Comparison of Chickpea Cultivars: Chemical Composition, Nutritional Evaluation, and Oligosaccharide Content

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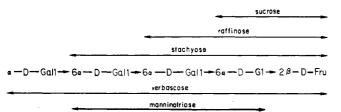
Twenty cultivars of the yellow and black chickpea (*Cicer ariethinum* L.), grown in the same climatic area, were compared. The two groups of seeds did not differ in protein and ash content. Black seeds had the highest fiber content and the lowest percentage of nitrogen-free extract. Black seeds had almost double the content of  $\alpha$ -galactosides as did yellow seeds. The ratio of sucrose to oligosaccharides was high in the yellow chickpea but equal in the black chickpea. Amino acid composition was almost uniform; all the seeds had methionine plus cystine as the first limiting amino acid. In vitro protein digestibility reached a mean value of 78% in the black seeds and 75% in the yellow seeds. The calculated protein efficiency ratio (C-PER) was similar and relatively low in all cultivars.

The chickpea is a legume of common consumption in Italy. Together with other legumes it has long been one of the most important protein sources of the rural population (Sarno and Stringi, 1980); the dry mature seed is traditionally cooked and eaten with cereals. Chickpea growing is also widespread in Central and South America, where it contributes considerably to protein requirements when animal sources are insufficient. The seed has a high protein content, about 20% (Aman, 1979; Khan et al., 1979), and is therefore well suited to human and animal nutrition. However, like all pulses, the chickpea lacks sulfur amino acids.

Flatulence is a common disturbance following legume consumption. Many investigators (Calloway et al., 1971; Fleming, 1981; Rackis et al., 1970) attribute it to a flatus factor, which is soluble in ethanol and is a complex mixture including oligosaccharides of the raffinose family. Murphy et al. (1972) found that oligosaccharides when consumed alone do not increase the carbon dioxide levels of the flatus. Hellendorn (1978) regards the flatus factor as consisting mainly of other glucidic components such as ethanol-insoluble compounds and insoluble starch.

Gas formation is due to anaerobic fermentation of undigested food by the intestinal microorganisms. In particular, the mammalian digestive system lacks the enzyme  $\alpha$ -galactosidase, which is able to break down the  $\alpha 1 \rightarrow 6$ bonds between the galactose molecules of  $\alpha$ -galactosides (Calloway et al., 1971).

The oligosaccharides of the raffinose family are  $\alpha$ -galactosylsucroses; the ones found in the chickpea (Åman, 1979; Sosulski et al., 1982) are raffinose, stachyose, and verbascose. Sosulski et al. (1982) reported the presence in the chickpea of large quantities of manninotriose, an  $\alpha$ -galactoside not belonging to the raffinose family. The composition of chickpea oligosaccharides is



Chickpea seeds may be divided into two groups distin-

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Table I.	Origin of	Chickpea Seeds	Analyzed

yellow	black		
seed	origin	seed	origin
1, Spagnoli <sup>a</sup>	Spain	11, Annigeri	India
2, Agrex Maroc <sup>a</sup>	Morocco	12, JGC-1	India
3, Altamura	Italy	13, Chafa	India
4, Pian di Scò <sup>a</sup>	Italy	14, CPS-1	India
5, Malocu	Italy	15, P-436	India
6, Cagnano Varano Aª	Italy	16, GW-5/7	India
7, Cagnano Varano Bª	Italy	17, BDN-9/3	India
8, Antella <sup>a</sup>	Italy	18, JG-74	India
9, Grossi d'Abruzzo <sup>a</sup>	Italy	19, ICCC-1	India
10, Leccese	Italy	20, 850-3/27	India

<sup>a</sup>Samples to be regarded as ecotypes.

guished by the color of the seed coat, light yellow or black. The color of the coat was described by other authors (Jambunathan and Singh, 1981) as salmon white and light brown. Generally, in Italy only light yellow seeds are employed for human consumption, because they are more digestible (Sarno and Stringi, 1980).

Jambunathan and Singh (1981) studied differences in composition existing between the two groups. They reported significant differences in fiber and mineral content (Jambunathan and Singh, 1981), in vitro protein and starch digestibility, and stachyose content (Singh et al., 1982).

This paper reports a comparison between 10 yellow Mediterranean chickpea cultivars and 10 black chickpea cultivars of Indian origin, all the samples being grown in the same climatic area. To identify any significant differences between the two groups, the chemical composition, including oligosaccharides and amino acids, and the in vitro protein digestibility were determined.

## MATERIALS AND METHODS

**Samples.** The 20 cultivars and ecotypes of the yellow and the black chickpea were all grown in the fields of the Experimental Farm of the University of Florence at Spedaletto (Italy) during 1980. The origin of seeds is reported in Table I. Representative samples of whole mature dry seeds were ground and analyzed.

**Experimental Methods.** The chemical composition of the samples was determined according to standard methods (AOAC, 1975). The crude protein content was calculated by using a factor of 6.25. Amino acids were determined according to Spackman et al. (1958) on an automatic analyzer after in vacuo hydrolysis of the samples with 6 N HCl for 24 h at 110 °C. In vitro protein digestibility was determined according to Hsu et al. (1977). The C-PER (calculated protein efficiency ratio) was calculated follow-

Table II. Chemical Composition (Percent) of Ch	ckpea Seeds
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sample	dry weight	protein ( $N \times 6.25$ )	ash	fat	crude fiber	nitrogen-free extract
yellow						
1, Spagnoli	90.17	21.09	3.06	6.80	4.95	54.27
2, Agrex Maroc	89.87	19.95	2.79	6.92	3.03	57.18
3, Altamura	90.63	19.80	3.00	7.30	2.80	57.73
4, Pian di Scò	90.40	20.20	2.94	7.38	3.61	56.27
5, Malocu	87.26	18.31	2.57	6.87	4.28	55.23
6, Cagnano Varano A	87.58	17.40	2.69	6.79	5.64	55.06
7, Cagnano Varano B	90.50	19.10	2.74	8.69	3.17	56.80
8, Antella	90.39	20.30	2.67	6.55	3.24	57.63
9, Grossi d'Abruzzo	89.64	18.40	2.73	7.18	3.16	58.17
10, Tipo Leccese	88.01	18.40	2.80	6.67	3.84	56.30
mean value	89.45	19.29	2.80	7.11	3.77	55.46
black						
11, Annigeri	89.30	16.70	2.71	6.96	9.91	53.02
12, JGC-1	87.81	18.46	2.71	5.90	8.85	51.89
13, Chafa	89.17	18.35	2.72	4.90	9.71	53.49
14, CPS-1	89.51	18.24	2.74	6.85	8.87	52.81
15, P-436	88.06	17.40	2.04	6.00	10.79	51.83
16, GW-5/7	89.24	19.35	2.52	7.42	7.67	52.28
17, BDN-9/3	89.36	19.64	2.66	6.29	9.75	51.02
18, JG-74	89.63	17.97	2.69	5.63	9.02	54.32
19, ICCC-1	87.57	17.82	2.59	5.72	8.86	52.58
20, 850-3/27	88.04	19.61	2.98	5.73	9.08	50.64
mean value	88.77	18.35	2.64	6.14	9.25	52.39

ing the mathematical model of Satterlee et al. (1979). Except for in vitro protein digestibility, two analyses were performed for each determination. Oligosaccharides were determined by high-performance liquid chromatography. (HPLC).

HPLC Carbohydrate Analysis. Soluble carbohydrates were extracted in 50% ethanol at 50 °C for 1 h. The mixture was centrifuged at 2792g for 10 min. The supernatant was passed through a 0.22- $\mu$ m Millipore filter and then used for injection.

Chromatographic separations of single sugars were performed on a Waters Associates HPLC apparatus by using a carbohydrate column ( $3.9 \text{ mm} \times 30 \text{ cm}$ , Waters Associates) and a precolumn with a mobile phase of acetonitrile-water (70:30) at a flow rate of 1.5 mL/min. The single carbohydrates were determined with a differential refractive index detector (Waters Associates Model R.401). A complete analysis was carried out in about 12 min.

Pure raffinose and stachyose were supplied by Merck, West Germany, and by BDH Chemicals, Ltd., Great Britain, respectively. Pure manninotriose and verbascose were not available commercially. Manninotriose was prepared by mild acid hydrolysis (0.01 M  $H_2SO_4$ , 15 min) of stachyose according to Åman (1979): the products of hydrolysis were manninotriose and fructose.

In order to point out any significant difference between the two groups of seeds, the yellow and the black ones, a statistical analysis has been carried out. For each determination, the data of the yellow seeds were compared with the data of the black seeds by the Student's t test (Sokal and Rohlf, 1973).

## RESULTS AND DISCUSSION

Table II reports the chemical composition of the seeds on an as is basis. The two groups of samples had substantially identical protein and ash contents. Fiber, fat, and nitrogen-free extracts showed significant differences (P < 0.01), yellow seeds having higher fat and nitrogen-free extract contents and a lower crude fiber content. The crude fiber difference is due to the fact that the black seeds are smaller than the yellow ones and they therefore present a higher percentage of hull and fiber.

Carbohydrate analysis was carried out to reveal any possible difference between the two groups with regard to

 Table III. k' of Carbohydrate Peaks Identified in HPLC

 Analyses

k'	carbohydrate	k'	carbohydrate
0.80	fructose	2.77	manninotriose
0.94	glucose	3.86	stachyose
1.23	sucrose	$4.20^{a}$	(verbascose)
2.31	raffinose		

<sup>a</sup> Assumed to be verbascose; no standard available.

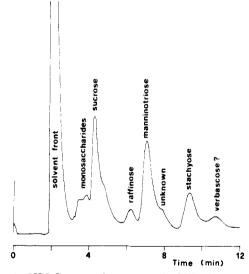


Figure 1. HPLC separation of sample GW-5/7 sugars on the carbohydrate column (Waters Associates). Mobile phase, acetonitrile-water (70:30); flow rate 1.5 mL/min; refractive index detector attenuation  $4\times$ .

oligosaccharides content, which has been associated with flatulence. The mobile phase (acetonitrile-water, 70:30) for HPLC analysis was selected to attain rapid elution of high molecular weight carbohydrates. Table III reports the k' of the peaks identified, and as an example Figure 1 shows the HPLC chromatogram of sample GW 5/7.

Monosaccharides and sucrose are of course eluted very close to each other when a mobile phase containing 30% water is used. These sugars were therefore estimated together against a standard mixture of equal quantities of glucose, fructose, and sucrose. Aman (1979) and Sosulski

## Table IV. Carbohydrate Composition (Percent) of Samples

	monosaccharides +				
	sucrose	raffinose	manninotriose	stachyose	total
yellow					
1, Spagnoli	6.37	0.09	2.05	0.44	8.95
2, Agrex Maroc	6.16	0.17	2.08	0.40	8.81
3, Altamura	6.67	0.21	1.84	0.58	9.30
4, Pian di Scò	5.60	0.11	2.13	0.68	8.52
5, Malocu	6.94	0.12	1.65	0.36	8.62
6, Cagnano Varano A	6.33	traces	1.56	0.37	8.26
7, Cagnano Varano B	5.88	0.10	1.84	0.35	8.17
8, Antella	2.58	0.15	1.24	0.61	4.58
9, Grossi d'Abruzzo	6.13	0.26	2.32	0.60	9.31
10, Leccese	6.27	traces	1.73	0.44	8.44
mean value	5.89	0.12	1.84	0.48	8.30
black					
11, Annigeri	3.38	0.26	2.54	1.22	7.40
12, JGC-1	3.32	0.30	2.58	1.37	7.57
13, Chafa	3.27	0.28	2.46	1.54	7.55
14, CPS-1	3.73	0.34	2.78	1.43	8.28
15, <b>P-4</b> 36	2.81	0.34	2.55	1.20	6.90
16, GW-5/7	3.07	0.30	3.04	1.19	7.60
17, BDN-9/3	3.41	0.17	2.65	1.48	7.71
18, JG-74	3.53	0.16	3.10	1.64	8.43
19, ICCC-1	3.33	0.20	2.62	1.36	7.51
20, 850-3/27	5.59	0.09	2.11	0.48	8.27
mean value	3.54	0.24	2.64	1.29	7.72

Table V. Amino Acid Composition (Milligrams per Gram of N) of Samples

	yellow													bla	ack					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
aspartic acid	699	708	717	683	711	692	685	683	789	679	705	691	683	694	694	692	669	694	680	697
threonine	218	214	215	224	217	225	216	207	231	217	218	218	211	220	219	210	210	215	188	213
serine	309	291	294	312	303	300	305	301	308	302	309	309	298	310	307	311	296	304	261	305
glutamic acid	1180	1215	1231	1284	1271	1265	1226	1206	1312	1297	1229	1253	1227	1211	1261	1263	1340	1228	1365	1191
proline	247	265	263	239	249	356	245	278	238	247	275	235	246	278	236	251	250	243	339	245
glycine	206	205	209	209	204	210	207	199	211	202	201	204	235	203	210	202	195	204	200	208
alanine	235	234	235	243	225	236	233	223	238	228	219	227	215	224	229	223	211	227	253	224
cystine	47	52	57	42	55	49	61	56	43	49	43	66	44	64	47	54	53	63	40	52
valine	250	261	274	250	259	257	268	261	253	249	266	247	253	262	256	260	239	293	258	247
methionine	62	51	67	60	56	63	68	67	49	64	57	61	54	64	64	59	58	55	51	63
isoleucine	266	258	275	263	268	261	276	260	258	256	261	257	251	253	265	260	247	271	264	266
leucine	474	469	477	476	458	459	477	465	471	474	471	464	456	450	471	460	439	455	463	476
tyrosine	139	125	139	98	128	92	116	124	88	137	91	134	119	139	116	118	137	133	93	141
phenyl- alanine	374	345	358	355	339	325	350	357	340	369	339	340	346	340	339	346	347	345	355	358
histidine	166	147	142	168	138	160	164	164	128	160	163	151	201	162	166	165	163	163	138	172
lysine	412	440	428	439	413	437	437	438	414	428	455	458	459	426	453	424	423	430	443	442
arginine	514	502	513	458	478	413	466	485	411	442	443	478	500	499	467	486	554	503	408	520
tryptophan <sup>a</sup>	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54

<sup>a</sup> Value from FAO (1970).

et al. (1982) report low concentrations of pinitol, myoinositol, galactopinitols, and galactinol in the chickpea: these may all be expected to be eluted immediately before and after the sucrose. This paper does not provide estimates for these compounds.

The peak eluted with a k' of 4.20 is probably that of verbascose, a trigalactosylsucrose, which should be eluted immediately after stachyose (a digalactosylsucrose). Pure verbascose was not available; therefore this peak could not be quantitifed. The carbohydrate composition of the samples on an as is basis is reported in Table IV. The major components of the seeds were found to be, in order, sucrose, manninotriose, and stachyose, as also reported by Sosulski et al. (1982) and Åman (1979). On the contrary, Rao and Belavady (1978) have shown raffinose to be a major sugar in 8 cultivars of the black chickpea grown in India, including two samples examined in the present study (Annigeri, Chafa).

Differences in composition between the two groups of samples are readily observed: they were significantly different (P < 0.01) with regard both to individual and to total sugars determined. The light yellow coated seeds have lower oligosaccharides and higher sucrose contents than black seeds. Total sugars are generally higher in yellow seeds.

Within the groups the composition is almost homogeneous except for the sample Antella (yellow) and sample 850-3/27 (black), especially with regard to sucrose content. A variation in stachyose content between the two groups was also reported by Singh et al. (1982), but the difference was not so evident as in the present findings.

Table V reports the amino acid composition of the samples. As found by Khan et al. (1979), chickpea cultivars have a nearly uniform amino acid composition and comparison between the two groups generally reveals no significant differences (Jambunathan and Singh, 1981). From Table V only the levels of leucine and lysine are found to differ significantly (P < 0.05) for yellow and black chickpeas. All seeds have methionine plus cystine as the first limiting amino acid [protein score based on the FAO/

Table VI. In Vitro Protein Digestibility (Percent) and C-PER of Samples

digestibility	C-PER	
ellow		
73.31	1.60	
74.21	1.27	
73.54	1.62	
76.69	1.29	
76.24	1.41	
74.89	1.41	
74.66	1.65	
77.03	1.66	
76.24	1.15	
73.42	1.37	
75.02	1.44	
lack		
76.47	1.28	
78.27	1.71	
78.61	1.28	
78.16	1.74	
80.08	1.62	
77.48	1.44	
79.40	1.46	
78.95	1.65	
79.74	1.16	
74.32	1.19	
78.15	1.45	
	ellow 73.31 74.21 73.54 76.69 76.24 74.89 74.66 77.03 76.24 73.42 75.02 lack 76.47 78.27 78.61 78.16 80.08 77.48 79.40 78.95 79.74 74.32	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

WHO (1973) scoring pattern]; the second limiting amino acid is generally value, except in samples JG-74, ICCC-1, Altamura, and Antella, all having threonine as the second limiting amino acid. Table VI reports the in vitro protein digestibility and C-PER of the samples; C-PER was calculated on the basis of digestibility and essential amino acid content. In vitro protein digestibility was significantly higher (P < 0.01) in black-coated seeds. Jambunathan and Singh (1981) have reported an opposite finding, with higher digestibility values for yellow chickpeas. However, these authors determined in vitro protein digestibility by a different method from that reported here. The calculated PER reported in Table VI was not significantly different in the two groups of samples.

In conclusion, the yellow- and black-coated seeds have similar nutritional characteristics except for in vitro protein digestibility, which is slightly higher in black chickpeas. With regard to chemical composition, significant differences are observed in crude fiber, in fat, in the nitrogenfree extract, and above all in carbohydrate content. The ratio of sucrose to oligosaccharides is higher in the yellow chickpea, while, in the black chickpea, the ratio is almost equivalent. Black-coated seeds, however, have almost double the content of  $\alpha$ -galactosides, sugars involved in flatulence phenomena.

**Registry No.** Sucrose, 57-50-1; methionine, 63-68-3; cystine, 56-89-3; fructose, 57-48-7; glucose, 50-99-7; raffinose, 512-69-6; manninotriose, 13382-86-0; stachyose, 470-55-3; verbascose, 546-62-3.

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