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EFFECTS OF ENZYME SUPPLEMENTATION ON THE NUTRITIONAL VALUE OF BARLEY IN CHICKEN DIETS

BY

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DEDICATED TO MY PARENTS

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ABSTRACT

Barley in some cases has a low nutritive value when fed to chickens, which in part may be attributed to its content of B-glucans. A series of experiments were conducted with broiler and Single Comb White Leghorn (SCWL) chicks to (1.) compare the effects on growth performance and nutrient retentions in SCWL chicks fed three enzyme preparations (Pectinex, Celluclast, T.v. Cellulase) when added at three concentrations to a barley-based diet, (2.) compare the response in growth and nutrient retentions in broiler and Leghorn chicks fed hulless barley (Scout) supplemented with Celluclast, (3.) determine the effects of enzyme supplementation on the nutritional value of two cultivars of barley (Scout and Minerva) that contain a high content of total and soluble B-glucans, and (4.) determine if the degree of response of broilers to enzyme supplementation of a barley-based diet (Bedford) was influenced by the duration of the feeding period.

In Experiment one, weight gains and feed consumption were improved ($P < 0.05$) to a greater degree with Celluclast while both Celluclast and T.v. Cellulase effectively improved ($P < 0.05$) the efficiency of feed utilization. Dry matter, fat, and protein retentions were improved ($P < 0.05$) in the presence of all three enzymes, with the effect being greater with Pectinex and T.v. Cellulase compared to Celluclast. The degree of response was the same ($P > 0.05$) for all concentrations of enzymes.

In Experiment two weight gains and efficiency of feed utilization of broilers and Leghorns were affected in a similar ($P > 0.05$) manner by

enzyme treatment during the first and second week of the study. The nutrient retentions, however, were affected to a greater degree ($P < 0.05$) in broilers than Leghorns.

The results from Experiment three showed that the addition of enzymes (Celluclast and SP-249) to barley-based diets at all three concentrations (0.05, 0.10, 0.20%) improved ($P < 0.05$) weight gain, feed to gain ratios and dry matter retentions to a considerable degree, with the effect being greater with Minerva as compared to Scout barley. Enzyme type (Celluclast vs. SP-249) and concentration had no effect ($P > 0.05$) on performance values.

In Experiment four, broiler chickens fed a barley-based diet that had a low content of B-glucan over a six week period did not respond to enzyme supplementation. It was therefore not possible to determine if the response of broilers to enzyme treatment, was influenced by the duration of the feeding period.

In most of the above studies growth improvements in chicks fed the enzyme supplemented diets were equal or superior ($P < 0.05$) to that obtained with the wheat control diets.

The results show that enzyme supplementation of barley-based diets with a high content of soluble B-glucans, significantly ($P < 0.05$) improved growth and nutrient retentions in both broiler and Leghorn chicks. Growth and nutrient retention improvements in some cases even exceeded that of birds fed wheat based diets. Growth improvements were dependent on; type of barley and enzyme used, while enzyme concentrations and bird-type had no effect. Nutrient retentions on the other hand were dependent on; bird-type, enzyme and barley-type. These

same performance values were generally not affected by enzyme concentration. It may be concluded that the nutritive value of certain barley-types may be improved by enzyme supplementation.

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B	beta
α	alpha
h	hour
kcal	Kilocalorie(s)
kg	Kilogram(s)
<	less than
>	greater than
g	gram(s)
°C	degree Celcius
%	percent
SE	standard error
IU	international units
ml	millilitre
S.S.	Sum of Squares
anova	analysis of variance

INTRODUCTION

Historically dietary fibre has been generally defined as being the residue of plant cell walls not digested by the alimentary enzymes of man. Theander and Aman (1979) have also proposed that dietary fibres include all non-starch polysaccharides plus lignin. Mixed linked B-(1->3),(1->4)-D glucans, also referred to as B-glucans, are non-starchy polysaccharides which are mainly present in cereals (Hesselman and Aman, 1985). In barley and oats, the endosperm cell walls contain large amounts of B-glucans which greatly influences the physical and nutritional properties of these grains (Wood, 1983).

In recent years there has been interest in increasing the consumption of cereals in human food in developed countries. This is related to their low fat and high starch and fibre contents of cereals (Cummings, 1978). Some positive effects of dietary fibre in human nutrition include a reduction in serum cholesterol and fat, which could aid in possibly reducing antherosclerosis and heart disease (Burkitt, 1976). Some health professionals believe that dietary fibre may provide protection from cancer of the colon and rectum (Spiller and Sorenson, 1976).

The general trend in human nutrition has been to increase the consumption of certain cereals due to the benefits associated with the dietary fibre that is present in these cereals. Animal nutritionists, in contrast have been reluctant to use these cereals as they tend to have a negative effect on nutrient utilization. A concern in the poultry industry is the deleterious effects on weight gains and

efficiency of feed utilization when cereals such as barley are used as the principal ingredient in poultry diets (Campbell et al, 1986). Removal of the hull from barley which is high in fibre through the development of hulless cultivars or by mechanical treatments does not eliminate the problem (Anderson et al 1961; Coon et al, 1979). The reduced feeding value of barley would have to be attributed to other factors, such as, B-glucans (Burnett, 1966) and tannins (Gohl and Thomke, 1976).

It has been proposed that B-glucans reduce growth and efficiency of feed utilization in poultry by increasing the viscosity of the intestinal fluids, thereby impairing nutrient digestion and absorption (White et al, 1983). B-glucans have been known to reduce the digestion and absorption of nutrients such as starch, fats, proteins, and minerals (Classen et al, 1985). Commercial enzyme preparations from Bacillus subtilus, Aspergillus niger, Trichoderma viride, Trichoderma reesei, and other sources have been employed to improve the nutritional quality of barley used for poultry feeds (Mannion, 1981; White et al, 1981; Hesselman et al, 1982; White et al, 1983). These enzymes contain high B-glucanase activities which can reduce the viscous components of barley (B-glucans). As a result, efficiency of feed utilization and weight gains are improved (Campbell et al, 1986).

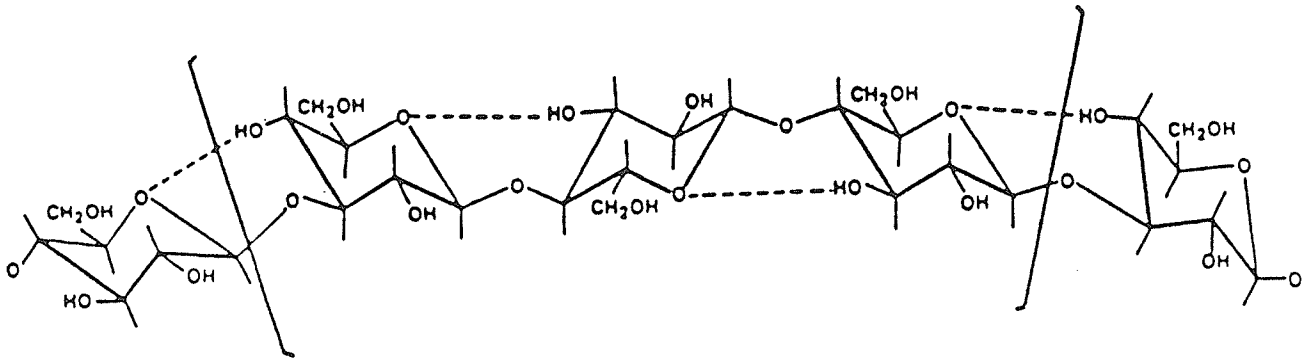
LITERATURE REVIEW

A. Structure of B-glucans in Cereal Grains

Barley and oat B-glucan molecules are unbranched polysaccharides which contain B-(1->3) and (1->4) linked glucopyranosyl units in the ratio of 2 and 3 to 1 (Figure 1; Wood, 1983). Despite the overall structural similarity of cereal B-glucans, there may be quite distinctive differences in physical properties, such as viscosity and solubility due to variations in molecular weights, protein content and sequence of linkages (Wood, 1983). The presence of B-(1->3)-linkages in the B-glucan chain results in irregularities in the molecular shape, which renders the B-glucans more susceptible to hydrolysis compared to the intransient cellulose, which is built up only of B-(1->4)-linkages (Bamforth, 1982).

Recent studies on the fine structure of B-glucans have revealed that about 90% of water-soluble B-glucan from barley endosperm was comprised of randomly arranged cellotriosyl and cellotetraosyl units, separated by single B-(1->3)-linkages (Figure 2; Staudte et al, 1983). The remaining 10.0% consisted of blocks of up to ten or more adjacent B-(1->4)-linkages (Fleming and Kawakami, 1977). In addition it has been reported that this polysaccharide contains sequences of two, three, or four continuous B-(1->3)-linkages, blocks of more than four B-(1->4)-linkages and regions having alternating B-(1->3) and B-(1->4)-linkages (Fleming and Kawakami, 1977).

B-glucans in barley and oats are found primarily in endosperm cell walls (Basic and Stone, 1981). In barley they constitute about 70% of



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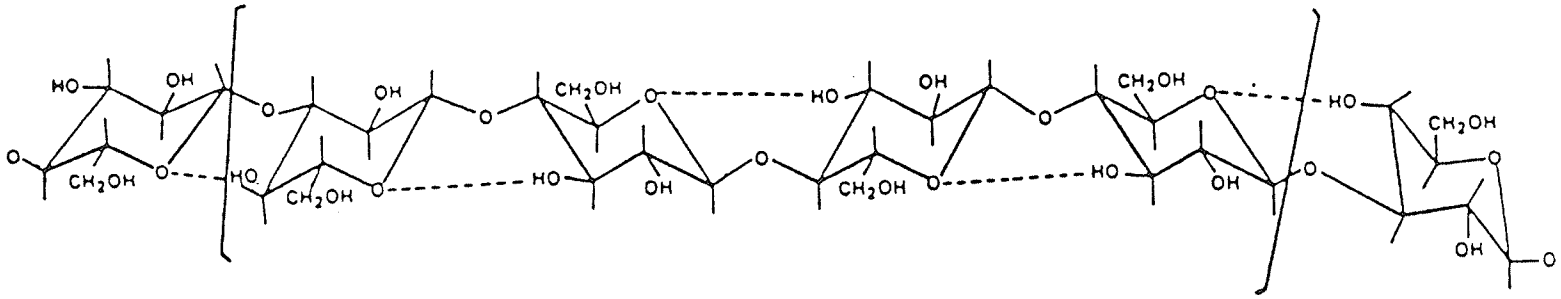

Fig. 1. Structure of cereal β-glucan

Adapted from Wood, 1983

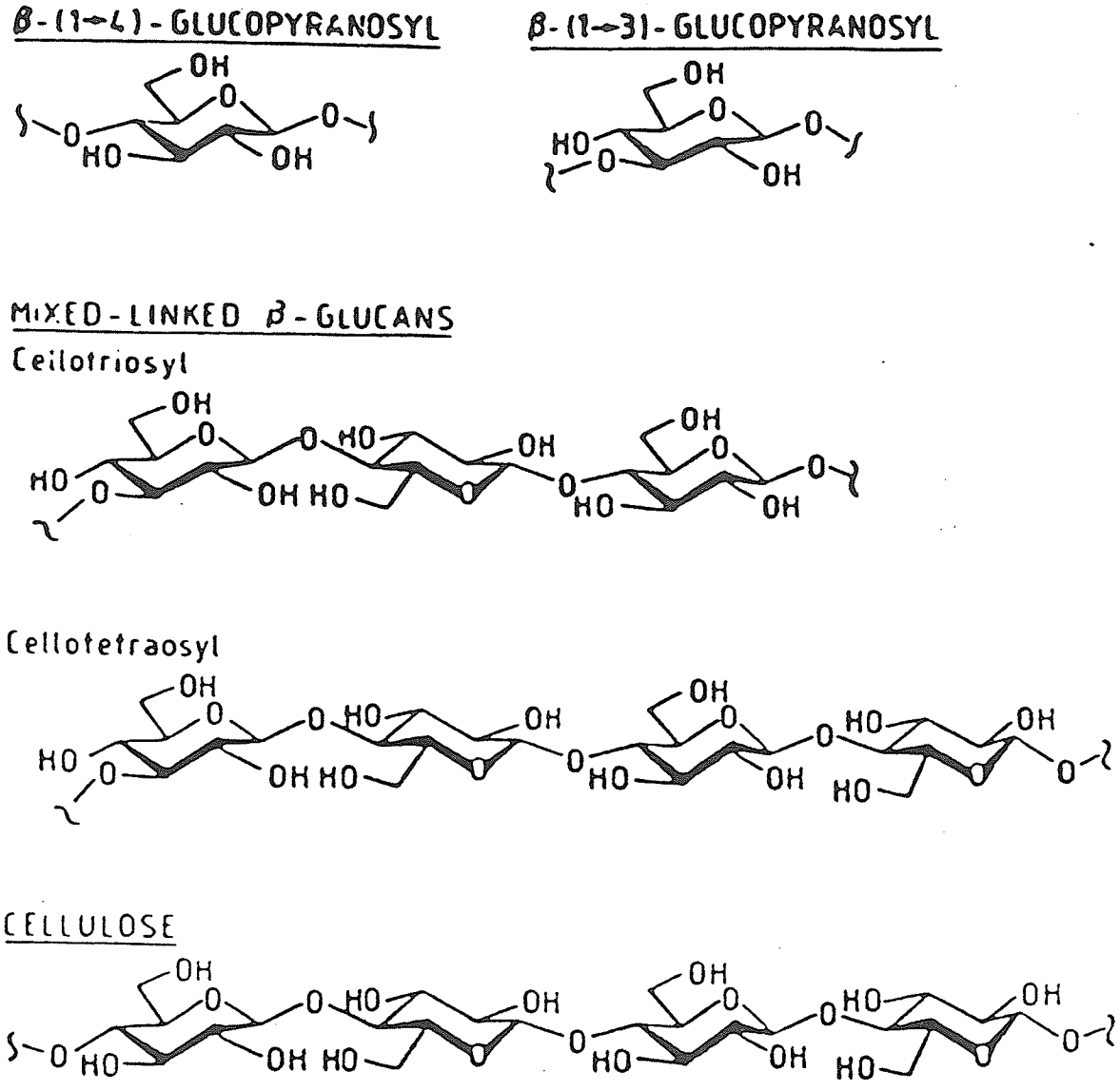

Fig. 2. Conformation Formulas of B-glucan Residues

Conformation formulas of β-(1->3)-linked glucopyranosyl residue, β-(->4)-linked glucopyranosyl residue, mixed-linked β-(1->3), (1->4)-glucans, including the cellotriosyl and cellotetraosyl residues, separated by single β-(1->3)-linkages and comprising about 90 % of the polysaccharides, and cellulose.

Adapted from Staudte et al, 1983

the starchy endosperm cell walls, the remainder being mainly arabinoxylans (25%), mannose containing polymers, protein and phenolic constituents (Basic and Stone, 1981). Significant differences in the thickness of endosperm cell walls of barley have been reported (Aastrup and Munck, 1985). For example, the cultivar Minerva has starchy endosperm cell walls more than twice as thick as its low B-glucan mutant, M737. Correspondingly, Campbell et al (1986) showed that the extract viscosity's of Minerva was over twice that of M737.

B. B-glucan Content and Occurance in Cereal Grains

The range of total B-glucan concentrations within cereal grains can vary from 2-10%, expressed on a dry weight basis (Wood, 1983). The contents of B-glucans in cereals is influenced by type of cereal, variety differences within cereals, differences in geographical locations, agronomic practices, and methods used for B-glucan determinations (Anderson et al, 1978).

Variations in total B-glucan content from cereal to cereal is evident when barley B-glucan (3-10%) is compared with wheat B-glucan (0.5-1.0%) (Table 1). It should be noted that B-glucan content measurements in this table were taken on different cultivars of barley. Anderson et al (1978) reported wheat, rice, and triticale had very low concentrations of total and soluble B-glucans, while barley and rye comparitively had high concentrations. The low metabolizable energy of barley for poultry, relative to other cereals, i.e. wheat, has been attributed to the suppression of nutrient digestion by the B-glucan component (Rickes et al, 1962; Potter et al, 1965; White et al, 1981). Thus higher concentrations of B-glucan seen in barley and rye as

Table 1 Recent Estimates of B-glucan Content in Barley, Oats, and Wheat by Various Methods

Cereal	Method[1]	B-glucan Content
Barley	a	6.20 - 7.24
Barley	b	3.64 - 6.44
Barley	b	3.16 - 5.78
Barley	b	2.00 - 6.36
Barley	b	1.93 - 6.81
Barley	c	4.5 - 8.2
Barley	d	5.43 - 8.62
Barley	d	4.85 - 8.62
Barley	d	6.13 -10.7
Barley	e	2.7 - 4.4
Barley	e	2.73 - 5.18
Barley	e	2.53 - 4.47
Oats (whole grain)	b	2.50
Oats (whole grain)	c	4.8 - 6.6
Oats (dehulled)	a	4.6
Wheat	a	1.4
Wheat	b	0.52 - 1.00

[1]a, total glucan-starch; b, B. subtilis (1->3) (1->4)-B-D-glucan 4-glucanohydrolase; c, cellulase method of Prentice et al (1980); d, cellulase method of Martin and Bamforth (1981); e, cellulase method of Martin and Bamforth (1981) modified to inactivate residual amyloglucosidase.

Adapted from Wood, (1983).

compared to wheat may be responsible for poor growth performance when fed to poultry.

Different varieties of each cereal have been shown to affect the total content of B-glucan in cereals. Table 2 shows that total B-glucan content can differ by as much as 30% among varieties of barley that have been grown under the same conditions.

Differences in geographical locations, with consequential differences in temperature and rainfall can also have an effect on the B-glucan content of cereals (Table 2). These environmental conditions affect B-glucan concentrations in cereals during the latter periods of plant growth (Aastrup, 1979b). In particular, drought conditions tend to elevate both soluble and total B-glucan deposition (Aastrup, 1979b; Hesselman et al, 1982). This may account for the regional differences in the apparent nutritional value of barley as a poultry feed. American barleys from western regions have long been considered inferior to those from more eastern regions, which is thought to reflect climatic influences on B-glucan concentration (Campbell et al, 1986). Lethbridge, Alberta which is comparatively drier than Winnipeg produced barley that has a much higher concentration of B-glucan than that produced in Winnipeg (Bendelow, 1975). The yearly rainfall averages for Lethbridge and Winnipeg are 269.2 mm and 411.0 mm, respectively. Aastrup (1979a) found that hot and dry growing conditions leading to an early harvest of barley resulted in an increased viscosity of acid extracts. Aastrup (1979b) also conducted a series of experiments to determine if rain treated plants had different extract viscosities and B-glucan contents from those of control plants. The results from the

Table 2 Total and 65°C Water-Insoluble B-glucan Content of Canadian Barley Cultivars Grown at Different Locations

Cultivar and Location	Total	65°C Water-Insoluble	65°C Water Soluble (by difference)
'Klages'			
Winnipeg	5.62 ± 0.02	2.46 ± 0.08	3.16
Lethbridge	5.16 ± 0.01	1.85 ± 0.03	3.31
'Betzes'			
Winnipeg	5.29 ± 0.10	2.54 ± 0.11	2.75
Lethbridge	5.46 ± 0.11	2.08 ± 0.06	3.38
'TR 904'			
Winnipeg	4.33 ± 0.03	2.23 ± 0.02	2.10
Lethbridge	3.77 ± 0.10	1.79 ± 0.02	1.98
'TR 406'			
Winnipeg	5.31 ± 0.04	2.15 ± 0.06	3.16
Lethbridge	5.11 ± 0.05	1.78 ± 0.02	3.33

Adapted from Anderson et al, (1978).

experiments showed that during the yellow and full ripeness stages, extract viscosities from all rain treated plants were significantly less ($P < 0.05$) than the control plants. During these stages of plant growth, the differences in the extract viscosities were paralleled by differences in soluble, insoluble, and total B-glucan contents (Aastrup, 1979b). These differences may best be explained on the basis of a lower rate of synthesis of B-glucans during the yellow and full stage ripeness in the rain treated plants as compared to the control plants. Coles (1979) found when the moisture contents of the seed declined by 40-50%, the accumulation rate of mixed linked B-glucans increased sharply in Lami and Triumph barley. The author stated that this result may have occurred as a result of the lack of starch accumulation in the barley grain.

Harvest conditions can also have an effect on extract viscosities on B-glucan contents. Aastrup (1979a) showed that a wet harvest results in barley flour being characterized by having a low extract viscosity and B-glucan content, while a dry harvest yields grains whose flour has a high extract viscosity and B-glucan content. In addition, Gohl et al (1978) found the viscosity of barley extracts were highest in grains harvested at yellow ripeness (Hesselman et al, 1981). Aastrup (1979a) reported a subsequent decline in barley extract viscosity from yellow to full ripeness. This reduction in barley extract viscosity is paralleled by a significant decline in the amount of soluble B-glucans but not in the insoluble B-glucans (Gohl et al, 1978). The structural nature of the soluble B-glucans in barley grains from yellow to full ripeness was similar (Aastrup, 1979a). Therefore, the reduction in

extract viscosity may have resulted from a lower percentage of soluble B-glucans being extracted in the acid flour extract and may have not been due to degradation of the soluble component. Some possible explanations for the reductions in soluble B-glucans from yellow to full stage ripenesses are (Aastrup, 1979a):

1. Continued synthesis of non-B-glucans which would reduce the relative concentration of B-glucan.

2. The soluble B-glucans may become insoluble.

3. The structure of B-glucans may be modified so that the resulting polymers are inaccessible to B-glucanases used for glucan determination.

Agronomic practices can also influence extract viscosity and B-glucan content of barley. Campbell et al (1986) observed an effect of seeding date (May 21 or June 14), fertilizer level, and location on extract viscosity. Prolonging the seeding date reduced extract viscosities of barley, presumably by delaying ripening into the cooler season (Campbell et al, 1986). Similarly, higher fertilization rates tended to reduce extract viscosities and B-glucan contents, which may also be explained on the basis of delayed harvest.

C. B-glucan Assays

Differences in B-glucan contents may also be derived from the wide ranges of methods used for the extractions and the determinations of B-glucans (Jorgensen and Aastrup, 1987).

1. Methods Based on Extraction

A majority of methods published for B-glucan analysis involve solubilization of the B-glucan polymer either in the native or degraded form (Jorgenson and Aastrup, 1987).

a) Viscosity Methods

Determining B-glucans by viscosity measurements of barley extracts is a simple method, and has been used as a screening technique in barley breeding programs (Greenberg and Whitmore, 1974; Bendelow, 1975; Morgan and Gothard, 1977; Aastrup, 1979b).

The final results of such methods may differ depending on the molecular weights of the extracted B-glucan and the co-extraction of other components which also may increase the viscosity (e.g. pentosans, starch). Variations in B-glucan content and extract viscosity may also be attributed to differences in the proportion of soluble and insoluble B-glucans (Wood, 1983). Possible actions of B-glucanases present in the barley during extraction, may also cause the content to be underestimated (Jorgensen and Aastrup, 1987).

b) Difference Methods

These methods are based upon the assumption that the only glucose polymers present in the extracts are derived from B-glucan or starch (Jorgensen and Aastrup, 1987). An estimate of the B-glucan content can be achieved by measuring the total carbohydrate content as glucose in an extract and subtracting that derived from the degradation of starch with amyloglucosidase. This method is considered accurate only if the extraction method employed does not extract too much of the starch

(Jorgensen and Aastrup, 1987). Wood (1983) has shown that some of the B-glucan which is present in the aleurone and sub-aleurone layer may still remain, even after repeated extractions.

2. Non-extraction Methods

a) Near Infrared Reflectance

Near infrared reflectance (NIR) is a well known non-destructive technique commonly used for determining B-glucans in the food and feed industry (Mcdonald, 1986). NIR analysis is based on the observation that major constituents within the cereal have a spectrum that is specific for the component to be investigated (B-glucan). A computor model can be used to eliminate the effects of interfering substances (Jorgensen and Aastrup, 1987).

b) Calcofluor Staining Used in Flour Suspensions

The fluorochrome, calcofluor, specifically stains B-glucans containing 1,4 linkages. The method involves staining intact cell walls of halved grains and visual inspection in UV light (Jorgensen and Aastrup, 1987). In a technique described by Jensen and Aastrup (1981), fine flour is stained by calcofluor suspended in glycerol and the fluorescence intensity is measured in a spectrofluorometer. A good correlation was found between the fluorescence intensity obtained from the flour suspensions and the total B-glucan content determined by the enzymatic method. Errors from this method may result from not all of the B-glucan cell walls being equally accessible to staining and therefore not stained uniformly (Jorgensen and Aastrup, 1987).