CENTRE D'ETUDE &
DE VALORISATION
DES ALGUES

CEVA

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CONTENT

This report describes results of the biodisponibility test of calcium for 2 samples : AlgaeCal product and Caltrate.

1.1- Materials and methods

Substances :
The following seaweed extracts or minerals were used :

- Caltrate 600 (Wyeth Consumer Healthcare inc.) Lot 344262 exp : 2006/07.
- Algae cal bulk powder sample fine grind (expedition : 2006/12/20).

Minerals :
Calcium : $CaCl_2$. $2H_2O$ from Fisher Scientific (Calcium chloride dihydrate)

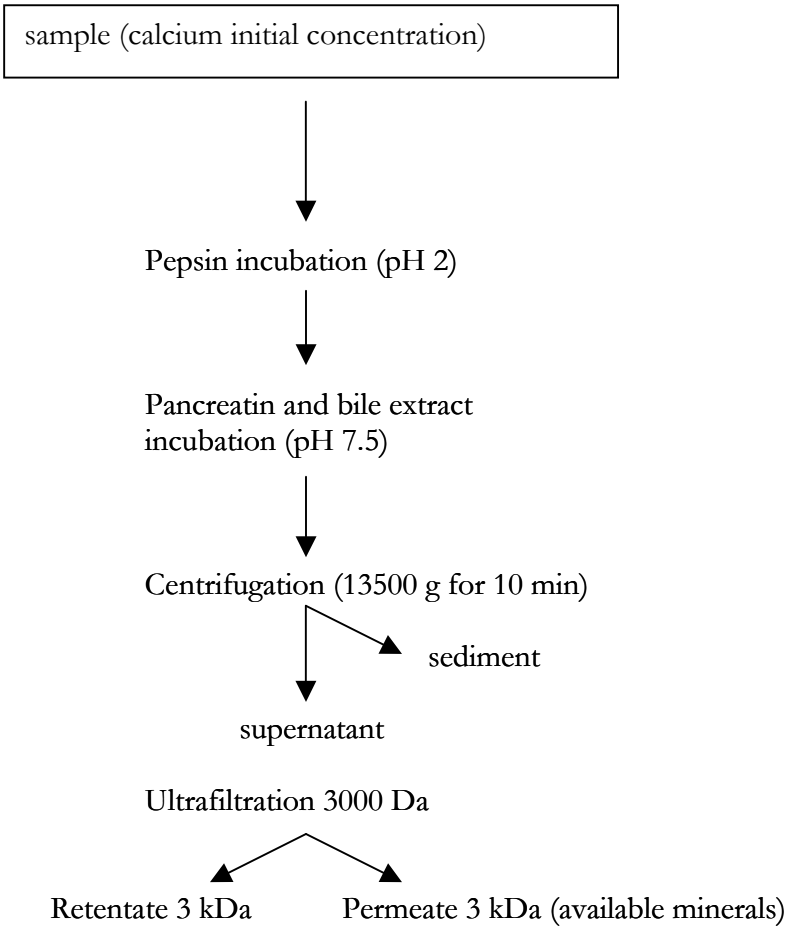
Enzyms :
Pepsin 1:2500 (from porcine stomach mucosa) from Sigma-Aldrich
Porcine bile extract from Sigma
Pancreatin (from porcine pancreas) from Sigma.

***In vitro* digestion experiment :**
The procedure was adapted from Garrett *et al.* 1999.
This technique has already been successfully used previously at CEVA in order to assess the *in vitro* chelating ability of alginates towards heavy metals (lead, mercury and cadmium) and aluminium. Results were used for the development of a new food complement called Xenosulf, which gives good *in vivo* results for human body detoxification.
The principle of the experiment is to analyze the quantity of soluble minerals (available minerals) after enzymatic and chemical hydrolysis simulating the gastrointestinal digestion.

The quantity of substances added are calculated on the basis of recommended caltrate mean intake : 600 mg to 1.2 g / day that gives 900 mg calcium per day in 2 litre of food bowl (total food volume).

Solutions of substances were prepared in saline solutions (120 mM NaCl) at the desired concentrations (calcium concentration of 450 mg/l).
The gastric digestion is simulated by acidification at pH 2 with 1M HCl and the addition of 2 mL of porcine pepsin (40 mg/mL in 0.1 M HCl). The homogenate was transferred to a clean bottle and incubated at 37°C in a water bath shaking at 95 rpm for 1 h.
Then, pH of the solution was raised to 5.3 by adding 1.0-1.3 mL of 0.9 M sodium bicarbonate followed by the addition of a mixture of bile extract and pancreatin (9 mL containing 2 mg/ml pancreatin and 12 mg/mL bile extract in 100 mmol/L sodium bicarbonate solution). Final concentrations of pancreatin and bile extract in the reaction mixture were 0.4 and 2.4 mg/mL respectively.
pH of each sample was increased to 7.5 by the addition of 1 N sodium hydroxide. Samples were incubated in shaking water bath (95 rpm) at 37°C for 2h to complete the intestinal phase of the *in vitro* digestion process.
Cooling on ice for 2 hours stopped the reaction (temperature decreased down to 4°C).
The mixture was then centrifuged (13500 g for 10 min).
The supernatant was ultrafiltered on a 3000 Da membrane in order to decrease 3-fold the initial volume.

Diafiltration was then achieved with 3 volumes of water.
Mineral analyses were then run on retentate 3kDa and permeate 3kDa.



Calcium analysis :
Total mineral concentrations in initial solutions and in permeate solutions were determined by means of flame atomic absorption spectrophotometry. Samples were diluted with lanthanum chloride to avoid phosphate interference.
Retentate solutions were mineralized before atomic absorption analysis.

1.2- Results

1.2.1- Mineral and calcium content

The mineral and the calcium content of both substances are expressed in table 1

	AlgaeCal	**Caltrate 600**
calcium g/100 g raw	30.4	31.0
dry matter g/100 g	99.6	98.4
mineral matter g/100 g	93.4	81.6

Table 1 : mineral and calcium content in AlgaeCal and in Caltrate 600

The richness of calcium in AlgaeCal is very interesting an unusual. By comparison, calcium content in *Lithothamnium calcareum* collected in France are lower :
- *Lithothamnium calcareum* from Iles des Glenans (South Britanny) is around 28-29%
- *Lithothamnium calcareum* from Brehat (North Brittany) is around 27-28%

The calcium content of caltrate 600 is lower than expected. The weight of a pill is around 1.824 g (n=20, STD = 0.0124). It means that the calcium intake by pill is precisely of 566 mg instead of 600 mg expected ("caltrate 600").

The mineral content in AlgaeCal is 93.4 g/100 g of raw product. Generally this type of algae is also very interesting for its magnesium content (not analyzed in this study). *Lithothamnium calcareum* collected in France contains 1.7 to 3.3 % magnesium. These two minerals represents the main mineral part of *lithothamnium* under carbonate form : 77 to 84 % calcium carbonate and 8 to 13% magnesium carbonate. Also minor elements mineral are present in *Lithothmanium* like (in decreasing order) : sodium, sulphur, strontium, iron, phosphorus, potassium, aluminium, manganese, boron, nickel, zinc, titanium, chromium, indium, copper (Blunden et al., 1997).
AlgaeCal contains 6.6 g organic matter /100 g of raw product. This part of organic matter is quite high. It could contain fibers and proteins. Organic content of *lithothanium* collected in France ranges from 4.2 to 6.8 % of raw product. It is important to notice that these organic matter content are higher than those found in literature (0.4 to 1.5% Augier, 1978).

The organic part of Caltrate 600 mg is also important : 18.4 g/100g raw product. This organic part is principally due to the excipient used for the coating of the pill (List of ingredients of caltrate 600: Calcium Carbonate, Maltodextin, Cellulose, Mineral Oil, Hydroxypropyl Methylcellulose, Titanium Dioxide, Polysorbate-80, Sodium Lauryl Sulfate, Magnesium Stearate, Stearic Acid, Crospovidone).

1.2.2- In vitro absorbability of calcium

The bioavailability of calcium is defined as the fraction of dietary calcium that is potentially absorbable by the intestine and can be used for physiological functions, particularly bone mineralization, or to limit bone loss (Gueguen & Pointillart, 2000). Different methods exist for measuring calcium bioavailability: from *in vitro* tests to animals or human *in vivo* tests (classical

balance studies, urinary excretion of an oral calcium load, measuring isotopes labelled in the blood, urine or bone, long-term evaluation of bone mineralization, measuring of biological markers in the blood or urine).
The *in vitro* tests can be used as the first test to predict future in vivo absorbability or to permit to do screening of different raw materials. However, the in vitro test doesn't take account the whole range of nutritional, physiological and ecological factors that influence absorption. To be more precise, we measure by our *in vitro* test the calcium absorbability and not the more general and physiological bioavailability.

After *in vitro* digestion and ultrafiltration at 3 kD the retentate and permeate are analysed for their mineral content and compared to the initial test solutions. All the data are given in table 2 and represented in figure 1.

The initial calcium concentration is calculated form the sample weight and the calcium analysis of raw substances.

		AlgaeCal	**Caltrate 600**
Initial calcium content	mg	141.5	127
Calcium content in sediment	mg	22.1	20.5
	% of initial calcium	15.6	16.1
Calcium content in permeate	mg	105.7	87.7
	% of initial calcium	74.7	69.1
Calcium content in retentate	mg	2.6	4.9
	% of initial calcium	1.8	3.8

Table 2 : Repartition of calcium in the different compartments

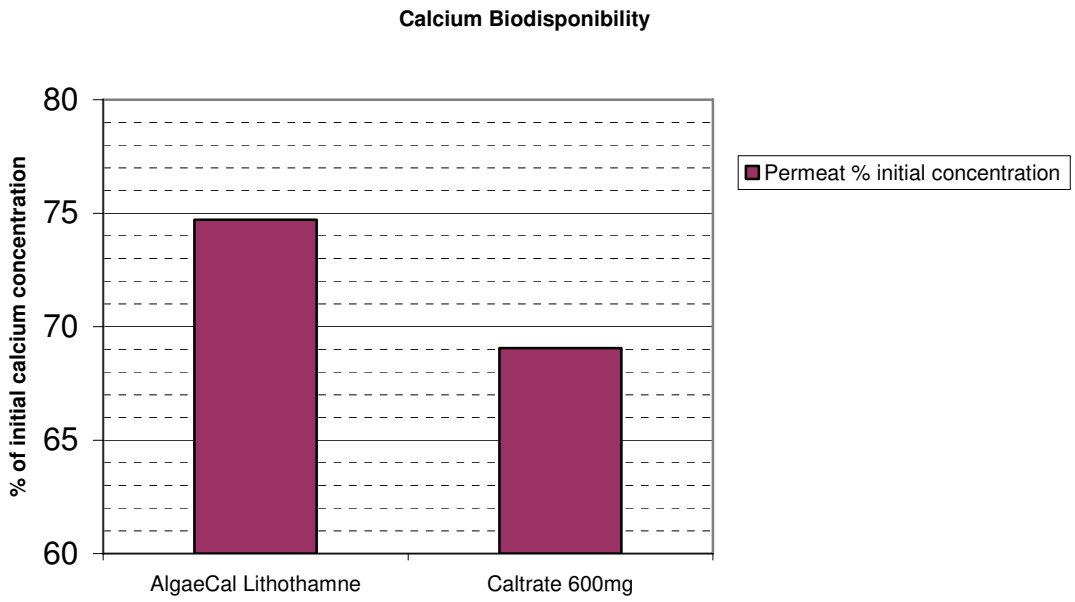


Figure 1 : *Comparison of calcium concentrations in AlgaeCal and Caltrate 600. Results are expressed as % of initial calcium content.*

Both products appear to have good calcium biodisponibility. They are found in the permeate at a concentration that accounts for about 75% of the initial concentration for Algae Cal and 69% for

Caltrate 600. The quantity of minerals going into permeate represent the quantity available for the organism. It appears that calcium biodisponibility for AlgaeCal is slightly higher than the one for Caltrate 600. Despite of a low difference between both products, it is significant for the test.

In comparison of other food calcium sources, calcium carbonate from AlgaeCal is an excellent calcium provider for the organism. We used the same test to compare calcium absorbability from different food : dairy products, vegetables and seaweeds. The most interesting food calcium sources were dairy products (especially yogurt with 43% calcium absorbable as the highest value), fruits (like orange 39%, pear 67% and cauliflower 31%) and spirulina 8%. By this *in vitro* test we showed that calcium content in spinach was not readily available by the organism because of the oxalic acid content (<0.3%).
By the same *in vitro* trial, AlgaeCal is ranked as better calcium sources than food products (even dairy products) with 75 % absorbable calcium.

To compare, a standard test realized with pure calcium chloride leads to a calcium content in the permeate representing 82% of the initial calcium content. The Caltrate 600 only reached 69% in our test. AlgaeCal reached 75%, very close to pure calcium chloride.
The majority of calcium present in AlgaeCal is readily absorbable for the organism.

1.3- Conclusion

AlgaeCal is a very interesting source of calcium because of its high and unusual calcium content : 30.4% calcium. Furthermore, it contains a quite important part of organic matter (equivalent to high values reported in *Lithothamnium* collected in France) : around 6%. It should contain mainly fibers and some proteins.
We can conclude that under physiological conditions and in the pH ranges existing in the jejuna segments (6.8-7.1), the calcium contained in AlgaeCal under carbonate calcium form is readily absorbable by the organism. We can say that AlgaeCal is a very good source of calcium for people. By this *in vitro* tests AlgaeCal appears as a better source of calcium than calcium Caltrate because of a better biodisponibility result, respectively 75% compared to 69%.
Forward-looking statement describing *in vivo* activity from these *in vitro* results may be subject to erroneous conclusions, as differences between AlgaeCal and Caltrat 600 results are low compared to natural variability of human metabolism and natural variability of AlgaeCal product.

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