FRONTIERS IN CARBOHYDRATE RESEARCH-1

FOOD APPLICATIONS

Edited by
Rick P. Millane
James N. BeMiller
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ELSEVIER APPLIED SCIENCE

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Rick P. Millane

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Rengaswami Chandrasekaran

Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, Indiana, USA

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Preface

In September 1988, the Whistler Center for Carbohydrate Research at Purdue University held the first of what is to be a continuing series of conferences on Frontiers in Carbohydrate Research. Developments in carbohydrate research as related to the food industry was the focus of this conference.

Research creates knowledge; knowledge brings about understanding; understanding creates choices; and choices create opportunities. A problem facing the scientific and technological community in this time of information explosion is in keeping abreast of developments and in transmitting that information in the proper form to those who must make decisions. The Frontiers in Carbohydrate Research conferences are designed to be a conduit of information on the chemical, physical, and biological properties of carbohydrates that are generally important in their practical applications.

At this time, there is a lively industrial interest in carbohydrates. The papers selected for presentation at the 1988 Frontiers in Carbohydrate Research conference are illustrative of developments in searches for new, useful gums; state-of-the-art techniques used for characterization of chemical and molecular structures and physical properties of polysaccharides; and chemical and physicochemical properties of carbohydrates.

This volume is a collection of those papers, save one. It contains descriptions of new plant and bacterial polysaccharides with potential usefulness in the food and other industries, descriptions of the conformations of polysaccharide molecules in solution and gels, descriptions of how various physical techniques are used to characterize polysaccharides, examples of how the principles and concepts of polymer science can be used to interpret those data, results of a study to determine the effects of sterilizing doses of gamma-irradiation on starch, a description of how cereal plants are protected against lethal ice crystal growth by certain of their polysaccharides, the latest information on nonenzymic browning, a discussion of the theory and design of sweeteners, issues involved in dietary fiber analysis, applications of acoustic-augmented drying, and suggestions of potential applications of cyclodextrins.

Thanked especially are the contributors to the conference, Rick P. Millane of the faculty of the Whistler Center who copy-edited this volume and made it ready for publication, Deborah D. Zerth and Terry L. Brown who typed the manuscripts, and Mark O'Neil for reproducing the figures.

JAMES N. BEMILLER
West Lafayette, Indiana, USA

List of Contributors

S. Arnott, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
N. Ayya, Monell Chemical Senses Institute, Philadelphia, Pennsylvania 19104, USA
J. N. BeMiller, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
D. A. Brant, Department of Chemistry, University of California, Irvine, California 92717, USA
R. Chandrasekaran, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
J. R. Daniel, Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907, USA
M. S. Feather, Department of Biochemistry, University of Missouri, Columbia, Missouri 65211, USA
R. B. Friedman, American Maize-Products Company, Hammond, Indiana 46320-1094, USA
I. Furda, General Mills, Inc., James Ford Bell Technical Center, Minneapolis, Minnesota 55427, USA
A. K. Gupta, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
A. R. Hedges, American Maize-Products Company, Hammond, Indiana 46320-1094, USA
P. K. Kindel, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, USA
H. Levine, Nabisco Brands, Inc., Fundamental Science Group, PO 1943, East Hanover, New Jersey 07936-1943, USA
S.-Y. Liao, Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824, USA
J. S. Marks, Department of Food Science, Purdue University, West Lafayette, Indiana 47907, USA
R. P. Millane, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
E. R. Morris, Department of Food Research and Technology, Cranfield Institute of Technology, Silsoe College, Silsoe, Bedford MK45 4DT, UK
E. U. Nzewi, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA
C. R. Olien, USDA, Agricultural Research Service, Department of Crop and Soil Sciences, Michigan State University, East Lansing, Michigan 48824, USA
P. Rayas-Duarte, Department of Food Science, Purdue University, West Lafayette, Indiana 47907, USA
J. H. Rupnow, Department of Food Science, University of Nebraska—Lincoln, Lincoln, Nebraska 68583, USA
S. J. Schmidt, Division of Foods and Nutrition, University of Illinois, Urbana, Illinois 61801, USA
L. Slade, Nabisco Brands, Inc., Fundamental Science Group, PO 1943, East Hanover, New Jersey 07936-1943, USA

T. A. Talashek, Kelco Division of Merck & Co., 8225 Aero Drive, San Diego, California 92123, USA

D. S. Wickramasingha, The Whistler Center for Carbohydrate Research, Purdue University, West Lafayette, Indiana 47907, USA

R. L. Whistler, Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907, USA

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STRUCTURE AND PROPERTIES OF THE WATER-EXTRACTABLE POLYSACCHARIDE OF MARIGOLD (*Tagetes erecta*) PETALS

JAMES N. BEMILLER, ASHOK K. GUPTA, and DHARSHINI S. WICKRAMASINGHA
The Whistler Center for Carbohydrate Research
Purdue University
West Lafayette, Indiana 47907 USA

ABSTRACT

Water extraction of the meal remaining after the commercial extraction of pigment from marigold (*Tagetes erecta*) blossoms yields a low-viscosity protein-polysaccharide that has emulsifying and emulsion-stabilizing properties similar to those of gum arabic. Pretreatment of the meal with an oxidant is necessary to obtain a preparation with minimal color. Light oxidation also increases the viscosity of the preparation. Structural analysis of the polysaccharide portion of purified protein-polysaccharide reveals a highly branched, acidic arabinoglucogalactan consisting of a heavily branched galactan backbone that is mainly (1→4) linked. Branches are largely at O-6. All arabinose occurs as arabinofuranosyl nonreducing end-units.

INTRODUCTION

There is a desire of food processors to have an alternative for gum arabic, both because it has unique and important properties and because its supply has been variable and uncertain.

The unique and important applications of gum arabic include such things as its use as a glaze on candy products, but especially its use in the preparation of spray-dried, free-flowing citrus oil powders (fixed flavors) and baker's citrus oil emulsions. The crucial, unique properties of gum arabic are its ability to form high-solids, low-viscosity solutions, its emulsifying and protective colloid properties, its ability to withstand relatively high temperatures during spray-drying, and its adhesive and film-forming properties.

Gum arabic is classified commercially as an exudate gum [1,2]; chemically it is a type-II arabinogalactan [3]. It is the natural exudate of various species of *Acacia* trees. Most of the world's supply comes from three areas in the Sahelian region of Africa. About 75% is produced by the Republic of Sudan; most of the remainder comes from Senegal, Mauritania, and Nigeria. It is produced by trees which take at least six years to establish. Because these *Acacia* trees grow in a belt just below the Sahara desert, and because the exudate masses are picked by hand, its supply is subject to climatic, economic, and political conditions in the growing region. Annual imports into the U.S. have varied between 6 and 32 million lbs (3-15 X 10^6 kg), the amount depending upon the world supply, with the average being about 25 million lbs (11 X 10^6 kg) per year. *Acacia* plantations have been planted and biotechnological techniques have been applied to improvement of gum-yielding species. Nevertheless, a dependable supply of a high-quality alternative is desired.

Two sources of a replacement are obvious: (a) another natural gum with similar properties and (b) a polysaccharide derivative with similar properties. Several modified starches, designed to be "emulsifying gums," are available. (Cognizance must be taken of the fact that a particular product may be an acceptable substitute for one specific application without being a general replacement.)

The goal of our research is to find or make a gum that is more than an acceptable substitute. To accomplish this goal, other gums with similar properties must be identified and their structures determined. The objective is to both find a polysaccharide(s) that can be used as gum arabic is and to establish the structural features related to its functional properties that will indicate other potential sources and required modifications.

To accomplish this objective, we decided to look in tissues in which polysaccharides seemed to have the ability to protect fat-soluble substances. The first tissue chosen was that of marigold (*Tagetes erecta*) flower petals. This is a progress report of our work to date with the water-extractable polysaccharide of marigold petals.

Marigolds are grown in various locations in the Americas (largely in Mexico) for the purpose of extracting xanthophyll from the blossoms for use as an additive to poultry feed and as a yellow food coloring. Annual cultivation varies, but current pigment demand requires approximately

200,000 acres (80,000 hectares). Blossoms are picked both by hand and by machine. In the preparation of the pigment, fresh blossoms are heated, then pressed. The resulting cake is dried, pelletized, and extracted with hexane. Annual production of the remaining dry (92% solids) meal, which is sold as feed, is approximately 40,000 tons.

PROCEDURES, RESULTS, AND DISCUSSION

Initially, in our investigation, it was determined that marigold flower polysaccharide (MFP) could be extracted from the solvent-extracted meal with warm (50-55°) water in a 9.5% yield (Figure 1). Neither extraction with alkali nor delignification increased the yield. The tan powder obtained after precipitation gave brown solutions. Methods were sought to reduce the color; successful ones will be described later.

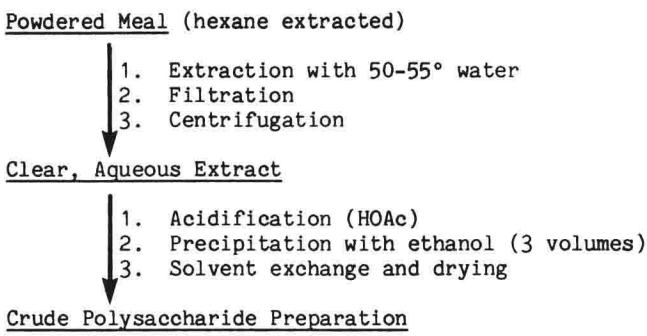

Figure 1. Isolation of marigold flower polysaccharide.

Two preparations of greatly reduced color have been tested for their ability to stabilize emulsions. Limonene was added to a polysaccharide solution in a ratio of 1:10 (v/v). This mixture was sonicated for 180 sec at power output of ~62 watts to form an emulsion [4]. Aliquots were then taken hourly and added to 1000 volumes of water. Turbidity was measured at 500 nm [5]. The results demonstrate that MFP is almost as good as gum arabic in stabilizing citrus oil emulsions (Figure 2) and much better than larch arabinogalactan (Figure 2b), a potential industrial gum once proposed as a substitute for gum arabic in the preparation of flavor oil-in-water emulsions [6].

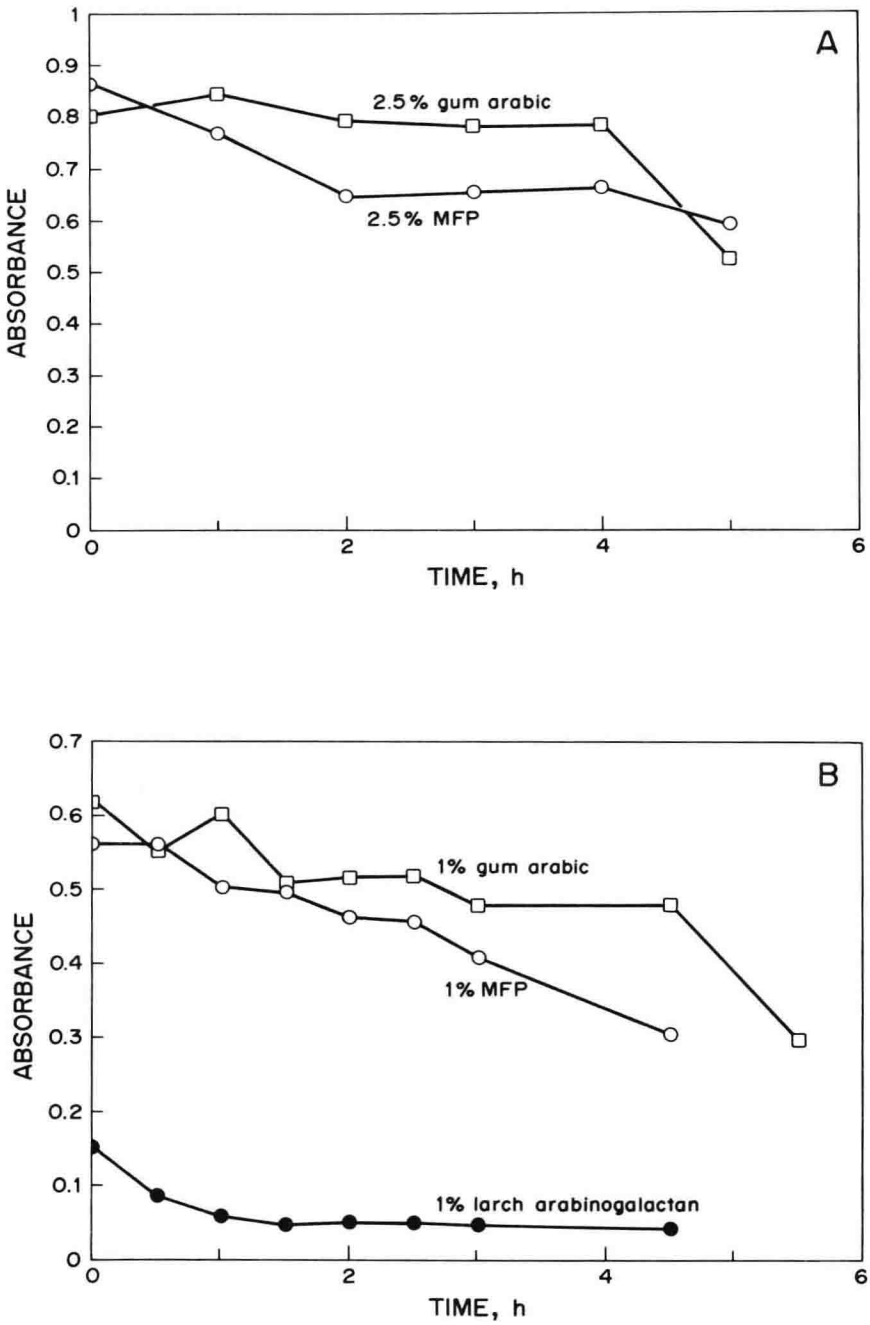

Figure 2. Stability of limonene-in-water emulsions (see text).

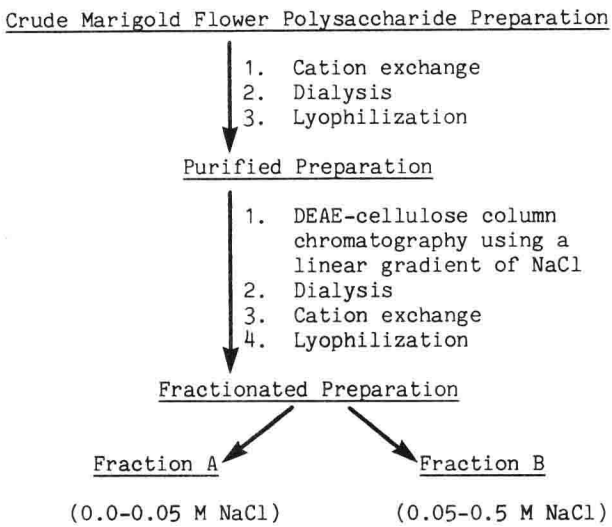

Figure 3. Purification and fractionation of marigold flower polysaccharide preparation.

The polysaccharide extracted from untreated meal with 50-55° water was purified by treatment with Amberlite IR-120[H^+] cation-exchange resin to remove cations, including proteins, and dialyzed (Figure 3). The polypeptide content of the purified product was 6.08 ± 0.17% (N X 6.25). Presumably, therefore, it is polysaccharide-protein [7-9]. For fractionation, a solution of purified polysaccharide (pH 2.7, indicating a rather acidic polysaccharide) was added to a column of DEAE-cellulose, which was then eluted with linear gradients of sodium chloride. One fraction (fraction A) eluting with a 0.0-0.05 M NaCl gradient and two fractions (B1 and B2) eluting with a 0.05-0.5 M NaCl gradient were obtained (Figure 4). The middle part of fraction A was used for structural analysis. Rechromatography of this material produced a single, sharp, symmetrical peak.

A portion of fraction A was reduced to convert the uronic acid(s) into neutral sugar(s) [11]. Acid-catalyzed hydrolysis (2 M trifluoroacetic acid, 100°, 5 h) and g.l.c. analysis of the neutral monosaccharides as alditol acetates [12] gave the following ratios of constituent sugars -- galactose:glucose:arabinose (mole %) : native polysaccharide 60:28:12; carboxyl-reduced polysaccharide 67:21:12.

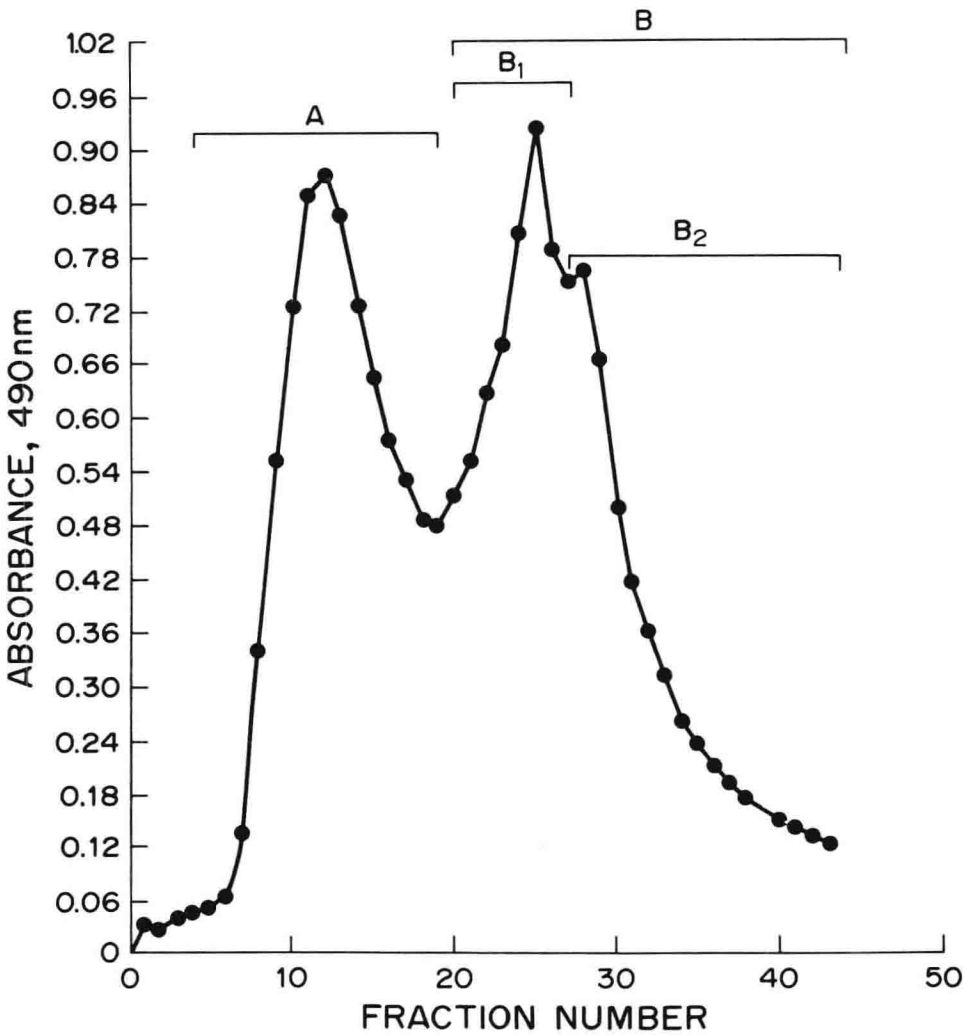

Figure 4. Fractionation of MFP on DEAE-cellulose: A = 0.0-0.05 M NaCl linear gradient; B = 0.05-0.5 M NaCl linear gradient. Carbohydrate content of each fraction was determined using the phenol-sulfuric acid reaction [10].

(Alditol acetates identified by retention times. Mole percentages calculated from peak areas with corrections for differences in molecular weights.) This data indicates that (a) the polysaccharide is an acidic arabinoglucogalactan and (b) the uronic acid is galacturonic acid. Paper chromatography also indicated galacturonic acid. A colorimetric analysis [13] indicated that the uronic acid content is 3.75%.

Arabinogalactan-proteins are common in the plant kingdom [7,8], but MFP is unusual in terms of known polysaccharides in containing glucose as a major component along with galactose and arabinose.

As with other polysaccharides of this type, MFP proved to be difficult to methylate completely. Eventually, complete methylation of the carboxyl-reduced polysaccharide (Fraction A) was realized by classic Haworth methylations (thrice), modified Haworth methylations (DMSO + NaOH + dimethyl sulfate, twice) [14], modified Purdie methylations (DMF + MeI + Ag_2O, thrice) [15] and a Hakamori methylation (DMSO + NaH + MeI, once) [16]. Hydrolysis, reduction, acetylation, and g.l.c./m.s. analysis of the partially methylated alditol acetates [17] gave the following results:

	Mole %	Substitutions
Unbranched chain units[a]		
2,3,6-Me_3-Gal	49.0	0-4
2,4,6-Me_3-Gal	5.8	0-3
2,4,6-Me_3-Glc	14.6	0-3
Branch points[a]		
2,3-Me_2-Gal	6.6	0-4, 0-6
3,4-Me_2-Gal	2.7	0-2, 0-6
2-Me-Gal	2.4	0-3, 0-4, 0-6
2,3-Me_2-Glc	2.7	0-4, 0-6
Non-reducing ends[a]		
2,3,5-Me_3-Ara	12.1	-
2,3,4,6-Me_4-Gal	3.8	-
2,3,4,6-Me_4-Glc	0.2	-

[a]As identified by retention time and mass spectra. Mole percentages calculated from peak areas.

The ratio of unbranched chain units to branches is ~4.6:1. These results indicate a highly branched polymer with a statistical rather than a repeating unit structure. Like the arabinogalactans, MFP seems to consist of a branched galactan framework. Like the arabinogalactans, the structure appears to be complex and heavily branched at 0-6 of the

galactosyl (and glucosyl) units. However, unlike the arabinogalactans, the backbone galactosyl units in MFP appear to be mainly (1→4) linked rather than (1→3) linked. All the arabinose occurs in the furanose ring form and as nonreducing end-units. The position of the galacturonic acid is unknown, but it may well also occur as non-reducing terminii (reflected in the reduced polymer as 2,3,4,6-tetra-*o*-methylgalactose).

Attempts to fractionate MFP preparations both with DEAE-cellulose (described earlier) and with Sephadex G-75 and G-100 (not described) indicated, not only that the preparations contained a spectrum of related molecules, but also that the color component was attached covalently to the polysaccharide. To reduce the color, several procedures were tried. Evidence was obtained that treatment of the meal with an oxidant before extraction gave products with less color than did bleaching of isolated polysaccharide or concurrent extraction and bleaching. Aqueous solutions could not be used for meal pretreatments because of the extractibility of the polysaccharide with water. It was determined that reducing agents had little, if any, effect on color. Of the pretreatments of the marigold flower meal with oxidizing agents, four are described here. All employed 70% ethanol to prevent solubilization of MFP. All resulted in increased yield of polysaccharide. In each case the polysaccharide was extracted and purified as described previously.

Pretreatment	Conditions	Yield	Solution color
1	NaOCl, 70% EtOH, HOAc to pH 5.0, r.t., 3 h	15%	brown
2	NaOCl, 70% EtOH, r.t., 3 h	16%	light brown
3	$NaClO_2$, HOAc, 70% EtOH, r.t., 2 h	11%	light yellow
4	$NaClO_2$, HOAc, 70% EtOH, reflux temp., 2 h	13%	very light yellow

Pretreatment 1 produced the least acceptable product in terms of color. It also was of higher viscosity (Figure 5b) than a preparation made without a pretreatment (Figure 5a), indicating that, perhaps, a cross-linking had taken place. Saponification of the native

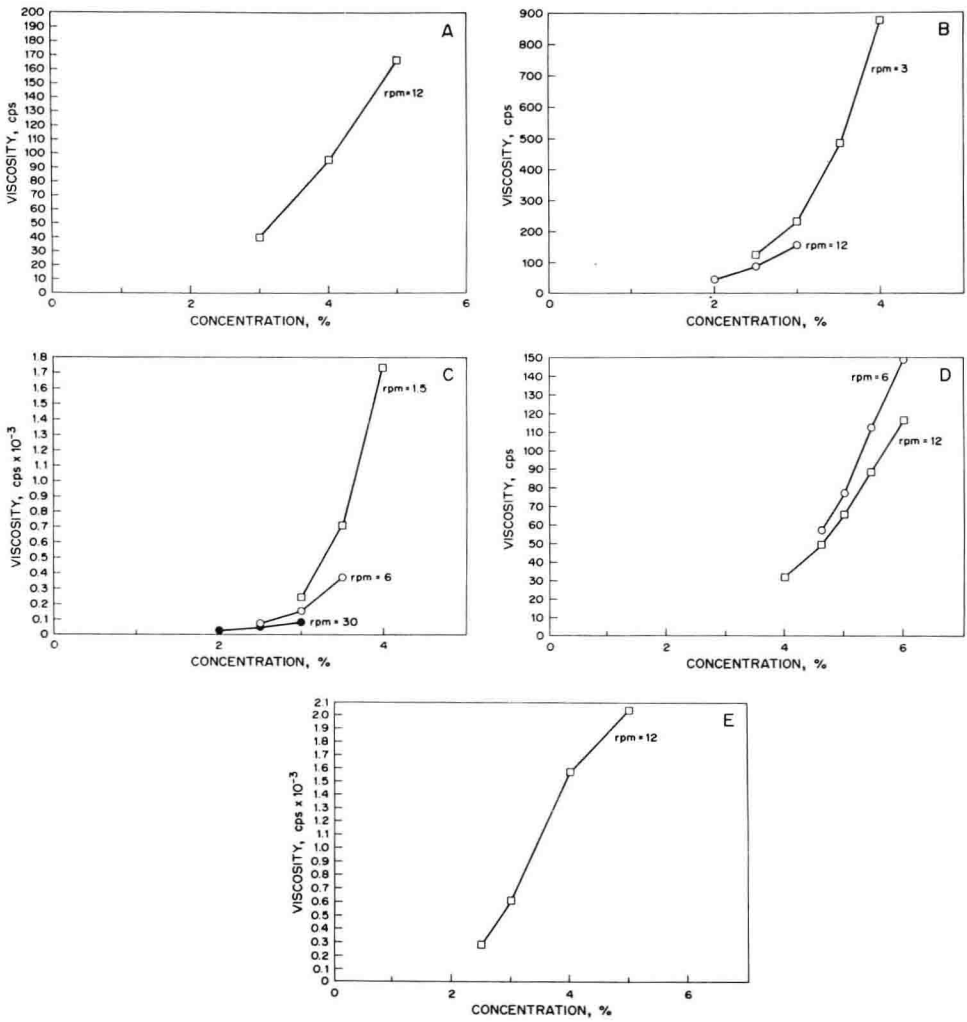

Figure 5. Viscosity as a function of MFP concentration: A = no pretreatment; B,C,D,E = pretreatments 1,2,3, and 4, respectively.