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Fate of Hydroxyapatite Nano particles during *In Vitro* Gastrointestinal Digestion

A thesis presented in partial fulfilment of the requirements for the degree of

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Abstract

There is an increasing change in population demographics towards an aging population in the world, which had led to the availability of various commercial nutritionally supplemented products. Hydroxyapatite (HA), with chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is an insoluble calcium salt used for calcium supplementation because of its similarity to the minerals found in human bone and teeth. The insoluble calcium salts are preferred over the soluble ones because of their high heat stability during milk processing under high heat treatment. However, the drawback of insoluble calcium salts is the tendency to sediment during storage resulting in unfavourable gritty texture. Thus, reduction in particle sizes into micron to nano-size improves the dispersion of these insoluble salts. However, the application of nano-sized particles in food products have raised concerns from both the regulatory organizations and consumers on the implications related to both the environmental and health safety aspects. Thus, the objective of the study is to determine the digestion behaviour of nano-sized needle/rod shaped HA (nHA) when added into skim milk during *in vitro* gastrointestinal digestion. Determination of calcium such as soluble and ionic calcium was conducted to determine the dissolution of nHA. The structural changes and the crystallographic changes of nHA were determined using electron microscopy and x-ray diffraction techniques. The results of *in vitro* gastric digestion showed presence of undissolved nHA particles even after 240 min of gastric and 120 min of intestinal digestion when examined under TEM, while the XRD analysis detected the presence of crystalline nHA in the first 120 min of gastric digestion. Thus, the possible mechanisms leading to the incomplete dissolution of nHA under acidic conditions of the stomach are discussed subsequently.

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List of figures

List of tables

List of abbreviations

°C	Degree(s) Celsius
%	Percent
CCP	Colloidal calcium phosphate
CaP	Calcium phosphate
EDS	Energy dispersive spectroscopy
g	gram(s)
GI	Gastrointestinal
h	hour(s)
HA	Hydroxyapatite
HGS	Human gastric simulator
L	Litre(s)
nHA	Nano hydroxyapatite particles
nHA blank	nHA_{blank}
nHA in milk	nHA_{milk}
mg	Milligram(s)
min	Minute(s)
ml	Millilitre(s)
mM	Millimolar (mmol.L-1)
mmol	Millimole(s)
mol	Mole(s)
Milk blank	$Milk_{blank}$
nm	Nanometre(s)
NMs	Nano materials
NPs	Nano particles
RDA	Recommended dietary allowance

SBF	Simulated biological fluid
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SM	Skim milk
TEM	Transmission electron microscopy
WP	Whey protein
WPI	Whey protein isolate
XRD	X-ray diffraction

List of appendices

Table of contents