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Mono- and Oligosaccharides in Fifteen *Vicia faba* L. Cultivars

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ABSTRACT

Fresh and dry mature seeds of fifteen cultivars or lines of faba bean were compared with respect to their mono- and oligosaccharide contents, in an attempt to determine the possibilities for selection in a breeding programme on the basis of some chemical characteristics. The raffinose content of the whole dry seed ranged from 0·12% to 0·29%, the stachyose content between 0·46% and 1·02%, the verbascose content, the principal α-galactoside, from 0·82% to 1·61% on a dry matter basis. These components occurred in seeds with more than 30% of dry matter, while fructose, glucose and sucrose regularly decreased during seed development.

INTRODUCTION

Faba beans are an excellent food for humans, being high in protein and easily digestible (Carnovale *et al.*, 1979; Scarascia Mugnozza & Marzi, 1979; Lattanzio *et al.*, 1983; Bhatty & Christison, 1984). However, the biological value of faba bean is negatively affected by the presence of antinutritional factors, i.e. trypsin inhibitors, haemagglutinins, tannins, and favism-inducing factors (Elias *et al.*, 1979; Mager *et al.*, 1980; Griffiths, 1981; Deshpande & Salunkhe, 1982; Elkowicz & Sosulski, 1982; Fernandez *et al.*, 1982; Lattanzio *et al.*, 1982; Arora,

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1983; Hill-Cottingham, 1983; Liener, 1983; Reddy *et al.*, 1985). Certain factors, such as trypsin inhibitors, haemagglutinins and condensed tannins, are readily destroyed by cooking or eliminated by pretreatments (dehulling, pre-soaking, etc.) (Deshpande *et al.*, 1982; Elkowicz & Sosulski, 1982; Marquardt, 1983).

In common with other legume seeds, faba beans also contain significant amounts of the oligosaccharides of the raffinose family. These oligosaccharides attract attention because of their known tendency to cause flatulence problems (Lee *et al.*, 1970; Cerning *et al.*, 1975; Macrae & Zand-Moghaddam, 1978; Liener, 1980; Quemener & Mercier, 1980; Sosulski *et al.*, 1982; Arora, 1983; Kennedy *et al.*, 1985). Even though Rockland (1968), searching with *Clostridium perfringens*, an intestinal anaerobe considered the primary source of flatus gases, found that stimulation of gas production by bean homogenate could not have been due to the presence of raffinose and stachyose, it is now widely accepted that the ingestion of these sugars as components of legumes is a major cause of flatulence. Some experiments with human subjects have demonstrated that legume species and cultivars exhibit wide variations in flatus production (Sanchez & Tucker, 1966; Liener, 1980; Arora, 1983; Hill-Cottingham, 1983). Using the rat model, with different legume seeds, flatulence was reported to be significantly and positively correlated with oligosaccharide and acid-hydrolyzable pentosan contents, but negatively correlated with starch and lignin contents. Removal of the oligosaccharides did not eliminate flatulence: some indigestible oligosaccharides and cell-wall fiber components also caused an increase in hydrogen production (Fleming, 1981).

Therefore, to utilize legumes as a more acceptable source of inexpensive proteins, it could be desirable to reduce the flatulence production. Possible approaches are: breeding of varieties low in these oligosaccharides, use of enzymes to reduce the oligosaccharide concentration (Reynolds, 1974) and soaking to germinate the seeds before cooking (Iyer *et al.*, 1980; Jood *et al.*, 1985).

In this study fifteen cultivars or lines of *Vicia faba* L., varying in size, protein content and yield, were grown under similar conditions and seeds were compared with respect to their carbohydrate content, in an attempt to determine the possibilities for selection in a breeding programme on the basis of this chemical characteristic. For five cultivars, the mono- and oligosaccharide contents were measured at various stages of seed development, in order to determine at what point the reputed gas-forming principles begin to appear between the immature green and dry mature seed stage.

EXPERIMENTAL

Plant material

All plants were sown on the same day and grown under similar conditions. Fresh seeds were harvested at different physiological stages, weighed and then analyzed for total dry matter and carbohydrate contents. Dry mature seeds, harvested at the end of the biological cycle, were also analyzed.

Sample preparation

For mono- and oligosaccharide determination, 10 g of fresh seeds, or 3 g of dry seeds, were first homogenized in a blender with 100 ml of ethanol:water (1:1) for 5 min and then refluxed for 1 h. The extracts were filtered and concentrated by removal of ethanol under reduced pressure at 30°C, passed through a 0·45 μm Millipore filter to prevent clogging of the column and then diluted (1:1) with acetonitrile.

HPLC

A Perkin–Elmer Series 2 liquid chromatograph, equipped with a Sigma 10B Data Station and a differential refractometer was used. Two different columns were used: a 4 mm (id) × 30 cm Waters Bondapak/Carbohydrate column (10 μm) eluted with acetonitrile:water (75:25 v/v), and a 4 mm (id) × 25 cm LiChrosorb–NH_2 (5 μm) column (Merck) eluted with acetonitrile:water (70:30 v/v). A 30 μl sample of the sugar standard or a 60 μl sample of each seed extract was injected by means of a Rheodyne valve. The flow rate of the elution solvent was 1·0 ml/min and attenuation was 2 × on the RI detector.

RESULTS AND DISCUSSION

The monosaccharides glucose, fructose and sucrose, and the α-galactosides raffinose, stachyose and verbascose, in fifteen faba bean cultivars were quantitated by LC. Despite the precautions of filtration with a Millipore filter and a precolumn, gradual plugging of the column by seed extracts occurred. With these precautions, however, resolvability of the sugars and column life were maintained through about 100 sample injections.

Table 1 shows the sugar content of the cultivars at the dry mature seed stage. It is important to note that the results reported in the Table have been obtained using calibration curves of the individual sugars, with the exception of verbascose. For this oligosaccharide a theoretical calibration curve was drawn by extrapolating the values for raffinose and stachyose.

The values obtained were broadly comparable with those obtained by Cerning *et al.* (1975), who have reported in more detail the range over which some of the carbohydrate fractions occurred in the beans from sixteen cultivars of *Vicia faba* originating from many parts of the world. Less than one-tenth of the total carbohydrate in the whole seed was soluble in aqueous ethanol: the main constituents of this soluble fraction were sucrose and a series of oligosaccharides—raffinose, stachyose and verbascose.

By the dry mature seed state, glucose and fructose had completely disappeared, while raffinose, stachyose and verbascose reached their highest levels. Verbascose was the major, and raffinose the minor, α-galactoside in all cultivars: analyses for these ranged from 0·82% to 1·61% and from 0·12% to 0·29% of dry matter, respectively. The stachyose content ranged from 0·46% to 1·01% of dry weight.

There appeared to be differences in the chemical characteristics of whole seed associated with phenotypic seed size. Significant positive correlation was found between seed weight and total α-galactoside content when the seed mean weight was lower than 1 g (correlation coefficient = 0·864; $P < 0{\cdot}01$). Only for cv. 'Eureka' did there appear to be no relationship: if this cultivar was removed from the correlation, the r value increased to 0·899. In the large-seeded cultivars (1 g and above in the seed mean weight of mature dry seed) this correlation was absent; the total α-galactoside content was almost constant. This difference is of interest in that the small-seeded cultivars are bred for processing (quick-freezing).

The results show that, probably, there was genetic variation among lines for total α-galactoside content, raffinose content, stachyose content and verbascose content. Thus, it may be feasible to select particular lines

TABLE 1

Sugar Content (Per cent on Dry Matter Basis) in Dry Mature Whole Seeds of Different Cultivars and Lines of *Vicia faba L.*

Cultivar or line	*Seed mean weight (g)*	*Per cent dry matter*	*Sucrose*	*Raffinose*	*Stachyose*	*Verbascose*	*Total α-galactosides*
Beryl	0·61	89·60	1·08	0·12	0·46	0·82	1·40
Eureka	0·79	89·50	1·53	0·21	0·69	1·25	2·15
Optica	0·83	89·68	1·10	0·19	0·60	0·88	1·67
Felix	0·84	89·42	1·22	0·14	0·58	1·17	1·89
Minica	0·91	89·16	1·47	0·18	0·66	1·19	2·03
Metissa	0·92	89·60	1·29	0·21	0·75	1·27	2·23
Feligreen	0·95	89·53	2·02	0·29	0·99	1·34	2·62
Ipro	1·07	89·58	1·38	0·26	0·89	1·26	2·41
MG 106364	1·10	89·02	1·44	0·26	1·02	1·30	2·58
MG 106361	1·16	89·44	1·55	0·19	0·73	1·61	2·53
Gemini	1·21	89·78	1·34	0·19	0·69	0·86	1·74
MG 103247	1·48	89·72	1·58	0·21	0·79	1·15	2·15
MG 103271	1·50	89·15	1·57	0·23	0·85	1·21	2·29
MG 103259	1·59	89·50	1·60	0·20	0·81	1·51	2·52
Reina Blanca	1·65	89·28	1·26	0·18	0·83	1·15	2·16
Mean ± standard error	1·10 ± 0·08	89·46 ± 0·06	1·43 ± 0·06	0·20 ± 0·01	0·75 ± 0·04	1·19 ± 0·06	2·16 ± 0·09
Coefficient of variation	28·87	0·25	16·52	21·67	20·07	18·31	16·72

Coefficients of variation for replicate analyses were 4·8%, 7·0%, 5·3% and 4·4% for sucrose, raffinose, stachyose and verbascose, respectively.

combining different proportions of single α-galactoside. Furthermore, conventional processing in distilled water (soaking and cooking) and soaking followed by autoclaving caused losses to a great extent (up to about 60%) (Iyer *et al.*, 1980; Jood *et al.*, 1985).

The sucrose content in dry mature seed was lower than in ripe green seed: it ranged between 1·08% and 2·02% on a dry matter basis. Figure 1 shows the variation of sucrose content during seed development and these variations are described by the function:

$$y = 386.x^{-1\cdot 31}$$

During the first stages of seed development (until seeds reach 30% of dry matter) the sucrose content rapidly decreased from 15% to 4·0% on a dry matter basis, then it decreased slowly to 1·0% on a dry matter basis in dry mature seed.

The variations of the other sugars were lower. Both glucose and fructose contents were about 2·0% on a dry matter basis in the first physiological stages (unripe green seed), then regularly decreased and disappeared in ripe mature seed (about 25% dry matter). The concentration of the α-galactosides was just the reverse of the glucose and fructose pattern. In fact, in all cultivars examined, until the colour of cotyledons was changing from green to pale yellow (30% dry matter), neither raffinose nor stachyose and verbascose could be detected in seeds. These oligosaccharides were detectable only in the later stages of seed development

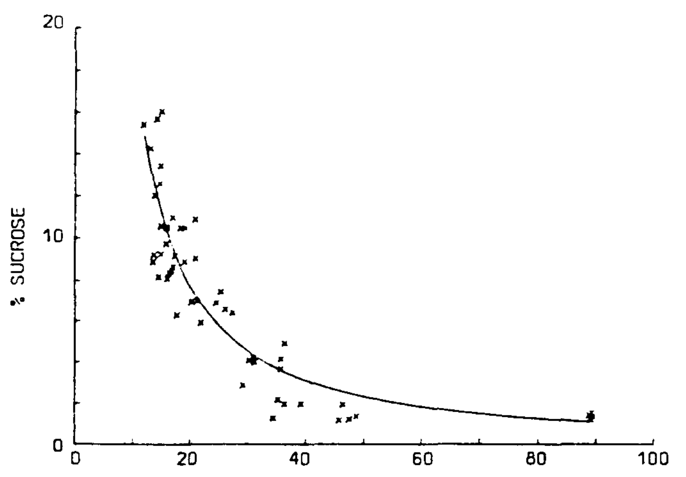

Fig. 1. Metabolism of sucrose during seed development.

after embryo development was complete: then their content increased regularly until the seed reached the dry stage. This result was in good agreement with the finding of Sanchez & Tucker (1966) who found, on feeding a panel of human subjects, that the flatus-principles appeared only after the beans reached full maturity.

CONCLUSIONS

Previous reports from our laboratory (Lattanzio *et al.*, 1982, 1983) have dealt with protein content and amino acid composition, as well as with vicine and convicine content of *Vicia faba* cultivars. On considering the nutritional value of this legume seed, no correlation was found between nutritional factors (protein content) and antinutritional factors (total α-galactoside and total alkaloid glycoside contents).

Furthermore, the total alkaloid glycoside content decreased during seed development and reached the lowest value in the dry mature seed, ranging at this stage from 0·80% to 1·32% on a dry matter basis. In addition, the protein content of the different cultivars at the dry mature seed stage ranged from 33·6% to 28·0% on a dry matter basis.

From the results reported here it appears likely that α-galactoside content is more likely to be a significant factor in mature seeds.

Cv. 'Beryl' showed the best nutritional characteristics, having a high protein content (30·0% on a dry matter basis) and a low antinutritional factor content (total α-galactoside = 1·40% on a dry matter basis and total alkaloid glycoside = 0·90% on a dry matter basis).

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