CHARACTERIZATION OF CARBOHYDRATE COMPLEX OF CEREALS AND ITS NUTRITIONAL IMPLICATIONS

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Summary

Two varieties of triticales, 3 of soft wheats, 2 of hard wheats and 3 of durum wheats were anlayzed for moisture, crude protein, ash, lipid fraction, acid and neutral detergent fibers, available and non-available carbohydrates, hemicellulose, lignin, reducing sugar and starch contents. Amylose / amylopectin ratios in starch were determined. The nutritional value was determined using Tribolium castaneum larvae and Japanese quail. The growth of test organisms was poorly correlated to the protein content of cereals. It was strongly correlated to the available and non-available carbohydrate contents.

I. INTRODUCTION

The plant breeders manipulate the genetic make up of cereals to develop new varieties for increased yields and pest resistence. The ultimate use of these cereals in the diets of humans and animals is of direct concern to the nutritionist. He evaluates the nutrient content of these cereals in terms of their protein content and amino acid profiles, nature of carbohydrates, lipids, vitamins and minerals, their availabilities, and the presence of any deleterious antinutrients. The evaluation of availability of nutrients has to be done by feeding trials with mice, rats,, or poultry and the sample size has to be large enough to conduct these experiments.

It is desirable to develop a bio-assay for screening a large number of samples which may be available in less than 10 g quantities. Insects, specially which infest cereals, deserve further study as test organisms. The rationale is that any cereal which does not support the optimal growth of pest insect larvae is either deficient in a nutrient or contains some deleterious antinutrient.

The larvae of red flour beetle (Tribolium castaneum) have proved quite useful as test organisms in evaluation of nutritive value of some carbohydrates, starches. and cereals (Vohra et al. 1978, 1979 a,b).

We are interested in the nutritional quality of cereals, particularly their carbohydrate complex. The results of a study on the chemical characterization of this complex and its. effect on the growth of Tribolium larvae as well as quail are presented in this report. .

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II. MATERIALS -AND METHODS

Milled rice samples with different amylose contents were obtained from Western Regional Laboratory, Berkeley, California. The wheats and triticales were grown in the University of California Agronomy Research Farm in Davis.

Moisture, ash, lipids, crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) were determined by the methods given in AOAC (1975) and as described by Robertson (1978). A factor of 5.8 was used to convert Kjeldahl nitrogen. to crude protein for wheats and triticales.

Starch was isolated from test samples as described by Wolf (1964). Amylose in starch was measured according to the procedure of Williams et al. (1958). Amylopectin was estimated by subtracting the value of . amylose from that of total starch.

The methods described by Southgate (1969 a,b) were used for the determination of unavailable carbohydrate and available carbohydrates. An'other enzymatic method suggested by Hellendoorn et al. (1975) was also used for determination of unavailable carbohydrates.

Unavailable carbohydrates were further fractionated into water soluble pentosans, cellulose, pcetic substances, hemicellulose and lignin asoutlined by Southgate (1969 b).

For bioassays, larval weights of Tribolium castaneum fed the test cereal diets (Table 1) from 6 days to 14 days of age were determined. The diets were ground finely 'to pass through a 100 mesh sieve. Diets containing 90% finely ground test cereal and 10% brewers yeast were also used in another study. Essentially, the procedure was the same as described previously by Vohra et al. (1979 a).

Day-old Japanese quail (Coturnix coturnix japonica) were fed the test cereal diets of the composition given in Table 1. A combination of a test cereal and DL-glutamic acid with an estimated protein equivalence of 59.5% obtained by multiplying its nitrogen content by 6.25 was used to obtain a total of 15% crude pro'tein. The gain in body weight of Japanese quail was determined. over an experimental period of 2 weeks.

TABLE 1 - Composition of test cereal diet

Ingredient	g/kg Diet D-1[1]	D-2	D-3	D-4	D-5	D-6	D-7	D-8
Test Cereal	862.2	866.4	822.6	859.7	856.5	848.5	830.6	849.2
Glutamic acid	47.8	43.6	87.4	50.3	53.5	61.5	79.4	60.8
Basal Mix[2]	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0

[1]Test cereals: D-1, Mapache; D-2, 6AT-204; D-3, H-Ra[2]-F_2 II 41593-IR-3M-1S-2M-0S; D-4, Nacozari 76; D-5, Anza; D-6, Mexicali 'S' * Chapala - 21563 Cross No. CD1894-3Y-0Y-0M (Mexichap); D-7, Mexicali 'S'* Flamingo's' cross No. 1895-12Y-1Y-0M (MexiFg); D-8, Mexicali 75.

[2]Contained : (in g), DL-methionine, 4.5; $CaHPO_4.2H_2O$, 27.0 $CaCO_3$, 10; soybean oil, 20; KCl, 2.97; K_2HPO_4, 4.95; $MgSO_4.7H_2O$, 3.97; NaCl, 5.5; $MnSO_4.H_2O$, 0.297; $CuSO_4.5H_2O$, 0.097; ZnO, 0.12; Co $(C_2H_3O_2).4H_2O$, 0.02; $Na_2MoO_4.2H_2O$, 0.009; KIO_3, 0.009; $Na_2SeO_3.5H_2O$, 0.00066; $FeSO_4.7H_2O$, 0.664; (in mg), mendione bisulfite, 1.5; thiamin HCl, 4; riboflavin, 8; niacin, 60; calcium pantolhenate, 25; folic acid, 1.2; biotin, 0.2; vitamin B_{12}, 0.01, pyridoxine HCl, 8; Choline chloride, 1300; BHT, 100; (in IU), vitamin A, 5000; vitamin D_3, 4500; vitamin E, 88.

III. RESULTS AND DISCUSSION

The results of a preliminary screening of a large number of wheat and triticale varieties (Table 2) suggested that Tribolium larvae developed significantly better on triticales and soft wheats than on durum wheats ($P \leq 0.01$). Hard wheats were intermediate between the soft and durum wheats but did not differ significantly from either of them. The correlation between the crude protein content of the test cereals and the larval growth was poor, especially for the soft and hard wheats.

There may be some constituent besides the protein content, which may have a better correlation with the larval growth.

TABLE 2 - The effect of different varieties of wheats and triticales on growth of *T. castaneum* larvae.

Cereal type (sample No.)	Mean Wt. gain larva, mg (Y)	Mean crude protein % (X)	For Y=M+NX (M)	For Y=M+NX (N)	Correlation coefficient (r)
Triticale (7)	3.09c*	13.63	3.76	-0.05	-0.4
Soft wheat (9)	2.97bc	14.81	3.04	-0.003	-0.06
Hard wheat (24)	2.80ab	14.03	2.69	+0.01	+0.17
Durum wheat (7)	2.60a	13.59	4.27	-0.12	-0.34

*Significant differences are denoted by different letters in this column ($P \leq 0.01$).

The preliminary data on the bioassay of some rice samples with Tribolium larvae (Table 3) suggest a significant correlation between the crude protein (X_1) or amylose (X_2) contents and the larval growth (Y) with r values of -0.86 and -0.79, respectively.

It implies that the carbohydrate complex is of equal importance in the nutritional evaluation of cereals.

The 'proximate analyses of some wheats and triticale samples which were further characterized for their carbohydrate complex are given in Table 4. Two of the soft wheat (Atlas 66 and NapH al/Atlas 66) were not acclimatized to Davis climate and the grains were shrivelled. This explains their significantly high protein content and low total carbohydrate concentration. No significant differences in lipid or ash contents of these samples were observed,

TABLE 3 - The effect of some varieties of rice with different amylose content on the growth of *T. castaneum* larvae

Rice variety	Wt. gain/larva mg (Y)	% Content in rice: Crude protein (X_1)	Amylose (X_2)
Calrose - 76	3.00	5.0	19.9
CP-231	2.83	6.8	16.6
Toro - 1974	2.77	5.9	19.9
LA - 110	2.33	6.9	25.8
IR - 8	2.20	7.4	25.7
Labilla 75	2.17	7.3	22.4

$Y = 4.70 - 0.33\ X_1 \qquad r = -0.86$

$Y = 4.26 - 0.08\ X_2 \qquad r = -0.79$

TABLE 4 - The proximate composition of test cereals

Test cereal	Moisture %	Crude protein %	Lipid %	Ash %	Total Carbohydrate % (by difference)
Triticale					
Mapache	9.13	14.22	1.63	1.47	73.55
6AT-204	8.60	14.44	1.57	1.38	74.01
Soft Wheat					
H-Ra^2 F_2	8.19	12.04	1.64	1.43	76.7
Atlas 66	8.23	17.48a	1.70	1.50	71.09
Nap H al/Atlas 66	8.09	20.29a	1.77	1.59	68.26
Hard Wheat					
Nacozari 76	8.98	14.09	1.41	1.41	74.11
Anza	8.64	13.92	1.52	1.35	74.57
Durum wheat					
Mexi Chap	8.43	13.49	1.39	1.40	75.29
Mexi Fg	8.05	12.50	1.44	1.58	77.44
Mexicali 75	8.54	13.52	1.60	1.52	75.98

[a]Significantly different from the rest of the samples ($P \leq 0.01$).

No significant differences in the amylose content of the starches from any of the test cereals were detected (Table 5). The ratio between amylopectin as determined by difference method and amylose was roughly between 3.2 to 4.5. As no significant differences were observed in amylose and amylopectin contents, both of these constituents of starch were lumped together with reducing and non-reducing sugars in the total available carbohydrate fraction of cereals.

TABLE 5 - Percentage of amylose and amylopectin in starch of some wheats and triticales

Test cereal	Percent		Amylopectin/ amylose
	Amylose	Amylopectin*	
Triticale			
Mapache	18.2	81.8	4.5
6AT-204	20.0	80.0	4.0
Soft Wheat			
H-Ra2 - F2	21.0	79.0	3.8
Atlas 66	19.0	81.0	4.3
Nap Hal/Atlas 66	20.5	79.5	3.9
Hard Wheat			
Nacozari 76	23.1	76.9	3.3
Anza	23.5	76.5	3.2
Durum Wheat			
Mexi Chap	19.5	80.5	4.1
Mexi Fg	22.1	79.9	3.6
Mexicali 75	21.0	79.0	3.8

*by difference between starch and amylose

Enzymatic method of Hellendoorn et al. (1975) for unavailable carbohydrate, which he calls dietary fiber, also estimated ash content of test cereals (Table 6). This explains why the values for unavailable carbohydrate were higher by this method than of Southgate (1969b) in which ash was characterized separately.

ADF is always less than NDF as determined by the methods of Van Soest (1966) because hemicellulose is not measured in ADF. It measures cellulose and lignin only. ADF and NDF were lower than unavailable carbohydrate. As pectins; gums and soluble pentosans were lost in solution in the detergent fiber methods, these methods are not very suitable for measuring total unavailable carbohydrate of cereals.

Significant differences in the unavailable carbohydrate contents were found for the two abnormal soft wheats and a hard wheat variety Nacozari 76 according to the method of Southgate (1969b).

Results of further fractionation of unavailable carbohydrate complex by the procedure of Southgate (1969b) are given in Table 7. The abnormal soft wheats had significantly higher levels of-water soluble pentosans, pectic substance and lignin than triticales, the normal soft wheat, and durum wheats. Hard wheats were not significantly different from other testsamples, but had a higher content of pectic substance.

TABLE 6 - Unavailable carbohydrate content of test cereals as determined by 4 different methods

Test cereal	% Unavailable Carbohydrate			
	Southgate (1969) method	Hellendoorn et al. (1975) enzymatic method	Van Soest 1966 NDF	ADF
Triticale				
Mapache	7.97a	10.44a	6.02a	2.89a
6AT-204	8.12a	11.07a	6.26a	3.20a
Soft wheat				
H-Ra2-F2	9.83a	11.30a	7.58a	3.38a
ATlas 66	13.25b	15.73b	10.11a	4.26a
Nap Hal/Atlas 66	13.86b	16.21b	10.16a	4.03a
Hard wheat				
Nacozari 76	12.39b	13.64ab	9.45a	2.93a
Anza	10.74a	13.51a	8.42a	3.47a
Durum wheat				
Mexi - chap	9.70a	11.32a	7.97a	3.17a
Mexi - Fg	10.41a	12.93a	8.23a	3.52a
Mexicali 75	11.80ab	13.94ab	9.20a	3.39ab

*Significantly different values ($P \leq 0.01$) are denoted by different letters in a column.

TABLE 7 - Fractionation of unavailable carbohydrate comples by Southgate method.

Test Cereal	% of test cereal					
	Total	Water soluble pentosans	Cell-ulose	Pectic sub-stance	Hemi-cellulose	Lignin
Triticale						
Mapache	7.97a	0.42a	2.37a	0.87a	3.25a	1.06a
6AT-204	8.18a	0.55a	2.43a	0.77a	3.43a	1.00a
Soft wheat						
H-Ra2-F2	9.83a	0.69a	2.80a	0.82a	4.44a	1.08a
Atlas 66	13.25b	1.30b	3.13b	1.01b	6.27a	1.54b
NapHal/Atlas 66	13.86b	1.41b	3.01ab	1.21b	6.61a	1.63b
Hard wheat						
Nacozari 76	12.39b	1.07ab	2.63a	0.97ab	6.68a	1.04a
Anza	10.74a	0.76a	2.86a	0.92ab	5.02a	1.18a
Durum wheat						
Mexi - chap	9.70a	0.90a	2.06a	0.88a	4.87a	0.99a
Mexi - Fg's	10.41a	0.59a	2.87a	0.85a	5.03a	1.07a
Mexicale 75	11.80ab	1.00a	2.69a	0.82a	6.07a	1.22a

Significant differences in a column are denoted by different letters ($P \leq 0.01$)

TABLE 8 - The effect of various constituents of cereals on the growth of T. castaneum larvae and Japanese quail.

Test Cereal	% Composition (determined)			Weight gain		
	Crude Protein	Carbohydrate Available	Carbohydrate Unavailable	per larva, mg		per quail, g
	(P)	(C)	(F)	(L_1)	(L_2)	(Q)
				(Yeast)	(Glutamic acid)	
Triticale						
Mapache	14.2	65.8	8.0	3.05c	1.57c	13.4b
6AT-204	14.4	65.9	8.2	3.13c	1.59c	13.7b
Soft wheats						
H-Ra^{2}F2	12.0	65.0	9.8	2.97bc	1.41bc	11.1ab
Atlas 66	17.5	50.9	13.3	2.76a		
NapHal/Atlas 66	20.3	46.9	13.9	2.80a		
Hard wheat						
Nacozari 76	14.1	62.9	12.4	2.88ab	0.87a	9.9a
Anza	13.9	62.5	10.7	2.85ab	1.15b	7.1a
Durum wheat						
Mexi chap	13.5	63.6	9.7	2.99bc	0.54a	7.0a
Mexi Fg	12.5	62.3	10.4	2.90b	0.71a	8.4a
Mexicali 75	13.5	61.0	11.8	2.79a	0.62a	6.2a

$L_1 = 3.69 - 0.07F \quad r = 0.89$

$L_2 = 2.98 - 0.19F \quad r = 0.68$

$L_2 = -11.71 + 0.2C \quad r = 0.83$

$L_2 = 0.49 + 0.11P \quad r = 0.23$

$Q = -2.3 + 0.88P \quad r = 0.26$

$Q = 22.9 - 1.3F \quad r = 0.70$

$Q = -84.3 + 1.48C \quad r = 0.90$

The data on weight gain of Tribolium larvae fed the test cereal diets with yeast indicate the best growth on triticales followed by a soft wheat (H-Ra^{2}F$_2$), durum wheats (Mexi chap and Mexi Fg), and hard wheats (Nacozari 76 and Anza). The poorest growth was on the soft wheats with the highest protein contents (Atlas 66 and NapHal/Atlas 66) and on the durum wheat (Mexicali 75) (Table 8). The correlation coefficient between crude protein and larval growth (Ll) was only 0.36. But the correlation coefficient between unavailable carbohydrate and larval growth was highly significant (r=0.89) for L_1=3.69-0.07F.

Due to a shortage of supply, the abnormally shrivelled-softwheats (Atlas 66 and NapHal/Atlas 66) were not tested with quail.

With a crude protein level of 15% in test diets, the triticales again were significantly superior than durum wheats or hard wheats in supporting the growth of either the Tribolium larvae or of Japanese quail. Soft wheat was intermediate between one hard wheat (Anza) and the triticales for larval growth. It was also intermeidate between triticales and the rest of wheat samples (PfO.O1)

The level of crude protein in test cereals was kept at 15% which is not optimal for the growth of Japanese quail., In our preliminary, study, if a crude protein level of 30% was used by incorporating a large amount of isolated soybean protein', the differences in the growth of' quail due to difference in the carbohydrate, complex of cereals were masked. Even for the estimation of protein quality of cereals, Fernandez et al. (1974) found it desirable to keep'the protein level of their diets at 14%. Our diets incorporated glutamic acid which was not deleterious for the larvae or for the Japanese quail, to bring the protein level to about 15%. On these diets, the growth of both the Tribolium larvae and of auail was poorly correlated to the protein content of the test cereals with correlation coefficients value of 0.23 and 0.26, respectively. The growth was significantly correlated to the available and unavailable carbohydrate contents. The respective correlation coefficients for Tribolium larvae were 0.83 and 0.68; and for quail 0.90 and 0.70. Under our conditions, the differences in growth of test organisms due to the carbohydrate complex of cereals are demonstrated more clearly with a low protein diet.

The overall data sugqest that the growth of Tribolium larvae and of quail was better, correlated to the available carbohydrate content'of these cereals (r=0.83, 0.90) followed by unavailable carbohydrate content (r=0.68 and 0.70), but poorly to the crude protein content (r= 0.23 and 0.26). A multiple regression using both the carbohydrates and protein did not imporve the correlation coefficient any further.

Tribolium larval growth is better on samples higher in amylopectin/amylose ratio (Vohra et al. 1979). Waxy maize and waxy rice are such samples, No'such significant differences in amylopectin/amylose ratio have been observed in wheats and triticales. .

The deleterious effects of high amylose starches from some legumes and tubers on the NPU of casein for rats have been also reported (Rao and Rao, 1978). This confirmed the earlier findings as reviewed by 'Vohra et al. (1979a) which suggested the importance of amylose/amylopectin ratios. .

The agreement between the biological evaluation between Tribolium larval growth and the growth of Japanese auail confirms the usefulness of bioassay with insects as a useful tool for nutritional screening of cereals.

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