

Effect of hydrocolloids on potential availability of calcium

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Summary – Interactions between calcium ions and hydrocolloids (kappa-carrageenan, sodium alginate, pectin, carboxymethylcellulose, locust bean gum, guar gum and gelatin) were evaluated at different pH levels (1, 4, 7 and sequential 1–7). Only kappa-carrageenan and alginate were found to bind appreciable amounts of Ca ions at pH 4 and 7 because of their anionic character. However, in the case of alginate, the affinity to bind Ca^{2+} decreased with increase of this hydrocolloid concentration at pH 7. In addition, when the pH was sequentially increased from 1 to 7, kappa-carrageenan did not bind Ca^{2+} as compared to sodium alginate. On the other hand, charged hydrocolloids (pectin and carboxymethylcellulose) as well as neutral hydrocolloids (locust bean gum, guar gum and gelatin) have little or no ability to interact with calcium under the various conditions studied.

calcium / hydrocolloid / interaction / pH / potential bioavailability / solubility

Résumé — L'effet des hydrocolloïdes sur la disponibilité potentielle du calcium. Les interactions entre le calcium et certains hydrocolloïdes (kappa-carraghénane, alginate de sodium, pectine, carboxyméthylcellulose, gomme de caroube, gomme guar et gélatine) ont été étudiées à différents pH (1, 4, 7 et séquentiel 1 à 7). De tous ces hydrocolloïdes seuls le kappa-carraghénane et l'alginate montrent une forte affinité vis-à-vis des ions Ca aux pH 4 et 7 à cause de leur caractère anionique. Cependant, cette affinité diminue à pH 7, dans le cas de l'alginate, avec l'augmentation de la concentration en ce polymère. D'autre part, après traitement séquentiel du pH, le carraghénane perd la capacité de fixer les ions Ca^{2+} comparativement à l'alginate. Quant aux autres hydrocolloïdes anioniques (pectine et carboxyméthylcellulose) et neutres (gomme de caroube, gomme de guar et gélatine), ils ne montrent que très peu ou presque pas d'affinité pour le calcium dans les conditions étudiées.

calcium / hydrocolloïde / interaction / pH / disponibilité potentielle / solubilité

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INTRODUCTION

During the last two decades, interest has focussed on the role of calcium in the prevention of diseases such as osteoporosis and hypertension (Albanese, 1977; McCarron, 1985). Several studies have postulated a relationship between osteoporosis and life-long low calcium intakes (Matkovic *et al*, 1979; Sowers *et al*, 1985).

The recommended daily allowance (RDA) for calcium is presently 800 mg for adults, but more than 1 200 mg Ca per day has been suggested for premenopausal women (Heaney, 1982). To achieve an adequate Ca intake, increased consumption of milk and dairy products has been recommended.

The absorption of calcium is influenced not only by physiological factors such as pregnancy, growth and lactation, but also by dietary components such as hydrocolloids whose thickening and gelling properties are widely exploited particularly in the dairy industry (Allen, 1982; Rizzotti *et al*, 1983). The presence of these substances in food products appears to affect the availability of mineral nutrients in general. The information presently available, however, is scant.

Recent *in vitro* studies have shown that mineral solubility under physiological pH conditions of gastrointestinal system is a good indicator of their bioavailability (Miller *et al*, 1981; Zemel, 1984; Wien and Schwartz, 1985). For example, both soluble and ionic calcium levels provide a satisfactory qualitative indication of Ca availability in foodstuffs (Kim and Zemel, 1986).

Several authors have also observed that certain hydrocolloids may affect the absorption of essential minerals. Harmuth-Hoene and Schelenz (1980) showed that both carrageenan and agar-agar depressed Ca absorption in rats while neither

sodium alginate nor guar gum did. The results of *in vivo* studies with alginate are thus far contradictory, showing interference with Ca absorption in some cases (Hodgkinson *et al*, 1967; Viola *et al*, 1970), while in others no effect has been observed (Millis and Reed, 1947; Carr *et al*, 1968). Low methoxylated pectin has been found to depress drastically the absorption and retention of Ca, Mg and Zn by the growing pig (Bagheri and Guéguen, 1985). Berner and Hood (1983) reported that sodium alginate preferentially bound Ca in the presence of iron and that alginate-iron complexes were disrupted during digestion. Locust bean gum and guar gum have been found to reduce zinc and iron solubility and hence their availability (Zemel and Zemel, 1985), while Camire and Clydesdale (1981) reported that pectin (low methoxylated) in aqueous solution bound more Ca than guar gum did.

The purpose of this study is to examine the effect of some hydrocolloids such as kappa carrageenan, gelatin, guar gum, pectin, sodium alginate, carboxymethylcellulose (CMC) and locust bean gum on calcium solubility under different pH conditions.

MATERIAL AND METHODS

Gelatin (Type B, from bovine skin), pectin (7.7% methoxylated), carboxymethylcellulose (sodium salt, medium viscosity), locust bean gum, kappa carrageenan (sodium salt), alginic acid (sodium salt), calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) and lanthanum chloride ($\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$) were purchased from Sigma Chemical Co, St Louis, MO. Guar gum was obtained from ICN Biochemicals, Cleveland, OH.

Ca-hydrocolloid mixtures were prepared by dissolving 0.2 g (0.2%) or 0.4 g (0.4%) of hydrocolloid in 80 ml distilled and deionized water, adjusting the pH to 1.4, or 7 with 0.1 N HCl or 0.1 N NaOH, and adding calcium chloride solution (32.3 mg) before bringing the volume to 100 ml.

They were incubated for 2 h at 37 °C in a shaker-bath. For sequential treatments, hydrocolloid solutions containing Ca were incubated at pH 1 for 2 h at 37 °C. The samples were then brought to pH 4, incubated for 1 h and 37 °C and finally the pH was raised to 7 and samples were incubated for one additional hour at the same temperature. For each pH treatment, standards containing only Ca were prepared according to the same procedure described above. To suppress phosphate interference, 0.5 g lanthanum was added to each sample. Total Ca was determined by digesting duplicate aliquots (10 ml) of each sample in 12 ml of HNO₃-HClO₄(3:1) in Kjeldahl flasks for 45 min, analysing by atomic absorption spectrometry (AAS) (Instrumentation Laboratory Spectrophotometer, model IL751, Wil-minton, MA). The remainder (80 ml) of the incubated sample was then centrifuged at 27 000 g for 1 h at 5 °C and the supernatant filtered on ashless Whatman #1. Ten ml duplicate aliquots

of the filtered supernatant were digested and analysed for soluble Ca by AAS as described above. Two other 10 ml aliquots were brought to 100-ml with deionized water. To each of these were added 2 ml 4 M KCl solution for ionic calcium measurement using a Ca ion-selective electrode (Model 93-21, Orion Research Inc, Cambridge, MA). Analyses were performed in duplicate throughout the experiment. All data were analyzed by analysis of variance, and Duncan's Multiple Range test was used to separate significantly different group means (SAS, 1986).

RESULTS AND DISCUSSION

Results obtained with 0.2% (w/v) hydrocolloid in the presence of calcium at three

Table I. Effects of 0.2% hydrocolloid solutions on soluble and ionic calcium at three pH levels. ¹ Total calcium; ² soluble calcium; ³ ionic calcium; ⁴ carboxymethylcellulose; ⁵ locust bean gum; ⁶ CaCl₂ without gum; ^{a-f} different letters in superscript denote significant differences in each column ($P < 0.05$).

Effets de solutions d'hydrocolloides à 0,2% sur les teneurs en calcium soluble et ionique à trois niveaux de pH.

Hydrocolloids	pH 1			pH 4			pH 7		
	Ca _{tot} ¹	Ca _{sol} ² (ppm)	Ca _{ion} ³	Ca _{tot}	Ca _{sol} (ppm)	Ca _{ion}	Ca _{tot}	Ca _{sol} (ppm)	Ca _{ion}
K-carrageenan	11.50 ±0.08 ^{bc}	11.30 ±0.09 ^c	11.33 ±0.08 ^{bc}	12.50 ±0.06 ^a	8.60 ±0.00 ^e	8.75 ±0.14 ^e	11.35 ±0.09 ^{cd}	7.13 ±0.10 ^e	7.13 ±0.11 ^d
Alginate	11.23 ±0.08 ^{cd}	11.20 ±0.09 ^{cd}	11.33 ±0.08 ^{bc}	12.72 ±0.05 ^a	6.22 ±0.29 ^f	6.32 ±0.13 ^f	12.82 ±0.09 ^a	3.52 ±0.19 ^f	2.62 ±0.03 ^e
Pectin	11.80 ±0.08 ^a	11.85 ±0.09 ^a	11.90 ±0.08 ^a	11.97 ±0.11 ^b	12.20 ±0.10 ^a	11.95 ±0.09 ^a	11.75 ±0.09 ^b	11.83 ±0.10 ^a	11.63 ±0.11 ^{ab}
CM4	11.30 ±0.08 ^{cde}	11.40 ±0.09 ^{bc}	11.38 ±0.08 ^{bc}	11.40 ±0.09 ^c	11.60 ±0.12 ^{bc}	11.63 ±0.09 ^{bc}	11.43 ±0.09 ^{cd}	11.48 ±0.10 ^{bc}	11.18 ±0.11 ^c
LBG ⁵	11.43 ±0.08 ^{bcd}	11.43 ±0.09 ^{bc}	11.48 ±0.08 ^b	11.05 ±0.09 ^d	10.97 ±0.12 ^d	11.18 ±0.09 ^d	11.20 ±0.09 ^d	11.40 ±0.11 ^{bc}	11.10 ±0.11 ^c
Guar gum	11.10 ±0.08 ^e	11.10 ±0.09 ^d	11.13 ±0.08 ^c	11.33 ±0.09 ^c	11.35 ±0.10 ^{cd}	11.68 ±0.09 ^{abc}	11.25 ±0.09 ^d	11.00 ±0.10 ^d	11.13 ±0.11 ^c
Gelatin	11.65 ±0.08 ^{ab}	11.65 ±0.09 ^{ab}	11.80 ±0.08 ^a	11.90 ±0.09 ^b	11.95 ±0.10 ^{ab}	11.88 ±0.09 ^{ab}	11.58 ±0.09 ^{bc}	11.70 ±0.10 ^{ab}	11.88 ±0.11 ^a
Control ^b	11.48 ±0.08 ^{bcd}	11.30 ±0.09 ^c	11.30 ±0.08 ^{bc}	11.35 ±0.09 ^c	11.40 ±0.10 ^{cd}	11.45 ±0.09 ^c	11.35 ±0.09 ^{cd}	11.20 ±0.10 ^{cd}	11.33 ±0.11 ^{bc}

pH values are summarized in table I. At pH 1, the amounts of total, soluble and ionic calcium are substantially the same for each hydrocolloid, indicating that the ions remained in solution rather than forming insoluble complexes with any of the polymers. At this pH, the carboxyl or sulfate groups of the anionic hydrocolloids are protonated. At pH 4 both ionic and soluble Ca levels decreased substantially in the presence of carrageenan and alginate, indicating that the polysaccharide functional groups became ionized, enabling them to interact with Ca, with approximately 50 to 60% of the Ca forming insoluble complexes. The precipitation of more calcium by alginate than by carrageenan may be due to their different chemical structures: the alginate molecule consisting exclusively of mannuronic and guluronic acid units, whereas carrageenan is characterized by repeating units of 1,3 galactose sulfate and 1,4 linked 3,6 anhydro-*D*-galactose, *ie* fewer ionizable groups in the latter (Sand, 1982). Differences in *pK* may also partly explain the affinity of Ca with respect to these hydrocolloids.

Pectin, CMC, locust bean gum, guar gum and gelatin did not appear to have irreversibly bound Ca^{2+} at pH 4, since no decrease in either ionic or soluble calcium levels was observed. Kohn (1975) has reported that addition of 1.5 mM Ca to pectin solutions led to the formation of strong intermolecular chelate bonds involving carboxyl groups. Rees (1982) has suggested an egg-box structure whereby calcium ions forming chelate bonds with oxygen atoms from both galacturonan chains fit into "cavities" in the structure.

As for CMC, in spite of the presence of carboxyl groups, the molecule did not affect soluble and ionic Ca. This is probably due to the low CMC concentration used in this work. As reported by Glicksman (1982), at lower concentrations, this cellu-

lose gum shows well-defined Newtonian behavior characterized by polymer chains which are widely separated, preventing thus interactions between Ca and opposite carboxyl groups. At pH 7, neither ionic calcium nor soluble calcium levels decreased in the presence of guar gum, locust bean gum, pectin, gelatin or CMC.

In the presence of carrageenan, the percentage of both amount of soluble and ionic Ca remained the same when the pH was increased from 4 to 7. With alginate, both soluble and ionic Ca decreased to less than 80% of total Ca at neutral pH. This may be due to a higher degree of charge in the ionization of carboxyl groups as compared to the sulfate groups of carrageenan.

As shown in table II, at a hydrocolloid concentration of 0.4%, all added calcium remained ionic at pH 1. These results are similar to those observed for 0.2% hydrocolloid level at the same pH. At pH 4, however, carrageenan and alginate reduced soluble Ca levels by 72 and 44% respectively, while the other hydrocolloids did not precipitate. These reductions were more pronounced than those with 0.2% alginate or carrageenan since more ionic groups were involved at the higher concentration. It should be noted that soluble and ionic Ca were equivalent in both cases, indicating the absence of soluble Ca-alginate or Ca-carrageenan complexes. With the pH increased to 7, the amount of insoluble alginate-bound calcium decreased, approximately 89% of the total Ca remaining in the soluble state. The substantial difference between ionic and soluble Ca levels (ionic Ca level < soluble Ca level) suggests that alginate formed soluble complexes with calcium. The precipitation of less calcium by 0.4% alginate at neutral pH may be due to the formation of aggregates in which most ionized carboxyl groups of mannuronic segments were free; the Ca^{2+}

Table II. Effects of 0.4% hydrocolloid solutions on soluble and ionic calcium at three pH levels. ¹ Total calcium; ² soluble calcium; ³ ionic calcium; ⁴ carboxymethylcellulose; ⁵ locust bean gum; ⁶ CaCl₂ without gum; ^{a-f} different letters in superscript denote significant differences in each column ($P < 0.05$).
Effets de solutions d'hydrocolloïdes à 0,4% sur les teneurs en calcium soluble et ionique à trois niveaux de pH.

Hydrocolloids	pH 1			pH 4			pH 7		
	Ca _{tot} ¹	Ca _{sol} ² (ppm)	Ca _{ion} ³	Ca _{tot}	Ca _{sol} (ppm)	Ca _{ion}	Ca _{tot}	Ca _{sol} (ppm)	Ca _{ion}
K-carrageenan	11.33 ±0.09 ^{cd}	11.40 ±0.07 ^{cd}	11.68 ±0.07 ^b	11.20 ±0.09 ^e	3.10 ±0.11 ^d	2.83 ±0.12 ^e	12.37 ±0.15 ^a	5.32 ±0.09 ^e	5.35 ±0.19 ^e
Alginate	11.53 ±0.09 ^{bc}	11.63 ±0.07 ^{bc}	11.50 ±0.07 ^b	11.63 ±0.01 ^c	5.10 ±0.11 ^c	5.30 ±0.12 ^d	11.55 ±0.09 ^c	10.33 ±0.11 ^d	8.48 ±0.11 ^d
Pectin	12.23 ±0.09 ^a	12.18 ±0.07 ^a	12.38 ±0.07 ^a	12.33 ±0.09 ^a	12.45 ±0.11 ^a	12.25 ±0.12 ^a	12.30 ±0.09 ^{ab}	12.10 ±0.11 ^a	11.30 ±0.11 ^b
CM ⁴	11.08 ±0.09 ^d	11.13 ±0.07 ^e	11.13 ±0.07 ^{cd}	11.23 ±0.04 ^e	11.38 ±0.11 ^b	11.00 ±0.12 ^c	11.35 ±0.09 ^{cd}	11.15 ±0.11 ^{bc}	9.80 ±0.11 ^c
LBG ⁵	11.18 ±0.09 ^d	11.23 ±0.07 ^{de}	11.30 ±0.07 ^e	11.35 ±0.09 ^{de}	11.28 ±0.11 ^b	11.30 ±0.12 ^{bc}	11.50 ±0.09 ^c	11.15 ±0.11 ^{bc}	11.28 ±0.11 ^b
Guar gum	11.18 ±0.09 ^d	11.13 ±0.07 ^e	11.05 ±0.07 ^d	11.55 ±0.09 ^{cd}	11.38 ±0.11 ^b	11.48 ±0.12 ^b	11.43 ±0.09 ^c	11.33 ±0.11 ^b	11.28 ±0.11 ^b
Gelatin	11.73 ^b ±0.09 ^b	11.70 ±0.07 ^b	11.63 ±0.07 ^b	12.05 ±0.09 ^b	12.15 ±0.11 ^a	11.90 ±0.12 ^a	12.03 ±0.09 ^b	11.98 ±0.11 ^a	12.28 ±0.11 ^a
Control	11.28 ^b ±0.09 ^{cd}	11.28 ±0.07 ^{de}	11.25 ±0.07 ^{cd}	11.18 ±0.09 ^e	11.10 ±0.11 ^b	11.28 ±0.12 ^{bc}	11.08 ±0.09 ^d	10.98 ±0.11 ^c	11.15 ±0.11 ^b

bonding sites for gelation with alginate occurring primarily on the guluronic segments (Sand, 1982). Increasing alginate or carrageenan concentrations had different effects on Ca solubility at pH 7. At a concentration of 0.4%, carrageenan precipitated with Ca to a much greater extent than alginate did, whereas at the 0.2% level, alginate caused a much greater drop in the soluble Ca level than did carrageenan. Moreover, at pH 7, pectin and CMC reduced ionic Ca levels by 15 and 10% respectively, indicating the formation of stable soluble complexes, while locust bean gum, gelatin and guar gum did not react with Ca.

Results obtained with 0.2 and 0.4% hydrocolloid levels following stepped pH

changes from 1 to 4 and 7 are illustrated in table III. Carrageenan, pectin, guar gum, and gelatin did not precipitate calcium at either the 0.2 or the 0.4% level. As compared to results shown in tables I and II, carrageenan did not precipitate Ca when the pH was sequentially raised from 1 to 7. This may be due to differences in ionic strength of the reaction media corresponding to single and sequential pH treatments. Depolymerization of this hydrocolloid is also known to occur in acidic solutions (Black *et al*, 1965). Alginate, on the other hand, reduced both soluble Ca and ionic Ca levels approximately by 55%, suggesting that most complexes formed were insoluble. These results are similar to those obtained in tables I and II at various pHs.

Table III. Effects of 0.2% and 0.4% hydrocolloid solutions on soluble and ionic calcium following sequential pH treatment (pH 1 4 7). ¹ Total calcium; ² soluble calcium; ³ ionic calcium; ⁴ carboxymethylcellulose; ⁵ locust bean gum; ⁶ CaCl₂ without gum; a-f different letters in superscript denote significant differences in each column (*P* < 0.05).

Effets de solutions d'hydrocolloïdes à 0,2% et 0,4% sur les teneurs en calcium soluble et ionique après traitement séquentiel du pH.

Hydrocolloids	0.2% hydrocolloid			0.4% hydrocolloid		
	Ca _{tot} ¹	Ca _{sol} ² (ppm)	Ca _{ion} ³	Ca _{tot}	Ca _{sol} (ppm)	Ca _{ion}
K-carrageenan	11.55 ±0.07 ^c	11.65 ±0.08 ^{cd}	12.05 ±0.11 ^a	11.55 ±0.15 ^b	11.55 ±0.09 ^b	12.03 ±0.07 ^b
Alginate	11.75 ±0.07 ^{bc}	6.53 ±0.08 ^e	6.55 ±0.11 ^f	11.83 ±0.15 ^b	5.90 ±0.09 ^d	6.00 ±0.07 ^f
Pectin	12.00 ±0.07 ^a	12.05 ±0.08 ^{ab}	12.03 ±0.11 ^{ab}	12.55 ±0.15 ^a	12.40 ±0.09 ^a	12.68 ±0.07 ^a
CMC ⁴	11.48 ±0.07 ^d	11.60 ±0.08 ^d	10.23 ±0.11 ^e	11.50 ±0.15 ^b	11.48 ±0.09 ^b	10.00 ±0.07 ^e
LBG ⁵	11.53 ±0.07 ^{cd}	11.68 ±0.08 ^{cd}	11.40 ±0.11 ^d	11.53 ±0.15 ^b	11.58 ±0.09 ^b	11.73 ±0.07 ^c
Guar gum	11.78 ±0.07 ^b	11.85 ±0.08 ^{bc}	11.70 ±0.11 ^{bcd}	11.50 ±0.15 ^b	11.30 ±0.09 ^{bc}	11.38 ±0.07 ^d
Gelatin	11.75 ±0.07 ^{bc}	12.10 ±0.08 ^a	11.78 ±0.11 ^{abc}	12.45 ±0.15 ^a	12.53 ±0.09 ^a	12.78 ±0.07 ^a
Control ^b	11.53 ±0.07 ^{cd}	11.45 ±0.08 ^d	11.50 ±0.11 ^{cd}	11.55 ±0.15 ^b	11.18 ±0.09 ^c	11.48 ±0.07 ^d

As for CMC, soluble complexes were formed with ionic Ca level being decreased slightly by 11%.

The results of this investigation clearly indicate that the Ca binding capacities of the hydrocolloids studied were dependent upon parameters such as the type and concentration of hydrocolloid, on pH and on ionic strength. Gelatin, locust bean gum and guar gum, which are neutral polymers, did not precipitate with calcium. This is consistent with the observations of Har-muth-Hoene and Schelenz (1980) who reported that neither of the latter two gums affected Ca absorption in growing rats. In spite of their anionic character, pectin, CMC, alginate and carrageenan differ in

their ability to bind calcium during stepped pH changes. Only alginate was found to bind appreciable amounts of Ca ions in an insoluble form, while pectin, carrageenan and CMC had no effect on Ca solubility. Calcium may therefore be less available in the presence of alginate.

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