# CHAPTER IV

## RESULTS AND DISCUSSION

Dietary fiber is believed to decrease the incidence of colorectal cancer, but not all types of fiber are equally protective. A number of recent studies have questioned the philosophy that a high fiber diet will do no harm, even it does not prevent colon cancer. An Australian study showed a correlation between cereal fiber intake and colon cancer (Potter and McMichael, 1986) and a British study showed an increased mortality from stomach cancer in vegetarian (Kinlen , Herman, and Smith, 1983)

Dietary fibers may be divided broadly into insoluble and soluble fibers, and there were evidences from animal experiments that the latter not only failed to protect against colorectal cancer but may enhance its development. Harris et al. (1993) found that commercial soluble fiber currently used as emulsifiers and stabilizer in the food industry namely, κ-carragenan, γ-galactomannan, (1→3,1→4)-β-D-glucan, gum arabic, pectin, polygalacturonic acid and sodium carboxymethylcellulose, maintained 1,8 dinitropyrene in aqueous solution and decreased its adsorption to α-cellulose which was used as an example of an insoluble dietary fiber. This phenomenon suggested the possible mechanisms by which soluble fibers might enhance the development of cancer. It was, thus,

enthusiatic to find out whether the same phenomenon would occur in the gastric-liked condition since it was contrast to a previous study which showed that some fibers were promised to inhibit the formation of mutagen which has nitrite salt as one of the precursors at pH 1-3 (Laohavechvanich, 1994).

**Nitrite Scavenging Activity**

The results in Figure 4.1 show the effect of commercial soluble fibers on the disappearnce of nitrite (320 μM) under simulated gastric conditions. All of them showed little or no effect on the nitrite concentration. It is contradicted to the results obtained by Laohavechvanich (1994) who reported that the fibers prepared from fruits and vegetables were efficient nitrite scavengers under acid condition. The fibers in their study were all insoluble fibers according to the method of preparation. Moreover, they also stated that a simple fiber α-cellulose, an insoluble fiber, did not have nitrite scavenging activity. Therefore, from the present experiment, it is revealed that only some fibers are benefit as nitrite scavengers to the consumer.

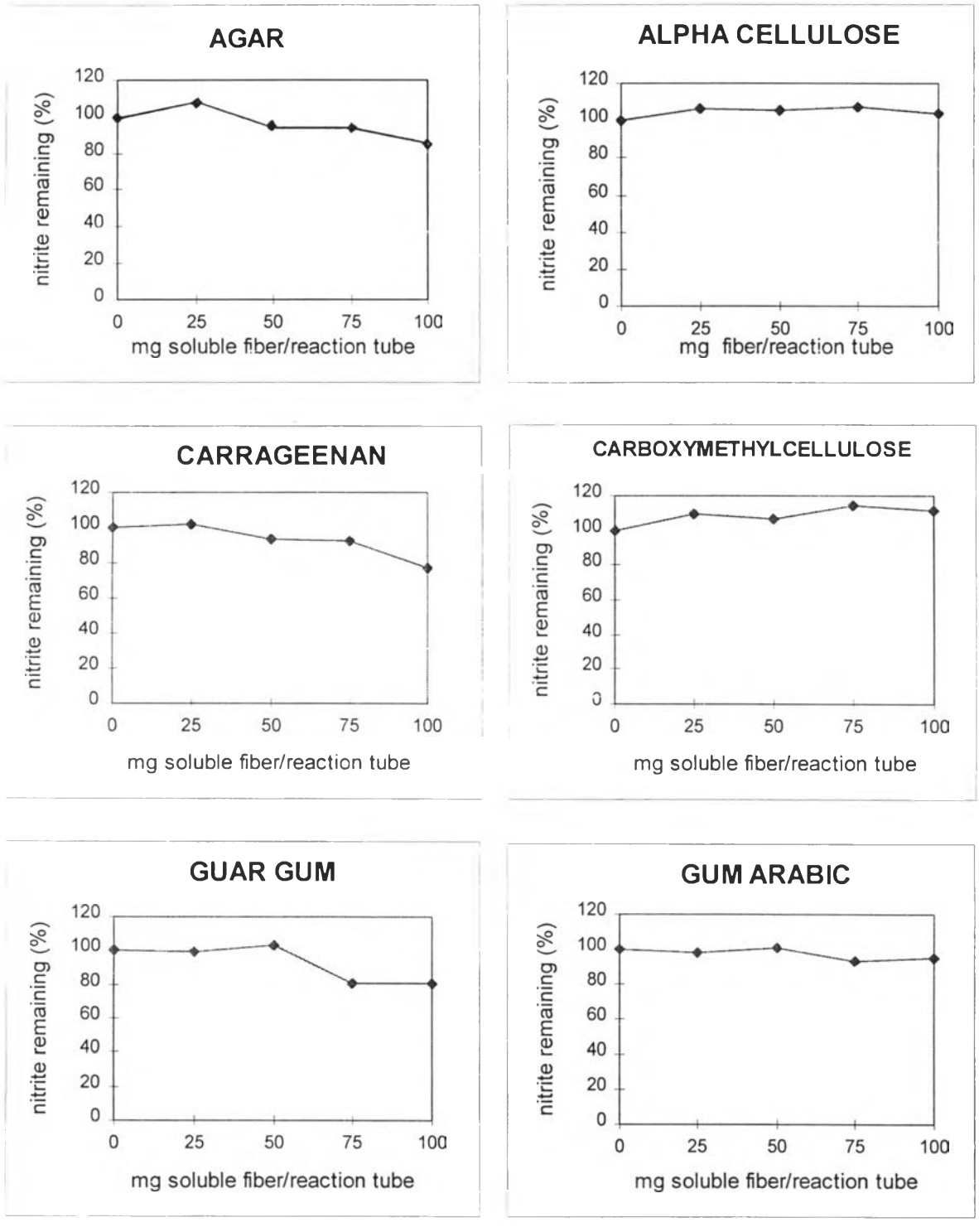



**Figure 4.1** The effect of polysaccharides on the disappearance of nitrite (320 μM)

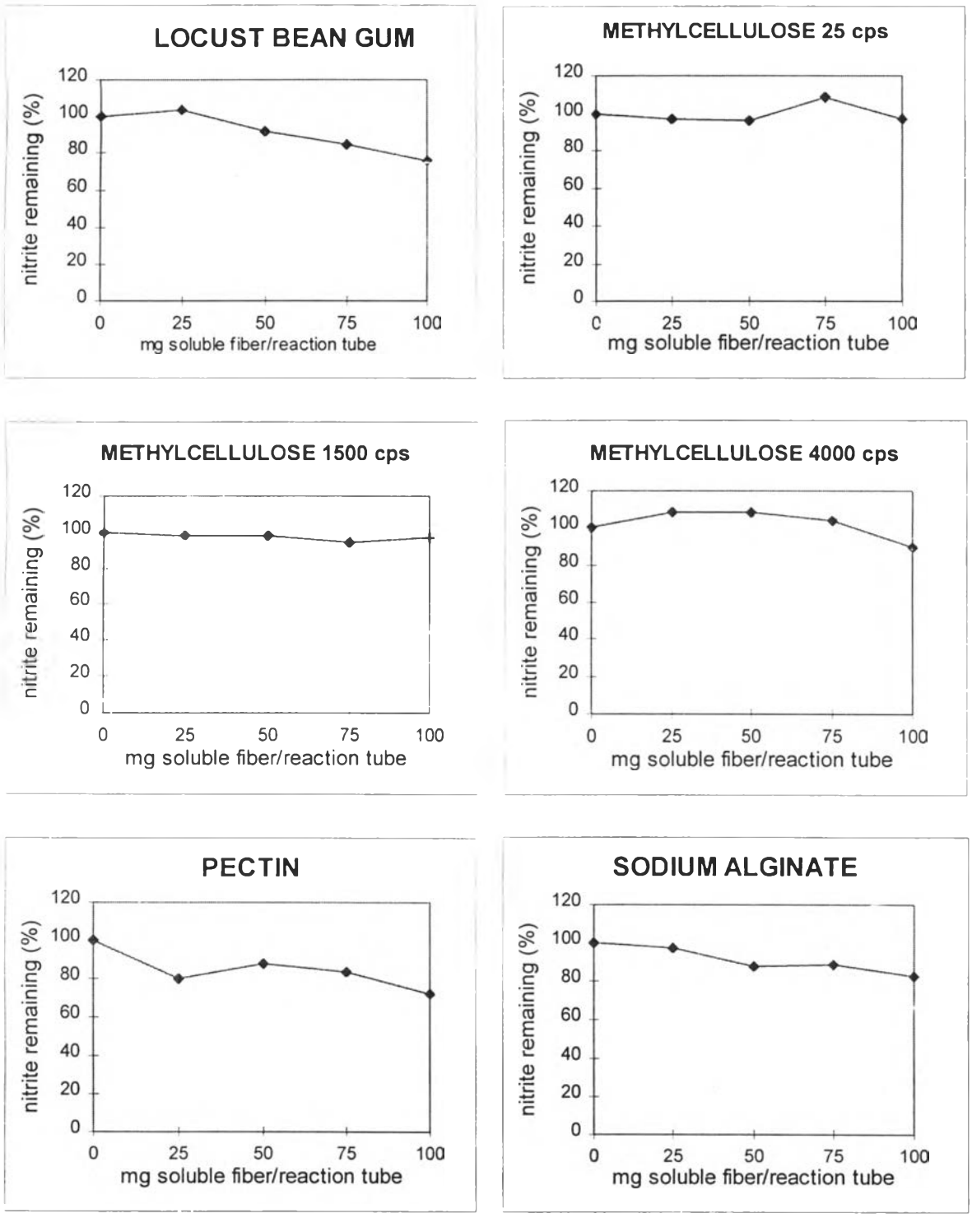



**Figure 4.1** (continue) The effect of polysaccharides on the disappearance of nitrite (320 μM)

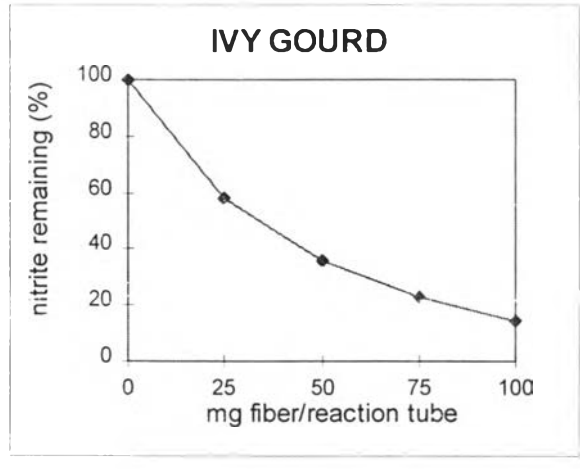


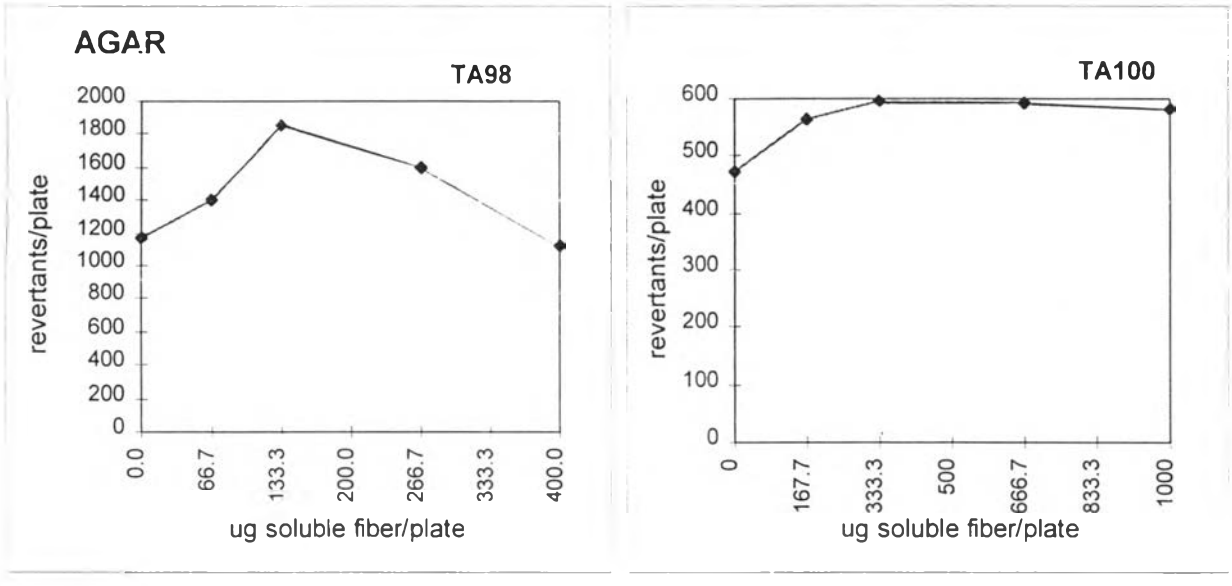

**Figure 4.1** (continue) The effect of polysaccharides on the disappearance of nitrite (320 μM)

**Effect of polysaccharides on mutagenicity of aminopyrene treated with nitrite**

As mention above, it is shown that commercial soluble fibers (polysaccharides) used in food industries had no nitrite scavenging activity. However, it is necessary to study whether these fibers have any effect on the mutagenicity of the aminopyrene-nitrite model. The effect of fibers on the incubation mixture of aminopyrene and nitrite are shown in Figures 4.2 to 4.14. It was found that fibers, namely agar, carboxymethylcellulose, guar gum, gum arabic, locust bean gum, methylcellulose 25 cps, 1500 cps, 4000 cps, and pectin, could not inhibit, but increase the mutagenicity of nitrite treated aminopyrene on *Salmonella typhimurium* TA 98 and TA 100 (Figures 4.2- 4.10). Four fibers, namely carrageenan, sodium alginate, xanthan gum, and α-cellulose exhibited their inhibitory effects against the mutagenicity of the model (Figures 4.11-4.14).

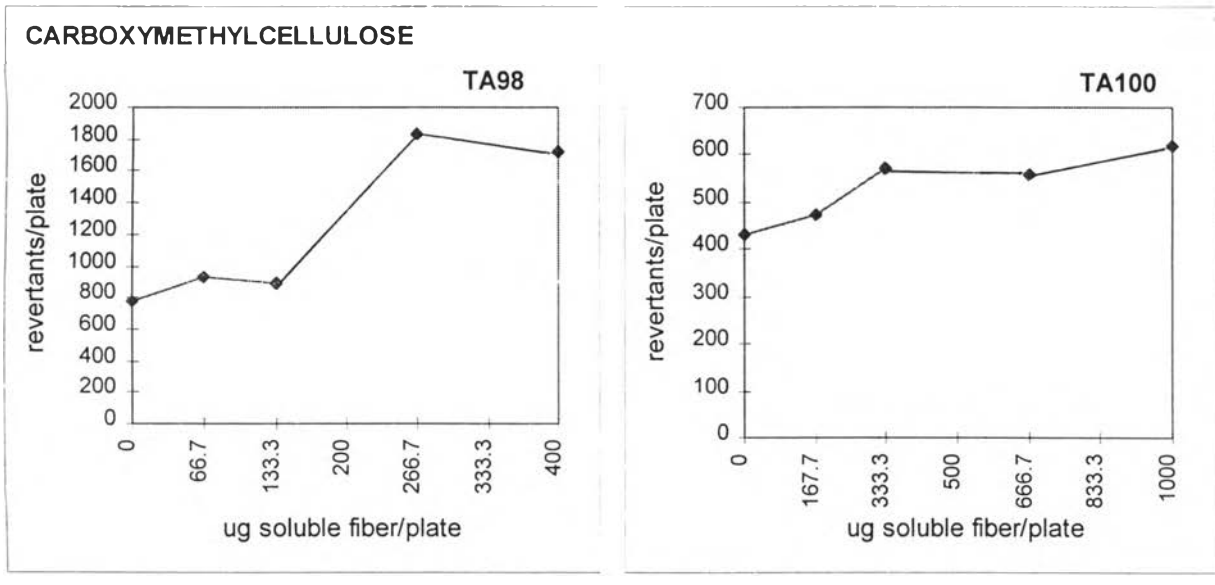
The inhibition on mutagenicity of the aminopyrene-nitrite model by α-cellulose did not confirm the result of Laohavechvanich (1994). This may due to the difference between the two study designs. Considering the amount of α-cellulose per tube of the two studies, it was found that they were nearly the same. However, the concentration of aminopyrene in the previous study was 4 mM which was higher than 0.05 mM of the present study. Therefore, in the previous study the newly formed mutagen occurred in the reaction with higher concentration of aminopyrene as the precursor might be too high to be reduced by limited amount of α-cellulose.

The finding that α-cellulose was effective antimutagenicity of nitrite-aminopyrene model in this study was also similar the study on the other mutagens. Roberton et al. (1990) found that increasing amounts of α-cellulose caused an increase in the loss of mutagenicity of 1,8-dinitropyrene on *Salmonella typhimurium* TA 98. The relationship between the loss of mutagenicity and the α-cellulose concentration was approximately logarithmic. Cellulose was also effective in binding 2-aminoanthracene and mutagens extracted from fried ground beef (Moorman, Moon, and Worthington, 1983)



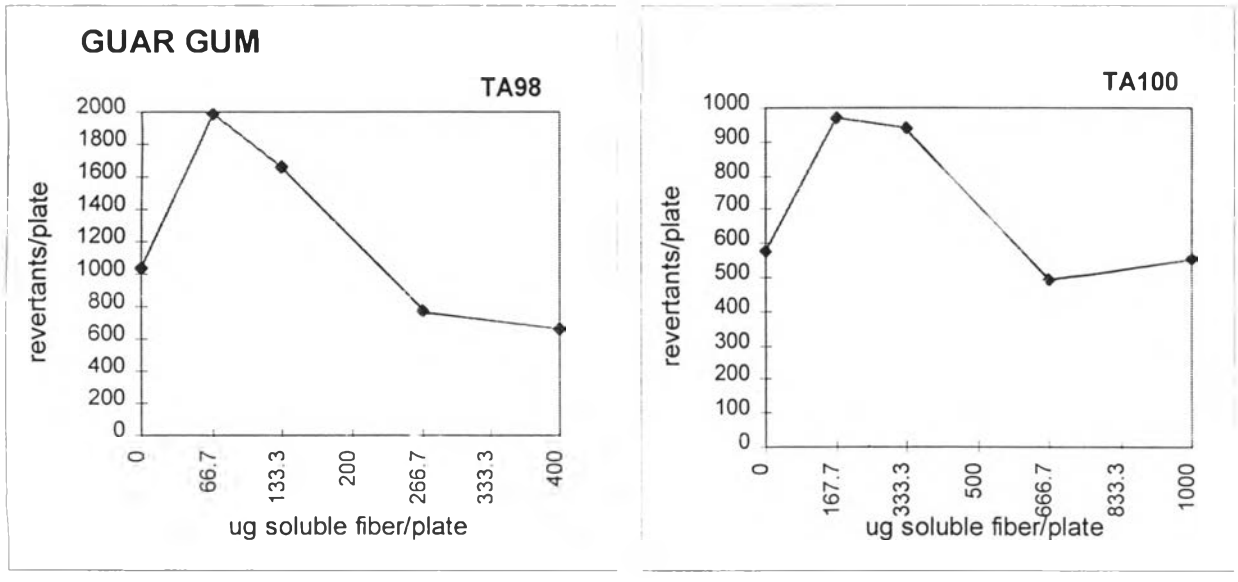

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Agar | 0.0 | 1176 (77) | 0.0 | 472 (88) |
| | 66.7 | 1400 (70) | 166.7 | 564 (22) |
| | 133.3 | 1858 (46) | 333.3 | 598 (13) |
| | 266.7 | 1595 (78) | 666.7 | 592 (13) |
| | 400.0 | 1125 (28) | 1000.0 | 582 (83) |
| | Spontaneous reversion | 30 (3) | Spontaneous reversion | 161 (4) |

**Figure 4.2** The effect of agar on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



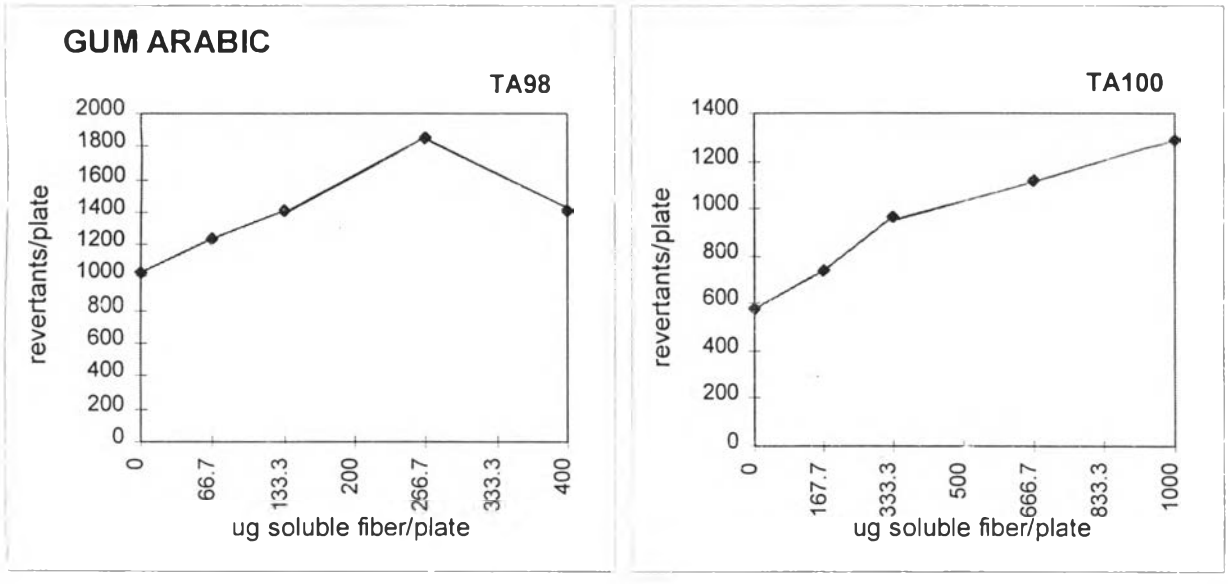

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Carboxymethyl- | 0.0 | 780 (90) | 0.0 | 433 (1) |
| cellulose | 66.7 | 933 (24) | 166.7 | 472 (11) |
| | 133.3 | 894 (20) | 333.3 | 576 (5) |
| | 266.7 | 1840 (28) | 666.7 | 561 (26) |
| | 400.0 | 1723 (37) | 1000.0 | 617 (35) |
| | Spontaneous reversion | 18 (1) | Spontaneous reversion | 143 (1) |

**Figure 4.3** The effect of carboxymethylcellulose on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



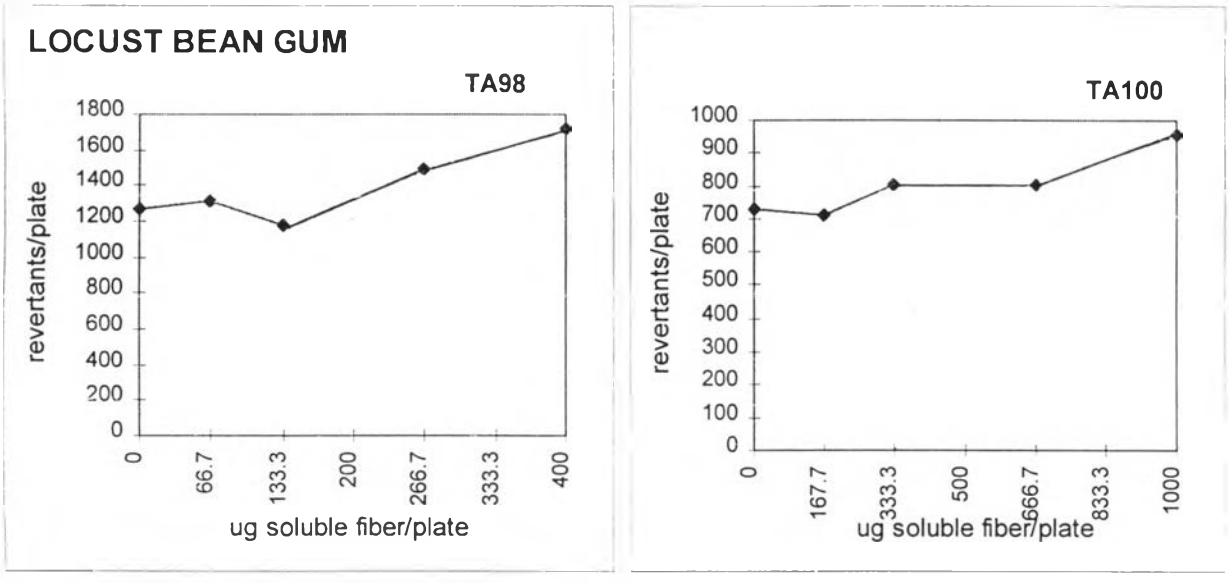

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Guar gum | 0.0 | 1035 (11) | 0.0 | 576 (35) |
| | 66.7 | 1983 (75) | 166.7 | 973 (10) |
| | 133.3 | 1660 (28) | 333.3 | 942 (169) |
| | 266.7 | 778 (30) | 666.7 | 492 (41) |
| | 400.0 | 658 (16) | 1000.0 | 556 (1) |
| | Spontaneous reversion | 22 (4) | Spontaneous reversion | 164 (1) |

**Figure 4.4** The effect of guar gum on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



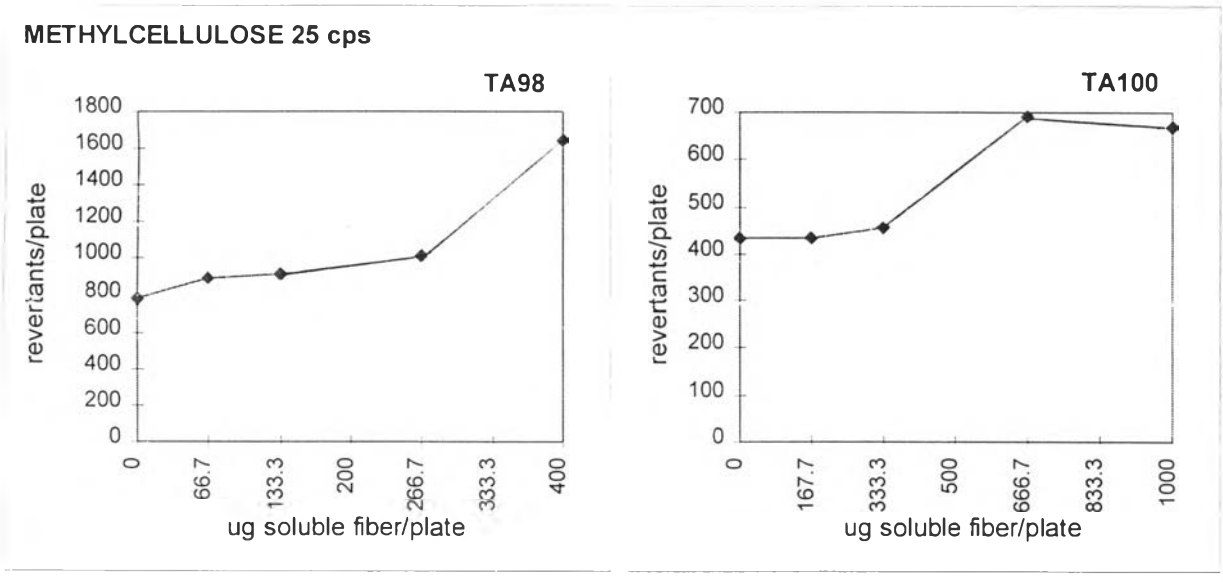

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Gum arabic | 0.0 | 1035 (11) | 0.0 | 576 (35) |
| | 66.7 | 1242 (25) | 166.7 | 742 (39) |
| | 133.3 | 1415 (35) | 333.3 | 966 (108) |
| | 266.7 | 1850 (42) | 666.7 | 1123 (24) |
| | 400.0 | 1412 (190) | 1000.0 | 1288 (14) |
| | Spontaneous reversion | 22 (4) | Spontaneous reversion | 164 (1) |

**Figure 4.5** The effect of gum arabic on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



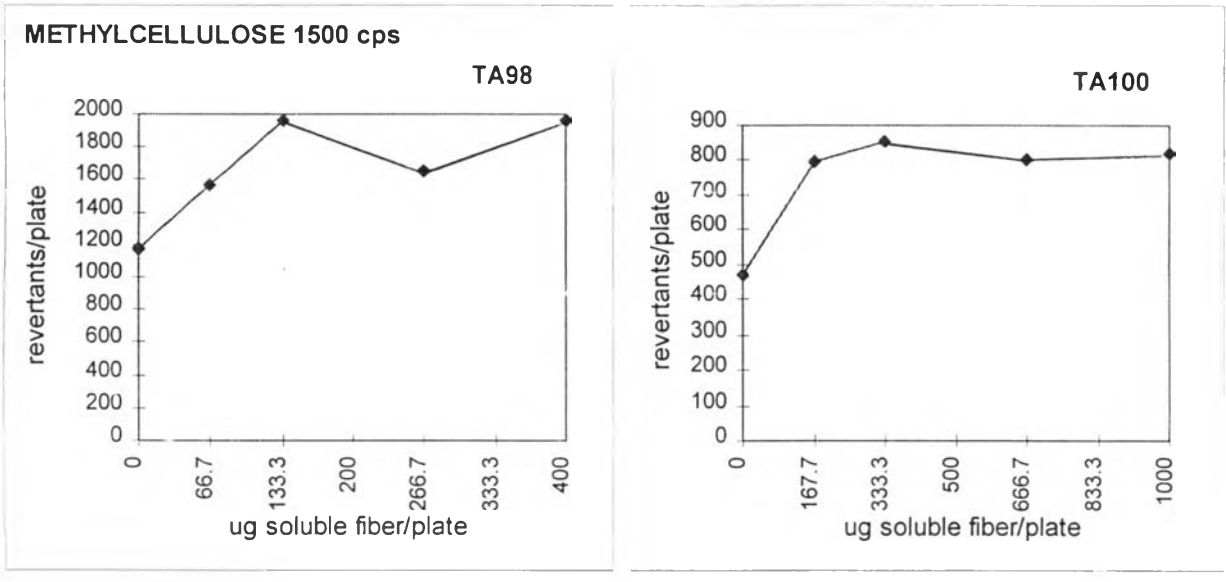

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Locust bean gum | 0.0 | 1271(94) | 0.0 | 730 (33) |
| | 66.7 | 1318 (154) | 166.7 | 712 (54) |
| | 133.3 | 1182 (88) | 333.3 | 802 (31) |
| | 266.7 | 1494 (111) | 666.7 | 802 (31) |
| | 400.0 | 1722 (126) | 1000.0 | 955 (35) |
| | Spontaneous reversion | 32 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.6** The effect of locust bean gum on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



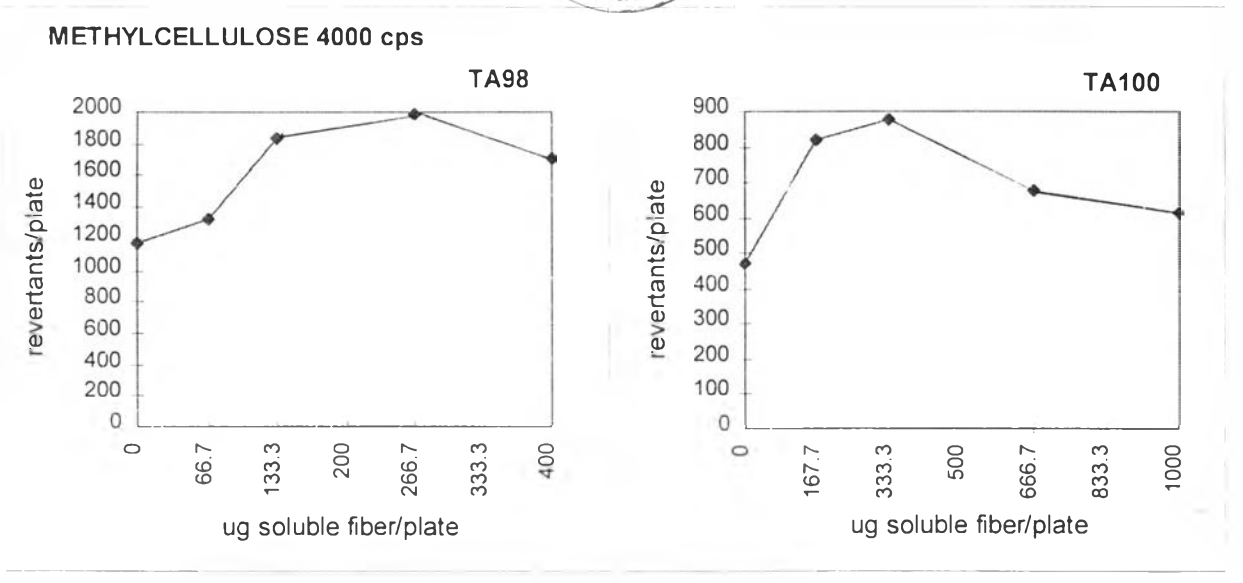

| Soluble fiber source | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Methylcellulose 25 cps | 0.0 | 780 (90) | 0.0 | 433 (1) |
| | 66.7 | 896 (34) | 166.7 | 433 (81) |
| | 133.3 | 918 (101) | 333.3 | 454 (6) |
| | 266.7 | 1020 (99) | 666.7 | 690 (8) |
| | 400.0 | 1640 (14) | 1000.0 | 668 (74) |
| | Spontaneous reversion | 18 (1) | Spontaneous reversion | 143 (1) |

**Figure 4.7** The effect of methylcellulose 25 cps on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



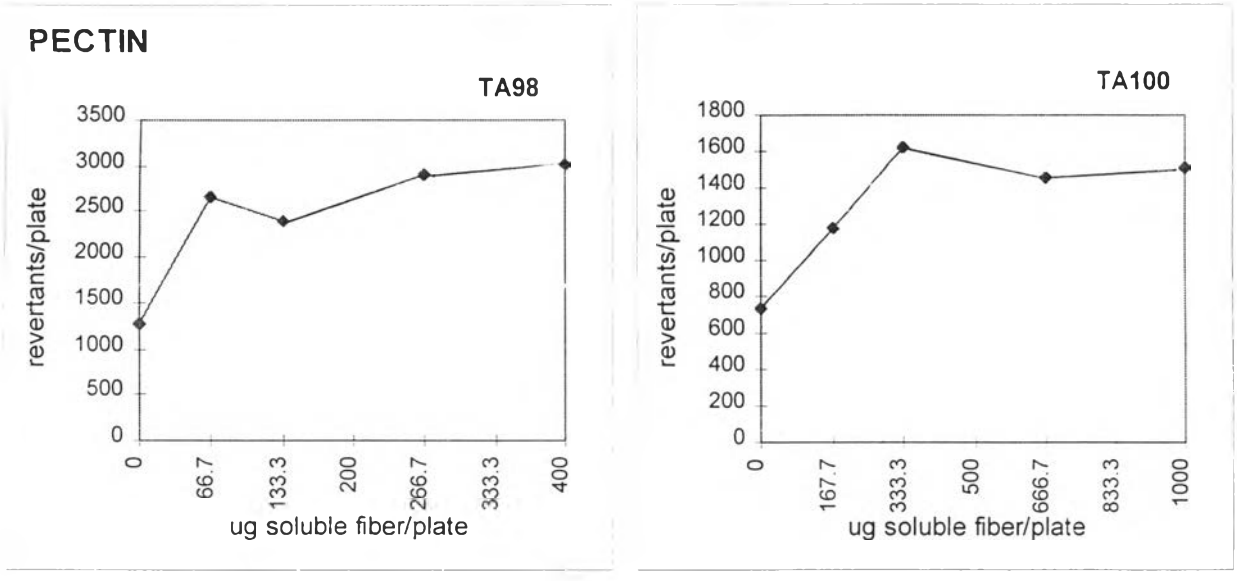

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Methylcellulose 1500 cps | 0.0 | 1176 (77) | 0.0 | 472 (88) |
| | 66.7 | 1565 (108) | 166.7 | 799 (30) |
| | 133.3 | 1964 (107) | 333.3 | 852 (33) |
| | 266.7 | 1656 (73) | 666.7 | 804 (6) |
| | 400.0 | 1960 (85) | 1000.0 | 818 (11) |
| | Spontaneous reversion | 30 (3) | Spontaneous reversion | 161 (4) |

**Figure 4.8** The effect of methylcellulose 1500 cps on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



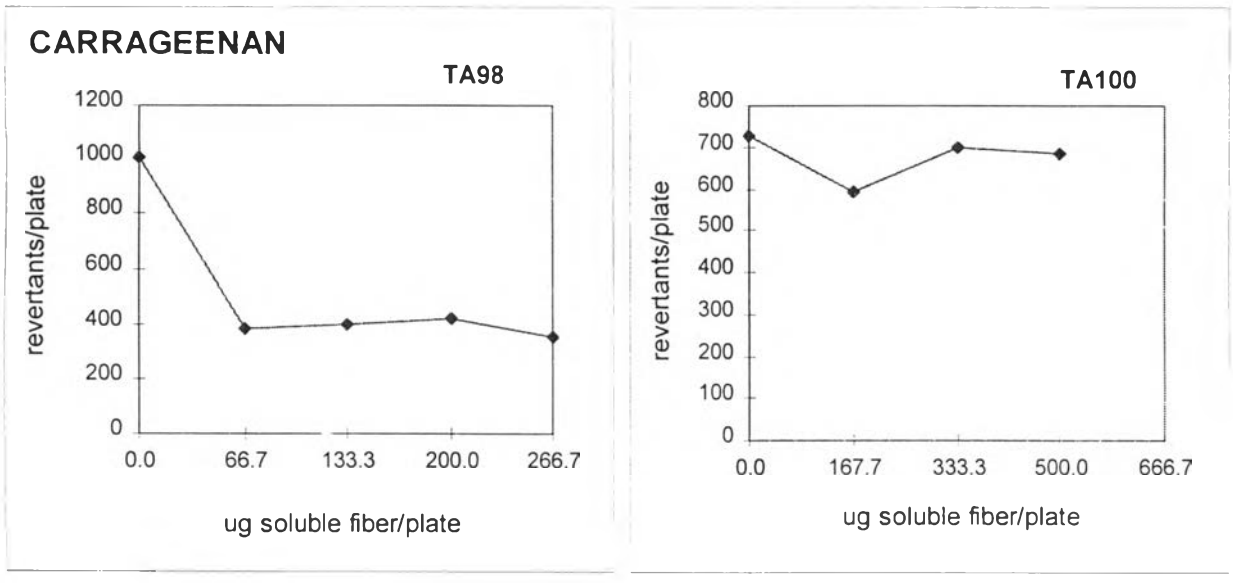

| Soluble fiber source | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Methylcellulose 4000 cps | 0.0 | 1176 (77) | 0.0 | 472 (88) |
| | 66.7 | 1325 (35) | 166.7 | 818 (23) |
| | 133.3 | 1839 (100) | 333.3 | 876 (13) |
| | 266.7 | 1990 (42) | 666.7 | 680 (62) |
| | 400.0 | 1703 (137) | 1000.0 | 618 (15) |
| | Spontaneous reversion | 30 (3) | Spontaneous reversion | 161 (4) |

**Figure 4.9** The effect of methylcellulose 4000 cps on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



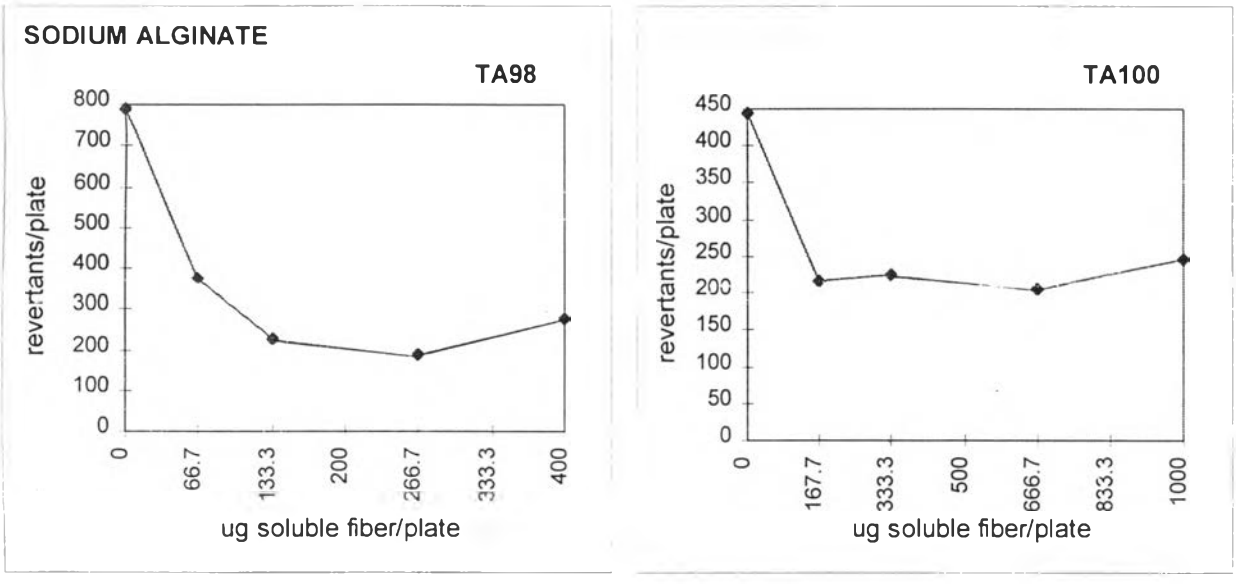

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Pectin | 0.0 | 1271(94) | 0.0 | 730 (33) |
| | 66.7 | 2672 (102) | 166.7 | 1175 (106) |
| | 133.3 | 2410 (212) | 333.3 | 1620 (85) |
| | 266.7 | 2900 (98) | 666.7 | 1455 (131) |
| | 400.0 | 3015 (162) | 1000.0 | 1515 (49) |
| | Spontaneous reversion | 32 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.10** The effect of pectin on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absencenn of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



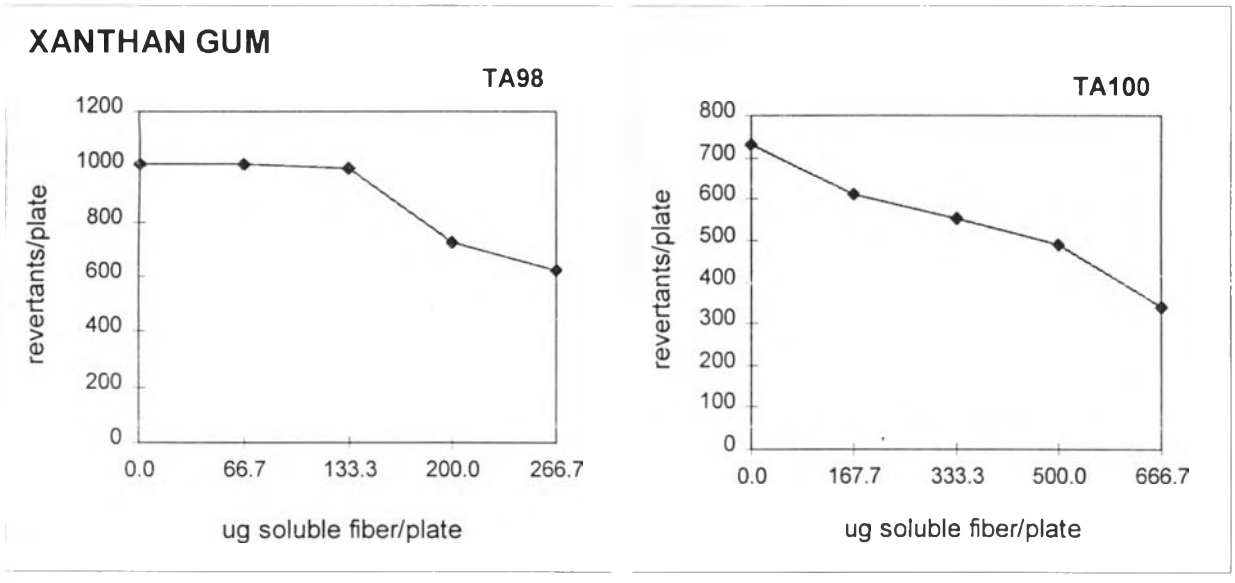

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Carrageenan | 0.0 | 1008 (94) | 0.0 | 730 (33) |
| | 66.7 | 380 (92) | 166.7 | 593 (76) |
| | 133.3 | 399 (93) | 333.3 | 698 (46) |
| | 200.0 | 420 (67) | 500.0 | 682 (59) |
| | 266.7 | 350 (42) | 666.7 | ND |
| | Spontaneous reversion | 36 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.11** The effect of carrageenan on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.



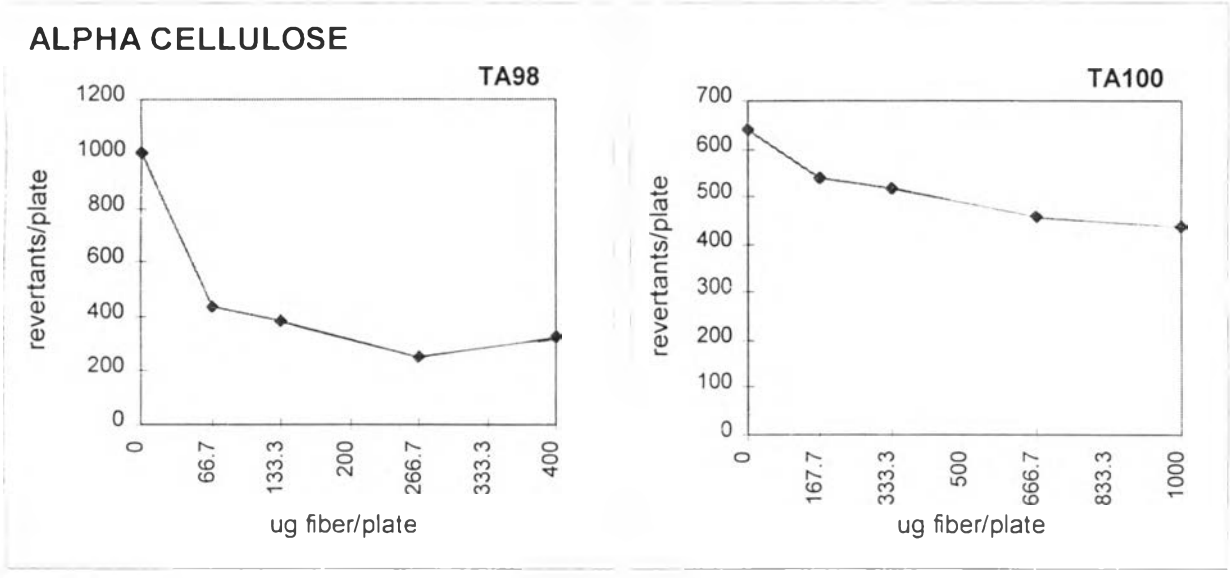

| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Sodium alginate | 0.0 | 788 (56) | 0.0 | 444 (18) |
| | 66.7 | 373 (53) | 166.7 | 218 (39) |
| | 133.3 | 226 (57) | 333.3 | 224 (20) |
| | 266.7 | 189 (29) | 666.7 | 206 (18) |
| | 400.0 | 278 (20) | 1000.0 | 248 (19) |
| | Spontaneous reversion | 36 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.12** The effect of sodium alginate on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.




| | TA98 | | TA100 | |
|---|---|---|---|---|
| Soluble fiber source | Amount of soluble fiber (μg/plate) | No.of revertants/plate | Amount of soluble fiber (μg/plate) | No.of revertants/plate |
| Xanthan gum | 0.0 | 1008 (94) | 0.0 | 730 (33) |
| | 66.7 | 1012 (69) | 166.7 | 611 (16) |
| | 133.3 | 992 (95) | 333.3 | 552 (65) |
| | 200.0 | 726 (83) | 500.0 | 490 (31) |
| | 466.7 | 620 (55) | 666.7 | 340 (11) |
| | Spontaneous reversion | 36 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.13** The effect of xanthan gum on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.




| | TA98 | | TA100 | |
|---|---|---|---|---|
| Fiber source | Amount of fiber (μg/plate) | No.of revertants/plate | Amount of fiber (μg/plate) | No.of revertants/plate |
| α-cellulose | 0.0 | 1008 (94) | 0.0 | 641 (104) |
| | 66.7 | 439 (16) | 166.7 | 537 (81) |
| | 133.3 | 388 (56) | 333.3 | 518 (40) |
| | 266.7 | 351 (28) | 666.7 | 458 (61) |
| | 400.0 | 328 (69) | 1000.0 | 436 (88) |
| | Spontaneous reversion | 36 (6) | Spontaneous reversion | 132 (4) |

**Figure 4.14** The effect of α-cellulose on the mutagenicity of the incubation mixture of 10 μl of 0.05 mM aminopyrene and 250 μl of 2 M nitrite (pH 3.0, 4 h) in the absence of activating system. Data expressed as means (and standard deviations in parenthesis) of the revertants per plate.

**Aminopyrene Binding Strength of Fibers**

Since nitrite scavenging activity was not a characteristic of the fibers in this study; therefore, the binding to aminopyrene was of interest to be explored. Fiber-mutagen binding is suggested as one mechanism of action which may prevent mutagenesis. The results of the present study divided the fibers into two groups.

The first group contains agar, carboxymethylcellulose, guar gum, gum arabic, locust bean gum, and pectin (Figures 4.15-4.20). These results came out according to the experimental design in Figure 3.3 which suggested that aminopyrene was bound to the fibers at a certain amount and left the unbound aminopyrene reacted with sodium nitrite, and then became mutagenic. When a portion of 1 ml of the mixture was removed and made up to the original volume (portion B) it was found that the mutagenicity of the solution was nearly the same as of the portion A. It was, thus, suggested that the binding was reversible due to the concentration of aminopyrene in the environment.

The second group of fiber (methylcellulose 25 cps, methylcellulose 1500 cps and methylcellulose 4000 cps) had a different characteristic (Figures 4.21-4.23). The mutagenicity of portion C was not different from that of portion A and the mutagenicity of portion B was not recovered after the addition of water suggesting that aminopyrene was firmly bound to such fibers.

The results of the first group indicated a possible mechanism by which soluble fibers may enhance mutagenesis of the aminopyrene-nitrite model. The fiber may maintain aminopyrene (a hydrophobic mutagen precursor) in some specific manner and presumably released it to the solution to interact with nitrite continuously. It is obvious that the products of nitrosation of compounds other than nitroamines in gastric liked condition are composed of some direct mutagens (Kikugawa and Nagao, 1991). Direct mutagens are generally less stable than indirect mutagens in the aqueous environment. Agents such as nitrosamide, nitroscurea and ethylenediamine are chemically stable only in the anhydrous state (Williams and Weisburger, 1991). Since the incubation time was 4 hours before the reaction mixture was stopped, the total direct mutagen that occurred in the reaction mixture of nitrite and aminopyrene may have had a less chance to interact with the DNA of the tester strains than that of the reaction mixture containing fiber of which the aminopyrene was proposed to be released from the fiber continuously.

The ability of soluble-fiber polysaccharides to act as emulsifiers and stabilizers may explain why they were effective in maintaining the concentration of aminopyrene in aqueous solution after the addition of distilled water to the reaction mixture (Figures 4.15-4.20) An explanation based on hydrogen bonding may also be advanced as to how soluble fibers maintained aminopyrene, the hydrophobic molecule, in aquoues solution. In solutions of uncharged fibers, many internal

hydrogen bonds occurred between hydroxyl groups. The internal hydrogen bonds reduced the number of hydroxyl groups available to interact with water, and hydrophobic pockets could be envisaged in which there was nothing on the surface to from hydrogen bonds with water. Aminopyrene may be adsorbed in these pockets and was released when its concentration in the medium was decreased.

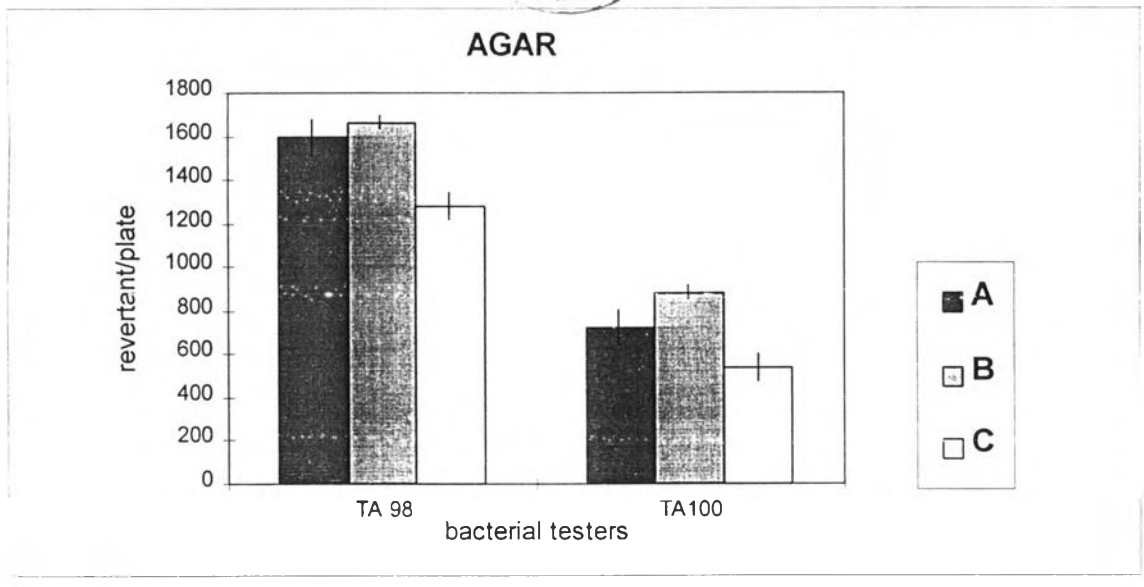
The present results also supported Harris et al. (1993) who revealed some possible mechanism that soluble fibers enhanced carcinogenesis in the presence of insoluble fibers. They suggested that the ability to maintain hydrophobic carcinogen in the medium reduced the possibility that insoluble fiber could scavenge the toxicant. After the toxicant was released from the soluble fiber or the fiber was degraded by intestinal bacteria, the toxicant would react with the target organs.

Studying on the aminopyrene binding strength, it was suggested that three types of methylcellulose (25, 1500 and 4000 cps) were better aminopyrene scavengers than carboxymethylcellulose. The latter released some portion of the trapped aminopyrene to the diluted environment indicated by the number of revertant per plate between the results of portions A and B.

The present study has shown that most of the soluble fiber could increase the mutagenicity of nitrite treated aminopyrene. Freudenheim et al. (1990) showed that no association between the amount of fiber and the reduction of colorectal cancer was found for soluble cereal grain fibers. In addition, a number of animal

experiments showed that soluble fibers usually enhanced, rather than prevented, carcinogenesis. For example, pectin increased the number of tumors induced by the carcinogen 1,2-dimethylhydrazine (Bauer et al., 1979) and failed to protect against the direct-acting carcinogen N-nitroso-N-methylurea (Watanabe et al., 1979). Roberton et al. (1991) indicated that the poor adsorption of 1,8-dinitropyrene (DNP) by cell wall preparations from potato flesh and immature cabbage leaves may results from the release of water soluble pectin fibers (soluble fiber) which compete with insoluble components (insoluble fiber) for the adsorption of DNP, thus, increasing the mutagenicity to such compound.

In conclusion, the Ames assay was not directly extrapolatable to food/human systems; however, it was supportive of the view that the increase or reduction of mutagenicity in this experiment was due to binding of the mutagen and depended on the particular fiber.



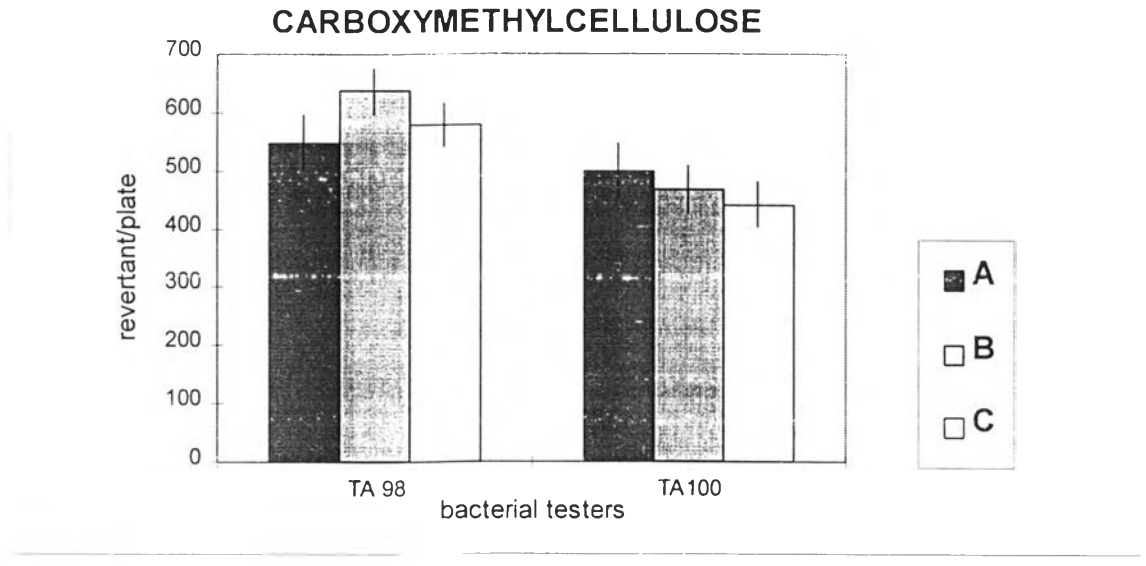

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 1600 (83) | 462.2 | 720 (32) |
| B | | 1665 (35) | | 886 (25) |
| C | | 1280 (64) | | 539 (65) |
| | Spontaneous reversion | 34 (2) | | 163 (15) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.15** The aminopyrene binding strength of agar. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



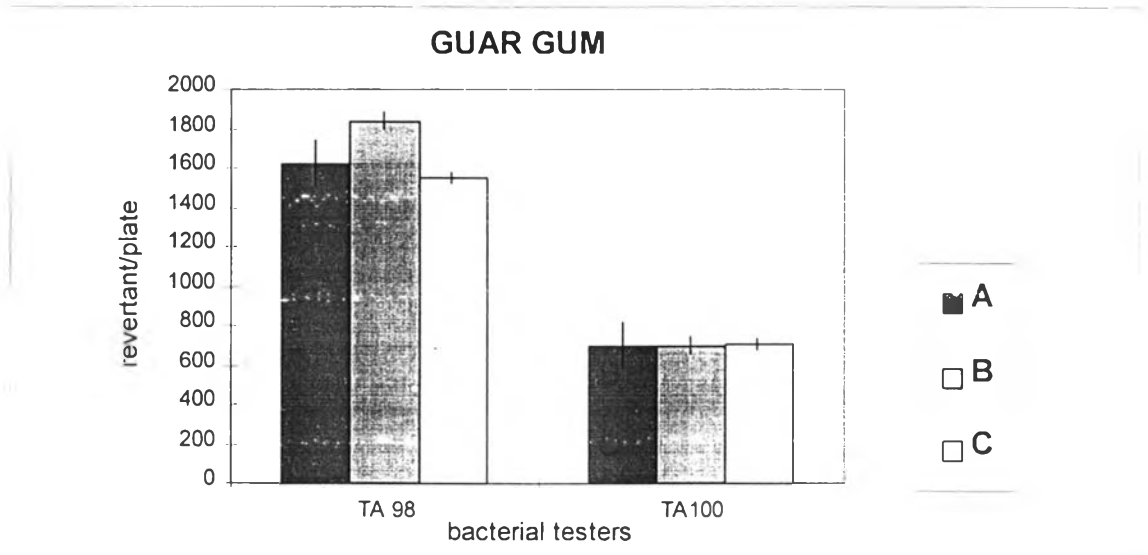

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 547 (49) | 462.2 | 500 (60) |
| B | | 636 (41) | | 467 (44) |
| C | | 578 (39) | | 441 (54) |
| | Spontaneous reversion | 44 (7) | | 125 (6) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.16** The aminopyrene binding strength of carboxymethylcellulose. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



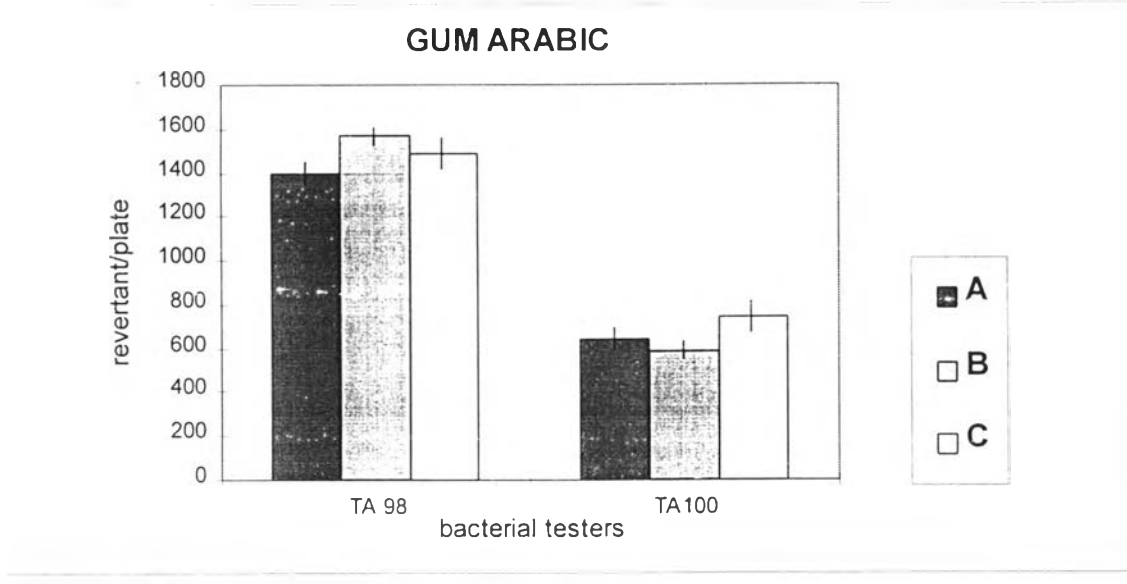

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 1622 (118) | 462.2 | 698 (77) |
| B | | 1840 (47) | | 699 (64) |
| C | | 1547 (33) | | 709 (18) |
| | Spontaneous reversion | 34 (2) | | 163 (15) |

A: Mixture of sooluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.17** The aminopyrene binding strength of guar gum. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



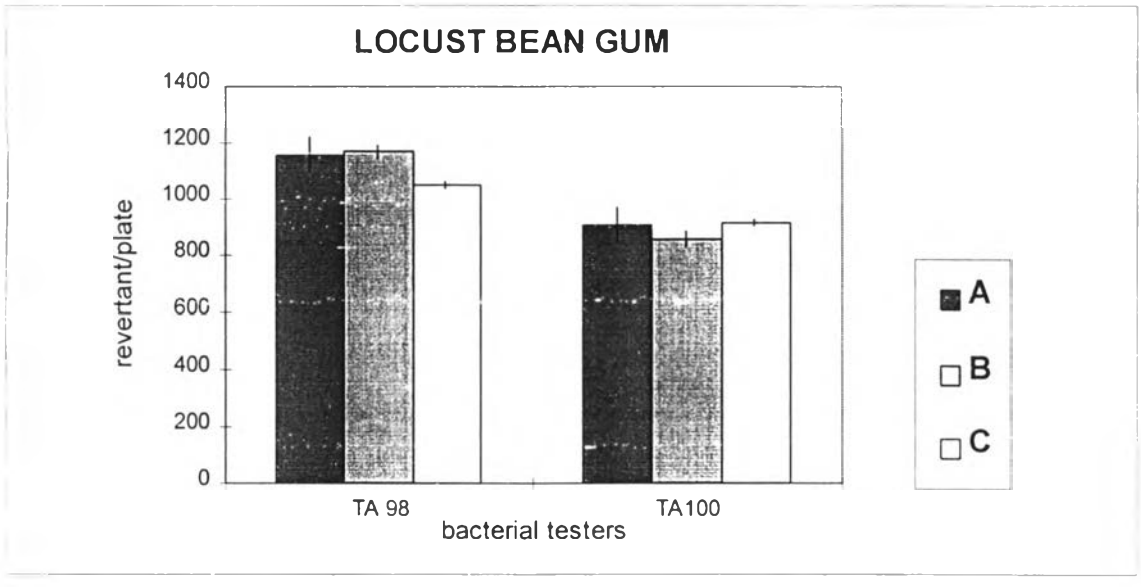

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 1399 (54) | 462.2 | 642 (34) |
| B | | 1568 (40) | | 592 (1) |
| C | | 1486 (73) | | 740 (26) |
| | Spontaneous reversion | 34 (2) | | 163 (15) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.18** The aminopyrene binding strength of gum arabic. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



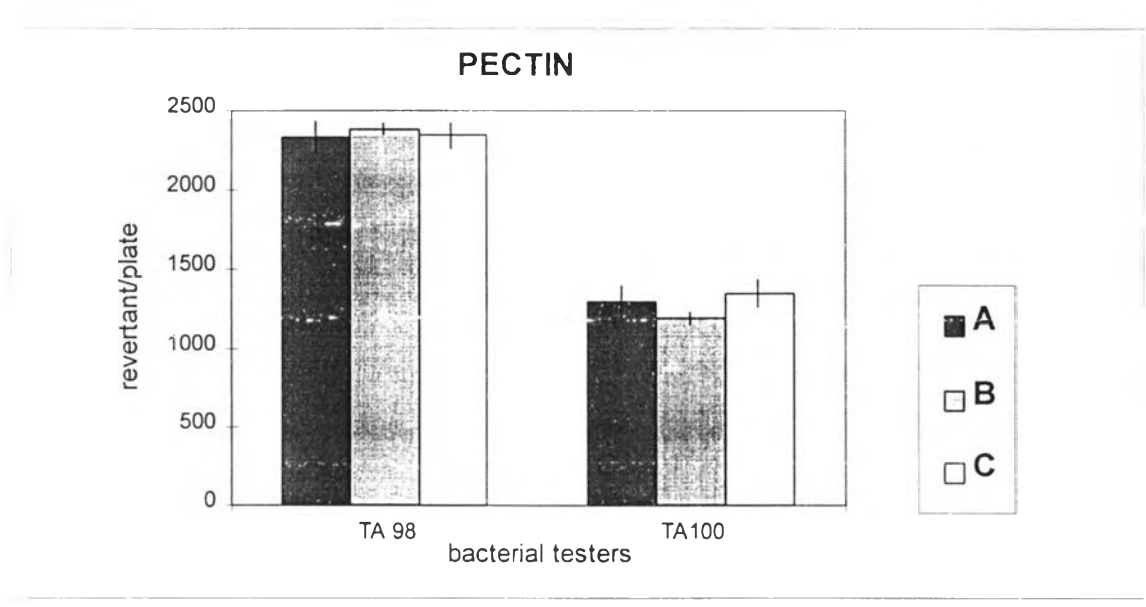

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 1158 (115) | 462.2 | 906 (62) |
| B | | 1168 (54) | | 860 (28) |
| C | | 1048 (30) | | 915 (15) |
| | Spontaneous reversion | 34 (2) | | 163 (15) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.19** The aminopyrene binding strength of locust bean gum. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



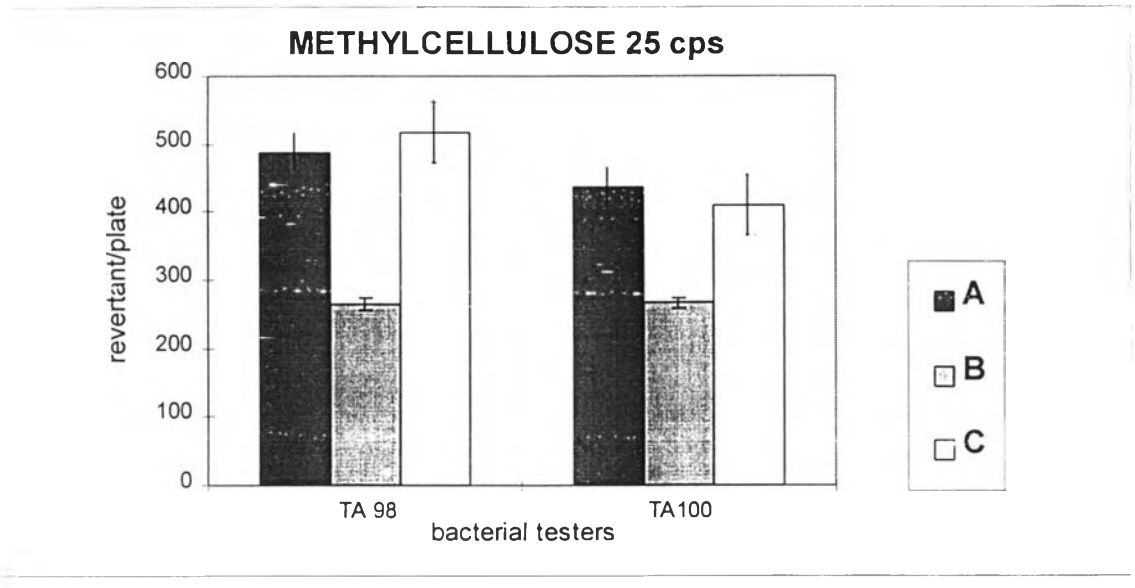

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 2335 (107) | 462.2 | 1293 (73) |
| B | | 2385 (35) | | 1195 (21) |
| C | | 2340 (85) | | 1345 (106) |
| | Spontaneous reversion | 34 (2) | | 163 (15) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.20** The aminopyrene binding strength of pectin. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



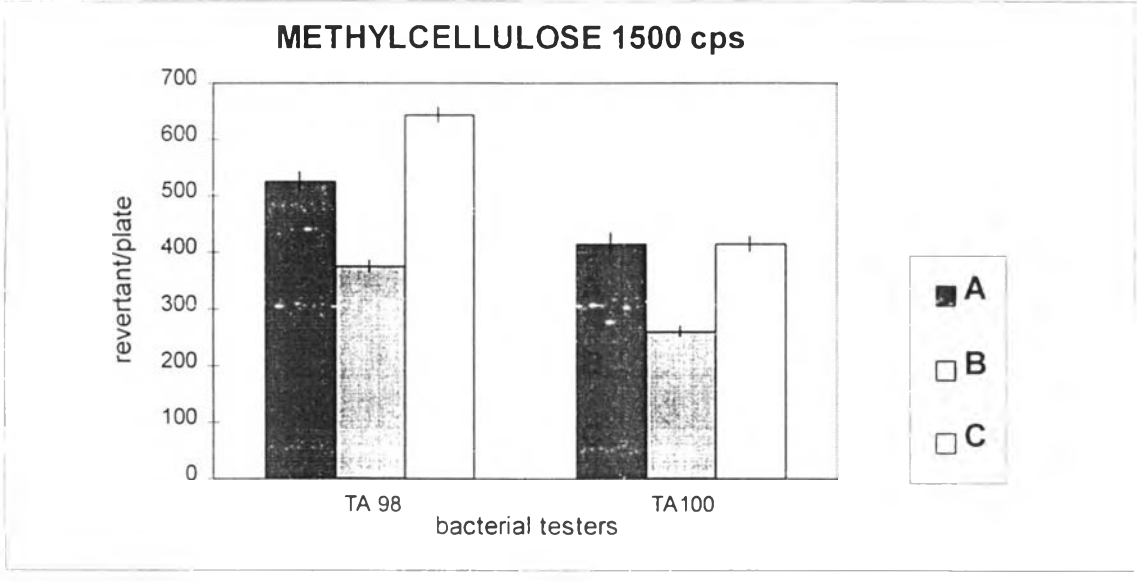

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soiuble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 488 (36) | 462.2 | 438 (29) |
| B | | 264 (8) | | 266 (7) |
| C | | 517 (45) | | 410 (44) |
| | Spontaneous reversion | 44 (7) | | 125 (6) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.21** The aminopyrene binding strength of methylcellulose 25 cps. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.



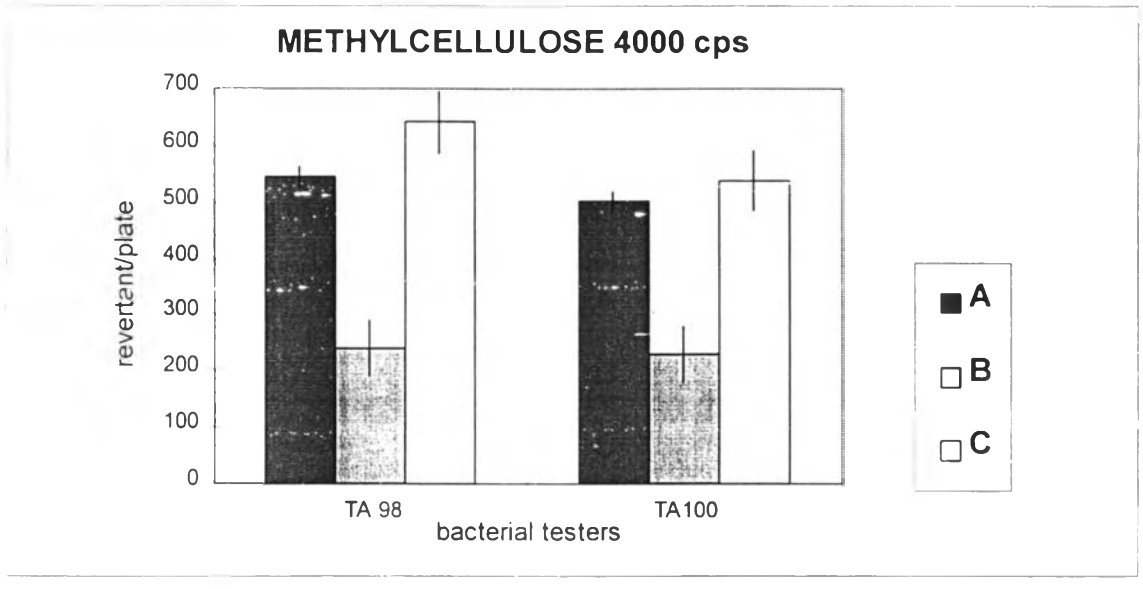

| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 524 (46) | 462.2 | 416 (18) |
| B | | 376 (47) | | 262 (10) |
| C | | 644 (13) | | 413 (14) |
| | Spontaneous reversion | 44 (7) | | 125 (6) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.22** The aminopyrene binding strength of methylcellulose 1500 cps. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.




| | TA98 | | TA100 | |
|---|---|---|---|---|
| | Amount of soluble fiber (μg/plate) | No. of revertants/plate | Amount of soluble fiber (μg/plate) | No. of revertants/plate |
| A | 184.9 | 544 (20) | 462.2 | 501 (76) |
| B | | 240 (49) | | 230 (30) |
| C | | 641 (55) | | 538 (6) |
| | Spontaneous reversion | 44 (7) | | 125 (6) |

A: Mixture of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next transferred 1 ml of the mixture to react with sodium nitrite, and, then, incubated the mixture for 2 h.

B: Remaining mixture of soluble fiber and aminopyrene (from A) was added with1 ml of sterile water pH 3.0, next incubated the mixture at 37 °C for 1 h, then transferred 1 ml of the mixture to react with sodium nitrite, and finally incubated the mixture for 2 h.

C: Second reaction mixture tube of soluble fiber and aminopyrene was incubated at 37 °C for 1 h, next discarded 1 ml of the mixture and incubated for additional 1 h; then take 1 ml of the mixture to react with sodium nitrite 2 h.

**Figure 4.23** The aminopyrene binding strength of methylcellulose 4000 cps. Data expressed as means (and standard deviations in parenthesis) of revertants per plate.