Food Bioscience 23 (2018) 1–8

Contents lists available at ScienceDirect

Food Bioscience

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

Lipolysis kinetics of milk-fat catalyzed by an enzymatic supplement under simulated gastrointestinal conditions

Irene Peinado*, Virginia Larrea, Ana Heredia, Ana Andrés

Universitat Politècnica de València, Research Institute of Food Engineering for Development, P.O. Box: 46022, Valencia, Spain

ARTICLE INFO

Keywords:
Milk "in vitro" digestion
Milk-fat lipolysis
Kinetics
Pancreatic insufficiency
Supplementation therapy

ABSTRACT

Pancreatic insufficiency is a clinical manifestation characterized by the in-ability of the pancreas to release enough pancreatic enzyme into the small intestine, necessary to digest intraluminal nutrients. The lack of digestive enzymes leads to the difficulty to absorb nutrients, which drives in infants, to malnutrition and lack of growth and development, due to the loss of calories. These patients generally need oral administration of enzymes to favor lipolysis and absorption of lipids from foods. However, there are a number of food related factors (matrix, type of fat, etc.) and digestive environment (intestinal pH, bile concentration, among others), which will influence the digestibility of nutrients.

In this study, an "in vitro" digestion model was used to characterize the kinetics of the lipolysis of milk-fat catalyzed by an enzymatic supplement. Different intestinal conditions (pH (6, 7 and 8) and bile concentrations (1, 5 and 10 mml L^{-1})) were simulated, using a fixed concentration of supplement. Gastro-Intestinal conditions, significantly affected lipolysis. High pH and bile concentrations were translated into low values of the Michaelis-Menten constant and high values of the catalytic constant. The kinetic parameters obtained from this work allowed estimating the dose of enzymatic supplement required to optimize the lipolysis of milk-fat under different intestinal environments, sufficient and insufficient pancreatic conditions.

1. Introduction

Exocrine Pancreatic insufficiency (EPI) is an associated disorder, which occurs in several diseases including pancreatic cancer, chronic pancreatitis (CP), cystic fibrosis (CF) and because of pancreatic surgery. EPI may occur due to loss of functional parenchyma (atrophy), blockage of the pancreatic duct, or postprandial asynchrony (Sikkens, Cahen, Kuipers, & Bruno, 2010). In EPI, the obstruction of the pancreatic duct produces an insufficient secretion of sodium bicarbonate and pancreatic juice, containing digestive enzymes. Besides this lack of digestive enzymes, the decrease of pancreatic juice may also cause variations within the intestinal pH, this leading to nutrients mal-digestion and mal-absorption (Layer & Keller, 2003; Naikwade, Meshram, & Bajaj, 2009; Whitcomb et al., 2010). In this scenario, the hydrolysis and absorption of lipids are the most jeopardized, due to pancreatic lipase is the main responsible of lipolysis (Sikkens et al., 2010).

Pancreatic Enzyme Replacement Therapy (PERT) consists on the oral administration of an enzymatic supplement of exocrine pancreatin to promote nutrients digestion and absorption (Armand, Fieker & Philpott, 2011). Even tough PERT has led to a large improvement of fats digestion and absorption, satisfactory levels of fat absorption are not often achieved. While the current guidelines for CF recommend an enzyme dose of 2000–4000 Lipase Units (LU)/ g fat (Turck et al., 2016), the optimal doses are still uncertain since they depend on food factors as well as on gastrointestinal (GI) conditions. Nowadays, the only available parameters to guide health professionals on adjusting the prescribed doses are based on the overall fat content of the meals or on patients body weight (Turck et al., 2016).

Individual factors such as gastric empting time, intestinal pH, intestinal transit, etc., may affect fat digestibility (Borowitz, Gelfond, Maguiness, Heubi, & Ramsey, 2013; Rovner, Schall, Mondick, Zhuang, & Mascarenhas, 2013). The decrease of pancreatic and/or bile secretion into the small intestine is frequently observed in some GI diseases (Layer & Keller, 2003; Whitcomb et al., 2010). As a consequence, the duodenal pH becomes more acidic (around pH 6) than in healthy person (around pH 7), while bile concentration might decrease even 10 fold (1 mM) compared to a healthy adult (10 mM) (Aseeri et al., 2012; Borowitz et al., 2013).

On the other hand, factors related to foods such as fat content, type of fat, or food matrix can influence the enzyme activity. Therefore, the rate and extent of lipolysis will depend on the kinetic parameters of the enzyme for each substrate and medium characteristics. The pH-stat

* Corresponding author.
E-mail address: irpeipar@gmail.com (I. Peinado).

https://doi.org/10.1016/j.fbio.2018.02.011
Received 21 August 2017; Received in revised form 28 January 2018; Accepted 24 February 2018
Available online 26 February 2018

titration method is, in association with static systems, a classical approach that allows for monitoring the intestinal stage of "in vitro" digestions by directly providing the dynamics of the reaction (Li, Hu, & McClements, 2011; Mat, Le Feunteun, Michon, & Souchon, 2016).

Lipolysis is an interfacial reaction and where the rate of the reaction depends on the emulsion characteristics (i.e. droplet size, concentration of fat…). In the majority of studies published until this date, the amount of free fatty acids (FFAs) released under simulated intestinal conditions has been monitored using formulated emulsions. That means that the characteristics of these emulsions were known and pre-designed (i.e. fat concentration, droplet size, concentration of surfactant…) (Charoen et al., 2012; Lesmes & McClements, 2012; Mat et al., 2016; Waraho, McClements, & Decker, 2011). This approach makes sense since, in fact, lipids are most often consumed in the form of oil in water emulsions (milk, sauces…) characterized by their formulation and process conditions. However, one has to take into account that transformations during digestion may lead to changes in the oil/water interface area (Giang et al., 2015; Mat et al., 2016). Those changes will lead to unknown and maybe less favorable characteristics of the emulsions that will have different consequences on lipolysis, thus the importance of monitoring the reaction using food systems instead of predesigned model systems.

The novel approach proposed in this work is based on enzyme kinetics methodology. As stated above, health and nutritional status of patients with pancreatic insufficiency strongly depends on the precise doses of enzyme supplements. In this sense, a complex food such as milk, a processed o/w emulsion where the fat globules are dispersed within the aqueous phase, has been used to estimate the saturation substrate concentration for a certain amount of enzyme under "in vitro" simulated conditions.

The aim of the present work was to explore the above-explained approach to analyze the influence of some GI factors (pH and bile concentration) on the pancreatic lipase's affinity for milk-fat. The parameters obtained from this approach will describe the enzymatic supplement performance on milk-fat lipolysis and will contribute to better adjust the required dose for an optimal digestion.

2. Materials and methods

2.1. Materials

Pancreatic enzyme supplement (Kreon® 10,000 LU), was kindly donated by "Hospital Universitari Politècnic La Fe" (Valencia, Spain). Each capsule contains 150 mg of porcine pancreatic enzyme as gastro-resistant microspheres equivalent to 10,000 lipase units, 8000 amylase units, and 600 protease units. The other chemicals used for the "in vitro" digestion: pepsin from porcine gastric mucosa, bovine bile extract, KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$ $(H2O)_6$, $(NH_4)_2CO_3$ y $CaCl_2$ and Triton X-100 were obtained from Sigma-Aldrich Chemical Company (St Louis, MO, USA). NaOH (1, 0.1 and 0.05 N) and HCl 1 N, were acquired from *AppliChem Panreac*. Full fat milk (3.6% f.m.) was purchased at a local supermarket.

2.2. Experimental design

The digestion of emulsified lipids depends on different parameters such as their compositional and structural properties (Armand, 2007; Li & McClements, 2010; Li et al., 2011; Zhu, Ye, Verrier, & Singh, 2013), the composition and the surface area of the interface surrounding, or the droplet size (Borel et al., 1994; Li & McClements, 2010; Li et al., 2011), as well as the enzyme's affinity for the interfacial layer (Giang et al., 2015; Hur, Decker, & McClements, 2009). The velocity of the reaction might be correlated with the concentrations of substrate [S] and enzyme [E] as follows (Eq. (1)):

Table 1
Volume of milk samples (mL); Mass of milk samples (g); fat concentration on the final digestion mixture (g/L) and substrate concentration (mmol of milk-fat/L) used for each one of the pH-bile combinations.

Sample	Milk volume (mL)	Milk weight (g)	Fat concentration ($g\,L^{-1}$)	[S] ($mmol\,L^{-1}$[a])
1	1,33	1,37	1,40	1,66
2	2,70	2,78	2,66	3,24
3	4,07	4,19	3,87	4,71
4	5,38	5,54	5,00	6,02
5	6,76	6,96	6,01	7,32
6	8,07	8,31	6,95	8,46
7	10,78	11,11	8,80	10,64
8	13,50	13,91	10,32	12,58
9	16,18	16,67	11,73	14,28
10	18,86	19,43	13,00	15,82

[a] To simplify palmitic acid molar weight was used to calculate the molar concentration of milk-fat.

$$r = \frac{k_{cat} \bullet [E]_o \cdot [S]}{km + [S]} \quad (1)$$

where, r is the reaction velocity (μmol/ mL min); k_{cat} is the catalytic constant (s^{-1}); k_m is the Michaelis-Menten constant (mM), *[S]* is the substrate concentration (mM), and $[E]_O$, is the initial enzyme concentration (mM).

The kinetic parameters of the lipolysis reaction of milk-fat during the duodenal digestion processes were estimated. At this purpose, milk samples representing ten different amounts of fat substrate (Table 1) were digested for each one of the experimental conditions, intestinal pH-bile concentration. Full fat milk (3.6% f.m.) was used for the experiments (1 L package for each experiment), so the amount of fat for each experiment was calculated according to the initial amount of fat in the mil package. Furthermore, this experimental design allowed for assessing the influence of intestinal conditions (pH and bile concentration) on the lipolysis reaction. Every experimental condition was assayed at least in triplicate.

2.3. "in vitro" digestion process

The methodology used for the present study was based on the harmonized static "in vitro" protocol published by Minekus et al. (2014) with some modifications. Summarizing, for the oral stage, the milk sample was mixed with Simulated Salival Fluid (SSF) in a ratio 1:1 (v/v) at 37 °C. After that, for the gastric stage, oral bolus was mixed with the Simulated Gastric Fluid (SGF) in an oral bolus: SGF ratio of 1:1 (v/v), the pH was adjusted to 3 with HCl, and the mix was shaken at 37 °C for 2 h in an incubator chamber Selecta (JP Selecta SA, Barcelona), using an Intell-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia), to agitate the samples head- over-heels at 55 rpm. Finally, for the intestinal stage, the gastric chime was mixed with the Simulated Intestinal Fluid (SIF) to obtain a final ratio of gastric chime to SIF of 1:1 (v/v). The pH was then adjusted to 7 with NaOH and the mix continued to be shaken at 37 °C for 2 h. However, in the present study, in order to analyze the influence of the intestinal pH and bile concentration on milk-fat lipolysis, some modifications were made. Porcine pancreatin was replaced by the enzymatic supplement Kreon® (0.21 g/L, 8.27 LU/ mL in the final digestion mixture). The experimental design consisted of two different variables (pH and bile concentration) at different levels. Three different levels were used for intestinal pHs (6, 7, 8); these values were chosen as they belong within the optimum working pH for the enzyme (Kreon®), as well as for being close to the physiological duodenum conditions (Etienne-mesmin & Denis, 2012). Moreover, two levels were used for the duodenal bile concentrations (1, and 10 mM). From the different possibilities, the combination pH 7/ bile 10 mM, was considered as the standard, while pH 6/ bile 1 mM, could correspond to