Food Chemistry 211 (2016) 17–26

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A study by ^{1}H NMR on the influence of some factors affecting lipid *in vitro* digestion

Bárbara Nieva-Echevarría, Encarnación Goicoechea, María J. Manzanos, María D. Guillén *

Food Technology, Faculty of Pharmacy, Lascaray Research Center, University of the Basque Country (UPV/EHU), Paseo de la Universidad n° 7, 01006 Vitoria, Spain

ARTICLE INFO

Article history:
Received 11 December 2015
Received in revised form 11 April 2016
Accepted 4 May 2016
Available online 4 May 2016

Chemical compounds studied in this article:
Ammonium chloride (PubChem CID: 25517)
Glucosamine hydrochloride (PubChem CID: 2830832)
Glucose (PubChem CID: 5793)
Glucuronic acid (PubChem CID: 94715)
Glycerol (PubChem CID: 753)
Monosodium phosphate (PubChem CID: 23672064)
Sodium bicarbonate (PubChem CID: 516892)
Sodium sulfate (Pubchem CID: 24436)
Urea (PubChem CID: 1176)
Uric acid (PubChem CID: 1175)

Keywords:
^{1}H NMR
Lipolysis
In vitro digestion
Fish lipids
Bile salts

ABSTRACT

This article focuses on the impact of several experimental factors, including gastric acidification, intestinal transit time, presence of gastric lipase, sample/digestive fluids ratio, concentration and nature of the enzymes in intestinal juice, and bile concentration, on the extent of *in vitro* lipolysis when using a static model that simulates human digestion processes in mouth, stomach and small intestine. The study was carried out by Proton Nuclear Magnetic Resonance (^{1}H NMR). This technique provides a complete molecular picture of lipolysis, evidencing for the first time, whether preferential hydrolysis of certain glycerides over others occurs. A lipolysis degree similar to that reported *in vivo* was reached by varying certain variables within a physiological range; among them, bile concentration was found to be crucial. The holistic view of this ^{1}H NMR study provides information of paramount importance to design sound *in vitro* digestion models to determine the bioaccessibility and bioavailability of lipophilic compounds.

1. Introduction

In recent years, *in vitro* digestion has attracted great interest in multiple fields, including food technology and nutrition, and has become a valuable research tool in studying the bioaccessibility and bioavailability of relevant nutrients and toxic compounds (Garrett, Failla, & Sarama, 1999; Goicoechea, Brandon, Blokland, & Guillén, 2011; Roman, Burri, & Singh, 2012; Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005). However, gastrointestinal digestion is a very complex and dynamic process where ingested food components are submitted to mechanical forces and to digestive juices until they are transformed into small bioavailable molecules, some of which can also be metabolized by the gut microbiota. Thus, an accurate reflection of the human physiological environment within the digestive tract in order to mimic naturally occurring events is very difficult and the performance of *in vitro* digestion can be influenced by several experimental factors.

The *in vitro* digestion models proposed in the literature greatly differ in their complexity level, varying from static to dynamic, and from one step procedures to models that simulate sequentially all of the digestive process, that is, those taking place in the mouth, stomach and gut, including colonic fermentation (Kong & Singh, 2010; Li, Hu, & McClements, 2011; Minekus, Marteau, Havenaar, & Huis in 't Veld, 1995; Molly, Woestyne, & Verstraete, 1993; Versantvoort, Van de Kamp, & Rompelberg, 2004). Depending on the research topic and objectives of the study, a wide variety of conditions has been assayed. Therefore, differences can be

* Corresponding author.
E-mail address: mariadolores.guillen@ehu.eus (M.D. Guillén).

http://dx.doi.org/10.1016/j.foodchem.2016.05.021
0308-8146/

observed between the proportions of samples/digestive fluids, the composition of digestive juices, the transit times performed in each step, or the intensity of the mechanical forces applied (Hur, Lim, Decker, & McClements, 2011). Recently, an attempt to homogenize experimental conditions for *in vitro* digestion was made (Minekus et al., 2014). Nevertheless, the first requirement for all the *in vitro* methodologies should be to mimic *in vivo* macronutrient digestion extent (Golding & Wooster, 2010; Hur, Decker, & McClements, 2009), and for this purpose it is of paramount importance to analyze the influence of the factors affecting it.

Regarding lipid digestion, the human body shows a high efficiency for this process. After the enzymatic hydrolysis that takes place in the stomach and mainly in the small intestine, >95% of dietary triglycerides are absorbed as monoglycerides or fatty acids (Golding & Wooster, 2010). *In vitro* lipolysis levels reported in the literature are usually far lower than those occurring *in vivo,* especially with regard to fish lipids (Martin, Nieto-Fuentes, Señoráns, Reglero, & Soler-Rivas, 2010; Marze, Meynier, & Anton, 2013; Zhu, Ye, Verrier, & Singh, 2013). The high resistance of long chain polyunsaturated acyl groups to *in vitro* hydrolysis by pancreatic lipase could explain the low rates of lipolysis reported for fish oils (Bläckberg, Hernell, Bengtsson, & Olivecrona, 1979). Thus, the improvement of lipolysis under *in vitro* conditions is a challenge that deserves a deeper knowledge of the factors affecting lipase activity. Furthermore, this deeper knowledge of lipid digestion would help in the future to design food products with specific performance during digestion; that is to say, with special properties like targeted delivery.

In this context, the effect of different experimental factors on lipid *in vitro* digestion extent is studied in this paper, in order to find those conditions under which a lipolysis degree similar to that occurred *in vivo* is reached. The starting point method was that described by Versantvoort et al. (2004, 2005). Although initially designed for assessing bioavailability of food mycotoxins, it has since been employed for several purposes, mainly related to lipid research, such as the study of microstructural changes in emulsified lipids (Hur et al., 2009), the fate of toxic compounds resulting from lipid oxidation (Goicoechea et al., 2008, 2011), the influence of the cheese matrix on lipid digestion (Lamothe, Corbeil, Turgeon, & Britten, 2012), the effects of antioxidants on lipid oxidation during digestion (Tarvainen, Phuphusit, Suomela, Kuksis, & Kallio, 2012), the digestion of fish oil emulsions (Marze et al., 2013), and milk macronutrient decomposition (Kopf-Bolanz et al., 2012). Attention was paid to different experimental factors, including gastric acidification, intestinal transit time, presence of gastric lipase, sample/digestive fluids ratio, intestinal enzymes concentration and bile concentration. Their influence on the lipolysis advance was quantified by means of Proton Nuclear Magnetic Resonance (^{1}H NMR). This technique was selected because it was previously successfully employed to quantify triglycerides, diglycerides, monoglycerides, fatty acids and glycerol in lipid mixtures and to evaluate the advance of lipolysis during *in vitro* digestion (Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2014, 2015).

2. Materials and methods

2.1. Samples, reagents and enzymes

Farmed European sea bass (*Dicentrarchus labrax*) specimens were purchased from a local supermarket. After cleaning, gutting, filleting and skinning, they were submitted to *in vitro* digestion. The average weight of a fillet was 252.9 ± 22.0 g and their average lipid content was 8.2 ± 1.0% (ww).

Reagents and enzymes for the preparation of digestive juices were acquired from Sigma-Aldrich (St. Louis, MO, USA): *Aspergillus oryzae* α-amylase (10065); pepsin from porcine gastric mucosa (P7125); lipases from *Aspergillus niger* (534781) and *Candida rugosa* (62316); pancreatin from porcine pancreas (P1750); lipase type II crude from porcine pancreas (L3126) and bovine bile extract (B3883).

2.2. In vitro digestion experiments

The starting point for this study was the *in vitro* digestion model developed by Versantvoort et al. (2004, 2005) for the fed state. The composition of digestive juices (saliva, gastric, duodenal and bile) is given in Table 1. Just before the *in vitro* digestion experiments, the juices were heated to 37 ± 2 °C. The fish sample was prepared by mincing in a grinder, to simulate mechanical disintegration that occurs in the mouth. The digestion experiment started with the addition of 6 ml of saliva to 9 g of minced sea bass sample. After 5 min of incubation, 12 ml of simulated gastric juice (GJ) were added and the mixture was rotated head-over-heels at 40 rpm for 2 h at 37 ± 2 °C. Thirty minutes after starting the gastric digestion, pH was set between 2 and 3 with HCl (37%), simulating the gradual acidification of the chyme that occurs *in vivo*. After 2 h of gastric digestion, 2 ml of sodium bicarbonate solution (1 M), 12 ml of duodenal juice (DJ) and 6 ml of bile juice (BJ) were added. Subsequently, pH was set between 6 and 7, and the mixture was rotated again at 40 rpm and incubated at 37 ± 2 °C for 2 h.

The influence of some experimental factors on the fish lipolysis was evaluated. These were: gastric pH, intestinal residence time, presence of lipase in the GJ, sample/digestive fluids ratio, enzymatic composition of the DJ and bile concentration in the BJ. Although each variable can be affected by the others, the influence of each experimental factor on the lipolysis extent was studied sequentially, keeping the rest of the experimental conditions constant but including the selected conditions for the factor previously tested. This selection was made considering the improvement of lipolysis, the reflection of physiological conditions, as well as practical and economical reasons. Each digestion experiment was carried out in triplicate, except for that using a lower amount of

Table 1
Composition of the juices employed in the model described by Versantvoort et al. (2004, 2005) used as a starting point in this study.

Components	Saliva	Gastric Juice (GJ)	Duodenal Juice (DJ)	Bile Juice (BJ)
KCl (mmol/l)	12.02	11.06	7.57	5.05
NaCl (mmol/l)	5.10	47.09	119.98	89.99
$NaHCO_3$ (mmol/l)	20.17	–	40.33	68.86
NaH_2PO_4 (mmol/l)	7.40	0.22	–	–
NH_4Cl (mmol/l)	–	5.72	–	–
KH_2PO_4 (mmol/l)	–	–	0.59	–
Na_2SO_4 (mmol/l)	4.79	–	–	–
KSCN (mmol/l)	2.06	–	–	–
$MgCl_2$ (mmol/l)	–	–	0.53	–
$CaCl_2{\cdot}2H_2O$ (mmol/l)	–	2.72	1.36	1.51
HCl (37%) (ml/l)	–	6.50	0.18	0.15
Urea (mmol/l)	3.33	1.42	1.67	4.16
Glucose (mmol/l)	–	3.61	–	–
Glucuronic acid (mmol/l)	–	0.10	–	–
Uric acid (mmol/l)	0.09	–	–	–
Glucosamine hydrochloride (mmol/l)	–	1.53	–	–
Bovine serum albumin (g/l)	–	1.00	1.00	1.80
Mucin (g/l)	0.025	3.00	–	–
α-amylase (g/l)	0.29	–	–	–
Pepsin (g/l)	–	2.50	–	–
Pancreatin (g/l)	–	–	9.00	–
Pancreatic lipase (g/l)	–	–	1.50	–
Bile (g/l)	–	–	–	30.00
pH	6.8 ± 0.2	1.3 ± 0.2	8.1 ± 0.2	8.2 ± 0.2