NITROGEN APPLICATION, PROTEIN CONTENT AND QUALITY OF WHEAT*

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Abstract

Protein content and loaf height of micro wheat loaves were compared with spring wheat cultivars grown in seven different localities of the Winter Rainfall Region during 1979. Some of the cultivars were also grown inside growth rooms at 15°/10°C day/night and 12 hours day length at low and high nitrogen level in order to eliminate the influence of environment and cultrual practices on protein content.

High protein content was related to favourable loaf height in a few localities only. On most localities cultivars produced fairly high protein content but low loaf height.

The loaf height obtained for four cultivars grown inside growth rooms at low nitrogen level was indicative of the loaf height obtained by the specific cultivar at high nitrogen level.

Production of too low a loaf height at low protein content might be associated with an unfavourable loaf height at high protein level. The increase in loaf height for per cent increase in protein content as obtained inside growth rooms might be considered an objective method of screening potential wheat lines for release as classes A or B wheat.

Gluten components, glutenin and gliadin were quantitatively determined for one cultivar.

Introduction

Regulations by the Wheat Board specify that a new wheat cultivar, upon release, be classed A or B based on baking quality. The norms for quality evaluation are bread volume, protein content, water absorption, physical dough properties, flour extraction, colour and milling properties.

Such information is collected for two or more seasons from different localities before the release of a new wheat line as a class A or B cultivar. A higher price is fixed for A than B wheat regardless of the area of production.

The miller is concerned about the place of production of the wheat he is receiving and grades incoming consignments according to his own standards, which comprise the falling number as an indication of the extent of germination which could have taken place, and mixograph curves. The latter demonstrate dough development time, protein level and dough tolerance. Classification of the specific cultivars received by the miller has less significance to him than the place of origin of the wheat.

Environment has an overriding influence on quality. Important environmental factors are level of soil fertility, available nitrogen, rainfall, atmospheric humidity and prevailing temperature, especially during kernel filling (Moss, 1973). These factors determine the protein content of the flour which is claimed the most important single factor causing variation in bread volume within the same cultivar (Pomeranz, 1973).

The purpose of this research is to try and establish to what extent environment overshadows the heritable differences of wheat cultivars as far as the uptake and utilisation of nitrogen are concerned. The underlying aim is to develop procedures for objectively rating quality potential of a genotype.

Methods

Experiment 1

Twelve wheat cultivars were grown in randomised blocks at seven localities in the winter rainfall region from May to October in 1979:

Cultivars

Cultivars	Class	Cultivars	Class
Elrina (El)	B	Flameks (Fl)	A
Gourits (Go)	A	Inia (In)	A
Janitor (Jan)	B	Kasteel (Ka)	A
Lee Mida (LM)	A	Liesbeeck (Li)	B
Raven (Ra)	A	Skemer (Sk)	A
SST_2	A	SST_3	A

Localities

SWARTLAND:	Morreesburg	(Mo)
	Malmesbury	(Ma)
RÛENS:	Boontjieskraal	(Bo)
	Dunghye Park	(DP)
	Sersantsrivier	(Sr)
	Jonaskraal	(Jo)
Welgevallen Experimental farm		(WG)
		Stellenbosch

The experiments received the same fertilizer treatments at planting supplemented with regular nitrogen top dressings.

Experiment 2

Cultivars El, Go, Li, Ra and SST_3 were grown in pots in growth rooms at 15°/10°C day/night, 12 hours daylength and 70% RH at two nitrogen levels, namely, the equivalent of 189 and 100 kg N/ha, respectively. The same quantity of nitrogen (equivalent of 42 kg/ha) was applied to both treatments at planting while the number of top dressings during growth differed — the low level treatment received two and the high level treatment five top dressings, the equivalent of 29,4 kg N/ha per top dressing.

Experiment 3

Cultivar Li was grown in pots in a glass house at 18°/12°C day/night and natural photoperiod from May to September at Welgevallen. Phosphate and potassium were applied in quantities dictated by soil analysis while nitrogren was added at the following growth stages and at quantities indicated in Table 1.

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TABLE 1: Nitrogen treatment of wheat cultivar Liesbeeck at successive growth stages

Treatments	Soil application (with limestone ammonium nitrate)		Foliar application (with urea)		
	With planting	Thirty days after emergence	At growth stages (gs)*		
			15	21	24,5
1	45 kg N/ha				
2	45 kg N/ha	plus 45 kg N/ha at gs. 6			
3	45 kg N/ha		45 kg/ha		
4	45 kg N/ha			45 kg/ha	
5	45 kg N/ha				45 kg/ha
6	45 kg N/ha		45 kg/ha	45 kg/ha	45 kg/ha

* The key for growth stage (gs) indentification of wheat by Craven (1966) was followed. Leaf production terminates at gs 6, spikelet initiation at gs 10, spikelet differentiation at gs 15, the flag leaf appears at gs 16,5 and anthesis occurs at gs 22.

Quality testing of the flour, obtained by milling the kernels with a small Miag mill for protein analysis and a Quadrumat Junior mill for gluten component analysis, involved the following:

(i) Total protein content by macro Kejldahl
(ii) Mixograph curves
(iii) Separation of protein components glutenin, gliadin, albumin and globulin with sephadex G200 column chromatography as described by Meredith and Wren (1966).
(iv) Dry gluten content by means of the Glutomatic 2100 (these are not reported in this paper).
(v) Baking of micro bread loaves by the Grain Technology Section of the Wheat Control Board, Pretoria. This determination was not done for experiment 3 due to a small grain mass harvested from individual treatments.

Results and discussion

Experiment 1

The protein content and loaf height of the micro loaves, as an indication of loaf volume, were plotted for the twelve cultivars over the six farms and for the farms over cultivars. Quadrant lines were drawn at 75 mm loaf height and at 13% protein.

These values are considered the definition lines between class A and B wheat. The Wheat Board sets a limit between 73 to 85 mm for loaf height and 12% flour protein for A wheat. In this study the protein limit was raised to 13% since macro Kejldahl analysis was done on the contents of whole kernels and not extracted flour.

The four quadrants represent the following quality division in terms of protein content and loaf height (Table 2):

Quadrant (Quad) I	Low protein High loaf
Quad II	Low protein Low loaf
Quad III	High protein High loaf
Quad IV	High protein Low loaf

TABLE 2: The grouping of 12 wheat cultivars grown on six localities in the Winter Rainfall Region in 1979 according to protein content and loaf height.

Locality	Low protein High loaf Quad I	Low protein Low loaf Quad II	High protein High loaf Quad III	High protein Low loaf Quad IV
Ma (Swartland)				El (B) LM (A) Fl (A) Li (B) Go (A) Ra (A) In (A) Sk (A) Jan (B) SST_2 (A) Ka (A) SST_3 (A)
Mo (Swartland)			El LM Fl Li Go Ra In Sk Jan SST_2 Ka SST_3	
Bo (Rûens)		Ka LM Li Ra		El Jan Fl Sk Go SST_2 In SST_3

Table 2 continued

DP (Rûens)		Ra SST_2		El Ka Fl LM Go Li In Sk Jan SST_3
SR (Rûens)		El Li Go Ra In SST_2 Ka SST_3 LM		Fl Jan Sk
Jo (Jonaskraal)			Fl LM In Sk Go SST_2	El Li Jan Ra Ka SST_3
Wg (Stellenbosch)			Fl Sk In SST_2 Go	El Li Jan Ra Ka SST_3 LM

Swartland localities
All cultivars produced high protein on Ma and Mo but in the former case the effect of protein was not reflected in high loaf height as was the case on Mo.

Rûens localities
High protein content was obtained for all twelve cultivars on Jo, for ten cultivars on DP, eight cultivars on Bo and only three cultivars on Sr. Despite high protein content, low loaf heights were produced by six of these cultivars on Jo, eight cultivars on Bo while all cultivars on Sr yielded low loaf height.

All twelve cultivars produced high protein on Wg but only five of these measured high loaf height.

A positive relationship between protein content and loaf height was obtained only for the twelve cultivars on Mo, six cultivars on Jo and five cultivars on Wg. These results are contrary to the findings of Finney (1978) that bread volume increases linearly with protein content within the same cultivar. The dominating influence of the environment of specific localities and cultural practices on protein content and loaf height has been illustrated by the above findings.

Experiment 2

The influence of environment by a specific locality was eliminated by growing four of the cultivars Go, El, Ra and SST_3 inside growth rooms and testing the effect of increased nitrogen application on protein content and loaf height.

The protein content and loaf heights at low and high nitrogen application are illustrated in table 3.

Loaf height of all four cultivars was increased when protein content was raised. El showed the biggest increase in both protein content and loaf height while SST_3 responded the least. It seems that loaf height obtained at a lower protein level is indicative of the height of the loaf at a higher protein content.

Differences in the slope of the curves in Figure 1 mean that some cultivars showed a larger increase in loaf height per percent increase in protein than others.

The loaf height produced at a protein content of, say, less than 12% and the slope of the regression line for loaf heights obtained between low and high protein of cultivars, or lines, grown under controlled environment, might be regarded as a measuring standard for class A or B wheat.

TABLE 3: Loaf height and protein content of wheat cultivars Gourtiz (Go), Elrina (El), Raven (Ra) and SST_3 grown inside growth rooms at 15°/10°C day/night, 12 hours daylength and high and low nitrogen application

Cultivars	Class	Protein content %	Percentage difference	Loaf height (mm)	Percentage difference
Go	A	18,8 11,7	61	74 67	10
El	B	20,2 12,1	67	73 62	18
Ra	B	16,1 9,7	64	82 73	12
SST_3	A	16,4 11,7	40	70 67	4

Comparison of loaf height, or volume, at different protein levels of cultivars/lines grown under controlled conditions and in different production localities over seasons, might serve as a selection measure for identifying genotypic ability on the uptake and utilisation of nitrogen.

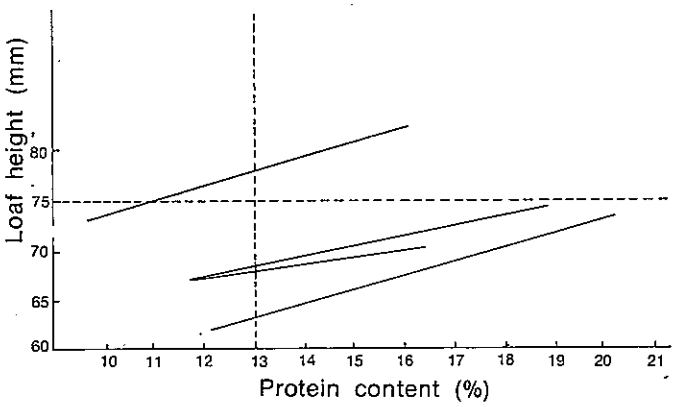

Fig 1: Loaf height and protein content of wheat cultivars Gourits (1), Elrina (2), Raven (9) and SST_3 (12) grown inside growth rooms at hight and low nitrogen nutrition.

Experiment 3

The observations in experiments 1 and 2 illustrate that the influence of high protein content on loaf height is dependent on the environment and cultivation practices of the specific locality and also on the cultivar. The miller is basically interested in the gluten fraction of the protein which can vary from 50 to 85% of the total protein content.

Differences in bread volume between cultivars are mainly due to gluten protein and the composition thereof. Gluten fractions identified in wheat are gliadin and glutenin. The former is soluble and the latter insoluble in ethylalcohol. Glutenin constitutes about half the total protein in wheat flour; it is stiff and tough, less inclined to stretch and determines dough development time which reflects water absorption capacity. Gliadin is less viscous and is responsible for gas retention during fermentation, thus influencing bread volume.

Favourable bread volume is related to a longer dough development time, as determined by the Mixograph and a higher gliadin fraction. The latter fraction differs quantitatively for the flour of cultivars producing different loaf volumes.

Application of the nitrogen fertilisation levels to cultivar Li at successive growth stages inside a temperature controlled glass house was aimed at finding out how total protein and more specifically the different gluten components, are affected.

The absorption curves obtained at 280 nm for gluten components, glutenin and gliadin and the water soluble flour protein components, albumin and globulin, as obtained by chromatographic separation of the residue of a 4 gram flour sample in a 700 mm x 16 mm sephadex G200 column, are demonstrated in Figure 2. Component identification was done as accurately as possible and the surface under the curves outlining the separate components is demonstrated in Figure 2. The surface areas determined for the individual components are stipulated as percentages on different histograms, in Figure 3, while total protein analysed for the various treatments is given in Table 4.

Increase in protein content of the kernels depended on the stage of application. Nitrogen dressings before and after anthesis (treatments 4 and 5) increased protein content significantly as compared with the control. Application of three consecutive nitrogen dressings before and after anthesis during kernel filling (treatment 6), caused an increase in protein content of 39% over and above the control treatment. It would be possible to still increase the protein content above 17,83% with higher dressings especially during kernel filling. Time of application appears to be the important consideration in this regard.

The question to be answered is whether gluten quantity, or components thereof, are likewise augmented to benefit the miller and baker. They are interested in a linear increase in gluten content or insoluble protein fraction with an increase in kernel protein.

Nitrogen treatments 1 to 4 favoured the glutenin fraction with a concomitant increase in protein content up to 14,5%. Gliadin synthesis, on the contrary, was benefitted by treatments 5 and 6 and it appears that this has happened at the expense of glutenin build up. The water soluble albumin and globulin protein components were reduced in quantity to a greater or lesser degree respectively. These soluble protein components play a rather insignificant role in bread volume. The cultivar Li is apparently capable of incorporating extra nitrogen in the gliadin component of gluten, a property which should favour bread volume with high nitrogen dressings.

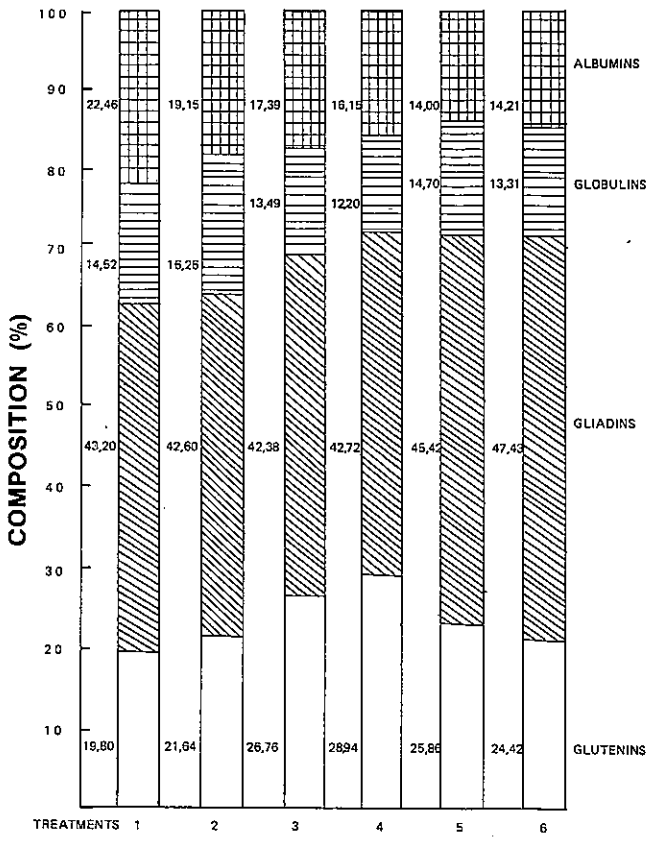

Fig 3: Composition of protein components as determined by gel filtration of flour samples of samples of the wheat cultivar Liesbeeck which received different nitrogen applications at successive growth stages inside a temperature controlled glass house.

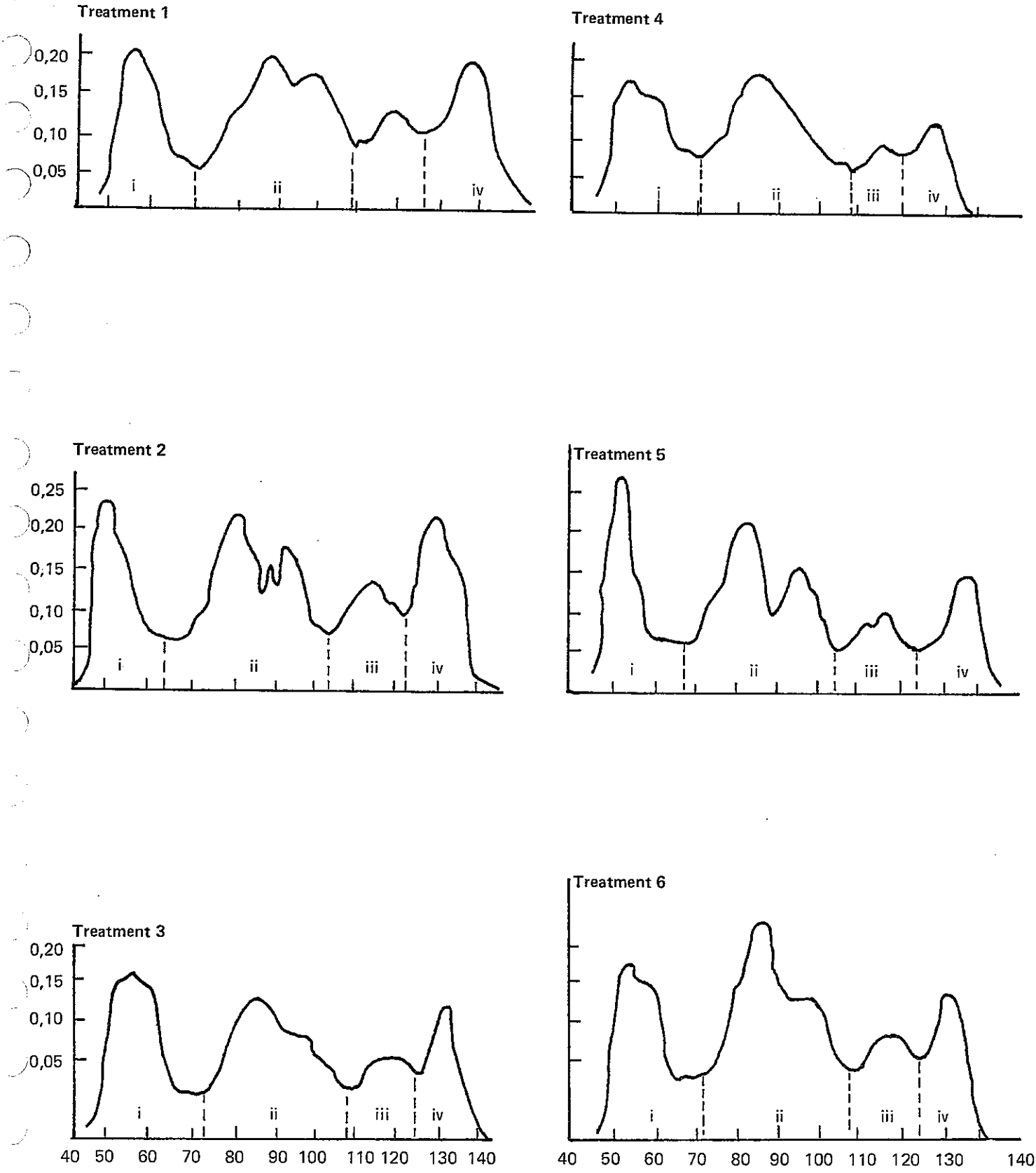

Fig 2: Chromatograms of components of endosperm proteins of wheat cultivar Liesbeeck after separation by gel filtration. (i = glutenins, ii = gliadins, iii = glubilins, iv = albumins)

TABLE 4: Protein content of wheat cultivar Liesbeeck (Li) which received different nitrogen treatments at successive growth stages (gs)

Treatment	Nitrogen Treatments — Time of application	Protein Content %
6	Soil applied at planting (45 kg N/ha), control treatment	12,78
2	Control treatment and soil applied at gs 6 (45 kg N/ha)	13,52
3	Control treatment and foliar spray at gs 15 (45 kg N/ha)	13,88
4	Control treatment and folair spray at gs 21 (45 kg N/ha)	14,49
5	Control treatment and foliar spray at gs 24,5 (45 kg N/ha)	14,70
6	Control treatment and foliar spray at gs 14,8 (45 kg N/ha), gs 20,5 (45 kg N/ha) and gs 23,8 (45 kg N/ha)	17,83
	LSD (0,05)	1,30

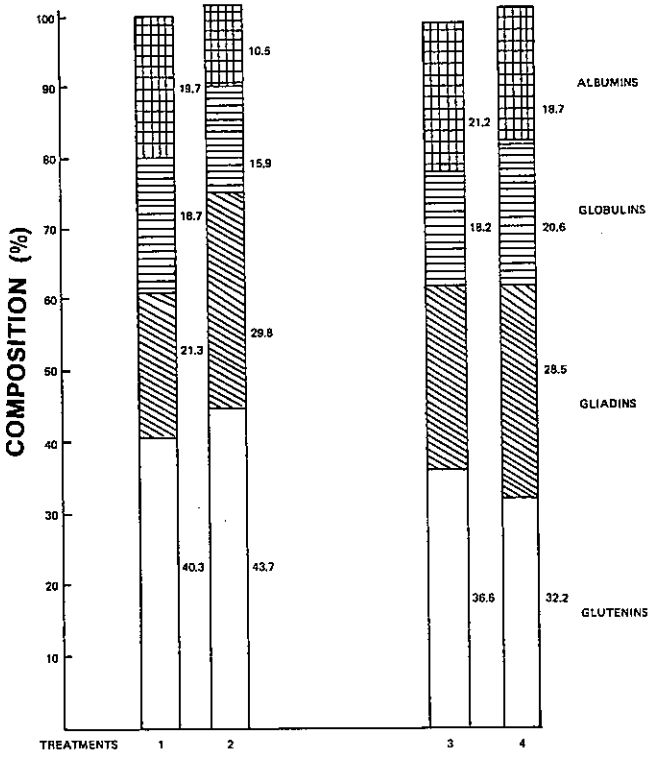

Fig 4: Composition of protein components as determined by gel filtration of flour samples of wheat cultivar Liesbeeck grown inside growth rooms at low and high nitrogen application (treatments 1 and 2, respectively) and grown on localities Jonaskraal and Moreesburg (treatments 3 and 4).

The fact that the glutenin component was reduced when the protein content reached a certain peak is in accordance with findings reported in literature (Bushuk *et al,* 1978). The effect of high protein content of some cultivars is reflected in the mixograms, in that a reduction in dough development time, or peak time, sets in after a certain protein level has been reached.

The protein components were also analysed for Li grown inside a growth room at 15°/10°C day/night at 12 hours daylength and receiving high and low nitrogen dressings as was described in experiment 2. The results are demonstrated in Figure 4. High and low nitrogen application increased the protein content to 15,8% and 13,1% respectively. The glutenin component was raised from 40,3 to 43,7% and the gliadin fraction from 21,3 to 29,8%. Both the albumin and globulin fractions were reduced as a result of high nitrogen application.

Analysis of wheat samples of Li drawn from experiments grown in Mooreesburg (Swartland area) and Jonaskraal (Rûens area) in 1979 was as per (Figure 4). The protein content analysed for Mo and Jo were 17,1 and 14,1% respectively. Similar to the glass house experiment the glutenin content for Mo, as compared with Jo, was reduced at a protein content over 17% while the gliadin component was increased, The albumin percentage of Mo was also decreased which agrees with the glass house results while no explanation can be offered for the increase in globulin content at Mo.

These results demonstrated the overwhelming influence of local environment and cultural practice on protein content and loaf height of different wheat cultivars. This finding should be borne in mind when samples of potential wheat lines under consideration for release and classification are drawn in different production regions for quality evaluation.

The deduction from experiment 2 is that application of a "nitrogen stress" under controlled conditions inside growth rooms and glass houses can aid in identifying and screening for inherent bread quality traits such as loaf height and volume. The merits of the baking test on wheat with low protein content due to insufficient nitrogen dressings inside growth rooms, as compared with the response obtained when sufficient nitrogen is applied, should be assessed.

Determination of the effect of increased protein content due to nitrogen application at different growth stages throws light on the favourable effect of gluten components, glutenin and gliadin. In Li the glutenin content is apparently reduced in favour of gliadin when the protein content reaches a certain level. The ratio between glutenin and gliadin components seem to differ depending on the environment, ie growth room, glass house or outside localities. The cause of these discrepancies should first be explained before gluten component analysis by means of column chromatography is accepted as a positive procedure for quality evaluation of potential wheat lines. The analytical procedure nevertheless holds promise for substituting present empirical practices such as the drawing of commercial samplings and obtaining an average score of physical dough and bread properties.

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