Determination of Neutral Detergent Fiber, Hemicellulose, Cellulose, and Lignin in Breads[1]

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ABSTRACT

Forty-four different kinds of breads were analyzed with the neutral detergent method of Van Soest. The neutral detergent fiber (NDF) was treated with the α-amylase from *Bacillus subtilis* to ensure complete starch digestion. Hemicellulose, lignin, and cellulose were estimated by further extraction with acid detergent, permanganate, and 72% sulfuric acid. Results were expressed as percent of the dry weight. The dry weight accounted for 60–65% of the fresh weight but was about 10% higher in raisin bread. The NDF content was approximately 1% in white bread, 4.6% in 60% whole wheat bread, and 7.2% in 100% whole wheat bread. NDF in almost all other breads ranged from 1 to 4%, with a mean of 2%. In general, the amount in each fraction was proportional to the total NDF: 65% insoluble hemicellulose, about 25% cellulose, and the remaining part mostly lignin. The NDF content of the breads suggests that the replacement of white bread by breads of higher fiber content would be a practical way to gradually increase the intake of dietary fiber.

Cereal foods are probably among the best sources of dietary fiber. Unfortunately, hemicelluloses, which are the major constituents of cereal fiber, have been seriously underestimated in crude fiber determinations (Kelsay 1978, Van Soest and Robertson 1977). Detailed analysis of the fiber content of important cereal foods such as bread is therefore needed.

We determined the neutral detergent fiber (NDF) content of various breads every three months for a year and also measured the amounts of the main fractions.

MATERIALS AND METHODS

The NDF of various breads was determined by the methods of Goering and Van Soest (1970), modified by an amylase digestion step to remove starch. Hemicellulose, lignin, and cellulose were estimated by further extraction with an acid detergent, permanganate, and 72% sulfuric acid.

Preparation of the Sample

Fresh breads were bought from supermarkets or grocery stores in Ottawa. A sample (five slices) of bread was weighed, freeze-dried, and reweighed. The dried bread was ground to pass through a 20-mesh screen and stored in a screw-cap bottle. Immediately before analysis, 10 g of the stored sample was freeze-dried and reweighed to measure the uptake of moisture during storage.

Analytical Procedures

Neutral Detergent Method. Approximately 0.5 g of dry sample (weighed to within 0.1 mg) was refluxed in a Berzelius beaker containing 50 ml of neutral detergent solution. The refluxing solution was similar to that used by Goering and Van Soest (1970) except that decahydronaphthalene and sodium sulfite were omitted. Sodium sulfite was omitted to prevent the partial degradation of the lignin fraction (Van Soest and Robertson 1977). After 30 min, 50 ml of neutral detergent and 3 ml of enzyme solution (α-amylase from *Bacillus subtilis*, Sigma A6505, 2% w/v, filtered) were added. The solution was refluxed for another 30 min, and the contents were filtered through a tared Gooch crucible on a filtering manifold. The residue was rinsed with hot distilled water (90–100° C). Next, the crucible was filled with hot distilled water followed by 3 ml of α-amylase solution. After 15 min, the residue was washed with hot distilled water and then acetone. The crucible containing the residue was dried overnight at 100° C and weighed hot. The weight of the crucible was subtracted to obtain the net weight of NDF.

Acid Detergent Method. The crucible containing NDF was placed in a beaker suitable for refluxing (Goering and Van Soest 1970). Acid detergent solution (100 ml) was added to the beaker and refluxed for 60 min from onset of boiling. The external wall of the crucible was carefully washed inside the beaker with hot distilled water, and the crucible was placed on the filtering manifold. The contents of the beaker were filtered, and the residue was washed with hot distilled water and then with acetone. The crucible was dried overnight at 100° C and weighed hot. The difference between NDF and acid detergent residue was used as an estimate of insoluble hemicellulose.

Sulfuric Acid and Ashing Method. The crucible containing the acid detergent residue was placed in an enamel pan and half filled with 72% sulfuric acid. Glass rods were used to stir and wet all particles. Asbestos was not used. The crucible was replenished with sulfuric acid at hourly intervals. After 3 hr, the remaining sulfuric acid was removed by suction and the residue was thoroughly washed with hot distilled water. The crucible was then dried overnight at 100° C and weighed hot. Cellulose was calculated as the loss in weight from the acid detergent residue. The residue was ashed in the crucible for 4 hr at 525° C, held overnight at 100° C, and weighed hot. The lignin content was calculated as the loss in weight upon ashing.

Permanganate Method. This method was an alternative to the sulfuric acid procedure for measuring lignin and cellulose. The crucible containing the acid detergent residue was placed in an enamel pan and treated for 90 min with a single 25-ml portion of permanganate solution (Goering and Van Soest 1970). After the aspiration of the remaining permanganate, the residue was treated with the demineralizing solution and washed with ethanol and then with acetone. The crucible was dried overnight at 100° C and weighed hot. The lignin content was calculated as the loss in weight from the acid detergent residue. The residue of the permanganate treatment was subjected to the 72% sulfuric acid treatment for 3 hr to separate cellulose from cutin. The washed residue was dried overnight at 100° C and weighed hot. Cellulose was then calculated as the loss in weight from the permanganate residue.

Hot Weighing Procedure. Goering and Van Soest (1970) reported that hot weighing is reproducible and superior to cold weighing. In this procedure, an air-forced oven set at 100° C is located near a single-pan automatic balance sensitive to 0.1 mg. Good precision requires reading the minimum weight within 30 sec. Equal time between crucibles is important; precision can be attained with practice. The following procedure was used:

1. The balance was adjusted at 0.0100 g.
2. An extra crucible was placed on the balance plate for 2 min to heat up the balance.
3. Control and sample crucibles were weighed, using the following sequence:

Time 0:	A crucible was removed from the oven and placed on the balance plate.

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Time 30 sec: The weight was recorded and the crucible removed from the balance.
Time 45 sec: The zero of the balance was recorded.
Time 60 sec: The next crucible was placed on the balance . . .

4. A control crucible was weighed every 10 samples. These crucibles were replaced in the oven and reweighed with each series of sample crucibles.
5. The "zero" deflection due to temperature change of the balance was subtracted for each weight. For example, if the weight of the crucible at 30 sec was 36.8708 g and if the "zero" of the balance at 45 sec was 0.0080 g, then the true weight of the crucible was 36.8628 g.

RESULTS

The dry weights of all breads were 60–65% of the fresh weights. The only exception was raisin bread, in which dry weight was nearly 10% higher throughout the year (data not shown). The NDF content was expressed as percent of dry weight, as shown in Fig. 1. NDF includes mainly insoluble hemicellulose, cellulose, and lignin. The NDF content of breads was less than 3% except for 60 and 100% whole wheat breads, which contained 4.6 ± 0.1% and 7.2 ± 0.2% NDF respectively. The white and 100% whole wheat breads were investigated more extensively; 10 different brands of each were studied. The individual NDF content of each brand of bread was collected every three months. Most NDF values were between 0.7 and 1.3% in white breads and between 6 and 8% in the 100% whole wheat breads. Seasonal variations in NDF values in bread were of much smaller magnitude than were variations among the different types of bread.

Figure 2 shows the insoluble hemicellulose content of breads as estimated by the difference between NDF and the acid detergent residue. A comparison with Fig. 1 shows that the hemicellulose content paralleled the total NDF content. The breads contained less than 2% insoluble hemicellulose in their dry matter, except the 60 and 100% whole wheat breads, which contained about 3 and 4.5% respectively. In these two categories, the mean insoluble hemicellulose content represented about two-thirds of the total NDF (Fig. 1).

The residue after extractions with neutral and acid detergents consisted mainly of cellulose and lignin. To separate these fractions, two procedures were employed, one on each of the duplicate residues (Fig. 3). The first procedure used sulfuric acid, which dissolved cellulose and left the lignin portion. Figures 4a and 5a show the values obtained. The second procedure used permanganate to remove lignin from cellulose and cutin and then sulfuric acid to remove cellulose from cutin. Figures 4b and 5b show the values obtained for cellulose and lignin, respectively. The very low values for cutin (less than 0.2%) are not reported here.

Cellulose in the 100% whole wheat breads represented 22 or 27% (Fig. 4) of the total NDF (Fig. 1), depending on the method. Because a single analysis was performed with each method and because low NDF values were obtained, comparison of the methods was difficult for the other types of bread. In general, the sulfuric acid method yielded slightly higher results. Cellulose in nonwhole wheat breads appears not to exceed 0.5% of the dry matter.

The lignin content of all breads (Fig. 5) was less than 1%. The two procedures yielded similar lignin values, which accounted for 11–12% of the total NDF in the whole wheat breads.

Bran bread also was investigated (Fig. 6), but because only one brand was available on the market, these results give only a rough indication of the amount of insoluble fiber in this type of bread. The mean NDF content over the year was more than 10% of the dry weight. The mean insoluble hemicellulose content accounted for 65% of the total NDF. The mean cellulose content represented 23 or 27% of the total NDF depending on the procedure used. Lignin values fluctuated somewhat, but most were around 1% of the dry matter, representing about 10% of the total NDF.

DISCUSSION

Dietary fiber is generally defined as the plant polysaccharides and lignin that are resistant to hydrolysis by digestive enzymes in the human upper gastrointestinal tract (Trowell et al 1976, Van

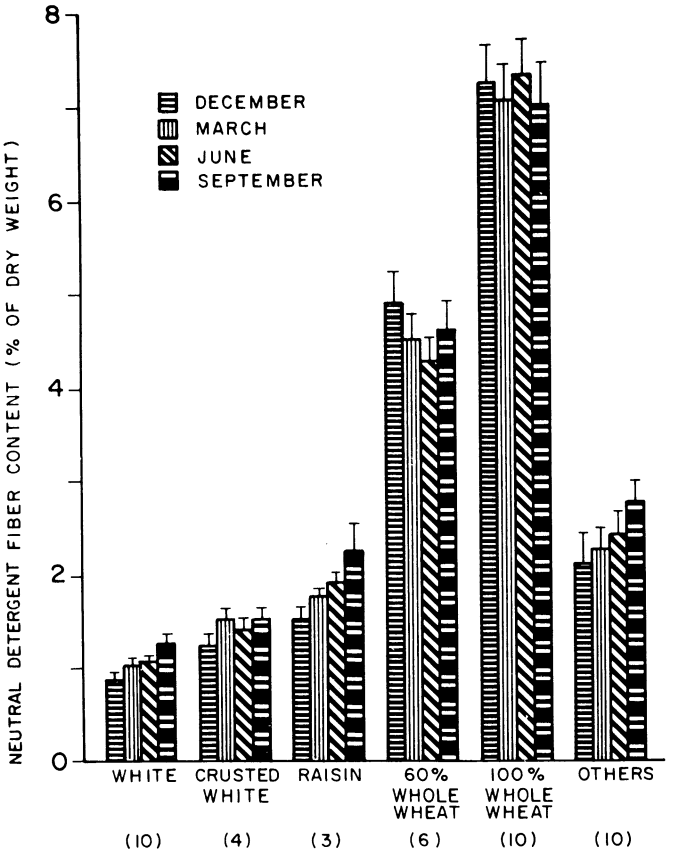

Fig. 1. Content of neutral detergent fiber (NDF) in bread. Each histogram represents the mean percentage in a type of bread at a given time of the year. Numbers in parentheses show the number of different brands analyzed at each time. Standard error of the mean is indicated by the error bars. Each sample was analyzed in duplicate.

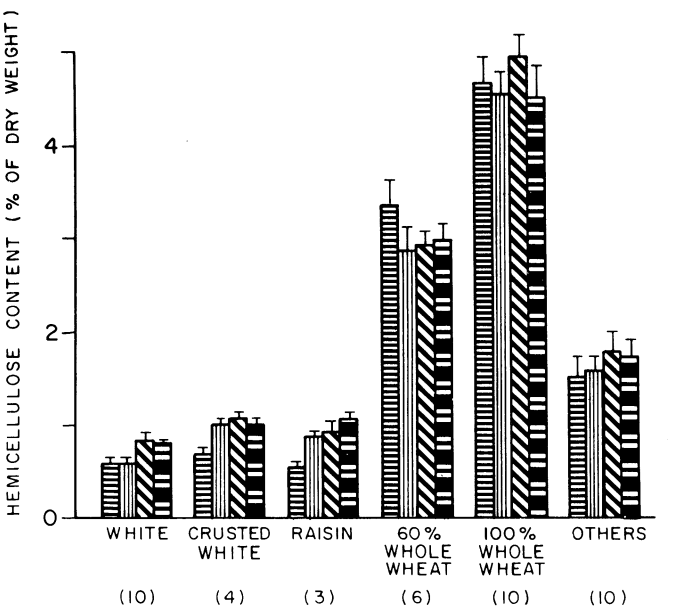

Fig. 2. Content of insoluble hemicellulose in bread as estimated by the difference between neutral detergent fiber and acid detergent fiber. Numbers in parentheses show the number of different brands analyzed at each time. Standard error of the mean is indicated by the error bars. Each sample was analyzed in duplicate.

Soest and McQueen 1973). The growing interest in the effects of dietary fiber on health has emphasized the need for data on the dietary fiber content of foods. The crude fiber method measures variable proportions of the total plant cell-wall constituents and underestimates dietary fiber, particularly in foods such as cereals that are rich in hemicellulose. The replacement of this old method has been advocated (Southgate 1976, Van Soest and McQueen 1973).

Methods to measure the components of dietary fiber in foods are available, but they are time-consuming and not practical for routine use. Schaller (1977) compared three different methods for analyzing fiber in white wheat bran: 1) the unavailable carbohydrate analysis of Southgate, 2) the enzymatic digestion method of Saunders, and 3) the neutral detergent method with an enzyme digestion (18 hr with α-amylase from porcine pancreas). The maximum difference between the values obtained by the three methods was only 2%. Because wheat bran is the main source of dietary fiber in the wheat breads analyzed here, the agreement between the methods suggests that the modified neutral detergent method can give a good estimation of these breads' dietary fiber. This method does not recover soluble pentosans that fit the dietary fiber definition, but because soluble fiber occurs only in trace amount in wheat (Southgate 1969), the total insoluble fiber content of wheat bread is close to its dietary fiber content. However, such good agreement among the three methods may not be found with other cereals and foods.

A rapid α-amylase treatment for analyzing NDF in high-starch foods has been suggested.[2] A thermoresistant α-amylase from *B. subtilis* (Sigma, A6505) digests most of the starch remaining in the neutral detergent residue within a minute. Complete digestion is assured with longer digestion time. Using whole wheat bread, we have compared the yield in this rapid method with that in Schaller's modified neutral detergent method (1977), which uses α-amylase from porcine pancreas (Sigma, 6880). The NDF values (mean and standard error of the mean; five analyses) were 7.10 ± 0.24 and 7.20 ± 0.18, respectively; the iodine test showed no colorable starch in either neutral detergent residue. Thus the yields were comparable and the rapidity of the bacterial α-amylase method appeared advantageous.

[2]P. J. Van Soest and J. B. Robertson, personal communication.

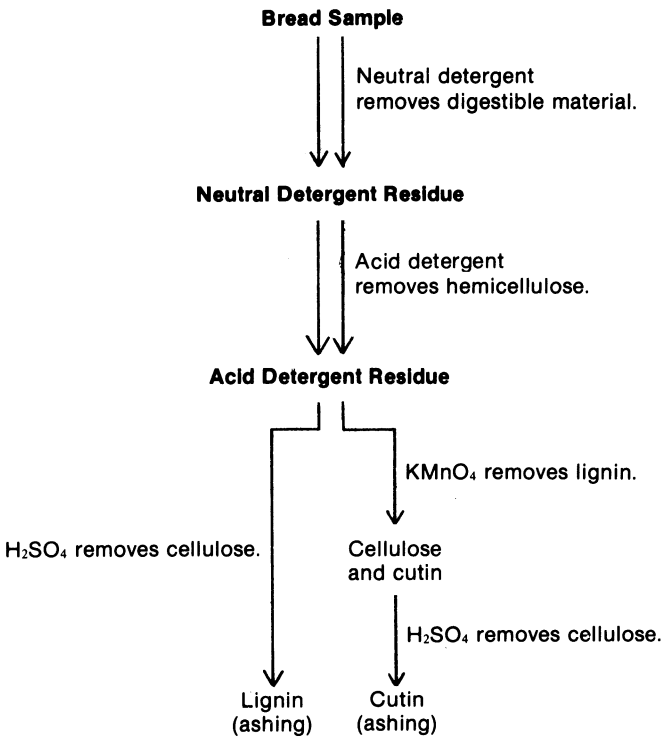

Fig. 3. Schematic summary of the procedures.

We then investigated whether under our conditions, the unpurified bacterial α-amylase preparation could attack the hemicellulose fraction. Because the porcine α-amylase does not attack this fraction (Schaller 1977), the yields of hemicellulose after digestion with each enzyme were compared. Long incubations (60 min or more) with the bacterial α-amylase caused loss of hemicellulose from wheat bran NDF. A mean loss of about 8% per hr occurred in that fraction over a 3-hr period, but no loss was apparent after an incubation lasting for only 20 min (unpublished data). In the present study, hemicellulose was in contact with the enzyme during the last 30 min of refluxing in neutral detergent and also for 15 min in the filtering crucible. The contact with active enzyme during the 30-min refluxing was short because the activity of the "heat resistant enzyme" was rapidly lost when the beaker was replaced on the hot plate. (Most of its activity disappeared within 2 min at 85° C). Therefore the total time during which the hemicellulose was in contact with active enzyme was about 20 min, and a significant loss of hemicellulose would not be expected after that treatment. This conclusion was confirmed in another comparison, using whole wheat bread and the same treatment (unpublished data). Thus only a small amount (probably less than

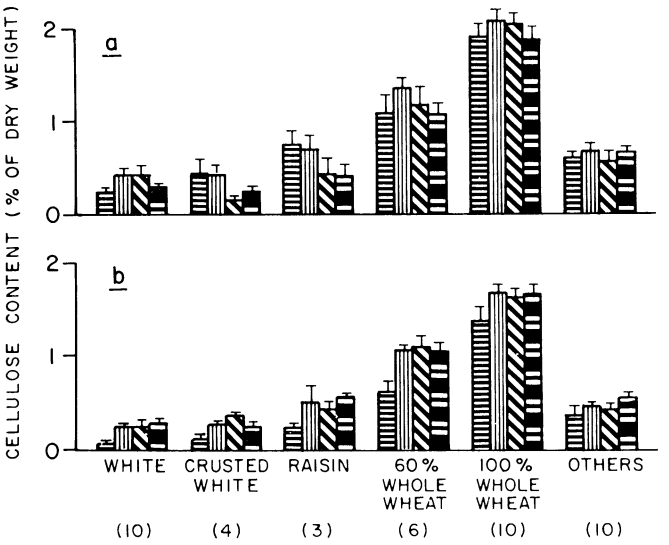

Fig. 4. Content of cellulose in bread as estimated by: **a**, the sulfuric acid method and **b**, the permanganate method. Numbers in parentheses show the number of different brands analyzed at each time. Standard error of the mean is indicated by the error bars. Single analysis was performed with each method.

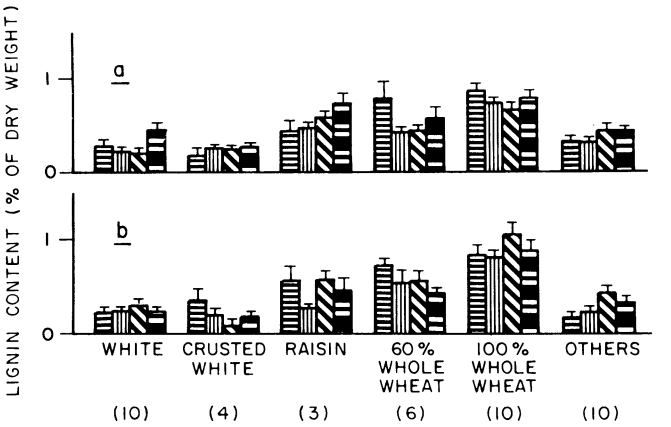

Fig. 5. Content of lignin in bread as estimated by: **a**, the sulfuric acid method and **b**, the permanganate method. Numbers in parentheses show the number of different brands analyzed at each time. Standard error of the mean is indicated by the error bars. Single analysis was performed with each method.

3%) of the hemicellulose fraction would have been lost under our conditions, and the analysis of sugars in the filtrate was not considered worthwhile.

Insoluble hemicellulose (Fig. 2) represented two-thirds of the total NDF in the 60 and 100% whole wheat breads. The same proportion also appeared in bran bread (Fig. 6). This proportion of hemicellulose is similar to that indicated by Southgate et al (1969) for wholemeal wheat.

The properties of hemicellulose may be important in human physiology. This fraction is largely degraded in the colon (Southgate and Durnin 1970, Williams and Olmsted 1936) and has been reported to increase fecal output (Hummel et al 1943, Williams and Olmsted 1936). Hemicellulose has other important properties such as ion-binding capacity and water-holding capacity.

According to Van Soest and Robertson (1977), the crude fiber method recovers approximately 15% of the hemicellulose. The fact that hemicellulose is the main constituent of dietary fiber in cereals underlines the deficiency of the old method, which associated the term "fiber" with "cellulose." Cellulose is the main constituent of crude fiber but, as indicated in Figs. 1 and 4, the cellulose fraction represents only about 25% of the total NDF of bread.

We used two procedures to estimate cellulose and lignin. In the first, sulfuric acid dissolved cellulose and perhaps some lignin, leaving lignin and cutin. In the alternate procedure, permanganate solution dissolved the lignin from cellulose and cutin, and then sulfuric acid dissolved cellulose from cutin. The permanganate method gives a better value for lignin, if penetration of the sample is rapid (Goering and Van Soest 1970); the small amounts of lignin in most foods are readily dissolved when the sample is properly ground.

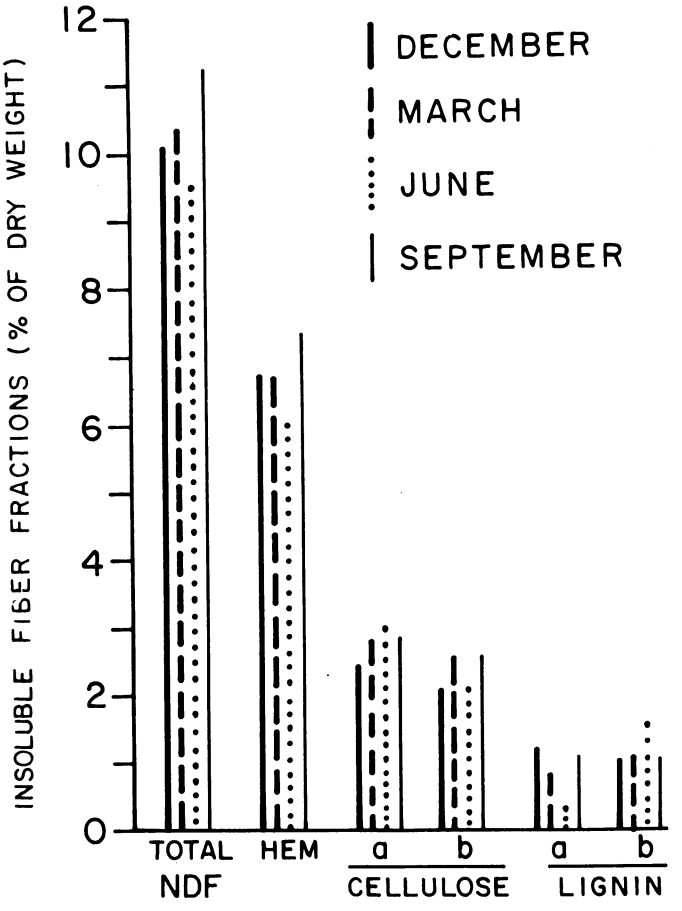

Fig. 6. Content of neutral detergent fiber (NDF) in a commercial brand bread. Values for total NDF and hemicellulose (HEM) are the mean of duplicate analyses. Cellulose and lignin were determined by: **a**, the sulfuric acid method and **b**, the permanganate method.

Although the values for lignin and cellulose by the two procedures are based on single determinations, they are in relatively good agreement (Figs. 4 and 5). Combination of the permanganate and the sulfuric acid methods seems preferable because it appears to give a more exact value for lignin and permits separation of cellulose from cutin. This latter fraction may be important in some other foods. In bread, cutin appeared to be less than 0.2% of the dry weight.

No significant variations appeared in the NDF content of the whole wheat breads (Fig. 1) at different times of the year. In some types of breads, there was a trend toward higher values from December to September. However, examination of individual values showed that the trend was not general; a much higher value in only a few brands changed the mean.

Eastwood et al (1973) showed that 16 g of wheat bran daily can approximately double the stool weight after three weeks of treatment, but other authors have suggested that much higher intake is needed to produce this effect (Cummings et al 1978). Nevertheless, 16 g of bran represents five to six tablespoonfuls, which is a large volume to ingest in a single meal. A supplement of bran at breakfast, although palatable for short periods, is not readily accepted as a permanent feature of the diet (Kahaner et al 1976).

On the other hand, almost everyone consumes bread at most meals. It is therefore interesting to compare the amount of NDF provided by a supplement of 16 g of bran daily to that provided by a normal intake of whole grain bread. Bran contains about 30–50% NDF, and the daily intake of 16 g of bran represents an intake of 5–8 g of NDF. Consumption of five slices of bread per day represents about 115 g of fresh weight and about 70 g dry weight. The data in Fig. 1 indicate that ingestion of five slices of white bread provides about 0.7 g NDF. Substitution of 60 or 100% whole wheat breads would provide about 3.2 or 4.9 g NDF respectively, with a net increase of about 2.5 or 4.2 g NDF respectively. Fig. 6 suggests that bran bread may provide about 7 g NDF. Thus, substituting bread with a higher fiber content for white bread provides a supplementary amount of NDF that may be partially or totally equivalent to the amount provided by a supplement of 16 g of bran daily.

Wholemeal bread is known to have a desirable effect on the physiology of the colon (McCance et al 1953), but it is not known whether fiber from bread has the same effect as fiber from bran. Indeed, some physical properties, such as particle size, may have been changed during processing. Nevertheless, the NDF content of the various types of breads suggests that the replacement of white bread by bread of higher fiber content is a good practical and palatable way to gradually increase dietary fiber intake.

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