Caroubin: A Gluten-like Protein Isolated from Carob Bean Germ

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ABSTRACT

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In 1935, Bienenstock and coworkers claimed the presence of gluten-like material in the germ of the carob seed. The viscoelastic properties of the water-insoluble proteins isolated from carob germ, which we propose to call caroubin, have been confirmed by dynamic (G' and G'') and static rheological measurements (texture profile analysis, viscoelastogram). Biochemical analyses showed important similarities (high glutamic acid content, size-exclusion HPLC profile, PAGE patterns of reduced and unreduced proteins) as well as large differences (high arginine and low cysteine and proline content of caroubin, carbohydrate composition) between caroubin and wheat gluten. Besides potential new industrial uses of carob seed, caroubin could be a valuable material to help us understand the physicochemical basis of the viscoelastic properties of plant protein complexes like wheat gluten.

From the classical work of Beccari in 1728, the unique nature of wheat gluten proteins has long been recognized and is thoroughly documented (Bietz 1992). However, Bienenstock et al (1935) were awarded a patent in which they claimed that "the germinal substance of the seed kernels of the fruit of the carob tree (*Ceratonia siliqua*) is rich in proteins that behave physico-chemically absolutely like wheat gluten proteins." They claimed an invention that relates to the manufacture of milled carob products for alimentary purposes and to the manufacture of foodstuffs that are rich in "gluten" proteins without using wheat products. To avoid a discoloration from the original yellow color of the germ flour into an undesirable greenish or green when water is added to the germ flour, they recommended adding dilute acid (citric acid) to the slurry. They also noted that the flour made up from germ absorbs 140–200% of its own weight of water and forms a dough of similar consistency to a wheat flour dough. According to these Hungarian scientists, an enriched protein fraction is easily isolated by hand-washing with water. Plants of the families of *Caesalpinaceae* or *Mimosaceae* are also a source of gluten-like proteins.

Fifteen years later, Rice and Ramstadt (1950) noted that a strong and coherent gluten is extracted from carob germ flour, provided that the flour is finely ground and that the proper amounts of water, time, and mechanical input are employed in the preparation of the dough. Using microbiological analysis, they found that the amino acid composition of carob gluten differs quantitatively from that of wheat gluten in that it contains much more arginine, aspartic acid, and lysine; less cystine, glutamic acid, and phenylalanine; and much less proline.

A few groups from Greece (Drouliscos and Malefaki 1980), Spain (Maza et al 1989) and Switzerland (Del Re-Jiménez and Amado 1989) have published some data on the solubility in water and on the amino acid composition of carob germ proteins in relation to nutritional value. The presence of trypsin inhibitors in carob seeds has been noted by Belitz et al (1982). None of these authors paid attention to the gluten-like material in germ meals. More recently, a margarine product containing carob bean meal proteins as a dairy ingredient substitute has been patented (Lander 1994).

These proteins may also help us understand how some proteins, such as wheat gluten proteins, form a viscoelastic material when hydrated. Moreover, carob germ flour, which accounts for ≈20% of the seed or water-insoluble proteins isolated from that flour, could have numerous uses in food industries as texturizing agents or in nonfood industries as biofilms. The potential for manufacture of bread and other cereal-derived foods suitable for people suffering from celiac disease deserves special mention.

This article reports some biochemical and physical properties of the gluten-like proteins isolated from carob germ flour, which we call caroubin (from the French word "caroube" for carob).

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MATERIALS AND METHODS

Materials

Wheat gluten was extracted by hand-washing (2.5% NaCl solution) of doughs prepared from two common wheat flours from the cultivars Florence Aurore (very strong dough with high tenacity) and Apollo (weak and extensible dough) using the method described by Mauze et al (1972).

Carob germ was kindly provided by Systems Bio-Industries (Boulogne Billancourt, France) from their plant in Morocco. It was finely ground into flour using a cyclotec laboratory mill (Tecator) to a final particle size of 100–200 µm. Protein, carbohydrate, lipid, and ash contents were, respectively: 58.9% (N × 6.25), 13.3%, 12.9%, and 6.8% on a dry basis. Caroubin was isolated from carob germ flour by gently stirring 20 g of germ flour with 200 mL of deionized water for 3 hr at room temperature and centrifuged 15 min at 5,500 × *g*. The supernatant was discarded and the two insoluble layers were separated. Caroubin A and B were, respectively, isolated from the upper and the lower layers by hand-washing (tap water) using the method described for the isolation of wheat gluten

Methods

Protein content was determined according to the Dumas procedure, using a nitrogen analyzer (NA2000, Fisons Instruments). Samples (10 mg) were run in triplicate. The conversion factor $k = 5.0$ from nitrogen to protein was calculated from the amino acid composition of caroubin A, as given in this article, supposing that the ratios of Asp/Asn and Glu/Gln and the content in Try are identical in gluten and in caroubin (the low k value is due to the high Arg content of caroubin).

Amino acid analysis was performed by column chromatography using an automatic amino acid analyzer (LC5000, Biotronik) after hydrolysis under vacuum at 110°C for 24 hr in 5.7*M* HCl.

SDS-PAGE of wheat gluten and caroubin was performed with or without reducing agent 2% mercaptoethanol as described by Payne and Corfield (1979) and as modified by Autran and Berrier (1984).

Size-exclusion (SE) HPLC was performed on lyophilized material solubilized in 0.1*M* sodium phosphate buffer (pH 6.9) containing 2% SDS (Dachkevitch and Autran 1989).

Total carbohydrate contents were determined in duplicate after acid hydrolysis, reduction and acetylation by gas liquid chromatography according to Blakeney et al (1983) and as modified by Hoebler et al (1989).

Crude fat of germ meal and of caroubin was extracted with hexane in a Soxhlet extractor and weighed after desiccation.

Ash content determinations were performed at 900°C according to Mauze et al (1972), using an incineration time of 3 hr.

The moduli of storage *G'* (elasticity) and of loss *G''* (viscosity) of wheat gluten and caroubin were determined (DMTA MK III Torsion, Rheometrics Scientific) according to the method developed for gluten proteins by Cornec et al (1994).

The TA-XT2 texture analyzer (Stable Micro Systems Ltd., UK) was used to measure elasticity, cohesiveness, and consistency of those materials from a stress time curve. The texture profile analysis (TPA) was performed on 1 g of wet gluten or caroubin shaped

TABLE I
Amino Acid Composition of Wheat Glutens (cv. Apollo and Florence Aurore), Caroubins A and B, and Carob Germ Proteins (g/100 g of amino acid)

Amino Acids	Apollo Wheat Gluten	Florence Aurore Wheat Gluten	Caroubin Fraction A	Caroubin Fraction B	Carob Germ Proteins[a]
Asp	3.5	3.2	7.8	7.9	8.0
Thr	2.7	2.8	3.0	3.0	3.8
Ser	5.1	5.1	4.5	4.4	5.3
Glx	38.3	39.0	31.8	32.1	26.7
Pro	9.9	10.3	3.0	2.9	4.0
Gly	2.8	2.8	4.1	4.0	5.0
Ala	2.4	2.3	3.6	3.4	4.4
Val	3.6	3.4	3.2	3.3	3.9
Cys	2.5	2.5	0.7	0.8	0.6
Met	1.7	1.6	0.4	0.3	1.4
Ile	3.3	3.1	2.6	2.6	3.1
Leu	7.3	7.4	6.5	6.6	6.4
Tyr	3.7	4.1	3.4	3.4	2.8
Phe	5.7	5.7	3.3	3.4	3.2
Lys	1.8	1.5	5.0	4.8	5.9
His	2.0	2.1	2.6	2.6	2.8
Arg	3.8	3.1	14.4	14.6	12.7

[a] Recalculated from the data of Del Re-Jiménez and Amado (1989).

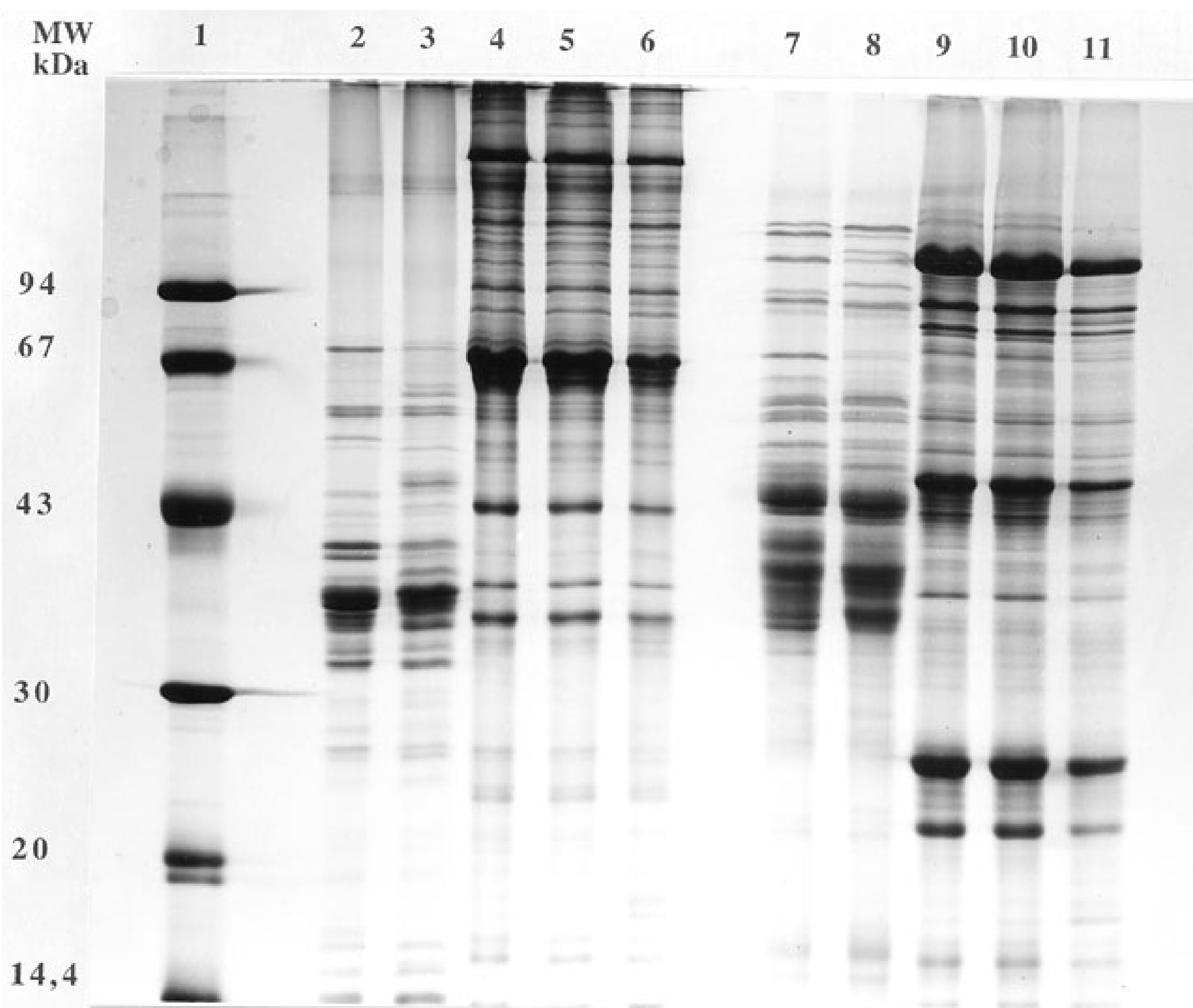

Fig. 1. PAGE of caroubins A and B and of wheat gluten (cv. Florence Aurore and Apollo). Lane 1: molecular weight markers (phosphorylase, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactalbumin); lanes 2 and 7: wheat gluten (Florence Aurore); lanes 3 and 8: wheat gluten (Apollo); lanes 4 and 9: Caroubin A; lanes 5 and 10: Caroubin B; lanes 6 and 11: total germ meal proteins. Lanes 2–6 contain unreduced proteins; lanes 7–11 contain reduced proteins.

by hand into a ball, using the following settings: speed 5 mm/sec; deformation (or strain) 20 mm. The probe was a parallel plate with a 25 mm diameter. Rest time between 2 cycles was 20 sec. Water contents of wheat gluten and caroubin before texture measurement were 60% (wheat gluten) and 45% (caroubin) on a wet basis.

The viscoelastic properties of thermoformed gluten and caroubin were determined using a viscoelastograph (Chopin, France) according to the method described by Damidaux and Feillet (1978). Wet gluten (1 g) was placed into a cylindrical cell (40 mm diameter, 15 mm height) closed at the two ends with a metallic cork and dipped into boiling water for 1 min. After cooling, the compressibility $C = (E - e_1)/E$, the elastic recovery $R = (e_2 - e_1)/(E - e_1)$ and the viscoelasticity index $VI = 10\ R/C$ were determined from the sample thickness versus time curve, where: E = thickness before loading; e_1 = residual thickness after loading (40 sec); and e_2 = final thickness after unloading (20 sec)

An Alveograph assay was performed as described by Mauze et al (1972) under conditions of constant dough water content (43.3 g of water for 100 g of dough) to measure the potential improving action of caroubin when added to wheat flour. In these assays, 1 or 2 g of caroubin A were added to 100 g of flour (cultivar Thésée).

RESULTS

The protein contents of caroubins A and B were 79.3% and 75.5%, respectively, on a dry basis. Other components present in the caroubin samples were: carbohydrates (2.8 and 3.9%), lipids (12.7 and 11.3%) and ash (6.8 and 7.2%). By comparison, typical wheat gluten composition is: protein (80%), starch (8%), lipids (7%), reducing sugars (2%), pentosans (2%), and ash (1%).

Amino Acids

Amino acid composition of caroubin A and B are given in Table I together with those of wheat gluten from Florence Aurore and Apollo. Compositions of caroubins A and B are very close, if not identical, showing large similarity between these two materials and confirming the early work of Rice and Ramstadt (1950). As compared to wheat gluten, caroubin is high in aspartic acid and basic amino acids (arginine and lysine), and poor in proline, cysteine, and methionine. The content of glutamic acid (Glu + Gln), while lower than that of wheat gluten, is nevertheless extremely high (about one-third of the total amino acid content). The low content of cysteine and proline in caroubin, considered to play important roles in determining structure and functionality of wheat gluten proteins, is worthwhile to note with regard to the viscoelastic properties of these two families of "gluten proteins."

Proteins

SDS-PAGE (Fig. 1) and SE-HPLC (Fig. 2) analyses demonstrated that caroubin is a mixture of a large number of proteins that are different in size, degree of polymerization, and electrophoretic mobility, covering an apparent molecular weight range from more than one million to several thousand. The overall composition of caroubin, reduced or not reduced, is fairly similar to that of gluten.

More than 50 proteins with different mobilities were separated by SDS-PAGE before reduction. On average, molecular weights of the caroubin proteins that entered the gel are higher than those of wheat gluten; a fraction of MW ≈ 70,000 is most prevalent. After reduction, three groups of subunits separate on the gel: one has molecular weights similar to high molecular weight subunits of glutenins (≈100,000 and over), another is in the range of low molecular weight glutenin subunits (70,000 to 30,000), and a third consists of low molecular weight (<30,000) proteins. The possibility that some aggregates were not solubilized in presence of reducing agents cannot be ruled out and needs further investigation.

The elution curves of caroubin A and wheat gluten (Apollo and Florence Aurore) in SE-HPLC consisted of approximately five

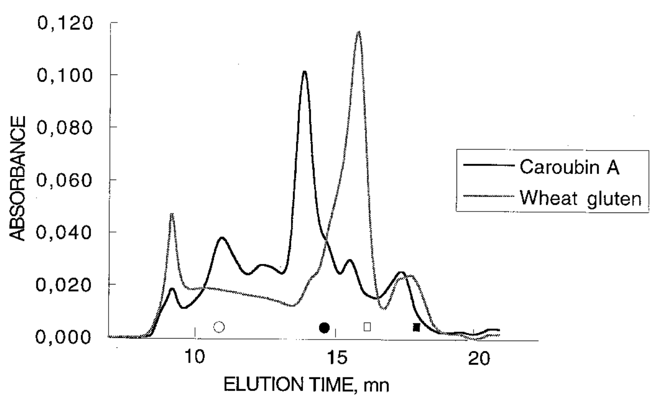

Fig. 2. Size-exclusion HPLC of unreduced caroubin A and wheat gluten (cv. Florence Aurore) extracted with sodium phosphate buffer containing 2% SDS. ○ = thyroglobulin, ● = bovine serum albumin, □ = carbonic anhydrase, ■ = cytochrome C.

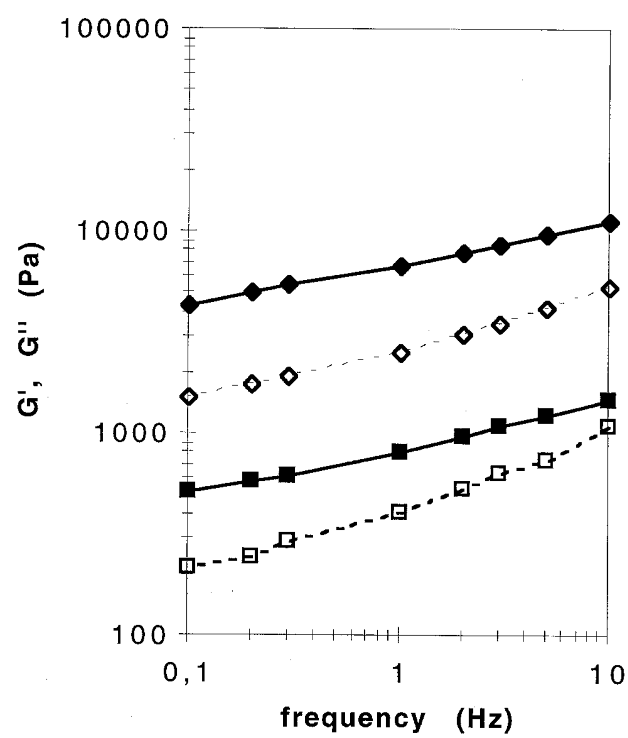

Fig. 3. Dynamic rheological analysis in shear of caroubin A and wheat gluten (cv. Apollo): frequency of the storage (G', ◆ = caroubin, ■ = gluten) and loss (G'', ◇ = caroubin, □ = gluten) moduli.

TABLE II
Percentage of SE-HPLC Fractions of Caroubin A and Wheat Gluten (cv. Apollo and Florence Aurore)

Fraction	Elution Time (min)	MW (Da)	Caroubin A	Florence Aurore Gluten	Apollo Gluten
F1	8.6–10.0	>1,000,000	5	7	11
F2	10.0–13.3	>200,000	33	19	22
F3	13.3–14.8	>65,000	40	9	9
F4	14.8–16.7	>20,000	10	54	45
F5	16.7–18.9	<20,000	11	11	12

fractions of different molecular weights. Amounts of each fraction were calculated as the percentage of total proteins in SE-HPLC peaks (Table II). The most striking difference between caroubin and wheat gluten is the high amount of fractions F2 and F3 (65,000 < MW < 1,000,000) in caroubin. Conversely, fraction F4 is more abundant in wheat gluten.

Carbohydrates

The neutral sugar composition of caroubin determined after acid hydrolysis is given in Table III. Most notable are the low content in caroubin of glucose (and therefore of starch, if present) and the relatively high content of arabinose.

Rheological Properties

The rheological behavior of caroubin A analyzed by dynamic assay in shear was close to that of wheat gluten (Apollo) (Fig. 3). The mechanical spectra (G' and G'' versus frequency) were qualitatively identical and typical of a network structure (Cornec et al 1994). Storage (G') and loss (G'') moduli of caroubin A were always higher than those of wheat gluten.

Viscoelastic behavior of caroubin was confirmed by texture profile analysis. Comparing a weak gluten (Apollo) to a strong (Florence Aurore) gluten, caroubin A showed higher elasticity, cohesiveness, and consistency. Elasticity of caroubin A was especially high and, therefore, its viscous modulus was rather low (Table IV).

TABLE III
Sugar Composition of Caroubin (% dry matter)

Sugar	Germ Meal	Caroubin A	Caroubin B	Gluten[a]
Arabinose	4.63	1.83	2.86	0.19
Galactose	2.52	0.23	0.27	0.57
Glucose	3.50	0.22	0.21	3.16
Mannose	2.08	0.17	0.19	Trace
Ribose	0.03	0.11	0.14	· · ·
Xylose	0.57	0.20	0.20	0.19
Total	13.33	2.76	3.87	4.11

[a] According to Saulnier et al (1997).

TABLE IV
Texture Analysis of Wheat Gluten Proteins and Caroubin A

Property	Apollo Wheat Gluten	Florence Aurore Wheat Gluten	Caroubin A
Elasticity	0.450	0.560	0.944
Cohesiveness	0.566	0.636	0.708
Consistency (g)	32.3	38.8	25.8

TABLE V
Compressibility, Elastic Recovery, and Viscoelasticity Index of Thermoformed Gluten[a] and Caroubin

	Apollo Wheat Gluten	Florence Aurore Wheat Gluten	Caroubin A	Caroubin B
Compressibility	0.66	0.65	0.27	0.29
Elastic recovery	0.24	0.32	0.59	0.49
Viscoelasticity index	3.6	5.0	22.0	17.0

[a] Freeze-dried gluten and caroubin were hydrated to allow easy handling of the gluten ball; 1.8 and 0.75 mL of water were added to 1 g of wheat gluten and caroubin, respectively.

TABLE VI
Alveograph Indices of Caroubin A in Wheat Flour

% Caroubin	Deformation Energy W (10^{-4} J)	Over-Pressure P (mm)	Abscissa of Rupture L (mm)	Swelling Index G (mL)
0	176	86	68	16.9
1	212	116	47	15.2
2	187	140	31	12.2

The effect of temperature on the viscoelastic behavior of caroubin was measured on thermoformed gluten (Table V). After dipping in boiling water for 1 min, caroubin still had a viscoelastic behavior like wheat gluten; its high viscoelasticity index and high elastic recovery are notable.

Caroubin was added to flour to evaluate its potentially improving effect in baking industries. Alveograph dough extensibility (L) and swelling index (G) decreased with addition of caroubin to the flour, while dough tenacity (P) and P/L ratio increased (Table VI). The dough strength (W) reached a maximum value (1% caroubin added) and then decreased (2% caroubin). This shows that adding caroubin to wheat flour has an important effect of the rheological properties of dough.

CONCLUSION

No significant differences were found between caroubin A and caroubin B, even though the layers from which they were isolated were easily distinguishable. The lower layer was brownish and nonuniform because of the presence of carob seed teguments. Since materials extracted from the two layers are almost identical, it appears unnecessary to separate these two layers in the future.

Caroubin is a complex aggregate of proteins of different molecular weights, ranging from ≈10,000 to several million. From our preliminary data, it appears that those proteins are joined through both noncovalent and covalent (disulfide) bonds. The ability of caroubin to form a viscoelastic material may result from protein interactions as for wheat gluten proteins. Components such as lipids and carbohydrates could also contribute to the viscoelastic properties of this material.

Marakis (1996) recently reviewed the present and potential uses of carob beans. Industrial gums from carob seed endosperm are most valuable in foods, paper, and cosmetics. Carob husk is used in animal feeds in some Mediterranean countries. Marakis did not, however, mention any industrial uses of germ meal and of its proteins. We have confirmed the work by Bienenstock et al (1935) that first noted that caroubin (or carob gluten-like proteins) could serve as a texturizing agent.

Wheat gluten and caroubin have many similar rheological properties, even if their biochemical compositions are different. The high content in caroubin of high molecular weight subunits might explain elasticity as in wheat gluten, but a higher content of SE-HPLC fraction F1 in caroubin would be expected to fit with the classical view on the relation between gluten elasticity and molecular size distribution. Considering the low cysteine content of caroubin, it is probable that the biochemical basis of caroubin viscoelasticity differs from that of gluten.

Caroubin is an extremely interesting and complex protein material, deserving of more attention. As a model protein, it may help explain the nearly unique viscoelastic properties of wheat gluten. From a nutritional point of view, the high content of basic amino acids in caroubin may make it valuable. We hope that this will encourage further investigation in these directions.

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