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Gel

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ABSTRACT

GEL

A method of providing a gel comprising gellan comprises the steps of fermenting a microorganism in a carbohydrate containing medium so as to yield a crude gellan gum fermentation broth and gelling the crude fermentation broth. The method may comprise the further step of deacetylating the gellan.

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COMPLETE SPECIFICATION

STANDARD PATENT

Application Number:

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Invention Title: **GEL**

The following statement is a full description of this invention, including the best method of performing it known to :- us

GEL

The invention relates to the use of crude or whole gum fermentation broths (still containing cells) in the preparation of gels suitable for incorporation into foodstuffs such as pet foods.

It is known to use carbohydrate fermentation products, such as gellan gum, to form gels for incorporation into foodstuffs. Gellan gum is commonly produced by fermentation of a microorganism of the genus *Sphingomonas*, such as *Sphingomonas paucimobilis*, in a medium containing a carbohydrate, such as glucose. The microorganism becomes intimately associated with the gellan gum produced during fermentation and is difficult to separate from it. Conventionally, crude or whole gellan gum fermentation broths are subjected to a number of washing, filtration and purification steps to remove the microorganism to provide substantially pure gellan in gellable form. There is considerable cost associated with carrying out these washing, filtration and purification steps. In addition, during the washing, filtration and purification processes a large proportion of the gellan gum, up to 50%, is lost.

It has now been found that gels suitable for use in foodstuffs may be formed from crude or whole gellan gum fermentation broths without the need for costly washing, filtration and/or purification processing steps. The crude broths contain in addition to gellan, the microorganism, other microorganism growth products and any unconsumed carbohydrate.

If desired, the gels formed from the crude or whole gellan gum fermentation broths may be deacetylated by, for example, pH manipulation.

Gels formed from the crude or whole gellan gum fermentation broths may be used directly in, for example, foodstuffs such as petfoods, either alone or in combination with other gelling agents such as xanthan gum, locust bean gum or cassia gum.

It has also been found that the propensity of gels comprising gellan to exhibit syneresis can be advantageously employed to concentrate the gels. Comminution of gels comprising gellan causes them to dewater and the separated water can then be easily removed by, for example filtration, to give concentrated gels.

By employing the tendency of gels comprising gellan to dewater by syneresis as a means for concentration, the water content of the gels can be easily reduced without the need for expensive and/or time consuming drying techniques. If desired, the resulting concentrated gels can be dried further, using conventional drying techniques such as vacuum drying, to give powders with water contents of less than about 5% by weight, which may be easily stored and transported. Such powders can then be reconstituted to form gels suitable for inclusion in foodstuffs such as petfoods.

According to the invention there is provided a method of providing a gel comprising gellan, comprising the steps of: fermenting a microorganism in a carbohydrate containing medium so as to yield a crude gellan gum fermentation broth; and gelling the crude fermentation broth.

Preferably, the crude gellan gum fermentation broth is gelled by heating.

Preferably, the microorganism is *Sphingomonas paucimobilis*, more preferably *Sphingomonas paucimobilis* strain ATCC 31461, which is commercially available.

Also according to the invention there is provided a gel formed by a method of the invention.

Also according to the invention there is provided a gel comprising gellan and the byproducts of the fermentation which produced the gellan.

Also according to the invention there is provided a foodstuff, such as a pet food, incorporating a gel according to the invention.

Also according to the invention there is provided a method of forming a dried powder from a gel comprising gellan, comprising the steps of: comminuting the gel to dewater it; removing the separated water to obtain a concentrated gel; and drying the concentrated gel to form a powder.

The invention will be further described, by way of example, with reference to the drawings in which;

Figure 1 is a graph of glucose concentration, viscosity, microorganism cell dry weight (CDW) and gellan dry weight (GDW) against fermentation time for the fermentation broth of Example 1;

Figure 2 is a graph of glucose concentration, viscosity, microorganism cell dry weight (CDW) and gellan dry weight (GDW) against fermentation time for the fermentation broth of Example 2;

Figure 3 is a graph of glucose concentration, viscosity, microorganism cell dry weight (CDW) and gellan dry weight

(GDW) against fermentation time for the fermentation broth of Example 3.

Figure 4 is a graph of the relative strength, elasticity and brittleness of gels formed from the crude fermentation broth of Example 1 by the methods of Examples 4 to 8;

Figure 5 is a graph of the relative strength, elasticity and brittleness of gels formed from the crude fermentation broth of Example 2 by the methods of Examples 4 to 8; and

Figure 6 is a graph of the relative strength, elasticity and brittleness of gels formed from the crude fermentation broth of Example 3 by the methods of Examples 4 to 8.

Example 1

A crude fermentation broth is made as follows:

Preliminary freeze-dried cultures of *Sphingomonas paucimobilis* strain ATCC 31461 are activated by the addition of sterile Yeast-Malt broth under sterile conditions and incubated for 30 minutes. Some of the cells from the resulting liquid are inoculated (streaked) onto plates of Yeast-Malt solid medium, which is solidified by addition of commercial Gellan ("Gelrite"). Isolated, pure cultures are then used to inoculate four shake flasks containing 250 ml of a fermentation medium consisting of 30 g/l glucose, 0.5 g/l K_2HPO_4, 0.1 g/l $MgSO_4.7H_2O$, 0.9 g/l NH_4NO_3, 0.5 g/l Yeast Extract and 1ml of a salt solution prepared by diluting 1.8g of $MnCl_2.4H_2O$, 2.487g of $FeSO_4.7H_2O$, 0.285g of H_3BO_3, 27mg of $CuCl_2$, 21mg of $ZnCl_2$, 74 mg $CoCl_2.6H_2O$, 23mg of $MgMoO_4$ and 2.1g of sodium tartrate dihydrate in 1 litre of deionised water. The shake flasks are then incubated at 30°C for one to two days. The resulting inoculums, with a total volume of 1

litre, are then added to a further 9 litres of the fermentation medium inside a 15 litre stirred bioreactor (B.Braun Biostat, ED ES10, Meslungen, Germany). Fermentation is carried out at a temperature of 30°C, a stirring rate of 500 rpm and an agitation rate of 2 vvm, with the pH of the broth controlled at 7.00 by automatic addition of 1M H_3PO_4 and 3M NaOH. The concentration of glucose and the viscosity of the fermentation broth along with the microorganism cell dry weight (CDW) and the gellan dry weight (GDW) in the fermentation broth are shown as a function of fermentation time in Figure 1

Example 2

A crude fermentation broth is made in the same way as in Example 1, using a fermentation medium consisting of 50 g/l glucose, 0.5 g/l K_2HPO_4, 0.1 g/l $MgSO_4.7H_2O$, 0.9 g/l NH_4NO_3, 0.5 g/l Yeast Extract and 1ml of a salt solution. The concentration of glucose and the viscosity of the fermentation broth along with the microorganism cell dry weight (CDW) and the gellan dry weight (GDW) in the fermentation broth are shown as a function of fermentation time in Figure 2.

Example 3

A crude fermentation broth is made in the same way as in Example 1, using a fermentation medium consisting of 30 g/l glucose, 0.5 g/l K_2HPO_4, 5 g/l $MgSO_4.7H_2O$, 0.9 g/l NH_4NO_3, 0.5 g/l Yeast Extract and 1ml of a salt solution. The concentration of glucose and the viscosity of the fermentation broth along with the microorganism cell dry weight (CDW) and the gellan dry weight (GDW) in the fermentation broth are shown as a function of fermentation time in Figure 3.

The crude fermentation broth of Example 1 contained the highest level of gellan production.

As seen from Figure 2, increasing the glucose concentration of the fermentation medium does not lead to increased gellan production.

In Example 3, increasing the Mg^{2+} concentration in the fermentation medium leads to decreased gellan production, but increased viscosity of the crude fermentation broth produced. The rate of growth of the inoculum is also decreased by increasing the Mg^{2+} concentration.

Examples 4, 5 and 6 describe the manufacture of gels from the crude fermentation broths of Examples 1, 2 and 3. In each of Examples 4, 5 and 6, gels were made from the broths of Examples 1, 2 and 3 after fermentation for different lengths of time. The table following the examples indicates the broths from which the gels were made.

Example 4

Gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths at 100°C for 20 minutes.

Example 5

Gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths at 100°C for 20 minutes, then allowing the broths to cool to room temperature, then heating the broths for a second time at 100°C for 20 minutes.

Example 5a

A gel was made from the crude fermentation broth of Example 3 by heating the broth at 100°C for 20 minutes, then allowing the broth to cool to room temperature, then heating the broth for a second time at 100°C for

20 minutes, allowing it to cool and then heating the broth for a third time at 100°C for 20 minutes.

Example 6

Gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths in the same way as in Example 4 and then adding a 1% solution of 25% weight by volume $CaCl_2$.

Example 7

Gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths in the same way as in Example 4 and then adding a 1% solution of 25% weight by volume NaCl.

Example 8

Deacetylated gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths at 100°C for 20 minutes, allowing the broths to cool to about 80°C and then increasing the pH of the broths to 10 through addition of NaOH. The broths were then held at 80°C for 10 minutes after which the pH was reduced to 7 through addition of HCl.

Examples 4 to 8 gave the following gel samples, the properties of which are shown graphically in Figures 4, 5 and 6.

Using the broth of Example 1 (lower glucose concentration than Example 2 and lower Mg^{2+} concentration than Example 3 in the fermentation medium):

		fermentation time in hours									
		29	41	54	66	72	90	90	90	90	90
Example	4	**A**	**B**	**C**	**D**	**E**	**F**				
	5							**G**			
	6								**H**		
	7									**I**	
	8										**J**

Using the broth of Example 2 (higher glucose concentration in the fermentation medium than Examples 1 and 3):

		fermentation time in hours									
		19	36	43	60	66	108	108	108	108	108
Example	4	**K**	**L**	**M**	**N**	**O**	**P**				
	5							**Q**			
	6								**R**		
	7									**S**	
	8										**T**

Using the broth of Example 3 (higher Mg^{2+} concentration in the fermentation medium than in Examples 1 and 2):

		fermentation time in hours												
		20	26	33	43	51	72	77	100	100	100	100	100	100
Example	4	**U**	**V**	**W**	**X**	**Y**	**Z**	**AA**	**BB**					
	5									**CC**				
	5a										**DD**			
	6											**EE**		
	7												**FF**	
	8													**GG**

The strength of each of the gels formed in Examples 4 to 8 was assessed by applying manual pressure to the gel.

The elasticity of each of the gels formed in Examples 4 to 8 was assessed by spreading the gels over a wide surface, (a Petri plate) and stretching them manually. The brittleness of each of the gels formed in Examples 4 to 8 was assessed by their tendency to crack. The results are shown in Figures 4, 5 and 6.

The addition of salts after the single heat treatment of Example 4 (samples A-F, K-P, U-BB), as in Examples 6 (samples H, R, EE) and 7 (samples I, S, FF), leads to slightly increased strength of the resulting gels compared to heating alone; the addition of divalent (Ca, Example 6) and monovalent (Na, Example 7) cations has the same effect. However, the presence of the extra Mg^{2+} ions in the crude fermentation broth (Example 3, samples U-GG) does not lead to increased gel strength for the gels formed from the crude fermentation broth of Example 3, despite the increased broth viscosity.

The strongest gel is produced in Example 8 (samples J, T, GG), where the broth was deacetylated after the heat treatment, especially when residual glucose, as in Example 2 (sample T), or extra Mg^{2+}, as in Example 3 (sample GG), was present.

The strength of the gel produced from a fermentation broth is related to the gellan concentration of the broth. For the crude fermentations broths of Examples 1, 2 and 3, this is clearly apparent in the first 30 to 40 hours, but becomes less apparent after about 40 to 50 hours.

Greater elasticity was observed for gels formed by heat treatment of the crude fermentation broths, as in Examples 4 and 5 (samples A-G, K-Q, U-DD), and/or for gels formed by the addition of salts after a single heat treatment, as in Examples 6 (samples H, R, EE) and 7 (samples I, S, FF). Deacetylated gels formed by Example 8 (J, T, GG) were found to be brittle.

Example 9

Gels were made from the crude fermentation broths of Examples 1, 2 and 3 by heating the broths in the same way as in Example 4 then adding 2 volumes of absolute ethanol and subjecting the product to centrifugation or mixing. The decolourisation carried out in Example 9 using absolute ethanol lead to precipitation of gellan and loss of structure and gelling ability.

Gels formed from the crude fermentation broth of Example 3, with high Mg^{2+} concentration, are significantly darker in colour than gels formed from the crude fermentation broths of Examples 1 and 2. Also, deacetylated gels formed in Example 8 are slightly darker than the gels formed in Examples 4 to 7 and 9. The colour of the gels formed was assessed visually.

The darker colour is probably due to the longer heat treatment employed in Example 8, and is possibly the result of caramelisation or Maillard reactions which produce dark coloured compounds. This may be advantageous in applications where a bright yellow gellan broth is undesirable.

~~CLAIMS~~

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of providing a gel comprising gellan, comprising the steps of:

fermenting a microorganism in a carbohydrate containing medium so as to yield a crude gellan gum fermentation broth; and

gelling the crude fermentation broth.

2. A method according to claim 1 further comprising the step of deacetylating the gellan.

3. A method according to claim 1 or 2 further comprising comminuting the gelled fermentation broth and removing the separated water.

4. A method according to any preceding claim wherein the crude fermentation broth is gelled by heating.

5. A method according to any preceding claim wherein the microorganism is *Sphingomonas paucimobilis*.

6. A method according to any preceding claim wherein the microorganism is *Sphingomonas paucimobilis* strain ATCC 31461.

7. A method according to any preceding claim wherein the carbohydrate is glucose.

8. A gel formed by a method according to any preceding claim.

9. A gel formed from a gellan gum fermentation broth wherein the broth has not been subjected to any filtration or purification steps prior to formation of the gel.

10. A gel comprising gellan and the byproducts of the fermentation which produced the gellan.

11. A foodstuff incorporating a gel according to any of claims 8 to 10.

12. A pet foodstuff according to claim 11.

13. A method of forming a dried powder from a gel comprising gellan, comprising the steps of:
comminuting the gel to dewater it;
removing the separated water to obtain a concentrated gel; and
drying the concentrated gel to form a powder.

14. A method substantially as described.

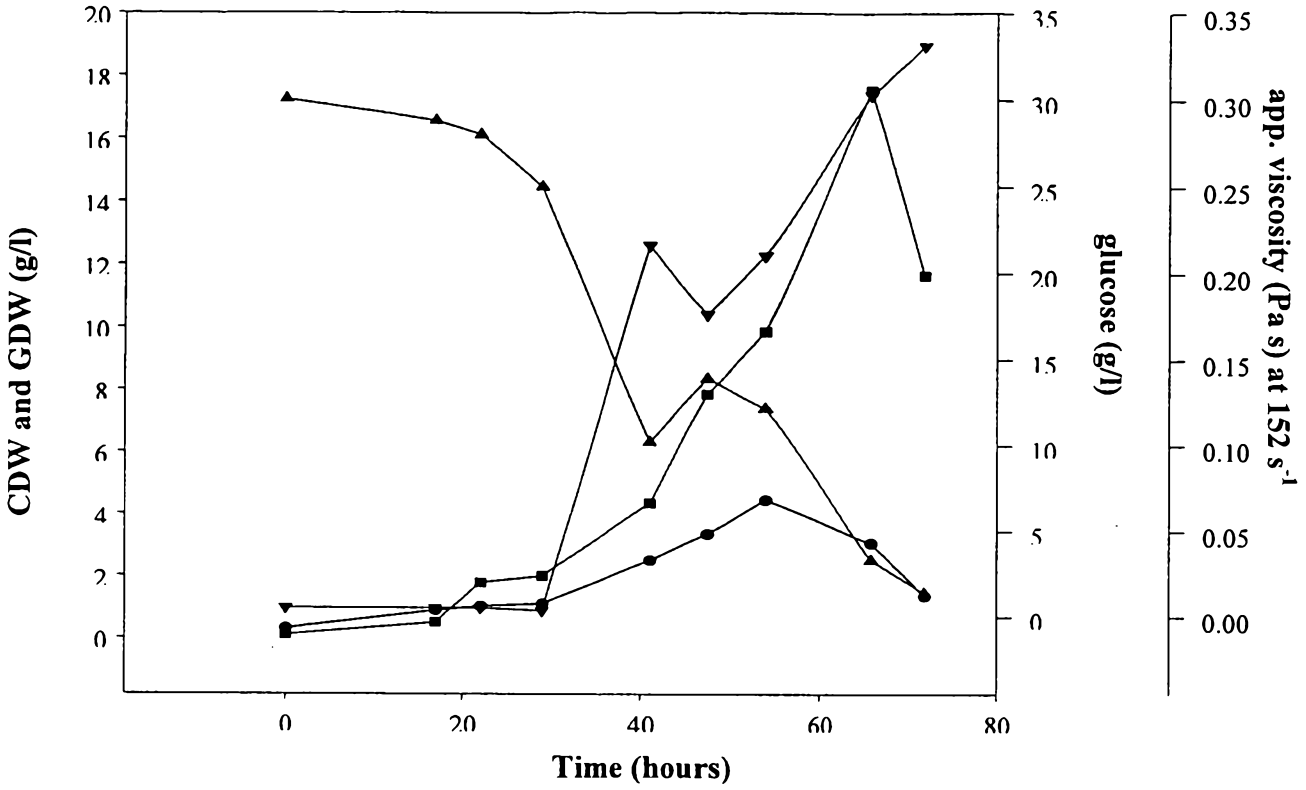
15. A gel substantially as described.

DATED this 27th day of March 2002.

MARS, INCORPORATED

WATERMARK PATENT & TRADEMARK ATTORNEYS
290 BURWOOD ROAD
HAWTHORN. VIC. 3122.

Figure 1



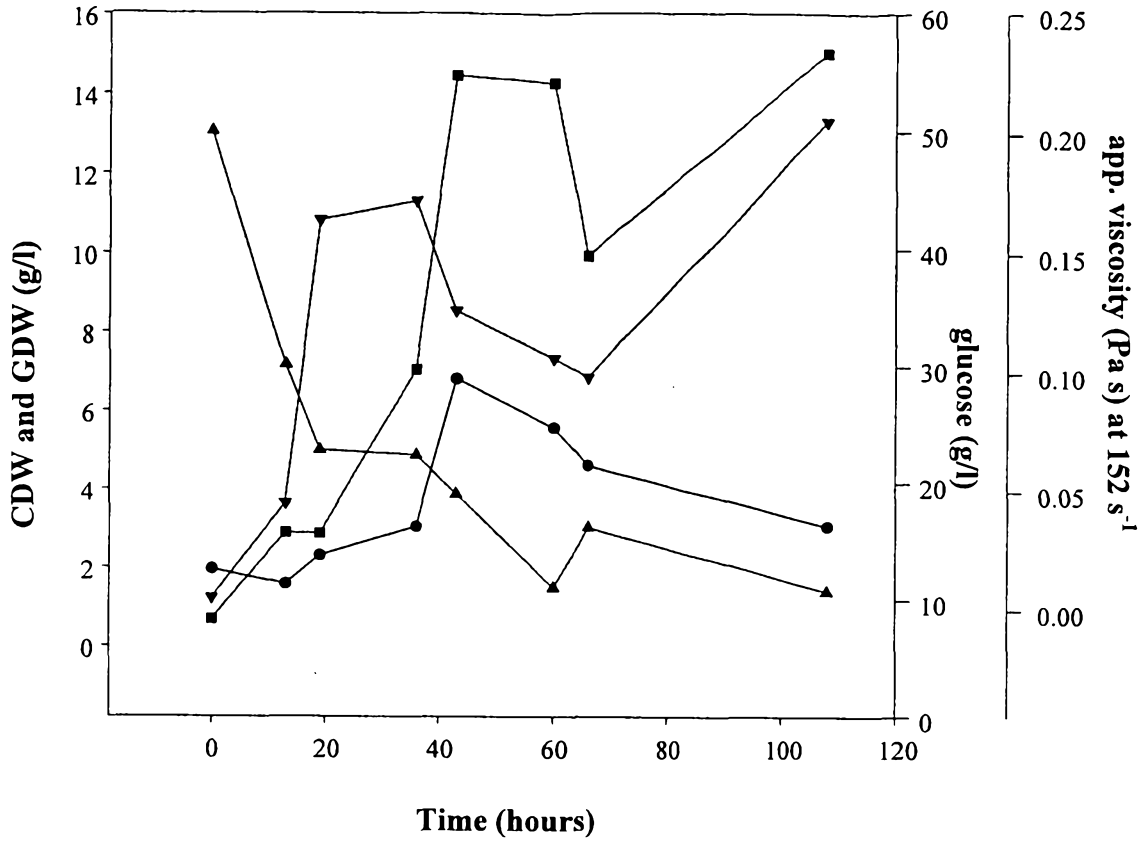
Time *vs* GDW (g/l)

Time *vs* Glucose (g/l)

Time *vs* Viscosity (Pa s)

Time *vs* CDW (g/l)

Figure 2



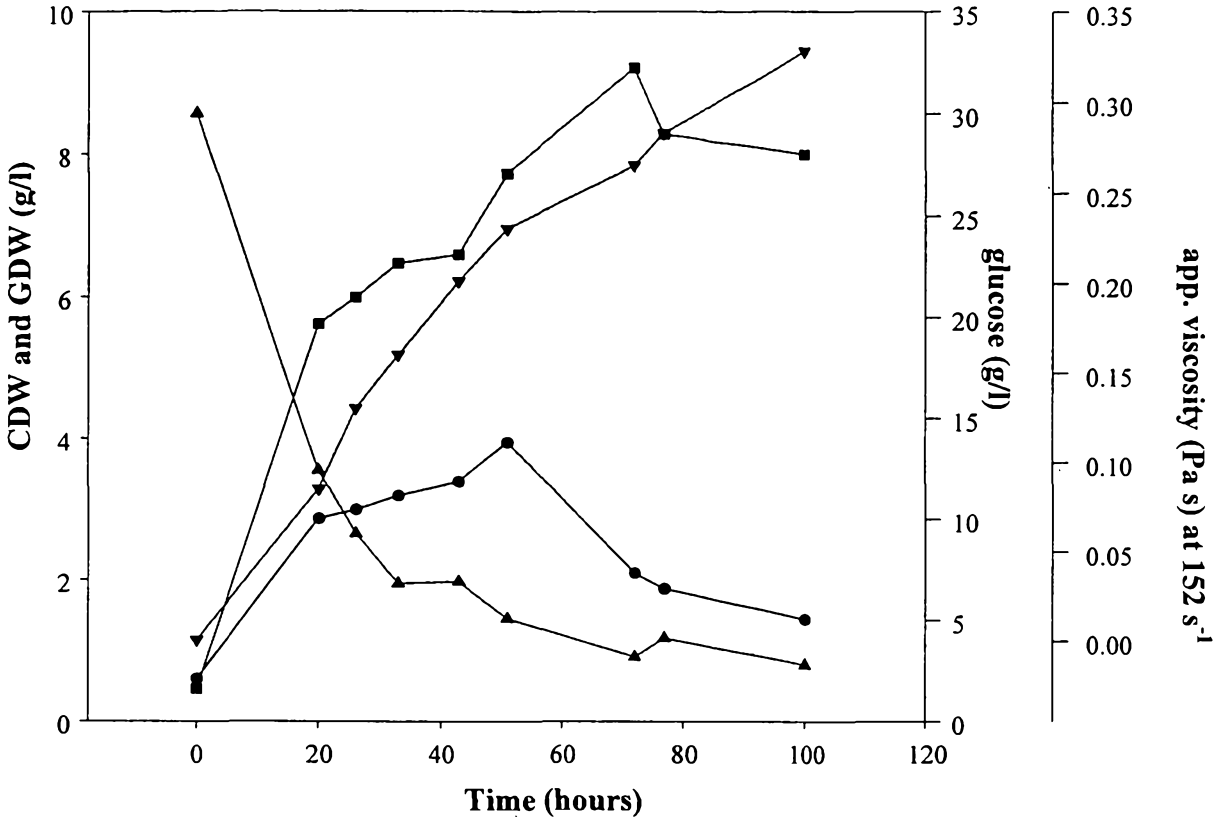
Time *vs* GDW (g/l)

Time *vs* Glucose (g/l)

Time *vs* Viscosity (Pa s)

Time *vs* CDW (g/l)

Figure 3



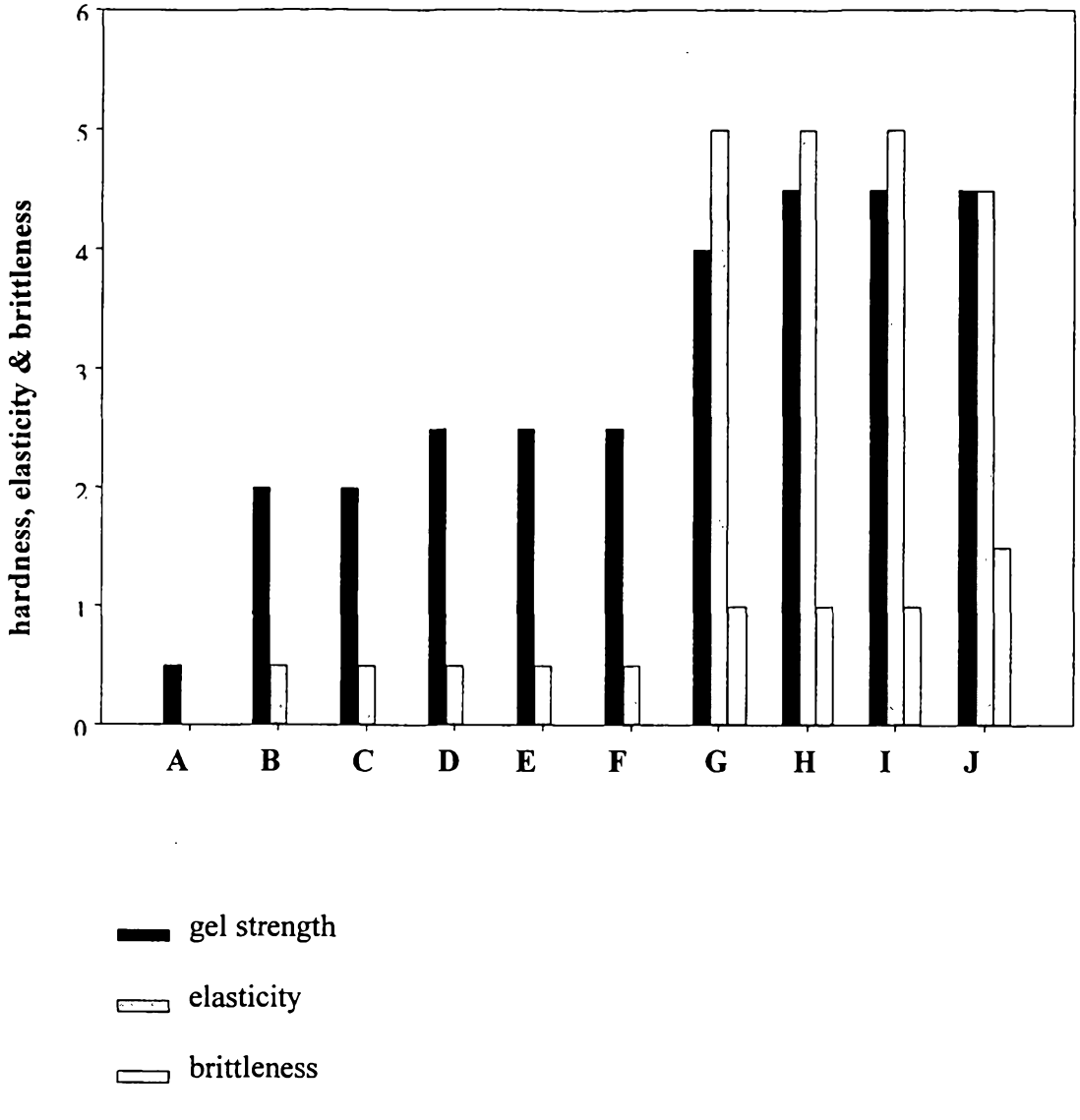
Time *vs* GDW (g/l)

Time *vs* Glucose (g/l)

Time *vs* Viscosity (Pa s)

Time *vs* CDW (g/l)

Figure 4


hardness, elasticity & brittleness
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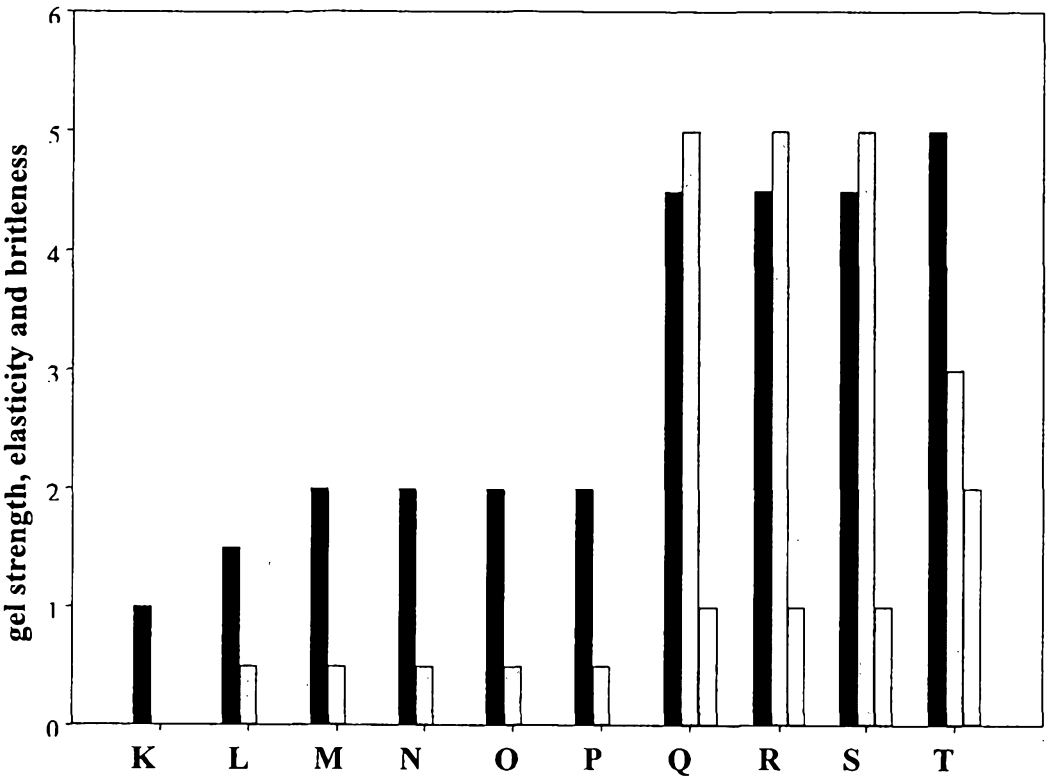
gel strength
elasticity
brittleness

Figure 5


gel strength, elasticity and britleness
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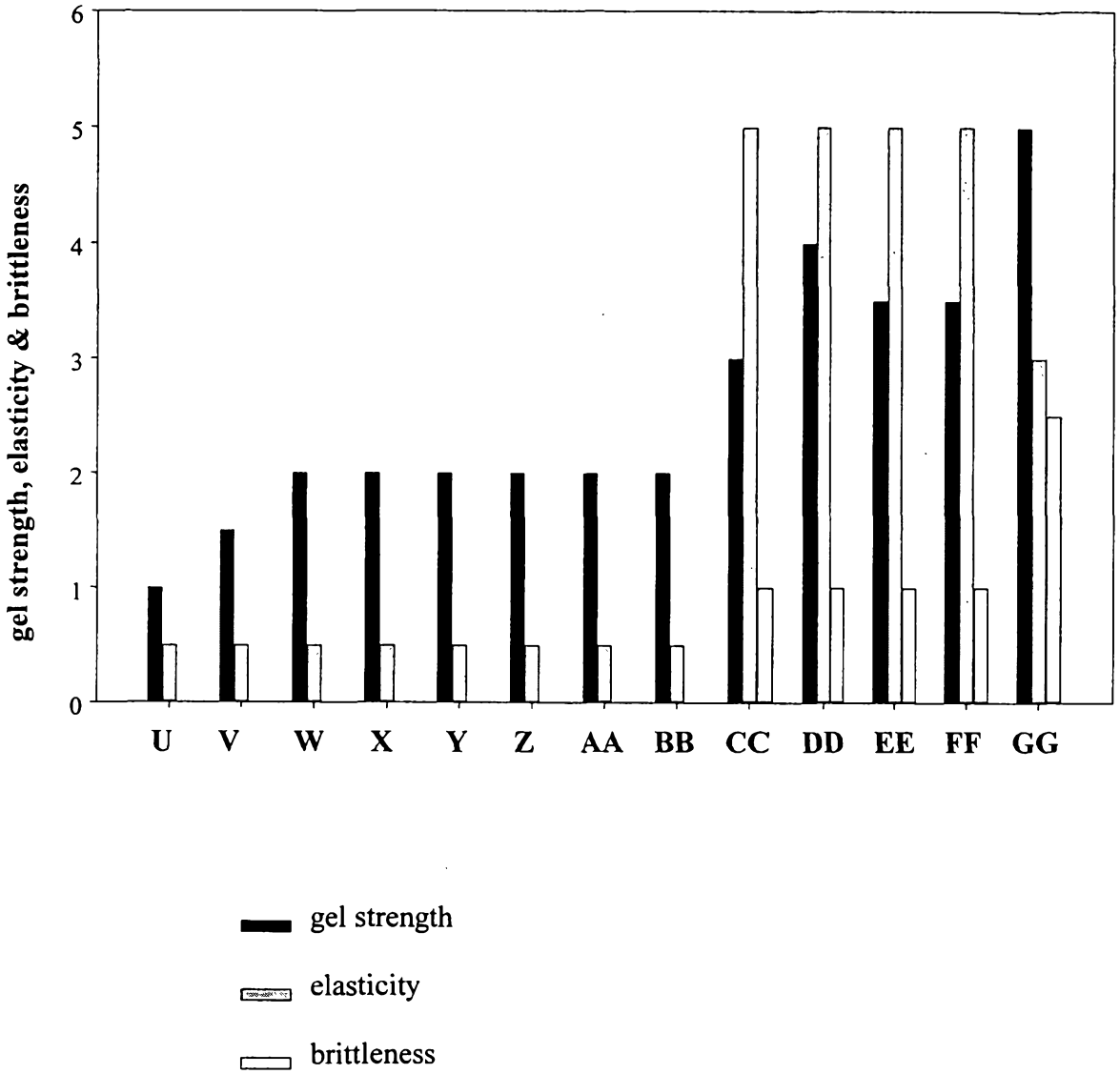
gel strength
elasticity
brittleness

Figure 6


gel strength, elasticity & brittleness
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