Chemical Constituents of Flour from Cytologically Synthesized and Natural Cereal Species

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Flours from synthetic species generally contained greater quantities of 80% alcohol-soluble sugars than two spring wheats and durum wheats. The soluble-sugar content of the synthesized bread wheat was somewhat lower. One of the tetraploid species contained the lowest amount of soluble sugar in the flour. Sucrose was present in greater quantities in the flour of most of the synthetic species and the durum wheats as compared to the hard red spring wheats. Oligosaccharides constituted the major part of the alcohol-soluble components in all of the varieties. The oligosaccharides were composed of maltotriose, -tetraose, and -pentaose since no fructose was released upon acid hydrolysis. No free uronic acids were detected. Small quantities of hemicellulosic and pectic substances were apparently present since hydrolysis of the extracted flour gave glucose, xylose, arabinose, and a uronic acid. The synthetic species generally contained greater quantities of soluble nitrogenous substances (amino acids) but were low in lipid content.

THE NATURE and relative quantities of chemical constituents in flours from common bread and macaroni wheats have been investigated. Minor varietal differences might be expected and have been observed. The composition of different cereal species varies, and seasonal climatic variations cause differences in the level of certain constituents within a variety. The successful development of cytologically synthetic cereal species has added a new dimension for biochemical investigations.

O'Mara reports (*10*) that one of the first cytologically synthesized "new" cereal species was obtained by Rimpau in 1888 when he was successful in combining the chromosomal complements (genomes) of rye (*Secale cereale*) and wheat (*Triticum* sp.). Interest in these new species, for which the name "Triticale" has been coined, has been renewed, and a large variety of these synthetic species have been produced and are now under investigation in this institution. Elsewhere, Muntzing (*9*) and Sanchez-Monge (*11*) are also actively pursuing this avenue. These new species are of particular interest to biological chemists in that this is an example in which the diploid rye genome (seven chromosome pairs) and the tetraploid wheat genome (14 chromosome pairs) exist as a unit in the single cells of the new species, namely Triticale. The combination of the foregoing two discrete species results in a hexaploid Triticale–that is, the addition of diploidy and tetraploidy gives hexaploidy. Octaploid Triticale were synthesized by the combination of diploid rye with common hexaploid wheat. The question of whether these alien genome combinations fully retain their biosynthetic integrity is under further investigation. In this communication, the results from certain carbohydrate, nitrogen, and other analyses are reported for a variety of synthetic cereal species. For comparison, the results for two common

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Table I. Alcohols and Ether-Soluble Constituents of Flours

	80% Alcohol-Soluble Carbohydrates						80% Alcohol Nitrogenous Material	
	Total sol. sugars, mg. per gram of flour	Component Sugars, % of Total						
Variety		Fructose	Glucose	Sucrose	Maltose	Oligo-sac.	Mg. N per Gram of Flour	Solubles (Fat), %
Pembina	17.2	4.6	5.1	11.1	6.0	73.2	3.7	1.14
Selkirk	16.7	1.2	0.9	15.5	3.9	78.6	2.0	0.93
Ramsey	18.5	2.4	2.4	30.9	8.1	56.6	15.2	1.25
Stewart	11.4	7.6	2.1	22.8	8.8	58.7	9.7	1.18
Fourex (rye)	27.0	2.3	1.8	15.3	5.3	75.2	8.0	0.61
6A49	15.3	2.5	7.1	16.9	7.7	65.5	4.3	0.67
4B113	14.8	2.1	0.5	16.9	12.1	68.3	14.3	0.52
4B121	11.6	1.2	0.6	19.9	10.3	67.8	6.3	0.46
4B249	20.3	0.1	1.9	23.3	7.7	67.1	6.2	0.87
4B276	20.6	0.5	1.2	21.8	6.8	69.5	15.1	0.86
8A112	13.3	2.8	2.5	20.7	4.5	69.4	9.7	0.74
8A125	21.2	1.5	1.9	26.0	6.3	64.2	7.3	0.32
6A190	33.0	3.2	3.7	28.4	2.7	61.9	12.7	0.91
6A20.1	26.7	4.8	5.4	25.1	2.9	61.8	14.0	1.46
6A20.2	25.3	3.8	5.3	30.2	5.0	55.7	12.5	0.53
6A66.1	29.3	4.0	2.6	24.2	4.6	64.5	8.4	1.45
6A66.3	24.3	2.1	2.3	22.2	5.3	68.2	7.1	0.45
6A67.1	24.8	4.4	1.2	20.4	7.7	66.2	11.2	...
6A67.2	25.7	7.8	4.5	28.1	5.7	53.7	7.0	0.43
6A67.3	19.3	2.5	2.9	26.5	4.1	64.0	4.1	0.58
6A67.4	25.7	1.3	4.1	23.7	3.3	67.5	5.6	0.53
6A67.5	21.6	1.9	4.9	18.9	3.5	70.7	4.1	0.73
6A67.6	25.7	1.1	0.9	21.3	3.7	72.9	4.2	0.67
6A67.7	25.7	1.5	2.4	21.7	3.5	70.9	7.6	0.83
6A67.8	20.5	1.5	1.9	24.5	3.8	68.1	5.6	0.66
6A67.9	21.0	1.3	0.9	20.9	3.8	72.9	7.8	0.87
6A67.11	27.5	2.3	2.3	20.7	3.7	70.9	4.8	0.66
6A67.12	31.0	1.9	3.6	22.5	4.1	67.7	6.3	0.62

spring wheats, two durum wheats, one variety of rye, and a synthesized bread wheat are reported as well.

Experimental Results

Alcohol Extraction. Samples of flour (3.00 grams) in cellulose extraction thimbles were extracted with 80% ethanol for 18 hours in a Soxhlet apparatus. The extract was evaporated to near dryness and the sirupy residue suspended in a small quantity of 95% ethanol. Identification of the sugars present and determination of the relative quantities of each was carried out by paper chromatography. Two solvent systems, *A*, ethyl acetate–acetic acid–water (8:2:2), and *B*, ethyl acetate–pyridine–water (9:2:2), were used. The presence of fructose, glucose, sucrose, maltose, maltotriose, maltotetraose, and maltopentaose was established by using a *p*-anisidine–trichloroacetic acid spray reagent. The identity of the latter three oligosaccharides was tentatively established by their characteristic chromatographic mobility and by acid hydrolysis which gave glucose exclusively. The relative quantities of the above sugars were determined by resolving a small quantity of the mixture chromatographically using Whatman No. 1 paper and solvent *A*. Guide strips were sprayed, the sugars eluted with water, and the eluates filtered through filters packed with glass wool to remove cotton linters. The sugar content was determined employing the phenol–sulfuric acid method (*4*). Total sugar, as glucose, was determined by suspending the sirupy residue in a suitable volume of water and employing the phenol–sulfuric acid procedure directly. The results of these determinations are summarized in Table I.

The amount of nitrogenous constituents soluble in 80% ethanol was determined by a colorimetric method (*8*) employing Nessler's reagent. These results are also presented in Table I. Preliminary chromatographic examination using Whatman No. 1 paper and solvent *A* as well as *n*-butanol–acetic acid–water (4:1:5) showed that at least 14 amino acids were present.

Hydrolysis of Alcohol-Extracted Flours. Samples (1.00 gram) of extracted flour were suspended in 1*N* H_2SO_4 (20 ml.) and the mixtures refluxed for 10 to 12 hours. The solutions were neutralized ($BaCO_3$), filtered, and the filtrates evaporated to sirupy residues. The component sugars were identified chromatographically as described previously, and in this manner, glucose, which constituted the major component, xylose, arabinose, and a uronic acid were shown to be present. The molecular ratio of these constituents was determined as described previously employing the phenol–sulfuric acid method. Crude protein was determined by the Kjeldahl method. The results are summarized in Table II.

Table II. Constituents of Flour Hydrolysates

Variety	Component Sugars, % of Total Carbohydrate				Kjeldahl Protein, %
	Glucose	Arabinose	Xylose	Uronic acid	
Pembina	91.8	1.5	1.5	5.1	15.1
Selkirk	95.1	0.1	0.5	4.1	15.2
Ramsey	93.3	1.1	1.1	4.4	15.7
Stewart	94.5	0.2	1.3	3.9	12.2
Fourex (rye)	91.1	1.9	2.2	4.6	18.5
6A49	94.7	0.5	0.3	4.4	17.2
4B113	83.1	3.2	1.1	12.5	14.7
4B121	84.3	3.7	0.4	11.7	14.6
4B249	91.1	2.1	1.6	5.2	18.2
4B276	92.5	1.2	0.4	5.8	15.6
8A112	89.1	4.3	0.7	5.9	15.9
8A125	89.8	2.1	1.9	6.1	18.6
6A190	86.6	3.9	1.3	8.1	17.0
6A20.1	88.3	1.2	6.0	4.4	17.3
6A20.2	89.1	2.1	1.3	7.4	16.8
6A66.1	92.5	1.1	1.4	4.8	15.6
6A66.3	90.1	2.7	1.8	5.4	16.3
6A67.1	94.9	0.1	1.6	3.3	15.0
6A67.2	90.5	2.7	1.3	5.5	14.9
6A67.3	92.2	2.5	1.5	3.7	14.8
6A67.4	90.9	2.4	1.4	5.3	14.8
6A67.5	90.0	3.2	2.0	5.7	14.6
6A67.6	91.8	2.2	1.5	4.4	14.8
6A67.7	91.7	1.8	1.5	4.9	14.5
6A67.8	91.7	1.8	1.7	4.7	14.7
6A67.9	90.0	1.7	1.9	6.3	14.7
6A67.10	90.9	2.1	1.8	5.1	14.7
6A67.11	90.7	2.9	1.7	4.7	14.6
6A67.12	91.8	3.7	0.6	3.8	15.9

Discussion

To facilitate the discussion, a brief generic description of the major types of synthetic and natural cereal species is given as follows:

4B113	*T. turgidum* var. *ramosa-megalopolitanum*
4B121	*T. turgidum* var. *plinianum*
4B249	*T. polonicum* var. *levisimum*
4B276	*T. persicum* var. *stramineum*
6A49	*T. aestivum* (synthesized from *T. turgidum* var. *nigro-barbatum* × *Ae. squarrosa*)
6A20	*T. durum* "Carleton"—*S. cereale*
6A66	*T. diccocoides*—*S. cereale*
6A67	*T. persicum*—*S. cereale*
8A112	*T. aestivum*—*S. cereale*

The 6A prefix denotes hexaploid rye–wheat Triticale, except in the case of the variety 6A49, which is a synthesized hexaploid bread wheat. The prefix 8A denotes octaploidy, and 4B denotes tetraploidy.

All of the hexaploid Triticale contained greater amounts of total alcohol-soluble carbohydrates than the hard red spring wheats (Pembina, Selkirk), the synthetic bread wheat (6A49), the durum varieties (Stewart, Ramsey), an octaploid Triticale (8A112), and two tetraploid wheats (4B113 and 4B121) (Table I). This higher soluble-sugar content of the hexaploid Triticale can be ascribed chiefly to their proportionately high sucrose content. The rye variety examined also contained a relatively high quantity of alcohol-soluble sugars and in this way was similar to the hexaploid Triticale. However, the sucrose content was relatively low and comparable to that found in Selkirk wheat. The sucrose content of the durum varieties was relatively high and comparable to that found in the hexaploid Triticale. The high soluble-sugar content of the hexaploid Triticale would appear to explain, at least in part, the rather dark crust colors that were obtained in baking tests when flour from these varieties were blended with that from spring wheats (*13*). Relatively high free sugar and amino acid contents have been shown to enhance the non-enzymatic browning reaction (*1–3*, *5–7*, *12*).

Hydrolysis of the soluble oligosaccharide material gave only glucose. The absence of fructose showed that no sucrose units formed part of the oligosaccharide molecules. The chromatographic mobility of the soluble oligosaccharide fraction indicated that it was composed of maltotriose, -tetraose, and -pentaose units. These fragments are probably intermediates in starch synthesis and/or enzymatic breakdown fragments.

Hydrolysis of the alcohol-extracted flours furnished glucose as the major component. Uronic acid, arabinose, and xylose were present in small and

variable quantities. The presence of the above three constituents indicates the occurrence of a small quantity of hemicellulosic material in the original flour. The relatively greater quantity of uronic acid may stem in part from pectic substances present in the flour or alternatively it may come from a rather acidic hemicellulose fraction. Attempts to characterize the starch of the rye-wheat Triticale have not yet been made but are projected. An interesting possibility arises in that an interaction of the rye and wheat genomes could possibly be present in a chemically detectable form.

Total nitrogen of the ether-extracted flour was determined and is expressed as crude protein. The values obtained were in close agreement with those which were obtained in some previous investigations (*13*). More detailed studies are underway on the flour proteins, and the results will be reported at a later date. The hexaploid Triticale generally contained more 80% alcohol-soluble nitrogenous substances than did the hard red spring wheat varieties and the synthesized bread wheat (6A49) (Table I). Some 6A-Triticale varieties (6A190, 6A20.1, 6A20.2, 6A67.1) and two of the tetraploid wheats (4B113 and 4B276) contained considerably more soluble nitrogen than the other species of these two classes. The two octaploid Triticale were also relatively high in soluble nitrogen as were the two durum varieties and the rye variety. Both the rye and durum wheat genomes may contribute toward this character although in some specific varieties (some of the 6A67 selections) a suppression seems to be evident. Paper chromatographic examination of the soluble nitrogenous fractions indicated the presence of at least 14 amino acids in varying relative quantities. This aspect will be further investigated using gas chromatographic techniques in conjunction with other available procedures.

Except for a few isolated exceptions, the hexaploid and octaploid Triticale were relatively low in fat and compared in this respect to rye. The durum varieties contained about twice as much fat. Whether the relatively higher fat content of a few Triticale is an expression of the durum wheat genome cannot be ascertained at this time, but it is an attractive possibility. A more detailed comparative study of the nature of the lipids is projected.

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FOOD ADDITIVES MEASUREMENT

Determination of Antioxidants in Certain Food Products and Packaging Materials by Gas Chromatography

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A method is described for measuring p.p.m. quantities of BHA and BHT in food products. The method includes a rapid extraction step and analysis with GLC utilizing 3,5-di-*tert*-butyl-4-hydroxyanisole (di-BHA) as an internal standard. The gas chromatographic column, containing a mixture of Silicone gum SE-30 and Tween 80, can be operated at a relatively low temperature (150° C.) with complete separation of the BHA and BHT from interfering compounds. The usefulness of the method is illustrated with data on ready-to-eat cereals, potato products, and waxed paper.

BUTYLATED HYDROXYANISOLE (BHA) and butylated hydroxytoluene (BHT) are added to many food products as freshness preservers. They may be added directly to the food or indirectly through the medium of packaging materials. Since these compounds act as free radical acceptors, small quantities of BHA and BHT very effectively protect foods from autoxidation. Present regulations allow 10 p.p.m. of the combination of BHA and BHT in potato granules and 50 p.p.m. in dry breakfast cereals. Therefore, a method for the determination of BHA and BHT in food products must be sensitive to at least the part per million level. In addition to sensitivity, a method suitable for quality control use must be rapid, accurate, and inexpensive.

Colorimetric procedures have been proposed by Anglin *et al.* (*2*); Filipic and Ogg (*6*); Lazlo and Dugan (*9*); and Sloman *et al.* (*12*). Hall and Clark (*7*) used infrared spectrophotometry to study complex mixtures of food antioxidants, and ultraviolet spectrophotometry was used by Phillips and Hinkel (*11*); Hansen *et al.* (*8*); and Berger *et al.* (*3*). Mitchell (*10*) suggested a paper chromatographic method for the analysis of mixtures of known antioxidant compounds, and Anderson and Nelson (*1*) and Buttery and Stuckey (*4*) have used gas liquid chromatography techniques. Sloman *et al.* (*12*) pointed out that most of these methods, except those based on GLC, work best at concentrations above 10 p.p.m.