

# Estimation of digestive stability and bioavailability of chlorophylls by an *in vitro* digestion/Caco-2 cell culture model



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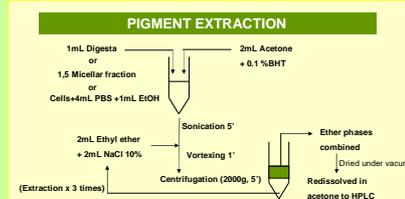
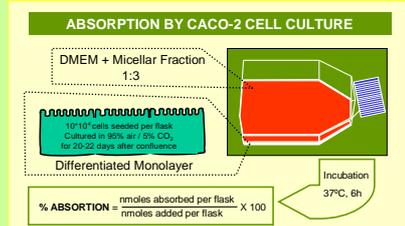
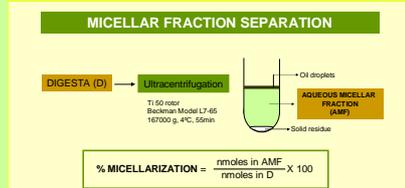
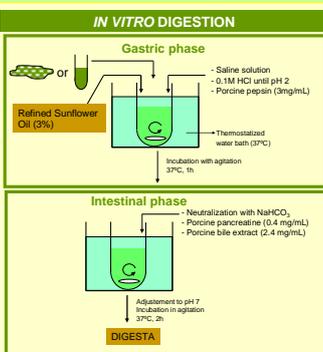
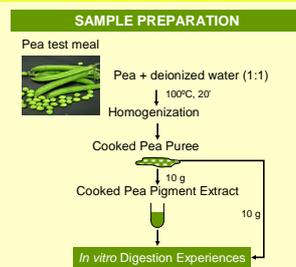


## INTRODUCTION

Epidemiological studies have consistently shown that the consumption of diets rich in fruits and vegetables is associated with a reduced risk of developing several chronic diseases such as cardiovascular disease and cancer. These evidences have taken to use important efforts in identifying the vegetal food components with biological activity, that although not being essential nutrients for the life, promote in our organism a greater protection against the implantation of certain pathologies. Within this group of compounds, generically referred as phytochemicals, the chlorophyll and carotenoid pigments stand out. These compounds enter to be part of our diet, interfere with the food, experience transformations associated to the process of the digestion and finally they are available for their absorption in the intestine. The pigment absorption takes place like the rest of lipophilic substances, in three basic steps: 1. disintegration of the ingested food matrix and liberation of pigments to gastrointestinal lumen, 2. dispersion and formation of the lipophilic emulsion and solubilization into micelles for transport and 3. incorporation by the enterocyte. Each one of these complex steps exert their influence in such way that a slight modification in any of them affects the bioavailability in a positive or negative way. Many factors have been found to affect carotenoid absorption once ingested. In some studies a smaller bioavailability of carotenoids such as beta-carotene has been attributed to the fibre presence. Although results are somewhat contradictory (depending on the pigment being studied), this fact served to establish that the rest of carotenoid components also contributes to modulating the absorbing amount. Respect to the chlorophylls very little is known about their bioavailability. Compared with carotenoids or the other phytochemicals present in the normal diet that have a relatively low chemical reactivity, chlorophylls are very susceptible to experience modifications during the digestion, preferably in four structural parts: the chelate, the ester bond of the phytol alcohol (C-17<sup>2</sup>), the isocyclic ring (C-13<sup>2</sup>) and the basic porphyrin structure.

**AIM.** The aim of this work was to study the effect of food matrix both on the chlorophyll pigment transformations and on their micellarization as a measure of bioavailability, using an two-stage *in vitro* digestion model. We also validate the above mentioned bioavailability measurement by evaluating the absorption of the micellized chlorophyll pigments by Caco-2 human intestinal cell cultures.

## MATERIALS AND METHODS



## RESULTS AND DISCUSSION

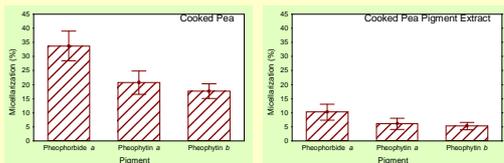
### Chlorophyll Pigment Transformation: Digestive Stability

Chlorophyll Pigment	PEA TEST MEAL (nmol in 5 g)		
	Household cooking Pea	Digesta	
		Cooked Pea	Cooked Pea Pigment Extract
Chlorophyll a*	417,71	-	-
Chlorophyllide a	7,27	-	-
Pheophytin a*	210,99	451,72	329,54
Pyropheophytin a	4,34	6,17	5,45
Pheophorbide a	9,41	5,15	1,11
Total Serie a	649,72	463,04	336,10
Chlorophyll b*	157,81	-	-
Chlorophyllide b	0,33	-	-
Pheophytin b*	36,75	182,4	157,45
Pyropheophytin b	0,02	0,98	2,22
Pheophorbide b	1,08	0,40	0,22
Total Serie b	195,99	183,78	159,88

\* Data includes epimer and allomerized derivatives

- During the gastric and intestinal simulated digestions of Cooked Pea and Cooked Pea Pigment Extract, the conversion of chlorophylls *a* and *b* in its respective pheophytins takes place, as well as certain degradation to colourless compounds, being the degradation of chlorophyll *a* greater than for *b*.
- Losses were more accused for Cooked Pea Pigment Extract (48.27% and 18.43% for chlorophylls *a* and *b*, respectively), than for Cooked Pea (28.73% and 6.23% for chlorophylls *a* and *b*, respectively).

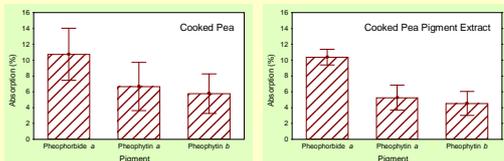
### Micellarization



Micellarization of chlorophyll pigments during digestion was:

- significantly greater ( $p < 0.0002$ ) for Cooked Pea than for Cooked Pea Pigment Extract.
- not statistically different ( $p > 0.05$ ) between Pheophytin *a*, and Pheophytin *b*, but significantly greater ( $p < 0.0002$ ) for pheophorbide *a*

### Absorption



Absorption of micellized chlorophyll pigments by Caco-2 cells was:

- not statistically different ( $p > 0.05$ ) for Cooked Pea than for Cooked Pea Pigment Extract.
- not statistically different ( $p > 0.05$ ) between Pheophytin *a*, and Pheophytin *b*, but significantly greater ( $p < 0.0002$ ) for pheophorbide *a*

## STATISTICAL ANALYSIS OF DATA

Data are expressed as mean  $\pm$  standard deviation for twelve replicates in each treatment. The data were analyzed for differences between means using one-way analysis of variance (ANOVA). The Duncan's multiple range test was used as a posthoc comparison of statistical significance. All statistical analyses were performed using the STATISTICA for Windows (StatSoft, Inc.1999). Statistical significance was set at  $p < 0.05$ .

## CONCLUSIONS

- During the gastric and intestinal simulated digestions of Cooked Pea and Cooked Pea Pigment Extract, the conversion of chlorophylls *a* and *b* in its respective pheophytins takes place, as well as certain degradation to colourless compounds, being the degradation of chlorophyll *a* greater than for *b*.
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## REFERENCES

- Garret D.A., Failla M.L., Sarama R.J. Development of an *in vitro* digestion method to assess carotenoid bioavailability from meals. *J. Agric. Food Chem.* 1999, 47, 4301-4309.
- Liu C.-S., Glahn R.P., Liu R.H. Assessment of carotenoid bioavailability of whole foods using a Caco-2 cell culture model coupled with an *in vitro* digestion. *J. Agric. Food Chem.* 2004, 52, 4330-4337.

## ACKNOWLEDGEMENTS

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