

EXAMPLE 2

Deacetylation and Clarification of the Heteropolysaccharide S-60

Clarification of the gum, while not necessarily for all uses, is of value when the gum is used as an agar substitute. Clarification can be accomplished before deacetylation (in the native state) or after deacetylation. Since deacetylation uses hot caustic, and clarification is done hot, the two procedures are easily and conveniently combined. Both deacetylation and clarification can be done with the beer or the dry polymer. For deacetylation, if the beer is used, the pH is adjusted to 10 with KOH, the solution heated to 90° C. for 15 minutes, the pH adjusted to pH 7 with dilute H₂SO₄, then clarified.

The general procedure for both deacetylation and clarification follows:

- A. A 2% solution of beer or gum is heated to 90° C.
- B. The pH is adjusted to 10 with KOH.
- C. The temperature of the beer or solution was maintained at 90°-95° C. for 15 minutes.
- D. The pH is adjusted to 6-8 with dilute HCl or H₂SO₄.
- E. Ten gms/liter of Super Aid were added to the material to be filtered.
- F. The material was filtered through a pressure filter unit (pre-heated) with approximately a 6 mm bed of Super Aid and approximately 20-30 psi, using a filter unit with an area of 136 cm².
- G. The filtrate is precipitated with isopropanol immediately to prevent gelation and the fibers dried at 50° C. for one hour or less.

When no deacetylation is necessary, the above procedure is followed, except that the pH is not raised; rather than holding at 90° C., the solution is immediately filtered, and then recovered.

Clarification is typically done on the potassium form; KCl can be added to a solution of previously made product as necessary.

EXAMPLE 3

Deacetylated, Non-Clarified S-60

S-60 fermentation liquor is heated to 90° C. and the pH adjusted to 10 by addition of 25% KOH. The temperature is maintained for 15 minutes, followed by neutralization with concentrated HCl. This liquor is then drained from the fermentor while hot and recovered with 2 volumes of 99% IPA. The fibers of deacetylated S-60 are collected, dried at 55° C. for one hour in a forced air tray drier, and then milled to a powder.

EXAMPLE 4

Clarification of Deacetylated S-60

Deacetylated heteropolysaccharide S-60 as prepared in Example 3 is reconstituted to a 1% concentration in deionized water using a Lightnin mixer for one hour followed by mixing on an Arti-Barinko with heating to 50° C. The solution is then centrifuged at 10,000 R.P.M. (GSA head) for 20 minutes in a Sorvall RC2-B refrigerated centrifuge keeping the temperature above 40° C. The supernatant is decanted off and sequentially filtered through Gelman type AN Hydrophilic Acropor membranes (293 mm) of porosities of 5 μ , 3 μ , 1.2 μ and 0.8 μ . The filtrate is added to approximately 3-4 volumes of 99% isopropanol, the fibers collected and dried briefly in the forced air tray drier at 55° C., followed by milling

to a powder. The product is the deacetylated, clarified S-60 gum of this invention.

EXAMPLE 5

Heteropolysaccharide S-60 Gelling Characteristics

A compilation of data comparing the native gum and the deacetylated gum, both in the K+ form and in the Ca++ form, with carrageenan and agar follows:

Type	Gel Nature	Belts sets	esis	Myster
Native S-60	Very Plastic	65-70° C.	65-70° C.	None
Deacetylated S-60				
K+ Gel	Brittle	90° C.	31-48° C.	45-60° C.
Ca ²⁺ Gel	Brittle	90° C.	45-50° C.	45-50° C.
Kappa Carrageenan	Brittle	40-85° C.	25-75° C.	15-20° C.
Agar*	Brittle	60-87° C.	32-39° C.	60° C.

*Bacteriological grade specs: gelling temperature range 33°-39° C. melting temperature 70° C. minimum. (Whistler's "Industrial Gums")
Minimum Gelling Concentration
Kappa Carrageenan - 0.38%
Agar - 0.048%
Deacetylated S-60 - 0.051%
(calcium gel)

Note above that there is a wide range of temperatures given for setting and melting of all the various types of gels. For agar, the variations are primarily due to type of seaweed while the kappa carrageenan the potassium ion concentration determines the gel characteristics. The gels of deacetylated S-60 are primarily characterized by the degree of deacetylation. With only a slight deacetylation the gels set at higher temperatures and are more elastic; in fact, a wide range of gel types from very elastic to very brittle is possible; depending on the degree of deacetylation. The gels appear to be more similar to agar than to kappa carrageenan, primarily because of the large hysteresis between setting and melting temperatures. It should be emphasized that they are difficult to melt and the gel-sol transition is difficult to observe. On the other hand, the gelling temperatures can be easily defined since the gels set sharply within a few degrees from incipient gelation to solid gel.

EXAMPLE 6

Agar Replacement Using Deacetylated Clarified S-60

Several different media are prepared as follows:

	Nutrient Agar
(A)	0.8% Nutrient Broth (Difco) 1.5% Agar (Difco)
(B)	0.8% Nutrient Broth (Difco) 0.2% KCl 0.9% S-60
	Trypticase Soy Agar
(A)	2.75% Trypticase Soy Broth (BBL) 1.5% Agar (Difco)
(B)	2.75% Trypticase Soy Broth (BBL) 0.2% KCl 0.9% S-60
	Potato Dextrose Agar
(A)	2.4% Potato Dextrose Broth (Difco) 1.5% Agar
(B)	2.4% Potato Dextrose Broth (Difco) 0.2% KCl 0.9% S-60
	YM Agar
(A)	2.1% YM Broth (Difco) 1.5% Agar (Difco)
(B)	2.1% YM Broth