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Coffee Dietary Fiber Contents and Structural Characteristics As Influenced by Coffee Type and Technological and Brewing Procedures

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Coffee brews contain considerable amounts of soluble dietary fiber, mainly low substituted galactomannans and type II arabinogalactans. Factors possibly influencing the content and structures of dietary fiber in coffee brews, such as type of coffee, roasting and grinding degree, and brewing procedure, were studied. In addition, several commercial samples such as instant espresso, instant coffee, instant cappuccino, decaffeinated coffees, and coffee pads were analyzed. The dietary fiber contents of the coffee brews ranged from 0.14 to 0.65 g/100 mL (enzymatic−gravimetric methodology), proving an influence of the factors investigated. For example, the drip brew of an arabica coffee contained significantly more soluble dietary fiber than the drip brew of a comparable robusta coffee, and depending on the brewing procedure, the soluble dietary fiber content of beverages obtained from the same coffee sample ranged from 0.26 to 0.38 g/100 mL. Dietary fiber contents of coffee brews were enhanced only up to a certain degree of roast. Drip brews of decaffeinated arabica coffees (commercial samples) contained significantly less dietary fiber than any non-decaffeinated drip brew investigated in this study. The observed differences in the dietary fiber contents were accompanied by changes in the structural characteristics of fiber polysaccharides, such as galactomannan/arabinogalactan ratio, galactose substitution degree of mannans, or galactose/arabinose ratio of arabinogalactans as analyzed by methylation analysis.

KEYWORDS: Coffee brews; dietary fiber; galactomannans; arabinogalactans; melanoidins; coffee type; degree of roast; grinding; coffee preparation; methylation analysis

INTRODUCTION

According to a widely accepted definition, dietary fiber is the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (*1*). Although dietary fiber is generally supposed to predominantly occur in solid foods, beverages may also contribute to dietary fiber intake. Beverages are partly enriched by manufacturers with soluble, ideally nonviscous soluble fibers, but they are also a source of natural fibers. Next to fruit and vegetable juices, coffee brews contain nondigestible polysaccharides that are per definition part of the dietary fiber complex (*2*−*4*). Galactomannans and type II arabinogalactans are typical polysaccharides found in coffee brews. Recent studies have shown that galactomannans and arabinogalactans, being part of the soluble dietary fiber complex obtained from a medium-roasted Colombian coffee brew, were readily fermented by human fecal microbiota (*4*). Although up to 85% fiber carbohydrates of the investigated coffee brew were degraded, differences in the fermentability of the different polysaccharides and between different structural features of the polysaccharides were observed; for example, structural units from arabinogalactans that are composed of (1→5)-linked arabinosyl units were least degradable. Whereas polysaccharides are the main components of the soluble dietary fiber complex of coffee brews, other components such as melanoidins, high molecular weight Maillard reaction products, are also partially detected as dietary fiber when the widely accepted enzymatic−gravimetric methodology is used. These complex, not readily understood high molecular weight coffee components are possibly degraded by the human gut microbiota (*2*) as also shown by using model melanoidins (*5*, *6*) that, however, most likely differ substantially in their chemical characteristics.

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Table 1. Defined Coffee Samples and Preparation of Coffee Brews

Coffea species/origin	roasting	grinding	brewing procedure	coffee/water	abbreviation[a]
robusta/India	medium	medium	drip brew[b]	50 g/L	RMMD
arabica/Brazil	medium	medium	drip brew[b]	50 g/L	BMMD
arabica/Colombia	light	medium	drip brew[b]	50 g/L	CLMD
arabica/Colombia	medium	medium	drip brew[b]	50 g/L	CMMD
arabica/Colombia	dark	medium	drip brew[b]	50 g/L	CDMD
arabica/Colombia	dark	fine	drip brew[b]	50 g/L	CDFD
arabica/Colombia	medium	medium	drip brew[c]	500 g/10 L	$CMMD_{10}$
arabica/Colombia	medium	medium	drip brew[c]	1000 g/20 L	$CMMD_{20}$
arabica/Colombia	medium	medium	French press, 3 min	50 g/L	$CMMF_3$
arabica/Colombia	medium	medium	French press, 6 min	50 g/L	$CMMF_6$
arabica/Colombia	medium	medium	stovetop espresso[d]	17.5 g/0.35 L	CMME
arabica/Colombia	dark	medium	stovetop espresso[d]	17.5 g/0.35 L	CDME
arabica/Colombia	dark	fine	stovetop espresso[d]	17.5 g/0.35 L	CDFE

[a] The four-letter abbreviations include information about the type of coffee (first letter; R, *robusta*; B, Brazil; C, Colombia), roasting (second letter; L, light; M, medium; D, dark), grinding (third letter; M, medium; F, fine), and preparation procedure (fourth letter; D, drip brew; D_{10}/D_{20}, large-scale drip brew, 10 or 20 L, respectively; F_3/F_6, French press, brewing time 3 or 6 min, respectively). [b] Costumary automatic drip brewing coffee machine. [c] Commercial automatic drip brewing coffee machine (up to 20 L). [d] Six-cup stovetop espresso maker.

The polysaccharide characteristics, especially the structural characteristics of arabinogalactans of green coffee beans, were shown to be somewhat dependent on the coffee species investigated (*7*). It was also demonstrated that the extractability and structures of the polysaccharides from roasted coffees are dependent not only on the type of coffee but also on the degree of roast (*8*, *9*). Consequently, coffee type and the degree of roast should also influence the dietary fiber contents and structural characteristics of fibers obtained from coffee brews. Therefore, the aim of our study was to investigate the influence of the mentioned parameters and also that of parameters such as degree of grinding and different coffee preparation methods on the dietary fiber contents of coffee brews. In addition, several commercially available coffees such as instant coffee and decaffeinated coffees and coffee preparations such as instant cappuccino were analyzed for their fiber contents. Because structural characteristics of coffee fiber components were shown to influence their fermentability, we also investigated the structural characteristics of the soluble fiber polysaccharides.

MATERIALS AND METHODS

Material. Arabica coffee from Colombia (roasted to light, medium, and dark color, respectively), arabica coffee from Brazil (roasted to medium color), and robusta coffee from India (roasted to medium color) were kindly provided by Tchibo Manufacturing GmbH Co. KG (Hamburg, Germany). The roast colors were determined using an in-house reflectance measurement system. Color values of 90, 75, and 60 scale divisions corresponded to light-, medium-, and dark-roasted coffee, respectively. The light/medium- and dark-roasted coffees represent those typically used for drip brews and espresso coffees, respectively.

These defined coffee samples were medium and/or fine ground with average particle sizes of 450 and 300 μm, respectively. The medium- and fine-ground coffees represent those typically used for drip brews and espresso coffees, respectively. All well-described coffee preparations used in this study are listed in **Table 1**.

Decaffeinated arabica coffees (100% Colombian, ground for use as filter coffee), coffee pads (arabica, ≥50% Colombian), and instant coffees products (coffee, cappuccino, espresso) were purchased from local stores or ordered from online shops. All commercially available coffees and coffee preparations investigated are listed in **Table 2**.

Coffee Preparation. Coffee preparation procedures were generally performed twice, and the resulting coffee beverages were combined for subsequent chemical analysis. To ensure most comparable experimental conditions, the same type of water (bottled water as purchased in a local store; 69 mg/L Ca^{2+}, 3.9 mg/L Mg^{2+}, 136 mg/L HCO_3^-) was used for all coffee preparations with the exception of brewing coffee in a large-capacity coffee maker (coffee urn). Drip coffee beverages were prepared using a standard coffee maker, no. 4 sized cone paper filters, 50 g of ground coffee, and 1 L of medium-hard water, resulting in about 900 mL of coffee beverage. Using the coffee urn, two different sized drip brews were prepared, a 10 and a 20 L brew. The large-scale drip brews were prepared using basket coffee filters (diameter = 535 mm, height = 203 mm). Because this coffee urn was attached to the internal water supply of the student house, it was not possible to use the standardized water. However, the used water was of comparable, medium hardness. Different from the preparation of the other coffee beverages, coffee preparations in the coffee urn were performed only once. Stovetop espresso coffee (Italian coffee or *moka*) was prepared in a 6 cup stovetop espresso maker using a coffee/water ratio of 17.5 g/350 mL. Preparation of coffee in a French press (50 g of coffee/L of water) was done by waiting 3 and 6 min, respectively, before pressing down the plunger filter. Commercially available coffee pads (arabica, at least 50% Colombian, 6.9 g of coffee per pad) were used to prepare brews in a coffee-pad machine. It was not possible to influence the coffee/water ratio that was determined to be 53 g of coffee/L of water. Pads were also opened, and released coffee powder was used to prepare a filter coffee brew as described above. Different from the preparation of filter coffee, French press coffee, and stovetop espresso brews, the preparation of the coffee-pad beverage was performed seven times, and the resulting beverages were combined. Because dietary fiber contents were determined using dry coffee powder following freeze-drying of the beverage, the coffee and espresso instant products were not prepared, but the powders were directly used for the analytical determination

Table 2. Commercially Available Products and Preparation of Coffee Brews

coffee brew	product specification	preparation
A	instant espresso	1.82 g/cup[a]
B	instant coffee	1.79 g/cup[a]
C	instant cappuccino, unsweetened	10 g/130 mL[a]
D	coffee pads, 100% arabica, ≥50% Colombia	pad coffee;[c] 6.9 g of coffee/130 mL of water
E	decaffeinated, 100% Colombia, medium ground	drip brew;[b] 50 g of coffee/L of water

[a] With regard to manufacturer's preparation instructions or calculated on the basis of amounts of servings provided by one package. Instant products were not prepared. Instant espresso and instant coffee powder were directly used for analysis. Instant cappuccino was defatted prior to analysis. [b] Using a costumary automatic drip brewing coffee machine. [c] Using a costumary coffee-pad machine. The ratio of coffee and water is predefined by the coffee pads and machine used.

and preparative isolation of dietary fiber. Due to its milk fat constituents instant cappuccino was defatted prior to dietary fiber determination and preparation. For defatting purposes, cappuccino powder (8 g) was dissolved in boiling water (70 mL), and the cooled solutions were extracted five times with *n*-hexane (20 mL).

Dry Weight Determination. Dry weights of coffee brews were determined gravimetrically following freeze-drying. Dried coffee preparations were used for chemical analysis.

Dietary Fiber Analysis. Analytical determinations were performed in duplicate using an enzymatic−gravimetric procedure (*10*), accompanied by a third "preparative" determination used for structural characterization purposes. Analytical and "preparative" determinations differed only in the final filtration procedure. Two blank values were run with each series of analytical determinations. A commercially available enzyme kit (BIOQUANT, Merck, Darmstadt, Germany) was used for both procedures. In brief, dried coffee samples (1.0 g) were dissolved or suspended in a 2-(*N*-morpholino)ethanesulfonic acid (MES)/tris(hydroxymethyl)aminomethane (TRIS) buffer (0.05 M, pH 8.3, 40 mL) and, if necessary, the pH was readjusted to 8.3. Following addition of a heat-stable α-amylase (50 μL, ≥4000 units/g), the solution was incubated for 30 min at 95–100 °C. After cooling to 60 °C, samples were incubated with protease (50 μL, ≥2.3 units/g) for 30 min. Following pH adjustment to 4.0–4.7, amyloglucosidase (150 μL, ≥15.0 units/mg) was added and the samples were incubated at 60 °C for another 30 min. The samples were filtered through beforehand tempered (525 °C) and weighed glass filter crucibles (porosity = 2, pore size = 40–90 μm) partially filled with Celite 545 (1 g, dried at 105 °C) using slight vacuum. The residues were washed twice each with H_2O (10 mL, 70 °C), ethanol [78 and 95% (v/v), 15 mL], and acetone (15 mL), dried for 15 h at 105 °C, and weighed. One glass filter crucible was used to determine protein and the second one for the determination of the ash content as detailed below. Insoluble dietary fiber contents were calculated from the weighed residues under consideration of the blank values, protein and ash contents: $w = 100 \times (m_R - m_P - m_A - m_B)/m$ [*w*, dietary fiber content (g/100 g); m_B, mass of the blank (mg); m_P, mass of protein in the residue (mg); m_R, mean of residue masses (mg); m_A, mass of ash in the residue (mg); *m*, mean of sample weights (mg)] with $m_B = m_{Rblank} - m_{Pblank} - m_{Ablank}$ [m_B, mass of the blank (mg); m_{Rblank}, mean of blank residue masses (mg); m_{Pblank}, mass of protein in the residue of the blank (mg); m_{Ablank}, mass of ash in the residue of the blank (mg)]. Deviating from the analytical insoluble fiber determination, fluted filter papers were used for the "preparative" isolation of dietary fiber. Washed and dried residues were ground using a pestle and mortar and used for chemical characterization studies.

Filtrates and water washings were combined to determine soluble dietary fibers. Ethanol [95% (v/v), preheated to 60 °C, 4 volumes] was added, and the precipitate was allowed to form for 1 h at room temperature. The precipitates were filtered as described for the analytical insoluble dietary fiber determination and washed three times with ethanol [78% (v/v), 15 mL]. Further washing of the residues was performed twice with ethanol [95% (v/v), 10 mL] and three times with acetone (10 mL). The residues were dried, weighed, and used for ash and protein determinations as described for insoluble fibers. Soluble dietary fiber contents were calculated as described for insoluble dietary fiber. "Preparative" isolation of soluble dietary fibers was performed by centrifuging (2000*g*) the precipitated soluble fibers instead of filtering through glass filter crucibles. The precipitates were washed as described, dried, ground, and used for chemical characterization studies. Coffee soluble dietary fiber contents were statistically compared using the two-tailed *t* test at the 0.05 level of significance.

Determination of Residual Protein and Ash Contents. Residual protein contents were analyzed as nitrogen × 6.25. Nitrogen contents were determined following protein digestion according to the Kjeldahl methodology. Deviating from the Kjeldahl methodology, nitrogen in the digests was analyzed spectrophotometrically according to the method of Willis et al. (*11*). Ash contents were determined gravimetrically by incineration at 525 °C for 5 h.

Characterization of Coffee Dietary Fibers. Preparativley isolated dietary fibers were used for the UV spectral and carbohydrate characterization studies. Aqueous solutions (0.25 mg/mL) of soluble dietary fibers were measured spectrophotometrically at 280 and 405 nm.

Determination of Total Carbohydrates and Carbohydrate Composition. The total carbohydrate content, calculated as the sum of anhydrosugars, was determined using the phenol−sulfuric acid method as described by Dubois et al. (*12*). Standard calibration curves were obtained using mannose and arabinose, respectively, as described in Gniechwitz et al. (*4*). In a different approach, neutral sugars were released by Saeman hydrolysis, as modified by Englyst et al. (prehydrolysis with 12 M H_2SO_4 for 5 min at room temperature, hydrolysis using 2 M H_2SO_4 for 60 min at 100 °C) (*13*) and analyzed as their alditol acetates by GC-FID (*14*). GC conditions used are detailed in Gniechwitz et al. (*4*).

Structural Characterization of Coffee Fiber Carbohydrates. Methylation analysis was carried out as described by Nunes and Coimbra (*8*) with minor modifications as detailed in Gniechwitz et al. (*4*). In brief, dried samples (2 mg) were dissolved in dry dimethyl sulfoxide (2 mL), and freshly powdered NaOH (100 mg) was added to the solution. Following sonication for 90 min at room temperature, samples stood for another 90 min at room temperature. Methyl iodide (1 mL) was added to the ice-cold solution. The mixture was sonicated for 30 min and stood for another 30 min at room temperature. Sodium thiosulfate (0.1 M, 3 mL) was added, and the methylated carbohydrates were extracted into chloroform (3 mL). The organic layer was washed with water (five times, 5 mL) and rotary evaporated. Following a repeated methylation, the remethylated material was hydrolyzed with trifluoroacetic acid (2 M, 1 mL) at 121 °C for 1 h. Following rotary evaporation, a solution of 20 mg of $NaBD_4$ in 0.3 mL of 2 M NH_3 was added. The mixture was allowed to react for 1 h at room temperature. The reaction was stopped by adding glacial acetic acid (0.1 mL), and partially methylated alditols were acetylated by adding 1-methylimidazole (0.45 mL) and acetic anhydride (3 mL). Following a reaction time of 30 min, decomposition of residual acetic anhydride by adding water (3 mL), and extraction of the partially methylated alditol acetates into dichloromethane (3 mL), these were structurally analyzed by GC-MS as decribed in Gniechwitz et al. (*4*). The quantification of partially methylated alditol acetates was carried out by GC-FID (*4*). Molar response factors according to Sweet et al. (*15*) were used for quantification.

Acetate Determination. Naturally occurring acetates from coffee fiber polysaccharides were determined enzymatically following alkaline hydrolysis as described by Nunes et al. (*16*) (0.33 M, 1 h, room temperature) and neutralization of the reaction mixture. Enzymatic determination was performed using a commercially available reagent kit (R-BIOPHARM, Darmstadt, Germany).

RESULTS AND DISCUSSION

General Considerations. AOAC Method 985.29 for the determination of total dietary fiber (*17*) has become a global standard method used for labeling food products. This enzymatic−gravimetric method has been modified several times to allow measurement of soluble and insoluble dietary fiber (SDF and IDF, respectively) and to ease the procedure using different buffers. In this study, the determination of SDF and IDF was carried out using a commercially available enzyme kit and the enzymatic−gravimetric method as improved by Lee et al. (*10*). By applying this methodology, all components, with the exception of minerals and residual protein, that are resistant to enzymatic degradation by α-amylase, protease, and amyloglucosidase and that either are insoluble in an aqueous solution or precipitate or coprecipitate in aqueous ethanol (76%) are regarded as dietary fiber. As expected, insoluble fiber contents were negligible in most coffee preparations as detailed later. Contrarily, the SDF contents of coffee brews ranged from 139 to 654 mg/100 mL (**Table 3**). However, as is well-known for this methodology, it cannot be ruled out that some nondigestible oligosaccharides or highly soluble polysaccharides might not precipitate in ethanol and thus not be measured as dietary fiber.

Although ethanol precipitation allows the isolation of a coffee fiber fraction containing fewer Maillard reaction products than

Table 3. Dry Matter, Soluble Dietary Fiber Contents, Contents of Nondigestible Soluble Carbohydrates of Coffee Brews, and Absorbance of Soluble Coffee Fiber Solutions at 405 and 280 nm

coffee brew	dry matter (g/100 mL)	SDF (mg/100 mL)	SDF noncorrected for residual protein (mg/100 mL)	SDF carbohydrates[a] (mg/100 mL)	SDF carbohydrates[b] (mg/100 mL)	SDF absorbance at 405 nm[c] (au)	SDF absorbance at 280 nm[c] (au)
RMMD	1.73	302 ± 14	324 ± 14	202 ± 8	203 ± 10	0.219	0.568
BMMD	1.58	297 ± 32	315 ± 32	212 ± 18	200 ± 14	0.184	0.489
CLMD	1.47	289 ± 2	310 ± 2	190 ± 1	189 ± 9	0.179	0.474
CMMD	1.58	350 ± 4	368 ± 4	254 ± 7	221 ± 5	0.206	0.509
CDMD	1.60	349 ± 9	369 ± 9	240 ± 7	234 ± 7	0.216	0.541
CDFD	1.61	369 ± 17	387 ± 17	263 ± 11	236 ± 9	0.183	0.500
$CMMD_{10}$	1.48	299 ± 8	320 ± 8	201 ± 9	182 ± 10	0.209	0.546
$CMMD_{20}$	1.31	259 ± 8	281 ± 8	176 ± 11	149 ± 5	0.244	0.612
$CMMF_3$	1.39	263 ± 9	284 ± 9	152 ± 6	150 ± 3	0.211	0.534
$CMMF_6$	1.39	268 ± 3	284 ± 3	157 ± 3	168 ± 8	0.216	0.559
CMME	1.64	375 ± 6	390 ± 6	278 ± 9	250 ± 4	0.187	0.495
CDME	1.66	393 ± 19	416 ± 19	274 ± 15	275 ± 9	0.197	0.504
CDFE	1.63	426 ± 3	446 ± 3	287 ± 9	266 ± 8	0.197	0.512
A	2.98[d]	654 ± 27	679 ± 27	501 ± 17	412 ± 20	0.197	0.523
B	1.19[d]	276 ± 6	288 ± 6	180 ± 5	187 ± 4	0.137	0.436
C	7.62	340 ± 20	462 ± 20	148 ± 8	140 ± 4	0.129	0.387
D	1.16	228 ± 12	232 ± 12	175 ± 8	153 ± 6	0.201	0.521
E	1.15	139 ± 5	147 ± 5	88 ± 6	76 ± 2	0.275	0.664

[a] Determined by colorimetric method as described by Dubois. [b] Determined as alditol acetates by GC-FID. [c] Aqueous solutions (0.25 mg/mL) of SDF, $d = 1$ cm. [d] Values are based on the postulation that the volume of one cup of instant coffee or instant espresso is 150 and 60 mL, respectively.

high molecular weight fractions solely obtained by dialysis or ultrafiltration, the coffee SDF fractions isolated in this study still displayed a slightly brown color. As a measurable indicator for Maillard reaction products the absorbance at 405 and 280 nm was determined (*18*) (**Table 3**). Because a certain proportion of Maillard reaction products may be covalently bound to coffee polysaccharides (*19, 20*), some of the chromophoric and UV active substances detected in the coffee SDF preparations are part of nondigestible polysaccharides and therefore dietary fiber constituents. Furthermore, rat feeding experiments with radioactively labeled model melanoidins indicated that high molecular weight Maillard reaction products are not absorbed in the small intestine (*21*). As mentioned before, melanoidins may be partially utilized by human intestinal microbiota (*2, 5, 6*), another requirement to be regarded as dietary fiber. Therefore, Maillard reaction products having their origin in plant material may add to the dietary fiber complex not only from an analytical point of view but also from a nutritional point of view, a hypothesis that, however, needs further proof and discussion.

Table 3 also shows the SDF carbohydrate contents, determined by the colorimetric phenol−sulfuric acid method and alditol acetate procedure. As described before (*4*), the SDF carbohydrate contents analyzed by the colorimetric method were generally slightly higher than the values obtained by determining individual alditol acetates. The differences between soluble fiber contents and fiber carbohydrate contents indicate that coffee soluble fiber contains significant amounts of unknown substances, as already indicated by their brown color and UV activity.

Non-protein-corrected SDF contents of coffee brews are also listed in **Table 3** because an unknown proportion of the Kjeldahl nitrogen may derive from Maillard reaction products, thus overestimating residual protein that is generally not regarded as part of the dietary fiber complex. However, the SDF contents that have not been corrected for residual protein are only slightly higher than the corrected values.

Díaz-Rubio and Saura-Calixto analyzed dietary fiber contents of three different coffee brews by using an alternative approach (*3*). Following enzymatic degradation of protein and starch, and isolation of the fiber fraction by dialysis (12–14 kDa molecular weight cutoff), retentates were hydrolyzed with sulfuric acid and SDF carbohydrates were measured colorimetrically using dinitrosalicylic acid. However, this methodology neglects an important part of coffee brew polysaccharides showing molecular masses between 3 and 10 kDa (*2*). Nonetheless, the SDF carbohydrate contents of the beverages investigated by Díaz-Rubio and Saura-Calixto (espresso, filtered, and instant coffee) were generally higher than the SDF contents determined in our study. For example, the SDF content of a drip brew obtained from a 70%/30% mixture of medium- and dark-roasted Colombian coffee was 34% higher than the SDF content we determined for a comparable drip-brewed coffee (CMMD; **Table 3**). This can be partially explained by a much higher coffee/water ratio used for coffee preparation, resulting in a much higher dry matter content (2.94 vs 1.58 g/100 mL). Such a high dry matter content may be realistic for espresso coffees, but does not reflect drip coffee beverages as usually prepared. The coffee/water ratio used in our experiments (50 g/L) seems to match realistic conditions as also confirmed by measuring the coffee/water ratio used in a popular commercial coffee-pad machine (53 g/L). In addition, using dialysis to isolate fiber polysaccharides, the SDF carbohydrates are determined in a fraction rich in Maillard reaction products, which may interfere with carbohydrate determination in the applied semispecific colorimetric method. Contrarily, coffee SDF fractions obtained by ethanol precipitation contain fewer Maillard reaction products.

Dietary Fiber Contents and Characteristics As Influenced by Coffee Type. To compare the dietary fiber contents of different coffee types, drip brews of two arabica coffees (wet-processed Colombian and dry-processed Brazilian; BMMD and CMMD, respectively) and one high-quality robusta coffee (wet-processed Indian, RMMD) were analyzed. All three coffee samples were characterized by the same roasting (as determined by color reflectance measurement) and grinding degree. All coffee brews contained only SDF and no IDF. **Table 3** shows that the brew obtained from wet-processed arabica coffee (CMMD) contained about 15% more SDF than the brew prepared with a comparable wet-processed robusta coffee (RMMD). Whereas this difference was statistically valid ($p < 0.05$), a lower SDF content of BMMD compared to CMMD

Table 4. Contents of Monosaccharide and Acetate Units of Nondigestible Soluble Carbohydrates in Coffee Brews

coffee brew	rhamnose (mg/100 mL)	arabinose (mg/100 mL)	mannose (mg/100 mL)	glucose (mg/100 mL)	galactose (mg/100 mL)	acetate (mg/100 mL)
RMMD	3	14	95	3	89	3
BMMD	2	13	108	3	74	2
CLMD	2	17	84	2	83	2
CMMD	2	16	116	2	85	2
CDMD	2	14	129	3	87	2
CDFD	2	15	128	2	90	2
$CMMD_{10}$	2	14	88	2	78	2
$CMMD_{20}$	1	12	69	2	65	2
$CMMF_3$	1	13	68	2	66	2
$CMMF_6$	2	14	78	2	72	2
CMME	2	18	139	2	90	2
CDME	2	15	165	3	91	2
CDFE	2	16	151	2	95	3
A	2	14	197	4	195	4
B	3	13	43	1	128	1
C	1	6	36	3	93	1
D	1	5	109	2	36	1
E	1	6	38	1	29	1

Table 5. Structural Characteristics of Soluble Dietary Fiber Galactomannans and Arabinogalactans of Coffee Brews As Determined by Methylation Analysis (Ratios Calculated on a Molar Basis)

		galactomannans	arabinogalactans			
coffee brew	galactomannan/ arabinogalactan ratio[a]	total Man*p*/ 4,6-Man*p* ratio	galactose/ arabinose ratio	3-Gal*p*/6-Gal*p* ratio	(3-Gal*p* + 6-Gal*p*)/ 3,6-Gal*p* ratio	T-Ara*f*/ 5-Ara*f* ratio
RMMD	1.2	26.1	3.1	4.5	2.5	2.7
BMMD	1.3	31.5	2.7	3.1	2.0	3.4
CLMD	0.9	26.5	2.1	2.9	1.9	3.4
CMMD	1.3	29.1	2.7	2.8	1.9	3.6
CDMD	1.5	32.6	3.2	3.0	2.1	3.7
CDFD	1.5	28.3	3.1	2.9	2.4	4.1
$CMMD_{10}$	1.1	26.8	2.7	3.0	2.4	3.2
$CMMD_{20}$	1.0	25.1	3.2	3.1	2.3	3.2
$CMMF_3$	1.1	27.8	2.7	3.1	2.0	3.5
$CMMF_6$	1.1	27.5	2.9	3.1	2.0	3.6
CMME	1.6	31.4	3.2	3.3	1.9	4.1
CDME	2.0	30.4	3.3	3.0	2.2	3.8
CDFE	1.6	31.4	3.0	3.0	2.0	3.8
A	1.1	26.6	8.3	2.9	2.7	3.7
B	0.4	22.9	5.6	3.7	2.7	2.6
C	0.5	29.9	8.2	4.5	3.5	3.6
D	2.8	30.8	3.6	3.0	2.7	2.7
E	1.3	31.4	2.5	2.5	2.2	2.7

[a] Galactomannans (mol %) were calculated as the sum of mannosyl residues (T-Man*p*, 4-Man*p*, 4,6-Man*p*) plus the proportion of 4,6-Man*p* (correlates to the proportion of T-Gal*p* side chains). Arabinogalactans (mol %) were calculated as the sum of arabinosyl residues (T-Ara*f*, 5-Ara*f*) and galactosyl residues (T-Gal*p*, 3-Gal*p*, 6-Gal*p*, and 3,6-Gal*p*) minus the proportion of T-Gal*p* attributed to galactomannans (correlates to the proportion of 4,6-Man*p*).

was not statistically confirmed. There was also no statistically significant difference in the SDF contents of RMMD and BMMD.

The higher SDF content of CMMD was associated with a higher amount of SDF polysaccharides compared to BMMD and RMMD. Also, a lower content of SDF mannosyl residues (**Table 4**) shows that RMMD contained fewer mannans than CMMD (95 vs 116 mg/mL), fully reflecting its lower content of nondigestible polysaccharides. These results are in agreement with findings of Nunes and Coimbra, who also observed that fewer galactomannans are extracted from robusta coffee than from arabica coffee (*8*).

In contrast to RMMD, the lower SDF polysaccharide content in BMMD compared to CMMD is equally due to lesser amounts of arabinogalactans and galactomannans, indicated by lower contents of SDF mannosyl, arabinosyl, and galactosyl residues (**Table 4**) and a nonetheless comparable SDF polysaccharide composition on a molar basis.

As shown in **Table 5**, the SDF galactomannans of BMMD and CMMD were characterized by a higher total Man*p*/4,6-Man*p* ratio than the galactomannans of RMMD, indicating that SDF galactomannans in the analyzed robusta coffee brew were more substituted than galactomannans from comparable arabica coffee brews. Similar trends were demonstrated for high molecular weight fractions from hot water extracts of roasted robusta and arabica coffees (*8*, *9*).

Methylation analysis also revealed different arabinogalactan structures from robusta and arabica coffee SDF. Whereas arabinogalactan structural characteristics from CMMD and BMMD were very similar, arabinogalactans from RMMD were less branched and contained lower amounts of arabinose as indicated by higher galactose/arabinose and higher (3-Gal*p* +

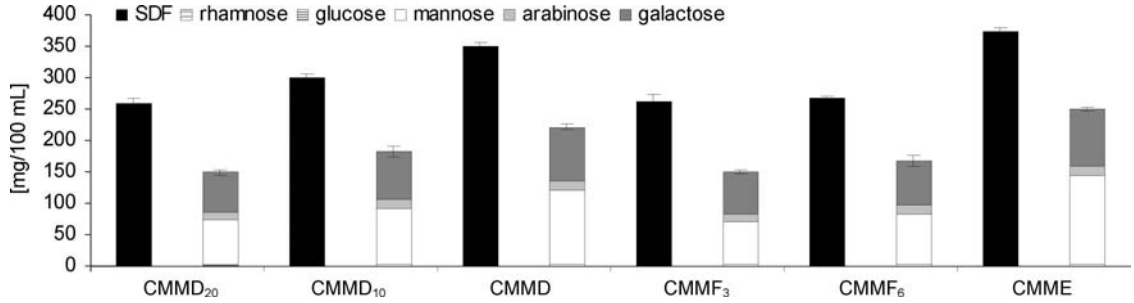

Figure 1. Contents of soluble dietary fiber (SDF) and contents of SDF monosaccharide residues in beverages obtained from medium-roasted, medium-ground Colombian coffee as influenced by brewing procedure: $CMMD_{20}$, large-scale drip brew (20 L); $CMMD_{10}$, large-scale drip brew (10 L); CMMD, drip brew; $CMMF_3$, French press (brewing time = 3 min); $CMMF_6$, French press (brewing time = 6 min); CMME, stovetop espresso; due to low contents, glucose and rhamnose are not distinguishable in this figure.

6-Gal*p*)/3,6-Gal*p* ratios (**Table 5**). In addition, RMMD arabinogalactans were characterized by a higher 3-Gal*p*/6-Gal*p* ratio, showing that the lower branching degree is due not only to a lesser amount of side chains containing arabinose but also to fewer side chains containing 6-Gal*p*. A lower T-Ara*f*/5-Ara*f* ratio of RMMD arabinogalactans compared to CMMD and BMMD indicates fewer single arabinosyl (T-Ara*f*) side chains or fewer side chains containing short arabinan sections.

In summary, arabica and robusta coffee beverages do not necessarily differ in their dietary fiber contents. Green coffee processing also seems to play a role. However, structural characteristics of SDF polysaccharides are probably determined by botanical variety.

Dietary Fiber Contents and Characteristics As Influenced by Roasting. The influence of roasting on the dietary fiber content of coffee brews was studied with drip brews of light-, medium-, and dark-roasted Colombian coffees (CLMD, CMMD, and CDMD, respectively), which contain only SDF but no IDF. As expected, the CLMD beverage was characterized by a significantly lower SDF content than CMMD or CDMD beverages (**Table 3**). However, CMMD and CDMD brews did not differ significantly in their SDF contents or in their SDF polysaccharide contents, indicating that more intense roasting results only to a certain point in higher SDF contents.

The lower yields of SDF polysaccharides observed for CLMD is explained by a lower galactomannan content as demonstrated by less SDF mannose (**Table 4**). With increasing degree of roast more SDF mannose was measured, whereas the arabinose and galactose SDF contents slightly decreased. Methylation analysis confirmed these data as shown in **Table 5**: galactomannan/arabinogalactan ratios increased in the order CLMD, CMMD, and CDMD ($0.9 < 1.3 < 1.5$). More intense roasting also led to increased total Man*p*/4,6-Man*p* ratios ($26.5 < 29.1 < 32.6$). This is in agreement with Redgwell et al. (*7*), who also observed that roasting of arabica coffee beans resulted in a decline in the substitution degree of galactomannans solubilized from cell wall material.

Increasing degree of roast also resulted in increasing galactose/arabinose and T-Ara*f*/5-Ara*f* ratios of SDF arabinogalactans. In addition, SDF arabinogalactans of CDMD were characterized by a higher ratio of (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* compared to CMMD and CLMD, showing that SDF arabinogalactans of the dark-roasted coffee brew were less branched than those of the medium- and light-roasted coffees. Comparable structural changes of arabinogalactans with increasing degree of roast are described by Nunes and Coimbra (*9*).

Dietary Fiber Contents and Characteristics As Influenced by Brewing Procedure. The influence of the brewing procedure on dietary fiber contents of coffee brews was investigated using the medium-roasted, medium-ground Colombian coffee sample (**Figure 1**). As shown in **Table 3**, distinct differences were observed for drip-brewed coffees of different preparation volumes: increasing the preparation scale (1 L of CMMD, 10 L of $CMMD_{10}$, 20 L of $CMMD_{20}$) led to a significant decrease in SDF contents, along with decreasing amounts of nondigestible polysaccharides. As indicated by the SDF mannose, arabinose, and galactose contents (**Table 4**), an increasing preparation scale resulted in both lower galactomannan and lower arabinogalactan yields of the coffee brew. However, the decrease in SDF mannose was more distinct than the decrease in SDF arabinose and galactose, as confirmed by methylation analysis for galactomannans and arabinogalactans (**Table 5**): the galactomannan/arabinogalactan ratio decreased in the order CMMD, $CMMD_{10}$, and $CMMD_{20}$ ($1.3 > 1.1 > 1.0$). Similar to the effects observed for different roasting degrees, decreasing galactomannan contents were accompanied with an increase of the mannan substitution degree, as indicated by decreasing total Man*p*/4,6-Man*p* ratios ($29.1 > 26.8 > 25.1$ for CMMD, $CMMD_{10}$, and $CMMD_{20}$, respectively). This shows that when the large-scale drip brewing machine was used, the decline in polysaccharide extraction is most pronounced for low-substituted mannans. The decrease in SDF extraction yields using the large-capacity coffee urn may be due to exceptionally short brewing times (10 min/10 L and 14 min/20 L) associated with shorter contact times of coffee powder and water.

French press coffees $CMMF_3$ and $CMMF_6$ (brewed for 3 or 6 min, respectively) also contained significantly less SDF and nondigestible polysaccharides compared to the same-scaled drip-brew CMMD (**Table 3**). Possibly, a continuous extraction drip brewing procedure is more effective than extraction in a French press, pouring all water on the coffee powder at once. The lower SDF carbohydrate contents of $CMMF_3$ and $CMMF_6$ compared to CMMD was caused by less extraction of both mannans and arabinogalactans (**Table 4**). However, the lower galactomannan/arabinogalactan ratio of the French press brews (1.1) compared to the drip brew (1.3) (**Table 5**) shows that declined extraction yields were more distinct for galactomannans than for arabinogalactans.

The SDF content of the French press coffee was not significantly enhanced by brewing for 6 min instead of 3 min (**Table 3**, $CMMF_3$ and $CMMF_6$), and similar structural characteristics were observed for the SDF polysaccharides of $CMMF_3$ and $CMMF_6$ (**Table 5**).

French press brews contained substantial amounts of IDF (118 mg/100 mL), probably due to fine particles passing the wire mesh filter. Methylation analysis revealed that IDF polysaccharides were mainly composed of not only galactomannans (69 mol %) but also arabinogalactans (15 mol %). Much higher

molar proportions of 4-Glc*p* were detected in IDF (15 mol %) than in SDF. Although it was shown that low amounts of 4-Glc*p* (1 mol %) are structural elements of roasted coffee galactomannans (*16*), the high molar percentage of 4-Glc*p* indicates the presence of cellulose in French press coffee IDF.

To compare different brewing procedures, the same medium-ground coffee was used. However, French press coffee is also prepared with coarse-ground coffee. IDF contents are probably less pronounced in these preparations.

Extraction in a stovetop espresso maker led to highest SDF and SDF polysaccharide contents (CMME, **Table 3**), because enhancing the temperature and the pressure increases the extaction yields. Next to SDF, minor amounts of IDF (23 mg/100 mL) were analyzed in the stovetop espresso brew, which were not chemically characterized. The significantly higher SDF content of CMME compared to CMMD occurred along with increased galactomannan extraction, whereas similar amounts of arabinogalactans were extracted (**Table 4**), as confirmed by methylation analysis (**Table 5**): SDF polysaccharides of CMME were characterized by a higher galactomannan/arabinogalactan ratio (1.6) than SDF polysaccharides of CMMD (1.3). Again, the enhanced galactomannan extraction was accompanied with a higher total Man*p*/4,6-Man*p* ratio (31.4), showing that more powerful brewing conditions allow for the extraction of additional, less substituted mannans. **Table 5** also shows that SDF arabinogalactans of CMME were characterized by higher ratios of galactose/arabinose, 3-Gal*p*/6-Gal*p* and T-Ara*f*/5-Ara*f*, compared to CMMD, whereas the (3-Gal*p* + 6-Gal*p*)/3,6-Gal*p* ratios were in the same range. This indicates that SDF arabinogalactans of CMME contain side chains which are composed of relatively less 6-Gal*p* and 5-Ara*f* residues. Díaz-Rubio and Saura-Calixto (*3*) also found higher SDF contents in espresso coffee than in filtered coffee. However, they did not use a stovetop espresso maker, but a commercially applied espresso machine. The stovetop espresso coffee CMME, which was analyzed to independently investigate the influence of the brewing procedure, does not reflect common espresso beverages, which are prepared with dark-roasted, fine-ground coffees. CDFE, representing a common stovetop espresso coffee, contained significantly higher amounts of SDF than CMME. However, it has to be kept in mind that stovetop espresso brews may strongly differ, depending on individually and regionally varying coffee/water ratios used for their preparation. Also, the type of espresso machine, especially the pressure and temperature used, influences the extraction yields and most likely SDF contents. Differences are not only expected to be found between stovetop espresso and commericially used espresso machines but also between different commercial espresso machines themselves.

Dietary Fiber Contents and Characteristics As Influenced by Grinding. The influence of the degree of grinding on the dietary fiber content was investigated by preparing drip brews and stovetop espresso brews of medium- and fine-ground coffees (CDMD/CDFD and CDME/CDFE, respectively). With both brewing procedures slightly higher SDF contents were measured in beverages from fine-ground coffee (**Table 3**). These brews also had slightly higher SDF carbohydrate contents. However, for both brewing procedures the observed differences in SDF contents were not statistically significant. Generally, an enhanced surface area as provided by a smaller particle size should improve coffee extraction. However, for the coffees investigated in this study the influence of grinding was not distinctive.

Dietary Fiber Contents and Characteristics of Selected Commercial Products. In this study, the instant espresso product provided the highest amount of SDF along with the highest amount of SDF carbohydrates (**Table 3**, A). However, the high SDF content of A is associated with a high dry matter content (provided that A is prepared as indicated by the amount of servings per package and assuming one cup of espresso is 60 mL). Hence, the SDF content of the instant espresso beverage strongly depends on the amount of espresso powder individually used for one cup. Contrary to the stovetop espresso brews CMME, CDME, and CDFE (each of them containing about 20 mg of IDF/100 mL), no IDF was found in the instant espresso.

The SDF contents of other instant beverages (instant coffee and cappuccino, B and C, respectively) were in the same range as observed for drip brews (**Table 3**) if manufacturers' preparation instructions were followed and assuming that one cup of coffee is 150 mL. However, major differences in polysaccharide characteristics were analyzed (**Table 5**). Because more intense extraction procedures, most importantly higher temperatures, are used in soluble coffee production to allow higher extraction yields, industrial coffee extracts contain additional polysaccharide fractions. For example, SDF polysaccharides of B and C showed a much lower galactomannan/arabinogalactan ratio, compared to all other coffee brews. The SDF polysaccharides of A also showed a lower galactomannan/arabinogalactan ratio than the stovetop espresso brew CDFE. In addition, SDF arabinogalactans from all three instant products were characterized by higher galactose/arabinose ratios (5.6–8.3) and (3-Gal*p* + 6-Gal*p*)/3,6-Galp ratios (2.7–3.5), indicating less branched arabinogalactans.

The coffee-pad beverage showed a low SDF content (**Table 3**, D). Even though the drip brew of the same coffee contained more SDF (0.27 ± 0.02 g/100 mL), the difference was not statistically significant. Therefore, these results do not allow any conclusion about the specific influence of the preparation in a coffee-pad machine.

An unexpected observation was made analyzing the drip brew of a commercially available decaffeinated Colombian coffee: it contained significantly less SDF (**Table 3**, E) than drip brews of non-decaffeinated Colombian coffee samples, whereas no major differences in the structural characteristics of SDF carbohydrates were observed (**Table 5**). Repeating this experiment with another commercially available decaffeinated Colombian coffee showed that the SDF content of the resulting drip brew (198 mg/100 mL) was also significantly lower, indicating that the decaffeination process may influence the polysaccharide extraction. However, further investigations analyzing defined decaffeinated and non-decaffeinated coffees from the same green coffee sample and studying the effect of different decaffeination procedures are required.

ABBREVIATIONS USED

One-letter abbreviations for commercial coffee samples: A, instant espresso; B, instant coffee; C, instant cappuccino; D, coffee pads; E, decaffeinated coffee. Four-letter abbreviations for coffee brews: first letter = type of coffee (R, robusta; B, Brazil; C, Colombia) second letter = roasting (L, light; M, medium; D, dark); third letter = grinding (M, medium; F, fine); fourth letter = preparation procedure (D, drip brew; D_{10}/D_{20}, large-scale drip brew, 10 or 20 L, respectively, F_3/F_6, French press, brewing time of 3 or 6 min, respectively). 5-Ara*f*, (1→5)-linked arabinofuranosyl residues; T-Ara*f*, terminally linked arabinofuranosyl residues; 3-Gal*p* (1→3)-linked galactopyranosyl residues; 6-Gal*p*, (1→6)-linked galactopyranosyl residues; 3,6-Gal*p*, (1→3,6)-linked galactopyranoysol residues; T-Gal*p*, terminally linked galactopyranosyl residues; IDF, insoluble dietary fiber; 4-Man*p*, (1→4)-linked mannopyranosyl residues; 4,6-Man*p*, (1→4,6)-linked mannopyranosyl residues; T-Man*p*,

terminally linked mannopyranosyl residues; MES, 2-(*N*-morpholino)ethanesulfonic acid; SDF, soluble dietary fiber, TRIS, tris(hydroxymethyl)aminomethane.

LITERATURE CITED

(1) DeVries, J. W. The definition of dietary fiber. *Cereal Foods World* **2001**, *46*, 112–126.

(2) Gniechwitz, D.; Reichardt, N.; Blaut, M.; Steinhart, H.; Bunzel, M. High molecular weight components from coffee beverages - degradation by human intestinal bacteria. In *Proceedings of the 21st International Conference on Coffee Science (Montpellier)*; Association for Science and Information on Coffee: Paris, France, 2006; pp 73–79.

(3) Díaz-Rubio, M. E.; Saura-Calixto, F. Dietary fiber in brewed coffee. *J. Agric. Food Chem.* **2007**, *55*, 1999–2003.

(4) Gniechwitz, D.; Reichardt, N.; Blaut, M.; Steinhart, H.; Bunzel, M. Dietary fiber from coffee beverage − degradation by human fecal microbiota. *J. Agric. Food Chem.* **2007**, *55*, 6989–6996.

(5) Ames, J. M.; Wynne, A.; Hofmann, A.; Plos, S.; Gibson, G. R. The effect of a model melanoidin mixture on faecal bacterial populations in vitro. *Br. J. Nutr.* **1999**, *82*, 489–495.

(6) Dell'Aquila, C.; Ames, J. M.; Gibson, G. R.; Wynne, A. G. Fermentation of heated gluten systems by gut microflora. *Eur. Food Res. Technol.* **2003**, *217*, 382–386.

(7) Redgwell, R. J.; Trovato, V.; Curti, D.; Fischer, M. Effect of roasting on degradation and structural features of polysaccharides in arabica coffee beans. *Carbohydr. Res.* **2002**, *337*, 421–431.

(8) Nunes, F. M.; Coimbra, M. A. Chemical characterization of the high-molecular-weight material extracted with hot water from green and roasted robusta coffees as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 7046–7052.

(9) Nunes, F. M.; Coimbra, M. A. Chemical characterization of galactomannans and arabinogalactans from two arabica coffee infusions as affected by the degree of roast. *J. Agric. Food Chem.* **2002**, *50*, 1429–1434.

(10) Lee, S. C.; Prosky, L.; deVries, J. W. Determination of total, soluble, and insoluble dietary fiber in foods − enzymatic−gravimetric method, MES-TRIS buffer: collaborative study. *J. AOAC Int.* **1992**, *75*, 395–416.

(11) Willis, R. B.; Montgomery, M. E.; Allen, P. R. Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. *J. Agric. Food Chem.* **1996**, *44*, 1804–1807.

(12) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.

(13) Englyst, H. N.; Quigley, M. E.; Geoffrey, J. H. Determination of dietary fibre as non-starch polysaccharides with gas−liquid chromatographic, high performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* **1994**, *119*, 1497–1509.

(14) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.

(15) Sweet, D. P.; Shapiro, R. H.; Albersheim, P. Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.* **1975**, *40*, 217–225.

(16) Nunes, F. M.; Domingues, M. R.; Coimbra, M. A. Arabinosyl and glucosyl residues as structural features of acetylated galactomannans from green and roasted coffee infusions. *Carbohydr. Res.* **2005**, *340*, 1689–1698.

(17) Prosky, L.; Asp, N. G.; Furda, I.; deVries, J. W.; Schweizer, T. F.; Harland, B. F. Determination of total dietary fiber in foods and food products: collaborative study. *J. AOAC Int.* **1985**, *68*, 677–679.

(18) Bekedam, E. K.; Schols, H. A.; van Boekel, M. A. J. S.; Smit, G. High molecular weight melanoidins from coffee brew. *J. Agric. Food Chem.* **2006**, *54*, 7658–7666.

(19) Nunes, F. M.; Reis, A.; Domingues, M. R. M.; Coimbra, M. A. Characterization of galactomannan derivatives in roasted coffee beverages. *J. Agric. Food Chem.* **2006**, *54*, 3428–3439.

(20) Bekedam, E. K.; De Laat, M. P. F. C.; Schols, H. A.; Van Boekel, M. A. J. S.; Smit, G. Arabinogalactan proteins are incorporated in negatively charged coffee brew melanoidins. *J. Agric. Food Chem.* **2007**, *55*, 761–768.

(21) Finot, P. A.; Magnenat, E. Metabolic transit of early and advanced Maillard products. In *Progress in Food and Nutrition Science*; Erikkson, C., Ed.; Pergamon Press: Oxford, U.K., 1981; pp 193–207.

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