

Chapter 3

Approaches to Static Digestion Models

Alan Mackie, Neil Rigby, Adam Macierzanka, and Balazs Bajka

Abstract It is not possible to look in detail at the wide range of static digestion methods that have been used to date. However, this section looks at some of the general approaches that have been used to look at the digestion of various nutrients and bioactives. I have focussed on the two main nutrients that undergo digestion in the upper GI tract, namely protein and lipid. In the case of protein, the research has largely been driven by the need to assess allergenic potential and the parameters used in such an assessment are given along with the justification provided by the authors for their choice. For the lipid digestion, we have drawn heavily upon the work of Julian McClements and colleagues who have been prolific in generating data in this area. The information provided highlights the fact that a wide range of methods are in use leading to a need for a single method, a role that can be filled by the Infogest method.

Keywords Infogest • Protein • Lipid • Allergy • Bioactive • Delivery

3.1 Introduction

Since the increase in interest in the health implications of specific foods or diets, there has also been an interest in how foods are digested and this has led to the development of a wide range of digestion methods and upper GI tract simulations. Of course the methods have been developed to address specific questions such as the allergenic potential of a protein or the delivery of fat soluble bioactives. Whilst many would argue that specific nutrients should not be considered in isolation, a reductionist approach can sometimes prove helpful. However, this does beg the question of how relevant some of the model digestion systems that have been used are to what happens in vivo after consumption of real foods. The macronutrients that are digested in the upper GI tract are protein, lipid and starch. For many reasons the digestion of both proteins and lipids have often been considered independent of

A. Mackie (✉) • N. Rigby • A. Macierzanka • B. Bajka
Institute of Food Research, Norwich Research Park, Colney Lane, Norwich NR4 7UA, UK
e-mail: alan.mackie@ifr.ac.uk

one another and the sections below will outline some of the approaches that have been used when considering the digestion of the micronutrients in isolation. In general, we would recommend that people using static simulations of digestion should use the Infogest model as described in Chap. 2 of this book. However, it is not always possible and so some of the circumstances under which other approaches may be appropriate are given below.

3.2 Static Models for Protein Hydrolysis

The ability of proteins to interact with the immune system in the gut causing intolerance such as coeliac disease and food allergy has led to a significant number of studies. One of the most highly cited articles using *in vitro* digestion is in the field of allergy. In the article by Astwood et al. (1996) a method is given for determining protein stability. In the article they used a single (gastric) phase of digestion involving simulated gastric fluid (SGF) containing pepsin at 3.2 mg/mL with an activity of 20,100 units in 30 mM NaCl at pH 1.2. The reason given for using these values was that they were “in line with recommendations from the US pharmacopeia”. When compared to the value of 2,000 U/mL at pH 3 that is recommended in the Infogest protocol, this seems very high, even if the units are not identical. Surprisingly perhaps, the Astwood article shows that proteins that were food allergens were generally not digested under these conditions. Indeed, this idea led to the use of pepsin resistance as a measure of the allergenic potential of a food protein (Eisenbrand et al. 2002). Under these circumstances it is argued that the method is merely an indicator of structural robustness rather than a precise simulation of how the protein would behave when it is consumed *in vivo*. It should perhaps be highlighted at this point that the allergenic proteins that were pepsin resistant tended to be those that were thought to sensitise via the oral route. There are a great many allergenic proteins, such as Ara h 1 (Vicillin-type 7S globulin from peanut) or Bos d 8 (casein from cows’ milk) that are very susceptible to hydrolysis by pepsin.

In studies undertaken to look at the digestion of allergenic proteins, it is common practice to add protease in a specific proportion relative to the amount of protein being digested. Certainly from the perspective of comparison it is useful to use a consistent activity of enzyme such as the 2,000 U/mL given above and a consistent protein concentration. For example in a ring trial comparing the digestion of milk proteins β -lactoglobulin and β -casein in different laboratories (Mandalari et al. 2009a), two regimes were used, a high and a low protease activity. The high protease used a pepsin activity of 10,560 U/mL based on haemoglobin as a substrate and a substrate concentration of 0.25 mg/mL, equivalent to a pepsin activity of 42,240 U/mg substrate. The low protease part used 165 U/mg of substrate. The pH used in the high protease phase was 1.2 whereas that used in the low protease phase was 2.5. The data from this study are very revealing in terms of comparison of data from different groups. As already stated elsewhere, if a comparison is to be made then the *in vitro* digestion methods employed must be standardised or at least comparable.

In this study, the methods used were nominally the same in all groups. However, the results varied significantly. For example, under the lower protease condition, β -casein was persistent for 10 min in 62 % of cases but 20 min in 26 % of cases and the remainder showed the protein persistent until either 5 or 40 min. After the simulated gastric phase the study also used a “duodenal” phase lasting 1 h at pH 7.5 or 6.5 for the high and low protease conditions respectively. For the high protease condition pancreatin was used at 12.8 mg per mg substrate and for the low protease condition, trypsin and chymotrypsin were used at 35.4 BAEE U/mg of substrate and 0.4 U/mg of substrate respectively. This is in comparison to the Infogest recommendations of 100 TAME U/mL and 25 U/mL for trypsin and chymotrypsin respectively. There is about a 100-fold difference between TAME and BAEE as a substrate with BAEE giving the higher values. The pancreatin concentration used is likely to have yielded trypsin activities around 8,000 BAEE units per mg of substrate.

In addition to different protease conditions, the study also looked at the effect of 3 mM phospholipid addition to the gastric stage of digestion. The results showed that the addition increased the resistance of β -lactoglobulin to simulated duodenal hydrolysis over 60 min. The mechanism by which this occurs was investigated in more detail in a related paper (Mandalari et al. 2009b). The authors also showed that thermal processing significantly decreased the effect. Such interactions highlight the importance of considering both the protein of interest and other components that may be present during and post consumption in vivo.

The safety assessment of genetically modified products requires consideration of various parameters including assessment of homology with known allergens using various in silico databases, IgE binding studies and resistance of the protein to digestion with simulated gastric fluid (Foster et al. 2013). In all such studies the standard approach has become the use of pepsin at 3.2 mg/mL in 0.03 M NaCl and pH 1.2 (Selgrade et al. 2009). Such amounts of pepsin will typically yield an activity of 10,560 U/mL, as indicated above. In a recent study investigating the safety of the protein osmotin, expressed in transgenic crops to enhance abiotic stress tolerance, the protein was shown to be resistant to pepsin digestion under standard conditions (Sharma et al. 2011). As result, osmotin was regarded as being a potential allergen. In addition to studying proteins for their potential detrimental effects, there has been significant study with regard to the release of bioactive peptides. For example, the group at the Institute of Food Research (CIAL) in Madrid have studied this extensively and in a recent publication they have shown the resistance of casein derived bioactive peptides. The method that they use to simulate adult digestion comprises two phases, gastric and duodenal. The gastric phase uses pepsin at 114 U/mL (11.4 U/mg of substrate) at pH 2.0 for 90 min. The small intestinal phase used Corolase (a pancreatic extract similar to pancreatin) at an enzyme to substrate ratio of 1:25. Given the pepsin activity recommended by Infogest of 2,000 U/mL this seem a little on the low side but it should be born in mind that the pH is also lower (2 rather than 3) and thus the activity of the pepsin in the actual experiment will be slightly higher (Okoniewska et al. 2000).

There has been a significant amount of study of the digestion of protein using the simulated adult gut. However, there have also been many studies of the breakdown

of milk proteins in the infant gut. For a good review of the conditions pertaining to the infant gut there is a recent article by the Bourlieu et al. (2014). This review gives a good idea of the physiological environment of the infant gut, both of premature and term infants. In a study where the digestion of protein was compared using infant and adult simulations (Dupont et al. 2010). The adult model used was similar to those given above with a gastric phase at pH 2.5, phospholipid and 182 U/mL pepsin followed by a duodenal phase at pH 6.5, containing 8 mM bile and chymotrypsin and trypsin at 0.4 and 34.5 U/mg of substrate respectively. For the infant model the following changes were made: The pH of the gastric digestion mix was adjusted at 3.0 instead of 2.5; the pepsin concentration in the gastric digestion mix was decreased by a factor of 8 and the duodenal digestion mix was altered by reducing the bile salt concentration by a factor of 4, while the PC, trypsin and chymotrypsin concentrations were reduced by a factor of 10. The proteins used for this comparison were β -lactoglobulin, β -casein and ovalbumin. One might expect that the lower concentrations of proteases used in the infant model would result in less extensive degradation of the three proteins used. Although this was found to be the case for β -casein and ovalbumin, the β -lactoglobulin was more extensively degraded by the infant than the adult digestion simulations. This was thought to be a result of the reduction in the protective effect that gastric phospholipid has on native β -lactoglobulin retarding digestion by trypsin and chymotrypsin. Surprisingly, no information is provided about the justification of the values chosen for the infant model. In a similar, more recent study of simulated gastric digestion of β -lactoglobulin and lactoferrin by a group in Israel, (Shani-Levi et al. 2013) the comparison between adult and infant used gastric pepsin activity of 240 and 210 U/mg of substrate respectively. The main difference between the two models was the way that the pH was lowered going from 6.5 to 3.5 over 4 h in the infant model as opposed to 4.5 to 1.5 over 2 h in the adult model. Needless to say there was little difference in the digestion of β -lactoglobulin but very significant differences in the persistence of lactoferrin, which is a much more labile protein.

Enzyme activity should be measured under the standard conditions recommended by the assay in order to be comparable with other measurements in the literature. However, it should be kept in mind that the activity of the enzyme on the substrate used in the simulation and under the conditions of the simulation is likely to be rather different. For this reason, the simulation should NOT aim to deliver a specific protease activity but rather to deliver a specified amount of active enzyme. This may be a subtle distinction but it has important consequences.

3.3 Static Models for Lipid Hydrolysis

In a similar way that *in vitro* digestion has been used in some cases to investigate protein digestion in isolation, a number of studies have concentrated on lipid digestion. For a review of this topic, there is an excellent article by Julian McClements (McClements and Li 2010) in which a large number of different study conditions

are given. Perhaps the main message for us from this review is that there is no consistency of approach and everyone uses their own model based on various different requirements and assumptions. Indeed, this very issue was the reason that Infogest was set up. The first stage of digestion may be considered the mouth but whilst there have been a number of studies looking at the behaviour of fat in the mouth (van Aken et al. 2007), as there is no lingual lipase produced in man there is no digestion of fat by endogenous enzymes in the oral cavity. Essentially all the work undertaken on lingual lipase has been done in rodents (Hamosh and Scow 1973) and this has led to the misconception that the same physiology applies to humans. Many studies do not include an oral phase for liquid systems containing fat (Borel et al. 1994; Fernandez et al. 2009; Hedren et al. 2002) or they include an oral phase that merely represents a resting phase after sample preparation (Beysseriat et al. 2006).

The next phase of digestion is the gastric phase containing human gastric lipase (HGL). However, this step is also often excluded from a static digestion focussed on lipolysis for a combination of reasons (Mun et al. 2007; Bonnaire et al. 2008). The most obvious reason is the pH that is being used in simulating the gastric phase is often too low for the HGL to be active as the activity drops rapidly below pH 2 (Ville et al. 2002). There is also the issue of what type of lipase to use as a substitute that has the same pH sensitivity and site specificity as HGL. Also lipolysis under gastric conditions may be considered difficult to follow as the fatty acids (FA) released are not fully dissociated and so not amenable to titration using the normal pH-stat methods. This can be corrected for at the end of the simulation by raising the pH to 9.0, assuring full dissociation of the fatty acids (Helbig et al. 2012). Those that do include a substitute HGL in their gastric simulations often opt for a fungal lipase such as that from *Rhizopus oryzae* (Day et al. 2014; Wooster et al. 2014). This lipase has been well characterised (Hiol et al. 2000), exhibits similar site-specific hydrolysis of triglycerides to that of HGL and is acid stable. However the 'optimal' pH of hydrolysis by *R. oryzae* lipase is 7.5 and the enzyme is only stable in the range pH 4.5–7.5. These values are different from the sensitivity of HGL which is said to have an apparent optimum at pH 4.5 and is still stable at pH 2 (Aloulou and Carriere 2008).

Regardless of the debate as to whether HGL or a substitute should be included in a gastric simulation, there is still the consideration of how much should be added. Recent work has used 0.2 mg/mL fungal lipase at pH 1.9 (Wooster et al. 2014), which given the activity determined by Hiol et al. (2000) of 8,800 U/mg is equivalent to 1,760 U/mL of SGF. As always the method used for the assay is important, and in this case it was against long-chain triacylglycerol plant oils and was determined with 20 mL of substrate emulsion prepared from 40 mL of oil in 400 mL of a 2 % solution of gum acacia prepared in distilled water. One lipase unit corresponded to the release one millimole of fatty acid per minute under assay conditions. This type of assay is difficult to repeat and is thus not comparable with the preferred standard method using tributyrin as a substrate (Carriere et al. 1993). The tributyrin method is preferred because the hydrolysis takes place mainly in solution and is thus not dependant on the surface area of substrate available in an emulsion. This makes it much more reproducible, at least in principle. The activity of HGL has been measured in humans

using the tributyrin assay and is around 1,000 U/mg with the activity in gastric fluid of about 100 U/mL in the fed state (Carriere et al. 1993).

In another recent study (van Aken et al. 2011), HGL was substituted by Amano Lipase A, a fungal lipase from *Aspergillus niger* that is quoted by Sigma as having an activity of 120 U/mg but the assay used is not quoted. In the article describing the study the authors go into some detail about the reason for their choice of this enzyme. The main reason for the choice was the broad pH stability meaning that the enzyme remains active at the low gastric pH. However such attention to detail is rare as the small intestine is quite correctly seen as the main site of fat digestion. Despite the lack of importance given to gastric lipolysis, it has been shown that in infants, HGL plays an important role in lipid digestion (Hamosh 1996). This is because in the neonate the production of pancreatic lipases is not fully developed. In a recent study of emulsion digestion using an infant simulation (Lueamsaisuk et al. 2014), fungal (*R. oryzae*) lipase was added at 16 U/mL and a range of pH was assessed (2, 3.5, 4.5 and 5.5). Despite the interesting results confirming the need for a full spectrum of enzymes, one of the main conclusions was the recommendation that outcomes based on in vitro digestion with fungal lipases should always be validated with at least one mammalian gastric lipase. As a final comment, we want to highlight the problems of pH sensitivity in the case of HGL substitutes. If sufficient lipase is added to a gastric simulation to give the relevant activity at the low gastric pH then when the pH is raised for the small intestinal simulation the lipase activity is likely to increase dramatically perhaps dominating the pancreatic lipases. This situation should be avoided.

As already stated the main site of fat digestion is the upper small intestine, duodenum and jejunum. This has led to simulation of this phase of digestion being the focus of most studies. There are three main factors that have been taken into account, enzymes (pancreatic lipase, colipase, etc.), bile (extract or specific composition) and pH. Starting with the simplest parameter, pH, the range of different values used is relatively narrow falling between 6.5 and 7.5 with the occasional study using values as low as pH 5.3 (Beysseriat et al. 2006), which is clearly of no physiological relevance. With this exception, values are generally physiologically relevant to the small intestine as a whole and allow the production of free fatty acids to be reasonably accurately followed by the pH-stat method (Helbig et al. 2012). In the pH-stat method the pH is monitored and any decrease caused by the formation of fatty acids is countered by addition of hydroxide. By monitoring the amount of hydroxide added, the amount of free fatty acid produced can be calculated. If the fatty acid is fully dissociated then the amount of hydroxide added is the same as the amount of fatty acid produced. Endogenous surfactants such as phospholipids and bile acids play a vital role in the hydrolysis and transport of lipids. Bile salts can adsorb onto fat droplets and can remove other materials such as proteins, emulsifiers and lipolysis products from the lipid surface (Maldonado-Valderrama et al. 2011). As a result they should be used in intestinal simulations. The question then arises as to what bile to use and the answer is not clear cut as can be seen from the recommendations in the Infogest method (Chap. 2). Table 2.2 in Chap. 2 shows an analysis of bovine, porcine and human bile using the method of Rossi et al. (1987). It is

clear from the table that whilst neither is a perfect match, the bovine bile is closer to human in composition.

3.4 Other Static Models

In addition to studying the digestion of proteins and lipids, static models of gastrointestinal digestion have been used for a range of other things. In particular, starch resistance has been studied using such models for many years (Ring et al. 1988; Wolf et al. 1999). However, the key to the functionality of resistant starch is its lack of digestion in the upper GI tract, thus the models have tended to focus on colonic fermentation. Despite this focus there have been some more recent articles that look at the digestion of starch in the upper GI tract. In an article by Wooster et al. (2014), an emulsion was combined with a range of different polymers including starch and the *in vitro* digestion simulated the small intestine with the use of pancreatin at 125 mg/mL but the activity was not assayed.

In addition to the three groups of macronutrients (protein, lipid and carbohydrate) food provides a wide range of other bioactive molecules and many of these have been studied using static simulations of the upper GI tract. For example the release of polyphenols from orange juice was assayed using a static digestion model (Gil-Izquierdo et al. 2001) in which the gastric phase was simulated for 2 h at pH 2 with 315 U of pepsin per mL of juice. The small intestinal phase was incubated for ~2.5 h at pH ~5 with 1 g pancreatin in 250 mL digesta and 6.25 g of bile extract. The conclusion was that although orange juice is a very rich source of flavanones, the concentration of compounds that are in a soluble bioaccessible form under the conditions of the small intestine, is probably much smaller but again the conditions were not those recommended.

Our final example looks at a GI simulation used to assess the iron availability from meals (Miller et al. 1981). The conditions used in this simulation are essentially identical to those used in the above simulation used to follow polyphenol release. It includes an interesting way of raising the pH between the gastric and small intestinal phases. Segments of dialysis tubing containing 25 mL water and an amount of NaHCO₃ equivalent to the titratable acidity measured previously were placed in the gastric sample. This was then sealed incubated in a 37 °C shaking water bath until the pH reached about 5 (approximately 30 min). This method provides the gentle rise in pH necessary for working with minerals. However the final pH is rather low compared to what might be expected *in vivo*. This method also highlights approaches that tend to be used out of context and one could argue that this might not be the most appropriate simulation for following the release of polyphenols from orange juice. When using a static model of digestion the parameters used should be appropriate to the question and physiologically relevant.

Open Access This chapter is distributed under the terms of the Creative Commons Attribution Noncommercial License, which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Aloulou A, Carriere F (2008) Gastric lipase: an extremophilic interfacial enzyme with medical applications. *Cell Mol Life Sci* 65(6):851–854
- Astwood JD, Leach JN, Fuchs RL (1996) Stability of food allergens to digestion in vitro. *Nat Biotechnol* 14(10):1269–1273
- Beysseriat M, Decker EA, McClements DJ (2006) Preliminary study of the influence of dietary fiber on the properties of oil-in-water emulsions passing through an in vitro human digestion model. *Food Hydrocolloids* 20(6):800–809
- Bonnaire L, Sandra S, Helgason T, Decker EA, Weiss J, McClements DJ (2008) Influence of lipid physical state on the in vitro digestibility of emulsified lipids. *J Agric Food Chem* 56(10):3791–3797
- Borel P, Armand M, Ythier P, Dutot G, Melin C, Senft M, Lafont H, Lairon D (1994) Hydrolysis of emulsions with different triglycerides and droplet sizes by gastric lipase in-vitro – effect on pancreatic lipase activity. *J Nutr Biochem* 5(3):124–133
- Bourliou C, Ménard O, Bouzerzour K, Mandalari G, Macierzanka A, Mackie AR, Dupont D (2014) Specificity of infant digestive conditions: some clues for developing relevant in vitro models. *Crit Rev Food Sci Nutr* 54(11):1427–1457
- Carriere F, Barrowman J, Verger R, Laugier R (1993) Secretion and contribution to lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology* 105(3):876–888
- Day L, Golding M, Xu M, Keogh J, Clifton P, Wooster TJ (2014) Tailoring the digestion of structured emulsions using mixed monoglyceride–caseinate interfaces. *Food Hydrocolloids* 36:151–161
- Dupont D, Mandalari G, Molle D, Jardin J, Leonil J, Faulks RM, Wickham MS, Mills EN, Mackie AR (2010) Comparative resistance of food proteins to adult and infant in vitro digestion models. *Mol Nutr Food Res* 54(6):767–780
- Eisenbrand G, Pool-Zobel B, Baker V, Balls M, Blaauboer BJ, Boobis A, Carere A, Kevekordes S, Lhuguenot JC, Pieters R, Kleiner J (2002) Methods of in vitro toxicology. *Food Chem Toxicol* 40(2–3):193–236
- Fernandez S, Chevrier S, Ritter N, Mahler B, Demarne F, Carriere F, Jannin V (2009) In vitro gastrointestinal lipolysis of four formulations of piroxicam and cinnarizine with the self-emulsifying excipients Labrasol (R) and Gelucire (R) 44/14. *Pharm Res* 26(8):1901–1910
- Foster ES, Kimber I, Dearman RJ (2013) Relationship between protein digestibility and allergenicity: comparisons of pepsin and cathepsin. *Toxicology* 309:30–38
- Gil-Izquierdo A, Gil MI, Ferreres F, Tomas-Barberan FA (2001) In vitro availability of flavonoids and other phenolics in orange juice. *J Agric Food Chem* 49(2):1035–1041
- Hamosh M (1996) Digestion in the newborn. *Clin Perinatol* 23(2):191
- Hamosh M, Scow RO (1973) Lingual lipase and its role in digestion of dietary lipid. *J Clin Invest* 52(1):88–95
- Hedren E, Diaz V, Svanberg U (2002) Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *Eur J Clin Nutr* 56(5):425–430
- Helbig A, Silletti E, Timmerman E, Hamer RJ, Gruppen H (2012) In vitro study of intestinal lipolysis using pH-stat and gas chromatography. *Food Hydrocolloids* 28(1):10–19. doi:10.1016/j.foodhyd.2011.11.007
- Hiol A, Jonzo MD, Rugani N, Druet D, Sarda L, Comeau LC (2000) Purification and characterization of an extracellular lipase from a thermophilic *Rhizopus oryzae* strain isolated from palm fruit. *Enzyme Microb Technol* 26(5–6):421–430
- Lueamsaisuk C, Lentle RG, MacGibbon AKH, Matia-Merino L, Golding M (2014) Factors influencing the dynamics of emulsion structure during neonatal gastric digestion in an in vitro model. *Food Hydrocolloids* 36:162–172
- Maldonado-Valderrama J, Wilde PJ, Macierzanka A, Mackie AR (2011) The role of bile salts in digestion. *Adv Colloid Interface Sci* 165(1):36–46

- Mandalari G, Adel-Patient K, Barkholt V, Baro C, Bennett L, Bublin M, Gaier S, Graser G, Ladics GS, Mierzejewska D, Vassilopoulou E, Vissers YM, Zuidmeer L, Rigby NM, Salt LJ, Defernez M, Mulholland F, Mackie AR, Wickham MSJ, Mills ENC (2009a) In vitro digestibility of beta-casein and beta-lactoglobulin under simulated human gastric and duodenal conditions: a multi-laboratory evaluation. *Regul Toxicol Pharmacol* 55(3):372–381
- Mandalari G, Mackie AM, Rigby NM, Wickham MS, Mills EN (2009b) Physiological phosphatidylcholine protects bovine beta-lactoglobulin from simulated gastrointestinal proteolysis. *Mol Nutr Food Res* 53(Suppl 1):S131–S139
- McClements DJ, Li Y (2010) Review of in vitro digestion models for rapid screening of emulsion-based systems. *Food Funct* 1(1):32–59
- Miller DD, Schricker BR, Rasmussen RR, Vancampen D (1981) An in vitro method for estimation of iron availability from meals. *Am J Clin Nutr* 34(10):2248–2256
- Mun S, Decker EA, McClements DJ (2007) Influence of emulsifier type on in vitro digestibility of lipid droplets by pancreatic lipase. *Food Res Int* 40(6):770–781
- Okoniewska M, Tanaka T, Yada RY (2000) The pepsin residue glycine-76 contributes to active-site loop flexibility and participates in catalysis. *Biochem J* 349:169–177
- Ring SG, Gee JM, Whittam M, Orford P, Johnson IT (1988) Resistant starch – its chemical form in foodstuffs and effect on digestibility in vitro. *Food Chem* 28(2):97–109
- Rossi SS, Converse JL, Hofmann AF (1987) High-pressure liquid-chromatographic analysis of conjugated bile-acids in human bile – simultaneous resolution of sulfated and unsulfated lithocholyl amides and the common conjugated bile-acids. *J Lipid Res* 28(5):589–595
- Selgrade MK, Bowman CC, Ladics GS, Privalle L, Laessig SA (2009) Safety assessment of biotechnology products for potential risk of food allergy: implications of new research. *Toxicol Sci* 110(1):31–39
- Shani-Levi C, Levi-Tal S, Lesmes U (2013) Comparative performance of milk proteins and their emulsions under dynamic in vitro adult and infant gastric digestion. *Food Hydrocolloids* 32(2):349–357
- Sharma P, Singh AK, Singh BP, Gaur SN, Arora N (2011) Allergenicity assessment of osmotin, a pathogenesis-related protein, used for transgenic crops. *J Agric Food Chem* 59(18):9990–9995
- van Aken GA, Vingerhoeds MH, de Hoog EHA (2007) Food colloids under oral conditions. *Curr Opin Colloid Interface Sci* 12(4–5):251–262
- van Aken GA, Bomhof E, Zoet FD, Verbeek M, Oosterveld A (2011) Differences in in vitro gastric behaviour between homogenized milk and emulsions stabilised by Tween 80, whey protein, or whey protein and caseinate. *Food Hydrocolloids* 25(4):781–788
- Ville E, Carriere F, Renou C, Laugier R (2002) Physiological study of pH stability and sensitivity to pepsin of human gastric lipase. *Digestion* 65(2):73–81
- Wolf BW, Bauer LL, Fahey GC (1999) Effects of chemical modification on in vitro rate and extent of food starch digestion: an attempt to discover a slowly digested starch. *J Agric Food Chem* 47(10):4178–4183
- Wooster TJ, Day L, Xu M, Golding M, Oiseth S, Keogh J, Clifton P (2014) Impact of different biopolymer networks on the digestion of gastric structured emulsions. *Food Hydrocolloids* 36:102–114