lations from Figure 1 show that 30% of the hydrocarbons are unsaturated, and the composition of the unsaturated portion is 24% $C_{31}$, 73% $C_{33}$ and 3% $C_{35}$. Streibel et al. (1) have reported 32% unsaturated hydrocarbons in Czechoslovakian beeswax with major components 2% $C_{29}$, 26% $C_{31}$, 60% $C_{33}$ and 2% $C_{35}$.

The composition of free acid methyl esters, calculated from Figure 1, is similar to that found earlier (2). In particular, as can be seen easily, the amounts of $C_{16}$ and $C_{18}$ acids are very small. Analysis using a large sample showed that the small peak between $C_{34}$ methyl ester and $C_{40}$ monoester has two partly resolved components, $C_{36}$ methyl ester and $C_{38}$ monoester in the approximate ratio 1:2. During column separation (2), the free acids are spread over a number of fractions so that some could be lost or the shortest chain acids could be retained by the column; the present method avoids these difficulties. Addition of stearic acid to beeswax would be very easily detected and estimated, since extremely little is present naturally.

The composition of the monoesters agreed very closely with that found before (2). Hydroxy esters, diesters and acid monoesters (as methyl esters) did not seem to interfere but may cause the rise in baseline. These esters of secondary alcohols give a much poorer response than esters of primary alcohols (2).

By adding a mixture of three internal standards, eicosane, methyl eicosanoate and octadecyl eicosanoate and comparing their areas with the total areas of hydrocarbons, methyl esters and monoesters, respectively, the percentages of these three groups of components in beeswax were calculated. The results agreed well with those found earlier (2) (in parentheses): hydrocarbons, 13.2% (14.0%); free acids, 13.2% (11.9%); and monoesters, 37.5% (34.7%). It should be noted that, though an acid value can be calculated from the free acid composition, it is lower than that found (16.9 compared to 19.0) because of the presence of acid mono- and polyesters (2).

Thus the amounts in the wax and the composition of the three major groups of components, ca. 65% of whole beeswax, can be obtained from one GLC analysis. The method should be suitable for comparing the compositions of a large number of waxes of different origin and for detecting and estimating adulterants.

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# Laundry Soil-Suspending Activity of Certain Microbial Polysaccharides[1]

## ABSTRACT

Certain extracellular microbial anionic heteropolysaccharides and phosphorylated mannans suspend laundry soil comparably with carboxymethyl cellulose. These biodegradable biopolymers have other properties compatible with components commonly used in detergent formulations.

Efficient laundering of cotton fabrics by use of synthetic detergents requires additives that hold the soil in suspension or prevent its redeposition, or both (1). Sodium carboxymethyl cellulose (CMC) acts in both ways: it is the standard for comparison of soil-suspending activity and is the most widely used antiredeposition agent (1). Other hydrophilic colloids that serve one of these functions, but usually not both, have been cellulosic derivatives or synthetic polymers (1). These, like CMC, are nonbiodegradable.

We have found that certain microbial anionic heteropolysaccharides and phosphorylated mannans compare favorably with CMC in tests for soil-suspending activity both in the absence and the presence of an anionic surfactant, using a water-based substrate, Aquablak B. These biopolymers, the extracellular occurrence of which permits production on an industrial scale (2), are biodegradable and compatible with most commonly used components of detergent formulations.

The microbial polysaccharides tested are identified in Table I. They are products of pilot plant-scale preparations or are derived from these by further treatment in the laboratory and have a 98% or higher purity. Samples tested are from different preparations of the respective strains. Three samples of commercial CMC served as controls; they were designed for soap and detergent formulations and were used without correction for possible inert material. Each value shown is the average of six determinations.

Tests for soil-suspending power were made by a modified procedure based on the methods of Bayley and Weatherburn (6) and of Weatherburn et al. (7). The soil-suspending agent, 2.5 g, was dissolved in 1 liter of water of 300 ppm hardness using a high-sheer Brookfield counter rotating stirrer. Aquablak B, 0.15 g, weighed on a 2 in. x 2 in. sheet of Saran Wrap, was added to the contents of the 1 liter stainless steel beaker in a Terg-O-Tometer and stirred in the Terg-O-Tometer until the carbon was removed. Four unsoiled cotton swatches 2.5 in. x 3.25 in. (Test Fabrics, Inc.) were added, and the suspension was stirred (110 cpm) for 20 min at 60 C. The swatches were withdrawn, rinsed in tap water and ironed dry. Reflectance was measured with a Photovolt meter relative to MgO as 100, and the per cent soil-suspending power was calculated from the average reflectance of the swatches before and after testing, as follows:

$$\% SP = \frac{R_{after} - R_{blank}}{R_{unsoiled} - R_{blank}} \times 100$$

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[2]Respective laboratories of the Northern and Eastern Marketing and Nutrition Research Divisions, ARS, USDA.

[3]Deceased.

TABLE I

Soil-Suspending Power of Microbial Polysaccharides

| Suspending agent and key references | Soil-suspending power, % Sample no. 1 | 2 | 3 | Composition of suspending agents[a,b] |
|---|---|---|---|---|
| CMC (standard) | 91 | 89 | 82 | DS: 0.65-0.85 |
| Microbial products[c] | | | | |
| Phosphomannans (3) | | | | Mannose-P ratio[d] |
| Y-1842 (4) | 98 | — | — | 2.5 |
| Y-2154 | 101 | 97 | 84 | 5.2 |
| Y-2448 (2,5) | 92 | 90 | 85 | 5.7 |
| Y-2579 | 85 | — | — | 12.7 |
| Y-411 | 78 | — | — | 26.3 |
| Phosphomonoesters, from phosphomannans[e] | | | | |
| Y-2154 | 80 | — | — | 4.3 |
| Y-2448 | 85 | — | — | 4.2 |
| Anionic heteropolysaccharides (2) | | | | Components |
| Y-1401 native | 92 | 84 | 81 | M, X, GA (18.5%), O-acetyl (7%) |
| Y-1401 deacetylated | 92 | 88 | — | |
| B-1459 native | 47 | — | — | G, M, GA (18%), PA (3%), O-acetyl (4.5%) |
| B-1459 deacetylated | 81 | 72 | — | |
| B-1973 native | 69 | — | — | G, Gal, MA (28.5%), O-acetyl (25%) |
| B-1973 deacetylated | 67 | — | — | |

[a]Anionic groups of the microbial-suspending agents are present as neutral potassium salts. These groups are: diester orthophosphate of phosphomannans; monoester orthophosphate of phosphomonoesters; and uronic and pyruvic acids of the heteropolysaccharides.

[b]Abbreviations: CMC, sodium carboxymethyl cellulose; DS, degree of substitution; G, D-glucose; Gal, D-galactose; M, D-mannose; X, D-xylose; GA, D-glucuronic acid; MA, D-mannuronic acid; PA, pyruvic acid ketal; and P, phosphorus.

[c]Phosphomannans and anionic heteropolysaccharides are designated by the NRRL strain number of the yeast (Y-) or bacteria (B-) producing them. All strains were provided by the ARS Culture Collection at the Northern Regional Research Laboratory.

[d]Phosphomannans are composed exclusively of D-mannose units and monopotassium orthophosphate in diester linkage.

[e]Phosphomonoesters are obtained by autohydrolytic depolymerization of decationized phosphomannans to their major building units, oligosaccharide monophosphates having degree of polymerization 4-6 (3,4).

Values were reproducible to about ±3%.

The test for soil-suspending properties in the presence of a detergent (Table II) is based on the method of Weatherburn and Bayley (8). Four unsoiled cotton swatches were placed in 1 liter of dispersion containing 0.10 g of Aquablak B (0.01%), 2.0 g (0.2%) of sodium dodecylbenzenesulfonate and 0.02 g (0.002%) of soil-suspending agent in water of 300 ppm hardness. The mixture was stirred in the Terg-O-Tometer as before. The results are expressed as average reflectance of the swatches relative to MgO as 100.

The soil-suspending activity of phosphomannans having a mannose-P ratio of ca. 5.7, or less, equaled or exceeded that for CMC controls, but activity diminished as this ratio increased (Table I). Thus activity appeared to be related directly to the degree of phosphorylation or the charge density. The three phosphomannan Y-2448 samples were vacuum drum-dried from solutions having pH values 6,9, 6.7 and 4.6, respectively (5). As is shown by the results for the phosphomonoesters, quite good soil-suspending activity persisted even after the macromolecular size of the phosphomannans had been eliminated and the diester orthophosphate structure had been changed to the monoester

TABLE II

Improvement of Soil-Suspending Activity of a Detergent

| *Soil-suspending agent* | Average reflectance *System A*[a] | *System B*[b] | Improvement of detergent, %[c] |
|---|---|---|---|
| Blank (hard water) | 87 | | |
| Sodium dodecylbenzenesulfonate, 0.2% | | 64.4 | |
| Carboxymethyl cellulose[d] | 22.0 | 73.1 | 13.5 |
| Phosphomannan Y-2448 | 26.1 | 71.0 | 10.2 |
| Phosphomannan Y-2154 | 27.6 | 72.4 | 12.4 |
| Polysaccharide Y-1401 | 28.7 | 71.6 | 11.2 |

[a]System A: S-S agent, 0.002%; standard soil, 0.01%.

[b]System B: system A + sodium dodecylbenzene sulfonate, 0.2%.

[c]Calculated from $\frac{R_B - 64.4}{64.4} \times 100$.

[d]Degree of substitution: 0.5.

form by autohydrolysis. These results also suggest that the charge is an important factor in the activity. Specific structural or spatial effects appear to be involved, also, as evidenced by: (a) the similarity in activity between phosphomannans Y-1842 and Y-2154, even though the mannose-P ratios differ by 50%; and (b) the great difference in activity between phosphomannan Y-2579 and a standard starch phosphate (43% soil-suspending power) which had a similar hexose-P ratio (glucose-P, 10.5; 1.76% P).

The anionic heteropolysaccharide Y-1401, both native and deacetylated, showed activity comparable with that of the CMC controls. Both native and deacetylated polysaccharides from strains B-1459 and B-1973 exhibited poor activity.

Even when used at a low concentration of 0.002%, the phosphomannans and polysaccharide Y-1401 maintained good soil-suspending power and significantly improved the detergency of the surfactant sodium dodecylbenzenesulfonate (Table II).

Several other considerations favor possible use in detergent formulations of certain phosphomannans and their phosphomonoesters, as well as the heteropolysaccharide from NRRL Y-1401. These biopolymers are biodegradable and physiologically inert; they do not form micelles; and they are compatible with inorganic salts, mild alkali and heat.

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