COMPOSITIONAL, NUTRITIONAL AND FUNCTIONAL EVALUATION OF INTERMEDIATE WHEATGRASS (*THINOPYRUM INTERMEDIUM*)

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ABSTRACT

'Oahe' Intermediate Wheatgrass (IWG) seed was found to have higher levels of protein (20.8%), fat (3.21%), and ash (2.64%) than wheat. The IWG protein is nutritionally limiting in lysine, as is wheat, but has higher levels of all other essential amino acids than wheat. A flour beetle larvae bioassay and chemical trypsin inhibitor and hemagglutinin tests demonstrated the absence of significant amounts of antinutrients. IWG kernels were milled with stone, impact, and roller mills. Stone milling resulted in a flour with farinograph characteristics more similar to those of whole wheat flour than did impact and roller milling. No gluten was found in IWG. Bread, muffins, cookies and cake containing various levels of IWG flour were evaluated by a sensory panel and judged to have very favourable appearance, texture, flavor and overall characteristics.

INTRODUCTION

The development of perennial grains as environmentally sound alternative crops for marginal lands has received recent attention amongst agronomists, plant breeders and environmentalists (Jackson 1985; Wagoner 1991). Such crops should form a good soil holding sod to minimize erosion and maintain soil fertility, have favourable grain producing qualities, and provide an acceptable yield. The grain should also be economically viable with establishment and production costs balanced by a break-even price competitive with conventional crops (Watt 1989). In 1983, the Rodale Research Center began testing a large

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number of perennial grains. Compositional and nutritional analyses contributed to the eventual selection of 'Oahe' intermediate wheatgrass (IWG) (*Thinopyron (Agropyron) intermedium*) as one of the apparent best candidates (Becker *et al.* 1986; Wagoner and Schauer 1989; Schauer 1990).

This report describes continuing efforts to evaluate the compositional and nutritional characteristics of 'Oahe' IWG grain and its suitability for use in food products for the marketplace.

MATERIALS AND METHODS

Seed Samples

'Oahe' Intermediate Wheatgrass (IWG) seed samples were obtained from production experiments performed by the Rodale Research Center (RRC). Crops grown in Eastern Pennsylvania in 1985 through 1988 and in Colorado in 1987 were tested. 'Chief' and 'Greenar' varieties of IWG and two varieties of pubescent wheatgrass, 'Greenleaf' and 'Mandan 759' were also evaluated. The seeds were harvested and field cleaned before final cleaning with a grain dockage tester. Visual inspection verified that this cleaning process removed virtually all contaminating plant debris and seeds of other species, as well as under- and over-sized sample seeds.

Wheat seed used for comparison stone milling tests was obtained from the California Wheat Commission; commercial whole wheat flour used in baking tests was purchased in local markets.

Composition

Representative samples of whole grain seed flour and mill fractions were milled to 100 mesh flour in a Udy mill (Udy Corp., Fort Collins, CO) prior to proximate compositional (AOAC 1980) and amino acid analysis. Amino acids in whole grain IWG were determined with a Durrum amino acid analyzer ion exchange method (Spackman *et al.* 1958), with the sulfur amino acids determined after perchloric acid oxidation (Moore 1963). Nitrogen to protein conversion factors were calculated as in Tkachuk (1969).

Starch was isolated (Knight and Olsen 1984) and the melting point determined by differential scanning calorimetry (DuPont Model 990) using excess water. Trypsin inhibitor activity was measured as described by Kakade *et al.* (1974), with one trypsin inhibitor unit (TIU) being defined as the amount of sample causing a 10% inhibition of porcine pancreatic trypsin and N-benzoyl-DL-arginine p-nitroanalide (BAPNA) (Sigma Chemical Co). Hemagglutinin activity was assayed with the method of Beeley (1985) using human erythrocytes, with one unit of activity being the minimum amount of flour causing agglutination in one hour.

Milling

Seed was milled to flour using three different mills. A fine flour (100 mesh) was prepared by milling with a Udy impact mill. Stone ground flour was prepared with a Morehouse Model 530 stone mill (Morehouse Industries, Fullerton CA) equipped with 5 in. stones set at a gap of 0.005 in. Endosperm flour, bran and high ash bran were obtained from a Brabender Quadrumat Jr roller mill (Brabender Instruments, South Hackensack, NJ). Bran fractions from the roller mill were further milled to a 100 mesh flour with the Udy mill.

Nutritional Evaluation

The *in vivo* nutritional value of IWG was compared to wheat using the larval method of Vorha *et al.* (1979). Samples of IWG or whole wheat flour were mixed with water and sampled raw, baked for 30 or 45 min at 177°C, or autoclaved at 110°C for 10, 20 or 30 min. The cooked and uncooked material was lyophilized and fed as 90% of the test diet, the remaining 10% being whole wheat flour. *Tribolium castaneum* eggs were collected, grown for 6 days on a basal diet, and acclimatized for 2 days on the test diet. Three replicates of 10 larvae each were weighed and fed the test diet for 6 days, after which they were reweighed and the mean weight gain per larva calculated for each diet. The results were compared to the weight gain of larva fed unbleached flour diet. See the cited method (Vohra *et al.* 1979) for additional details.

Product Formulation and Evaluation

The farinograph mixing characteristics of IWG whole grain flours and mill fractions were compared to a commercial whole grain flour using AACC methods (AACC 1976; Farinograph Handbook 1972). Absorption, Dough Development Time, Mixing Tolerance Index, Stability and Twenty Minute Drop were calculated as in AACC methods (1976). The IWG whole grain flours and mill fractions were tested for gluten using both the AACC hand washing and AACC machine washing methods.

Baked products were prepared using stone ground IWG flour in modified commercial recipes. Bread was made with 85 parts wheat flour, 15 parts IWG flour, and 5 parts vital wheat gluten. The only flour in muffins was stone ground IWG flour. Cookies were made with IWG flour, oatmeal, butter, sugar, chocolate chips and walnuts. Cake contained 1 part (w/w) IWG flour, 1 part whole wheat flour, 2 parts bananas and proportioned amounts of walnuts and other ingredients.

To evaluate the baked products, a 57 member sensory panel was assembled from laboratory staff volunteers. The judging experience of the panel members ranged from expert to novice; however, most participants had served on multiple panels throughout the year. The panel was supervised by expert staff using

established techniques. The products were hedonically scored for appearance, texture, flavor and overall characteristics, using a scale of 1 (very poor) to 9 (excellent). Results were tabulated and summarized graphically.

Cooking characteristics of blends of 10 and 25% whole grain IWG in brown rice were determined using criteria developed in this laboratory for evaluating wild rice. The rice/IWG mixtures were boiled for 50 minutes and sampled as indicated. Flavor, mouth feel, and appearance were evaluated by 5 experienced judges.

RESULTS AND DISCUSSION

Composition

The proximate composition of multiple lots of Oahe IWG is shown in Table 1. The protein content of IWG is higher than common cereal grains, but near results observed in other perennial grains (Becker et al. 1986). The carbohydrate content was determined by difference, so it reflects the protein and fiber observations. The fat and ash are within expected ranges.

The amino acid composition of IWG seed proteins is shown in Table 2. The essential amino acids for wheat (FAO, 1970) and the FAO Standard (1973) are shown for comparison. Lysine is the limiting amino acid in both IWG and wheat, resulting in a chemical score of 54. All other essential amino acids are substantially higher in IWG than in wheat.

The levels of trypsin inhibitor and hemagglutinin activity in IWG are probably not nutritionally significant. Uncooked IWG flour contained 0.01 TIU/mg N and a hemagglutinin titer of 0.0125. For comparison, wheat has 0.5–1.0 TIU/mg N and triticale has 4–7 TIU/mg N (19). Antinutrient levels in cooked IWG were not determined.

TABLE 1.
PROXIMATE COMPOSITION OF INTERMEDIATE WHEATGRASS

Grain	Protein[1] % ± sd n = 14	Carbohydrate[2] % ± sd n = 6	Fat % ± sd n = 6	Crude Fiber % ± sd n = 6	Ash % ± sd n = 3
Intermediate Wheatgrass	20.8 ± 1.8	61.7 ± 2.8	3.21 ± 0.44	1.69 ± 0.41	2.64 ± 0.21
Wheat[3]	14.3	68.6	1.9	3.4	1.8

1. Protein = Nitrogen X 6.25
2. Carbohydrate by difference.
3. NAS/NRC. 1964.

TABLE 2.
PROTEIN AMINO ACID COMPOSITION (g/16 g N) OF INTERMEDIATE WHEATGRASS,
WHOLE WHEAT FLOUR, AND THE FAO STANDARD

Amino Acid[1]	Intermediate Wheatgrass[2]	Wheat Flour[3]	FAO Standard[4]
Lysine	2.9 ± 0.2	2.9	5.4
Histidine	2.4 ± 0.2		
Threonine	3.5 ± 0.3	2.9	4.0
Cystine	3.4 ± 0.3	2.5	
Methionine	2.2 ± 0.3	1.5	
Cys + Met	5.6 ± 0.6	4.0	{3.5}
Valine	5.3 ± 0.5	4.4	5.0
Isoleucine	4.0 ± 0.3	3.3	4.0
Leucine	7.2 ± 0.5	6.7	7.0
Tyrosine	3.5 ± 0.3	2.9	
Phenylalanine	4.9 ± 0.4	4.5	{9.6}
Serine	5.4 ± 0.4		
Glycine	4.3 ± 0.2		
Arginine	5.2 ± 0.4		
Alanine	3.8 ± 0.3		
Aspartic Acid	5.1 ± 0.3		
Glutamic Acid	29.6 ± 2.2		
Proline	10.4 ± 0.9		
Chemical Score	54	54	100
Protein Conversion Factor[5]	6.75 ± 0.05	5.85	
Limiting Amino Acid	Lys	Lys	

1. N = 12.
2. Mean ± standard deviation.
3. FAO (1970).
4. FAO (1973).
5. N = 9.
Bracketed values are sums of related amino acids, Cys + Met and Tyr + Phe.

Larvae Bioassay

The results of the *Tribolium castaneum* bioassay are shown in Fig. 1 and 2. This assay has been successfully used to evaluate the nutritional characteristics of wheat, triticale, rice, corn, soy (Shariff *et al.* 1981), sorghum (Banda-Nyirenda *et al.* 1987), and eastern gamagrass (Bargman *et al.* 1989). With this assay, the nutritional value of uncooked IWG is identical to uncooked wheat flour (Fig. 1 and 2). Baking at 177°C for 30 min raised the nutritional value of both IWG
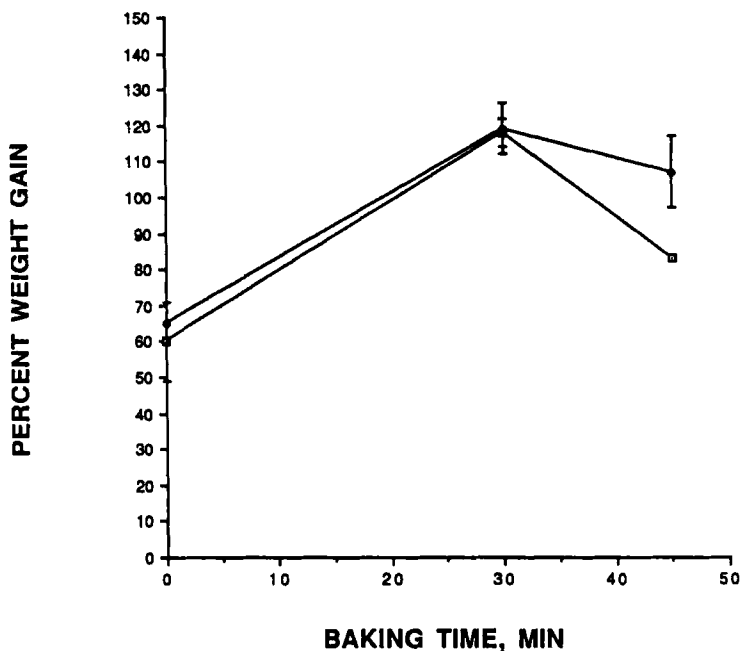
FIG. 1. WEIGHT GAIN OF LARVAE FED BAKED INTERMEDIATE WHEATGRASS
(SOLID BOXES) AND WHEAT (OPEN BOXES)

and wheat grains compared to unbaked samples at 0 min (Fig 1). This effect is probably due to the nutritionally beneficial changes associated with cooking, such as cellular disruption, protein denaturation, and inactivation of low levels of antinutrients. Baking for 45 min diminished the nutritional value of both IWG and wheat from levels observed at 30 min, perhaps due to formation of less digestible protein-carbohydrate complexes.

When autoclaved diets were fed, the nutritional value was initially increased, then was diminished by overcooking (Fig 2). Wheat required more autoclaving to attain maximum nutritional values (20 min vs 10 min for IWG), and IWG lost more nutritional value when overcooked.

These results demonstrate that IWG has nutritional characteristics which are similar to wheat, and overcooking is deliterious to both grains.

Milling

Both impact and stone milled whole grain IWG flour had textural character- istics comparable to similarly processed whole wheat flour. In neither case were processing difficulties encountered, other than mill adjustments for the smaller
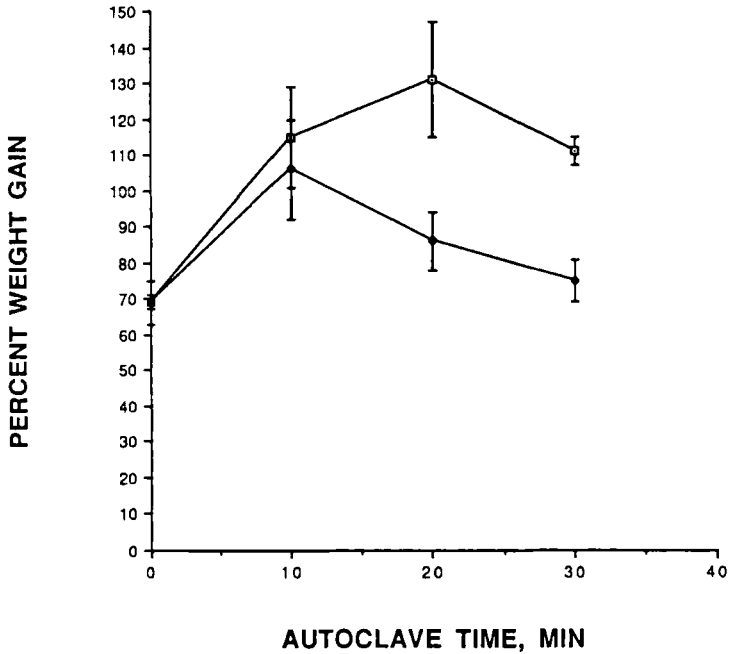
AUTOCLAVE TIME, MIN

FIG. 2. WEIGHT GAIN OF LARVAE FED AUTOCLAVED INTERMEDIATE
WHEATGRASS (SOLID BOXES) AND WHEAT (OPEN BOXES)

kernel size of the IWG. Roller milled endosperm flour from IWG seed had a
substantially lower yield (Table 3) and grayer color than other comparable cereal
grains. The grayer color is probably due to the larger amounts of bran, as
associated with the smaller seed size of the IWG. For example, varieties of IWG
seed other than 'Oahe' had a mean kernel weight of 4.7 mg in the 1988 and
4.4 mg in 1989; 'Oahe' samples averaged 5.1 mg and 4.9 mg kernel weights
for those years (Wagoner and Schauer 1989; Schauer 1990; Hanners *et al.* 1988),
compared to kernel weights of 20–32 mg for commercial hard wheat. Untempered
IWG seed shattered and gave very small amounts of pure endosperm flour.
Tempering the seed to 15% moisture improved typical roller mill endosperm
flour yields to about 38%. Stone milling tempered seeds gave more uniform
particles (data not shown).

Flour Characteristics

Stone milling of perennial grains produced flours with high absorptions, long
Dough Development Times (DDT), small Mixing Tolerance Indices (MTI), high
stability, and small Twenty Minute Declines (TMD) (Table 4); all characteristics

TABLE 3.
ROLLER MILL FRACTION YIELDS FROM PERENNIAL GRAINS, %

Grain	Endosperm Flour	High Ash Flour	Bran
Intermediate Wheatgrass.			
Oahe 1988	38.7	5.3	56.0
Oahe 1987	44.5	6.9	48.6
Chief	44.4	4.6	51.0
Greenar	46.1	5.2	47.8
Pubescent Wheatgrass			
Greenleaf	48.1	5.2	53.0
Mandan 759	38.1	4.2	57.8
Hard Red Spring Wheat			
Yecora Rojo	76.4	6.8	16.8

of a thick, viscous dough. It is known that particle size, which is larger in stone milled flours as opposed to impact or roller milled flours, may effect absorption, but has much less impact on the other characteristics. These thicker doughs form very dense breads, even when used as a minor ingredient in composite flours. Such stone milled flours make good ingredients in flours used for muffins or pancakes, where denseness may be an attribute.

Flours made by impact or roller milling do not have these farinograph characteristics and have thinner, pastier doughs and form more dense, compact products.

None of the perennial grains tested contained gluten forming protein. Although the literature is sparse, the genus is reportedly nonuniform (Shibaev 1937), with gluten being reported in some *Agropyron intermedium* samples and not in others.

Since the perennial grain kernels are smaller than wheat kernels, they have a greater surface area per gram of seed and consequently more bran and more dietary fiber. Components of dietary fiber are probably responsible for the viscosity characteristics of the whole grain flour doughs, as evidenced by the farinograms of the weaker doughs from endosperm flours. The chemically determined crude fiber as reported here is for comparison with literature values and has no correlation with dietary fiber.

TABLE 4.
FARINOGRAPH CHARACTERISTICS OF SEVERAL INTERMEDIATE AND PUBESCENT
WHEATGRASS GRAINS MILLED WITH DIFFERENT TYPES OF MILLS

Mill Type	Absorption %	DDT (min)	MTI (BU)	Stability (min)	TMD (BU)
INTERMEDIATE WHEATGRASS					
Oahe, 1988 crop					
Stone	63.4	4.6	90	4.7	60
Impact	64.0	1.6	190	0.7	228
Roller	63.8	2.4	140	1.2	170
Oahe, 1987 crop, Pennsylvania grown					
Stone	60.5	3.0	40	6.5	65
Roller	70.0	2.1	195	0.8	240
Oahe, 1987 crop, Colorado grown					
Stone	63.2	2.5	130	1.6	125
Oahe, 1987 crop, Endosperm flour					
	57.7	1.1	280	0.7	375
Oahe, 1988 crop, Endosperm flour					
	58.5	1.2	195	0.7	240
Chief, 1988 crop					
Stone	64.0	7.0	80	8.9	45
Impact	64.8	2.5	80	1.7	90
Roller	66.8	3.3	90	2.4	120
Greenar, 1988 crop					
Stone	64.0	5.0	40	4.4	50
Impact	65.6	2.4	123	1.3	145
Roller	67.7	3.2	115	1.4	145
PUBESCENT WHEATGRASS					
Greenleaf, 1988 crop					
Stone	63.9	5.0	10	12.2	10
Impact	65.6	1.8	120	1.0	135
Roller	68.3	2.4	150	1.5	180
Mandan 759, 1987 crop					
Stone	63.0	2.8	90	1.7	110
Impact	65.2	1.4	180	0.8	205
Roller	70.6	2.0	180	1.0	220
HRS Wheat, Yecora Rojo					
Stone	67.2	6.3	30	6.3	30

Absorption (%) = 2(x+y-50) where x=mls added and y=gms flour
DDT (Dough Development Time) = time of first peak
MTI (Mixing Tolerance Index) = Top of curve at first peak minus top of curve 5 minutes later
Stability = Difference in time between when top of curve reaches the 500 BU line and when it leaves the line.
TMD (Twenty Minute Drop) = Difference between the center of the curve at peak and center of curve 20 minutes later.

The differences in the farinographs of the endosperm flours from 'Oahe' IWG seed grown in two crop years are not significant. These doughs are weak, with little tendency to become viscous. The IWG starch has a gelatinization temperature of 62–68°C, midpoint 65.5°C as compared to wheat which ranges from

58–64°C, midpoint 61°C. Since the endosperm flours are predominantly starch and low in fiber, they would be expected to make composite breads that are lighter than those made from corresponding whole grain flours.

In a separate experiment, seeds from a single lot were grown in both Pennsylvania and Colorado (Wagoner and Schauer 1989). The resultant seeds appeared physically different. The Pennsylvania seeds were typical of those harvested from other lots and crop years while the Colorado grown seeds were about 20% smaller and appeared less developed. The farinograms of flours made from stone ground grain from Colorado were similar to farinograms of impact or roller milled flours in that they had shorter dough development times, larger mixing tolerance indices, lower stabilities and larger twenty minute declines. Not enough compositional data (dietary fiber and starch determinations) are available to fully define the differences, but climatic effects are obviously important. Proximate and amino acid analyses were similar (data not shown).

Baked Products and Their Sensory Evaluation

IWG performed well in the four baked products tested (Table 5). When used as 15% of the flour in bread, IWG imparted a distinct, nutty-grain taste. The loaf had a color and crumb appearance typical of whole grain breads; added gluten was required to produce loaf volumes comparable to whole grain breads. Most panel members rated the overall characteristics between fair and good (5–8), Appearance between good and excellent (7–9), Texture between fair and good (5–8), and Flavor between fair and good (5–8) (Fig. 3A). The bread had a rather coarse texture, which may have influenced judges preferring lighter breads.

The muffins made from 100% IWG flour were judged to have a good to excellent appearance (7–9) by nearly 50% of the judges (Fig. 3B); this is an impressive score considering the diversity of preferences expressed for other products. The muffins had good volume and a plump inviting appearance; they scored good to excellent for appearance (7–9) and overall (6–8), fair to excellent in texture (5–8), and fair to excellent in flavor (5–8).

Cookies made with IWG as the only flour ingredient were judged good to excellent (7–9) in appearance, good in texture and flavor (7–8), and good to excellent (6–9) overall (Fig. 3C). The cookies had a soft texture, broke easily without crumbling and a rather sweet taste.

The banana bread, which was 50% IWG-wheat, was judged good to excellent (7–9) for appearance, flavor and overall; texture scored only slightly lower (6–9) (Fig. 3D). The primary flavor component was banana; IWG and wheat moderated the banana flavor somewhat to produce a mildly banana-nutty flavor. The bread was moist without being soggy and had good crumb and color.

TABLE 5.
RECIPES FOR BAKED PRODUCTS USING INTERMEDIATE WHEATGRASS

Ingredient	gms	Ingredient	gms
BREAD			
Whole wheat flour, Gold Medal	80	Straight dough method	
Intermediate Wheatgrass	15	1. Basic fermentation 2 1/2 hours	
Vital wheat gluten	5	2. Floor time 20 min	
Brown sugar	60	3. Proof to height 45-55 min at 38°C.	
Shortening	40	4. Bake temperature 405°F for 25 min.	
Salt	20		
Fresh yeast	25		
Water	730		
Vitamin C	50 ppm		

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COOKIES		**MUFFINS**	
Intermediate Wheatgrass flour	100	Intermediate Wheatgrass	100
Oatmeal	90	Brown Sugar	35
Butter	106	Non-Fat Milk	80
Granulated Sugar	90	Egg White	28
Brown Sugar	70	Salad Oil	25
Whole eggs	50	Raisins	40
Vanilla	T	Baking Powder	3.5
Baking Powder	2	Salt	1.5
Baking Soda	2	Cinnamon	0.6
Salt	100		
Chocolate Chip	100		
Walnut	50		

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WHEATGRASS BANANA CAKE	
Intermediate Wheatgrass	50
Whole Wheat Flour	50
Banana	100
Sugar	85
Salt	0.6
Shortening	40
Eggs	45
Walnut	30
Baking Powder	1.5
Baking Soda	1.5

There were no obvious shelf-life differences between IWG and their all-wheat counterparts when products were held under similar conditions at room temperature for 7–10 days (data not shown).
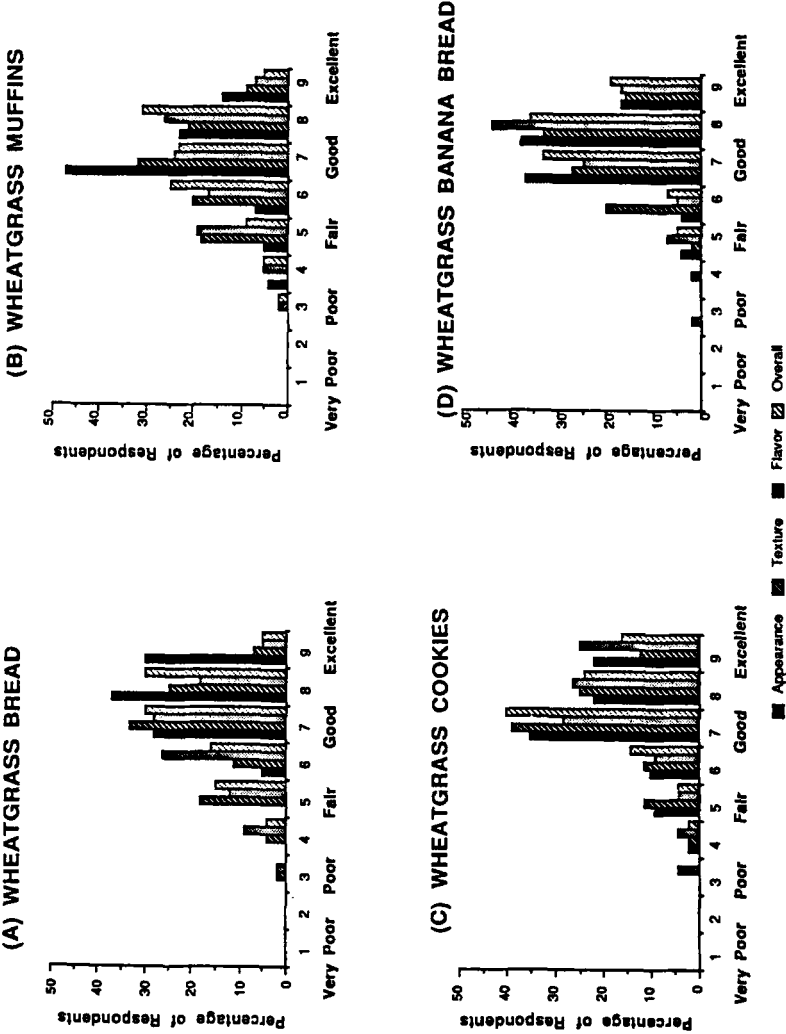
FIG. 3. SENSORY PANEL APPRAISAL SCORES OF BACKED PRODUCTS MADE WITH
WHOLE GRAIN INTERMEDIATE WHEATGRASS FLOUR
(A) Bread, (B) Muffins, (C) Cookies, (D) Banana Bread.

Cooked Kernel Characteristics

Whole kernel IWG were cooked in boiling water and sampled every 5 min during cooking. Minimum cooking time was about 20 min; the kernels were plump, soft and had a nutty flavor and clean bite with only about 10–15% split. Longer cooking reduced the bran flavor but resulted in more split kernels. Adding 10 or 25% IWG kernels to brown rice was judged to add a mild nutty flavor to the rice dish, similar to but more pronounced than wild rice.

CONCLUSIONS

The quantity of protein present in IWG and the amounts of amino acids in the proteins is superior to the cereal grains now commonly grown, the larvae bioassay indicates IWG is at least nutritionally comparable to wheat, and it has been shown that baked products can be made that are potentially very consumer attractive. All of these factors are supportive of continued consideration of IWG as an alternative perennial grain crop.

ACKNOWLEDGMENT

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