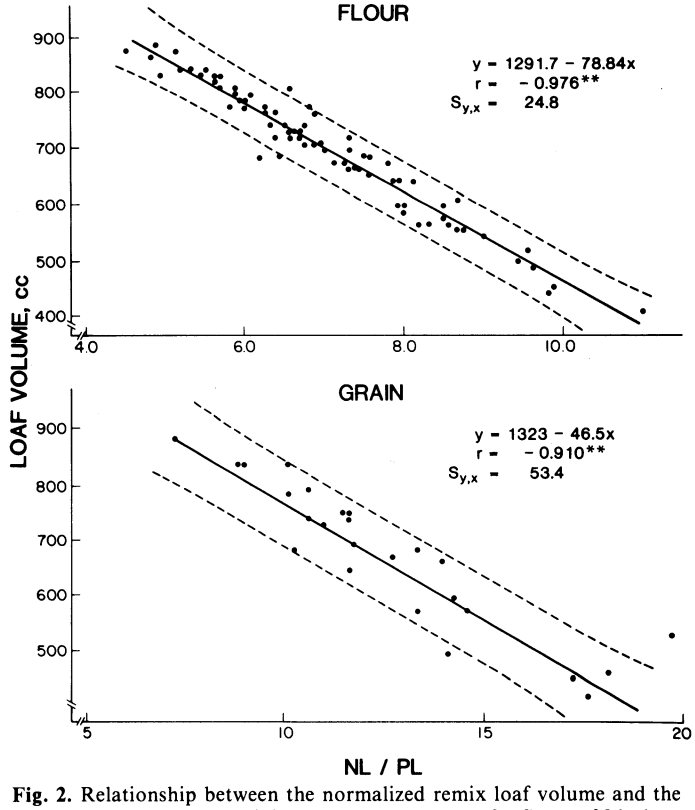

Fig. 2. Relationship between the normalized remix loaf volume and the ratio of nonpolar to polar lipids (NL/PL). **Top,** data for flours of 26 wheat varieties grown at three locations (n = 77; there was insufficient grain for one variety at one location). **Bottom,** data for whole grain meals for 25 varieties grown at one location (n = 25). Dashed curves indicate 95% confidence limits.

The most accurate estimation would be from NL/PL (Fig. 2). NL/GL also would give a good estimation (Table IV).

Prediction of RLV can be improved by using any of several possible multiple linear regression equations in which the independent variables of the equation are selected by the stepwise regression method (Table V). The three variable equations based on protein content, NL/PL, and NL/GL produced the best results for both flour and whole grain.

There is now substantial evidence that the free lipid fraction of wheat flour (Chung et al 1978, Pomeranz 1980), especially its glycolipid component (Pomeranz 1971), plays a significant role in the breadmaking potential of the flour. Many of the flour lipids, especially the glycolipids, form an integral part of gluten (Chung and Tsen 1975). It has been suggested that glycolipids, through hydrogen bonds and hydrophobic interactions, form a linkage between gliadin and glutenin molecules in gluten (Hoseney et al 1970). Protein-lipid complexes, which either exist in flour or are formed during doughmaking, appear to be involved in the aggregation of specific gluten proteins (Bekes et al 1983a,b). On the macro level, this aggregation could result in an improvement of the gas-retaining capacity of dough and thereby produce larger loaf volume and better crumb structure.

The present study investigated the relationships between loaf volume and various lipid fractions for 26 common spring wheats. As found previously for HRW wheat varieties (Chung et al 1982), significant correlations were obtained. It was shown by simple or multiple linear regression analysis that loaf volume can be estimated with a high degree of accuracy from several lipid parameters for flour or whole grain meal. The degree of accuracy is sufficient to make the estimation equations useful in screening plant breeders' populations. The estimation is better than those obtained by other indirect tests. The lipid analyses require a much smaller sample size than the baking test.

Effective application of the prediction equations presented herein will require rapid methods for the determination of the content of NL and PL or GL in flour or ground grain. Also, it remains to be determined if the significant statistical correlations result from a direct functional cause and effect relationship between certain flour lipids and breadmaking potential.

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