

Isolation and chemical evaluation of carob (*Ceratonia siliqua* L.) seed germ

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Abstract

The present investigation was undertaken in order to study the influence of the dehusking procedure on the germ meal composition of carob (*Ceratonia siliqua* L.) seeds and also to investigate its detailed composition and nutritive value. Meals of carob seed germ were obtained by acid treatment or boiling water treatment of the whole seeds. These procedures allowed to separation of the tight-fitting brown coat of the seed and the removal of the endosperm. Results indicated that the carob germ meal composition could be affected by the isolation procedures. Small reductions were observed in protein and lipid contents in germ meals from acid extraction. The analysis of the carob germ meal (containing fine fragments of husk and endosperm), which could be really obtained industrially, showed the following composition: moisture 8.3%, ash 6.5%, lipids (neutral and polar) 6.6% containing ~21% of polar lipids, crude proteins 54.7% and energy value 17.5 kJ/g.

Oleic acid (34.4%) and linoleic acid (44.5%) were the major fatty acids, while palmitic acid (16.2%) and stearic acid (3.4%) were the main saturated ones. Essential amino acids were present in very interesting amounts, according to the FAO standard except for a low content of tryptophan. The abundant protein fraction bands detected by SDS polyacrylamide gel electrophoresis showed that the carob germ protein fraction was extremely heterogeneous.

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1. Introduction

The carob tree (*Ceratonia siliqua* L.), also called algarroba, locust bean and St. John's bread, is a leguminous evergreen tree which grows throughout the Mediterranean region, mainly in Spain, Italy, Portugal and Morocco. The fruit pod (containing sweet pulp) gives, after removal of the seeds, the carob powder (Yousif & Alghzawi, 2000) often used as a chocolate or cocoa substitute (Nyerges, 1978; Brand, 1984). The seeds, covered with a tight-fitting brown coat, contain a white and translucent endosperm

(containing galactomannans), also called Carob gum, Locust bean gum (LBG) or E411. Locust bean gum is utilized in food and non-food industries for its ability to form a very viscous solution at relatively low concentration. It is also exploited for its synergy property with carrageenan, agar and xanthan to form stronger and more elastic gels (Goycoolea, Morris, & Gidley, 1995). World production of commercial carob seeds is about 32000 tons/year (Batlle & Tous, 1997). The processing of the seeds to yield the corresponding endosperm involves the removal of the husks and of the germ fractions, either by chemical or by thermo-mechanical treatment (Ensminger, Ensminger, Konlande, & Robson, 1994). The germs recovered as by-products of the seed processing are mainly used, after milling and heat treatment, as dietetic food or as animal feed (Batlle & Tous, 1997). There are

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no published studies on the influence of the separation procedures on the carob seed germ meals composition. The literature data on chemical composition of carob seed germ from Del Re-Jiménez and Amadò (1989) and Maza et al. (1989) showed that its nutritive value is high, due to the protein content (>50%). The germ oil (some 5–8%) is highly unsaturated (iodine value >100) and more than 50% of the protein is water-soluble. The aim of the present investigation was the study of the dehusking procedure (by acid or boiling water pre-treatment) effect on carob germ meal composition. Particular attention was paid to the lipid fraction, which was extracted by using a polar solvent (for polar lipids content) and analyzed by a GC method (for fatty acids composition). The amino acid composition (the sulphur amino acids included) was analyzed by cation exchange HPLC and tryptophan was analyzed by reversed-phase HPLC.

2. Materials and methods

2.1. Raw materials

The carob seeds used in this study were obtained from T.A.S.A., Parque Arboretum de Algarrobos (Malaga, Spain). The seeds had been gauged beforehand according to their length (ranging from 5.5 to 6 mm) and their thickness (ranging from 3.5 to 4 mm).

Three kinds of carob seed germs meals were isolated after acid or hot water treatments.

- (1) The *GermW* (pure germs, from boiling water extraction) was obtained after an aqueous thermal pre-treatment of carob seeds under the following conditions: 100 g (~780 seeds) of whole seeds immersed in 800 ml of boiling water during 60 min. Components (husk, endosperm and germ) of the swelled seeds, without tegument disruption, were then easily separated manually. A pure flour of germ was then obtained by drying (100 °C/30 min) and crushing the germ fraction.
- (2) The *GermA* (pure germs, from acid extraction) was obtained after an acid pre-treatment of carob seeds under the following conditions: 100 g of seeds in 60 ml of sulphuric acid (H₂SO₄/H₂O 60/40 v/v) at 60 °C during ~60 min. The mass containing the peeled seeds was washed extensively with water and rubbed to eliminate the carbonised husk, through a 2 mm sieve. The dehusked seeds was dried (100 °C/30 min) and then briefly crushed (10 seeds/2–3 s) with a laboratory mill (model MF 10, IKA, Staufen, Germany) to separate the two endosperms (or albumen) and to release the germ which was detached in pieces because it was more friable. The manual separation (facilitated by a preliminary sifting operation using a 1 mm sieve) of some large pieces of germs, made it possible to obtain a pure flour of germs after crushing.

- (3) The *GermAC* (contaminated germs, from acid extraction) was isolated by acidic pre-treatment, similar to *GermA*, and the removal (manual separation) of the endosperm pieces only. This germ flour was slightly contaminated with residues of husk and of endosperm, so it was closer to that obtainable by technological procedure. The flour thus obtained was similar to that obtained by Del Re-Jiménez and Amadò (1989).

The extraction procedures were performed at least in triplicate.

2.2. General methods

2.2.1. Chemical analysis

The moisture content of the germ meals was determined gravimetrically after heating the material (500 mg) in an oven at 105 °C for 24 h.

The ash content of the germ meals (3 g) was determined gravimetrically after dry mineralization at 600 °C for 12 h.

Lipids from the germ meals (5 g) were determined by using chloroform/methanol (2/1 v/v) mixture, as described by Folch, Lees, and Stanley (1957).

In order to establish the polar lipids content of the germ, neutral lipids were extracted in a Soxtherm S306 AK Automatic Extractor System Gerhardt (Gerhardt, Bonn, Germany) for 4 h, using 140 ml of petroleum ether (boiling range: 40–60 °C). After the automatic solvent removal phase, the extraction beakers (containing the extracted lipids) were dried in an oven at 103 ± 2 °C before being weighed.

The crude protein ($N \times 6.25$) content was estimated from 60 mg of germ meal by the Kjeldahl procedure, by nitrogen determination after mineralization (with a 1000 Kjeltabs MQ tablet and a Digestion System 20, 1015 Digester, Tecator AB, Höganäs, Sweden) and distillation (by a Kjeltac Auto 1030 Analyser, Tecator AB, Höganäs, Sweden).

Carbohydrates (nitrogen free extract) were estimated by difference.

The composition of total amino acids of the flours of germs (~100 mg containing ~10 mg of nitrogen) was obtained after hydrolysis under nitrogen with 6M HCl at 110 °C during 24 h and an HPLC analysis (Biochrom 20 Plus amino acid analyser, Pharmacia, Cambridge, UK) (Spackman, Stein, & Moore, 1958; Kaiser, Gehrke, Zumbalt, & Kuo, 1974). Norleucine (500 nM) was added as internal standard. The hydrolysates (30 µl) were injected on to a cation exchange column; the amino acids, separated by elution with suitable buffers of increasing pH, were detected with ninhydrin in a continuous flow photometric analytical system at 570 and at 440 nm (only for proline). Amino acid standard solutions (AA-S-18 from Sigma-Aldrich, Steinheim, Germany) (500 nM) also containing norleucine were separately injected to calibrate the analyzer and to calculate the response factors (used to calculate the amount of amino acid in the samples).

Sulphur amino acids (cysteine and methionine) were determined as cysteic acid and methionine sulphone, respectively, according to the Commission Directive 98/64/EC method (1998). A performic acid oxidation was done before the acid hydrolysis under nitrogen and an HPLC analysis (Biochrom 20 Plus, Pharmacia, UK).

Tryptophan was determined after alkaline hydrolysis of the proteins and a SP 8800 HPLC (Spectra physics, San Jose, CA, USA) analysis at 280 nm, with a XTERRA RP18 (4.6 × 150 mm; 3.5 μm) column, according to the Commission Directive 2000/45/EC method (2000). Column temperature was kept at 45 °C and the injection volume was of 5 μl. Elution rate was 1 ml/min with a mixture of solvent A (760 ml of sodium acetate buffer (0.07 M) /triethanolamine (0.025% v/v) adjusted to pH 4.5 with glacial acetic acid + 40 ml of methanol) and solvent B (acetonitrile containing 0.05% (w/v) trifluoroacetic acid). The following gradient (solvent A/Solvent B/minutes) was used: 0/100/0; 0/100/10; 50/50/5; 50/50/5; 0/100/5; 0/100/5. Separated tryptophan was quantified by using reference α-methyl-tryptophan.

Free amino acids were determined after precipitation (by sulfosalicylic acid) and separation (by centrifugation) of proteins, followed by an HPLC analysis (Biochrom 20 Plus, Pharmacia, UK) of the free amino acids, according to the Commission Directive 98/64/EC method (1998).

The composition of fatty acids was determined by GC by the proportioning of fatty acids methyl esters of the lipids prepared by saponification–esterification, according to the IUPAC Method No. 2.301 (1990). GC analyses were performed using a Hewlett–Packard 6890 series Gas Chromatograph System equipped with a HP-INNOWAX capillary column (30 m × 0.25 mm, film thickness 0.32 μm). Derivatized extracts (1 μl) in hexane were injected on-column. The oven temperature was programmed from 50 (isothermal for 1 min) to 150 °C at 30 °C min⁻¹ and from 150 to 240 °C (isothermal for 10 min) at 4 °C min⁻¹. Compounds were detected using a flame ionisation detector at 325 °C. Helium was used as carrier gas at a flow rate of 65 ml/min. Identification and quantification of fatty acid methyl esters were accomplished by comparing the retention times of the peaks with those of standards of Supelco 37 component FAME Mix 1 ml (Supelco Inc., Bellefonte, PA, USA).

2.2.2. Determination of the crude energy

Crude or gross energy (in kJ/g) was determined by the measurement of heats of combustion of carob germ meals

by means of a calorimetric bomb in the presence of oxygen (Adiabatic Calorimeter 1241 (PARR instrument company–Moline, Illinois, USA)). Benzoic acid was used as standard.

2.2.3. Electrophoretic analyses

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) of the proteins was performed, as described by Laemmli (1970), using a Hoefer SE 600 Ruby System (Amersham Bioscience, San Francisco, USA). A set of marker proteins with phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumine (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa) (LMW electrophoresis calibration kit; Pharmacia Biotech, Milwaukee, WI, USA) was used for determination of the molecular weights of the germ meal proteins. For the reduced condition, 18 mg of germ meal were mixed with 3 ml of SDS buffer containing 1.8% (w/v) of Tris (pH6.7), 2.5% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 10% (w/v) glycerol. The protein bands were stained with a Coomassie Brilliant Blue R 250 solution.

The molecular sizes of the bands were calculated on the basis of a standard curve.

Descriptive statistics were calculated and results expressed as means ± SD.

3. Results and discussion

3.1. Yield of extraction

Table 1 shows the results of the separation of carob seeds into their three components (husk, endosperm and germ) after acid or water pre-treatments. In the boiling water extraction procedure for GermW flour, the volume of seeds had greatly increased after the boiling water pre-treatment. So, the cuticle was easily broken and the germ easily separated (manually with a small spatula) from the endosperm.

In the acid pre-treatment for GermA and GermAC flours, the seed skin was burnt and the disintegrated (carbonised) husks were easily eliminated by washing and manual shearing. After dehusked seed drying, the germ and endosperm separation require, in this case, a milling. In the case of acid pre-treatment, fragments of hull remained attached to the dehusked seed. In particular, a thin skin layer around the dehusked seed, at the joint of the cotyledons, had most resisted the acidic attack. These resistant coats contaminated the germ and the endosperm flours

Table 1
Proportions of husk, endosperm and germ of carob seeds (in % of the fresh weight) isolated by acid and boiling water procedures

	Acid pre-treatment	Boiling water pre-treatment	Herald (1986)	Neukom (1988)
Husk	29 ^a –35 ^a	21–24	30–38	30–33
Endosperm	37–48	51–61	42–46	42–46
Germ	17–23	18–25	23–25	23–25

The whole seed of carob contains 9.5% of moisture.

^a By difference.

during the milling operation. So, only in GermAC flour, it could be seen the thin residues of the brown husk in the yellow carob germ meals. If the acid pre-treatment is prolonged, the acid may reach the germ between the two endosperms and partially hydrolyse it. So, it is important to follow the acidic attack cautiously.

In general, the results showed that the germ was the smallest fraction in carob seed and the germ yields from acid (17–23%) and water (18–25%) procedures were not significantly different. However, it appeared that the germ isolation procedure could affect the yield of extraction. Our results were close to those reported (23–25% of germ) by Herald (1986) and Neukom (1988).

3.2. Chemical composition

Results of the chemical composition of the three isolated flours of carob seed germ are presented in Table 2. Reference values of commercial carob germ are included in the Table, for comparison. The analysis showed small differences in the composition of the carob seed germ obtained by acid and water pre-treatment procedures. This indicates that the carob germ meal composition can be affected by the isolation procedures.

In the cases of GermA and of GermAC (both extracted after acid pre-treatment), the protein and lipid contents showed significant reduction in comparison to those of GermW (from water pre-treatment). This may be due to a partial degradation during the acid dehulling procedure. However, whatever the technique of extraction used, the carob germs meal showed a high content of proteins (54–67%). For, the inactivation of anti-nutritive substances, such as trypsin inhibitors (TI), generally contained in leguminosae seeds (Weder, 1986), a heating procedure (e.g. at 121 °C/25 min according to Martínez-Herrera, Siddhuraju, Francis, Dávila-Ortíz, & Becker, 2006) applied to the extracted germ meals would be a solution to make them suitable for food and feed products. Our extraction treatments with acid (at 60 °C/1 h) or water (at 100 °C/1 h), including the drying operation (at 100 °C/30 min), was probably not enough to completely inactivate the TI.

Considering the high level of carbohydrates (nitrogen-free extract), it would be interesting in a further work to determine the exact content of total carbohydrates (e.g. photometrically using the anthrone reaction).

The total lipid content (obtained by using a chloroform/methanol (2/1 v/v) mixture) compared to neutral lipid content (extracted by petroleum ether solvent) showed that carob germ meal contains ~1.1–1.7% of polar lipids (or constituted ~13–24% of total lipid content). Our results of GermAC (which can be obtained industrially) are closer to commercial carob germ data published by Del Re-Jiménez and Amadó (1989) except for lipids (neutral) content. This is perhaps due to differences in species and environmental factors.

The results showed that carob germ meals have a high energy value GermA (18.3 kJ/g), GermAC (17.5 kJ/g), GermW (17.9 kJ/g).

3.3. Composition in amino acids

3.3.1. Total amino acids composition

The composition of total amino acids of the flours of carob germs is presented in Table 3 with commercial carob germ reference values, for comparison. Accurate tryptophan and sulphur amino acid data is essential in order to obtain a complete amino acid profile from a legislative or economic point of view. In particular, determination of tryptophan concentration in feedstuff is essential, because tryptophan is, after lysine, cysteine and methionine, the amino acid most frequently found at limiting concentrations (Yust et al., 2004).

The analysis showed that GermAC (contaminated germ meal) had the lowest amino acid content. However, the three carob seed germ meals contain, in high concentration, all the known amino acids. Carob bean germ can be considered as a “complete protein” in which all the essential amino acids are present. Our cysteine and methionine values are higher than the values obtained by Del Re-Jiménez and Amadó (1989). These differences could be due to the performic acid oxidation, done before the acid hydrolysis in this work, in order to avoid the loss of the sulphur amino acids.

Table 2
Overall composition of carob seed germ meals (% on the basis of matter dry)

	GermA	GermAC	GermW	Del Re-Jiménez and Amadó (1989).
Moisture	6.5 ± 0.6	8.3 ± 0.1	5.9 ± 0.4	6.80
Ash	6.0 ± 0.4	6.5 ± 0.1	5.6 ± 0.1	6.59
Crude protein ($N \times 6.25$)	59.5 ± 0.3	54.7 ± 0.5	67.1 ± 0.4	51.68
Lipids (neutral and polar)	7.1 ± 0.1	6.6 ± 0.3	8.2 ± 0.6	–
Carbohydrates ^a (nitrogen free extract)	20.9 ± 0.4	23.9 ± 0.2	13.2 ± 0.4	–
Crude energy (kJ/g)	18.3 ± 0.4	17.5 ± 0.3	17.9 ± 0.3	–
Lipids (neutral)	5.4 ± 0.3	5.2 ± 0.3	7.1 ± 0.5	8.05
Polar lipids ^b	1.7 ± 0.2	1.4 ± 0.3	1.1 ± 0.6	–

Data are the means of triplicate analyses ± SD.

GermA (pure germs, from acid extraction); GermAC (germs contaminated with husk and endosperm fine fractions, from acid extraction); GermW (pure germs, from water extraction).

^a Calculated by difference.

^b Calculated by difference between total and neutral lipids.

Table 3
Amino acid composition of carob seed germ meals (in g amino acid/100 g of dry germ meal)

Amino acid	GermA	GermAC	GermW	Del Re-Jiménez and Amadó (1989).
Asp + Asn	4.6 ± 0.02	4.3 ± 0.2	5.1 ± 0.04	3.74
Thr	1.9 ± 0.01	1.8 ± 0.1	2.2 ± 0.02	1.73
Ser	2.7 ± 0.01	2.5 ± 0.1	3.0 ± 0.02	2.47
Glu + Gln	19.0 ± 0.2	17.2 ± 0.4	20.3 ± 0.3	12.6
Pro	1.9 ± 0.02	1.8 ± 0.1	2.1 ± 0.02	1.99
Gly	2.8 ± 0.1	2.5 ± 0.1	3.1 ± 0.02	2.32
Ala	2.4 ± 0.02	2.2 ± 0.1	2.7 ± 0.02	2.07
Cys – Cys	0.8 ± 0.02	0.6 ± 0.01	0.9 ± 0.04	0.25
Val	2.3 ± 0.1	2.1 ± 0.1	2.6 ± 0.02	1.85
Met	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.34
Ile	1.8 ± 0.1	1.7 ± 0.1	2.1 ± 0.01	1.44
Leu	3.5 ± 0.1	3.3 ± 0.1	4.0 ± 0.03	2.99
Tyr	1.8 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	1.20
Phe	1.5 ± 0.1	1.7 ± 0.1	2.0 ± 0.02	1.48
His	1.6 ± 0.01	1.4 ± 0.1	1.7 ± 0.01	1.12
Lys	3.2 ± 0.01	3.0 ± 0.1	3.6 ± 0.1	2.96
Arg	7.3 ± 0.1	6.7 ± 0.2	8.1 ± 0.1	5.80
Trp	0.5 ± 0.03	0.4 ± 0.1	0.5 ± 0.03	–

The values represent the means of triplicate analyses ± SD.

GermA (pure germs, from acid extraction); GermAC (germs contaminated with husk and endosperm fine fractions, from acid extraction); GermW (pure germs, from water extraction).

In addition, all the studied samples are characterized by a very low content of free amino acids (< 0.4%). This means that most of the amino acids come primarily from proteins.

3.3.2. Essential amino acids composition

The quality of carob seed germ protein was estimated (Table 4) by calculating the chemical score (CS), using the following equation: $CS = (a_i/a_s) \times 100$. This is based on comparison of the concentration ratio of the essential amino acid having the shortest supply a_i (restrictive amino acid) to the concentration of this amino acid in the standard a_s . The FAO/WHO (1991) reference pattern was used as the protein standard.

The analysis clearly indicated that essential amino acids were present in interesting amount according to the FAO/WHO standard, except for tryptophan, which is found at the most limiting concentration. Our results contrast with

the values obtained by Del Re-Jiménez and Amadó (1989) in which tryptophan was not analysed and cysteine and methionine, determined after the acid hydrolysis method, were found to be the limiting amino acids.

3.4. Composition of fatty acids

Fatty acid compositions of the oil extracted by chloroform/methanol (2/1 v/v) from the carob seed germ meals are given in Table 5 with commercial carob germ reference values, for comparison. These results confirm that oil from carob seed germ is rich in unsaturated fatty acids. Oleic (18:1n – 9) and linoleic (18:2n – 6) acids account for more than 78% of the total fatty acids in the three germ meals isolated. A previous study (Maza et al., 1989) also showed that the oil of *Ceratonia siliqua* L. seed germ contains oleic 38.5% and linoleic 43.6% acids at relatively high levels.

Table 4
Contents of essential amino acids (AA) in carob seed germ meals (in g amino acid/16 g N^a)

Amino acid	GermA		GermAC		GermW		FAO/WHO (1991) standard
	g AA ^b /16 g N	% of FAO	g AA/16 g N	% of FAO	g AA/16 g N	% of FAO	g AA/16 g N
Thr	3.2	94	3.2	94	3.2	94	3.4
Val	3.8	109	3.9	111	3.9	111	3.5
Met + Cys	2.4	96	2.3	92	2.4	96	2.5
Ile	3.1	124	3.2	128	3.1	124	2.8
Leu	5.9	89	6.0	91	5.9	89	6.6
Phe + Tyr	5.5	87	6.0	95	5.8	92	6.3
Lys	5.4	98	5.4	98	5.4	98	5.8
Trp	0.8	73	0.8	73	0.7	64	1.1
His	2.7	142	2.6	137	2.5	132	1.9
Chemical score	73		73		64		100
Limiting amino acid	Trp		Trp		Trp		

GermA (pure germs, from acid extraction); GermAC (germs contaminated with husk and endosperm fine fractions, from acid extraction); GermW (pure germs, from water extraction).

^a Calculated from crude protein content.

^b AA: amino acid

Table 5
Fatty acid composition (% of total FA content) of oils from carob seeds germ meals

Fatty acids	GermA	GermAC	GermW	Maza et al. (1989)
Myristic (C14)	–	–	–	0.1
Palmitic (C16)	15.4 ± 0.4	16.2 ± 0.1	15.7 ± 0.4	14.2
Palmitoleic (C16:1)	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	–
Stearic (C18)	3.4 ± 0.4	3.4 ± 0.5	3.5 ± 0.5	3.0
Oleic (C18:1)	35.3 ± 2.0	34.4 ± 0.1	34.7 ± 0.2	38.5
Linoleic (C18:2)	45.0 ± 0.1	44.5 ± 0.2	44.5 ± 0.3	43.6
Linolenic (C18:3)	0.7 ± 0.4	0.7 ± 0.2	0.6 ± 0.1	0.3
Arachidic (C20)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	–
Gadoleic (C20:1)	0.3 ± 0.03	0.3 ± 0.1	0.3 ± 0.04	–

The values represent the means of triplicate analyses ± SD.

GermA (pure germs, from acid extraction); GermAC (germs contaminated with husk and endosperm fine fractions, from acid extraction); GermW (pure germs, from water extraction).

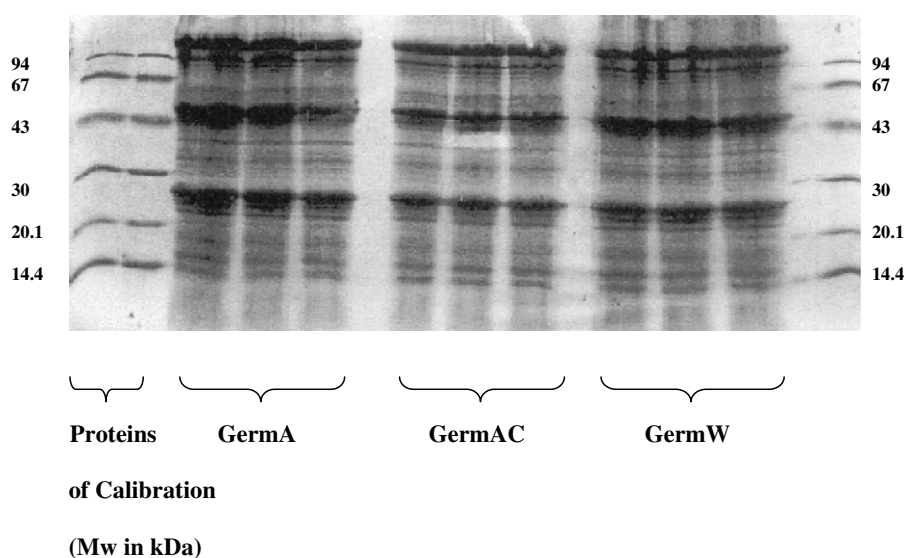


Fig. 1. Electrophoretograms of carob seed germ proteins fraction. GermA (pure germs, from acid extraction); GermAC (germs contaminated with husk and endosperm fine fractions, from acid extraction); GermW (pure germs, from water extraction).

Unfortunately, carob germ oil not contain sufficient ($n - 3$) fatty acids ($\sim 0.7\%$) to be considered as high nutritional quality oil.

3.5. Electrophoresis analysis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in order to determine the molecular weight distribution of the proteins of the isolated carob seed germ meals. The results are presented in Fig. 1.

The high number of protein fraction bands in the electropherograms means that the carob germ protein fraction is extremely heterogeneous. Among the different bands, our three germ preparations present proteins bands at approximately 95.5, 55, 26.3 and 13.8 kDa. These values are in agreement with results published by Del Re-Jiménez and Amadó (1989) who have worked at much lower concentrations and detected main protein bands at 91, 51, 25 and 14 kDa.

4. Conclusion

From the results, we can conclude that the carob seed germ meals, extracted after acid or boiling water pre-treatment of the whole seeds, preserve a considerable amount of proteins. Essential amino acids are present in interesting amount according to the FAO/WHO standard, except for tryptophan which is the limiting amino acid. Germ oil is highly unsaturated, but does not contain sufficient ($n - 3$) polyunsaturated fatty acids to be considered as very good nutritional oil. Nevertheless, a heat treatment of carob seed germ meals would make them interesting for nutritional purposes.

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