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Sangeeta Prakash, Qian Ma, Bhesh Bhandari

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Rheological behaviour of selected commercially available baby formulas in simulated human digestive system

Sangeeta Prakash*, Qian Ma and Bhesh Bhandari

School of Agriculture and Food Sciences, The University of Queensland, 4072, Australia.

*Correspondence author's email address: s.prakash@uq.edu.au

Abstract

A variety of formulas are available in the global market for infants (< 12 months old) who are not having access to mother's milk. The rheological properties of four different commercially available infant formulas – new born, anti-reflux, soy and lactose free, in an *in vitro* digestive system were investigated. The enzymatic saliva when mixed with the formulas did not influence their viscosity in the mouth possibly due to the short residence time. Systematic measurement (every 15 minutes) of viscosity during gastrointestinal digestion process revealed a decrease in viscosity as time progressed. The most interesting observation was that the viscosity of the anti-reflux formula was relatively higher compared to the other formulas throughout the simulated gastro intestinal digestion process. The results suggest that viscosity of the infant formula in the stomach may have a role to play in preventing gastroesophageal reflux.

Keywords

Rheology, particle size distribution, in-vitro digestion, gatro intestinal environment, soy, lactose-free, anti-reflux

Highlights

- Rheometer monitors the flow behaviour from mouth, through to stomach and intestine
- *In vitro* gastrointestinal digestion of infant formulas in a rheometer revealed a decrease in viscosity over time.
- Enzymatic saliva does not influence the viscosity of formulas in the mouth

1. Introduction

It is well known that mother's milk is the best nutrition for infants providing all the nutritive elements either for normal growth or for infants' digestive conditions. On an average (solid basis), breast milk consists of 42% carbohydrate (mainly lactose), 52% fat, 6% highly digestible protein and a number of protective and immunoregulatory components (Alles, Scholtens, & Bindels, 2004). However, when breast milk is unavailable or insufficient, infant formula is a vital substitute or supplement to guarantee infants' growth and development. The world infant formula represents 40% of the entire baby food market (Blanchard, Zhu, & Schuck, 2013). There is a worldwide demand for infant formula with an average growth of 8.2% per year and it is expected to increase to over 20% per year on average (Blanchard et al., 2013). A variety of formulas are available for infants less than 12 months old who are not drinking breast milk or consume as a supplement. Infant formulas vary in nutrients, calorie count, taste, and ability to be digested depending on the age of infant. The basic constituents of standard infant formula are carbohydrates, proteins, lipids, minerals, vitamins and nucleotides and is primarily made from milk of cow and/or other ingredients which have been proven to be suitable for infant feeding (Koletzko et al., 2005). For babies suffering from cows' milk allergy (intolerance to protein) and lactase deficiency (intolerance to carbohydrate lactose) there are soy based infant formula and lactose free infant formulas, respectively. The anti-reflux infant formula is thickened with starch or hydrocolloids (carob bean gum or κ-carrageenan) for infants who show gastroesophageal reflux. The range of carbohydrate, fat and protein (soy or cow) per 100 g solids of infant formulas found is 42-84g, 26.4-39g, and 10.8-19.00g respectively (Nasirpour, Scher, & Desobry, 2006).

The digestive system or gastrointestinal tract consists of the mouth, oesophagus, stomach, small and large intestines, along with glands like salivary glands, liver, pancreas, that secrete digestive juices including enzymes. The ingested fluid undergoes

digestion as it proceeds from mouth through to the stomach and intestine. Whole saliva secreted in the *mouth* is a dilute, viscous solution containing (infant saliva has a pH 6.0-6.2) electrolytes (sodium chlorides and sodium bicarbonate), proteins (mucin, α-amylase, lingual lipase and proline-rich proteins) and epithelial cells from the oral mucosa. The salivary α-amylase and the lingual lipase enzyme initiate the digestion of carbohydrate and lipids, respectively in the mouth but with no reported work is found about their effects. The chemical digestion begins in the *stomach*, wherein digestion of protein by pepsin occurs. Approximately 1–3 litres of gastric juice is produced in the stomach of an adult per day containing hydrochloric acid (pH ~1), water, salts, pepsin, mucous and bicarbonate ions (Campbell, 2012; Liang et al., 2010). HCl is produced by the parietal cells and gives gastric juice its characteristic acidic pH (pH 1–2) and chief cells secrete the digestive enzymes, pepsinogen and gastric lipase (Liang et al., 2010). Some studies have reported the infant stomach being at pH 4-5 unlike adult stomachs where the pH drops to < 2 (Li-Chan & Nakai, 1989; Rudloff & Lönnerdal, 1992). *In vitro* digestion study by Dupont et al. (2010) suggest protein digestion in adult and infant are identical, except that the protein hydrolysis rate for infants is slower due to the lower enzyme concentration. *Small intestine* consists of duodenum, jejunum and ileum. When fluid food reaches the duodenum it undergoes further enzymatic digestion and is subjected to pancreatic juice (secreted by pancreas and drained into duodenum), intestinal juice, and bile, the intestinal digestion takes place in an alkaline environment, pH 7.0-8.5 (Kaczkowski, 2002). Pancreatic juice contains three enzymes which break down carbohydrates (by amylase), lipids (by lipase, cholesterol esterase, phospholipase) and proteins (by trypsin, chymotrypsin). In the intestinal juice, there are enzymes to further break down peptides and disaccharide sugars like aminopeptidases, disaccharidases. The chymus then enters the jejunum where the digested breakdown products of carbohydrates, fats, proteins and most of the vitamins, minerals and iron are absorbed (Kaczkowski, 2002). The pancreatic lipase and bile salt concentration required for digestion of fat are very low in infants compared to adults (Lebenthal, Lee, &

Heitlinger, 1983; Lindquist & Hernell, 2010). Gastric lipolysis plays an important role in fat digestion for newborns than for adults. Salivary amylase are active in adult stomach but very low in new born babies (Rossiter, Barrowman, Dand, & Wharton, 1974), however babies are able to digest a reasonable amount of starch, due to the presence of significant concentration of glucoamylase or amyloglucosidase in the small intestinal mucosa, with its activity over 50% that of adults. Therefore, glucoamylase may become an alternate enzyme for starch toleration in infants (Lebenthal et al., 1983).

Formula-fed infants derive nutrition from reconstituted formula through digestion. Various physiological complications may prevent absorption of nutrients from formula like lacking of coordination in sucking and swallowing. Swallowing problems may originate from the characteristics rheological properties of food (Dantas et al., 1990). Viscosity of the milk influences its safe transport from the mouth to the stomach (Sopade, Halley, Cichero, and Ward (2007). It has been confirmed that thin fluid can cause a lack of coordination among sucking, breathing and swallowing in infants (Almeida, Almeida, Moreira, & Novak, 2011). The rheological property of the formulas may affect the absorption of the nutrients, bowel movement and clearing in the digestive tract. As per Graham (2006), use of thickening agents in infant formulas can reduce the gastro-oesophageal reflux. Thus, the knowledge of rheological properties can be valuable for design and evaluation of the infant formula for infants.

There are limited published reports in the literature (Infante-Pina, Lara-Villoslada, López Ginés, & Morales Hernández, 2010; Rottoli, Decarlis, Gianni, & Giovannini, 1997) about infant milk formulas and their rheological properties but no work has been reported on the flow behaviour of the infant formula in the digestive environment. So, the main focus of the current work is to study the rheological properties of selected typical infant formulas in a simulated digestive environment. This will provide insight into differences in the rheological behaviour of infant formulas with different formulations through gastrointestinal tract. During digestion, particle size distribution of

infant formula solids is subjected to transformation due to enzymatic breakdown. The physical state of various components in infant formula will be in colloidal, emulsion and soluble solution forms. In the process of digestion, these components can go through aggregation, dispersion and complete solubilisations (Singh, Ye, & Horne, 2009). Measurements of particle size distribution may show how different formula behaves in the digestive system. Since particle size influences the rheological behaviour of a product, the particle size distribution of infant formulas was also determined as it passed through the digestive system.

2. Materials and Methods

Infant Formula: Four commercially available infant formulas - Anti-reflux, new born, lactose free and soy having similar shelf life were purchased from the local supermarket. The main ingredients and nutrition of different formulas are as shown in Table 1.

Reconstitution of powder infant formula samples: The liquid formula was prepared from the powders as per the instruction provided on the containers. 8.7g of infant formula (solid weight) was dissolved in 60 mL of boiled deionised water in a 100 mL beaker by constant stirring with a magnetic stirrer. The sample was then held in a water bath maintained at 37°C.

Preparation of artificial saliva: Artificial saliva was prepared from $NaHCO_3$, K_2HPO_4 $3H_2O$, NaCl, KCl, $CaCl_2$, mucin and α-amylase as described by (Hong, Duncan, Dietrich, & O'Keefe, 2006). The pH of the artificial saliva was adjusted to 6.8 with drop wise addition of 6N HCl solution. Artificial saliva was used within 1 hour of formulation.

Preparation of simulated gastric fluid and intestinal fluid: Simulated gastric fluid was prepared from NaCl, HCl, pepsin while simulated intestinal fluid was prepared from K_2HPO_4, NaOH and NaCl, Bile extract and pancreatin as described by Gallier, Ye, and

Singh (2012).

Digestion in mouth, stomach and intestine: The digestion procedure adapted in artificial saliva, simulated gastric and intestinal fluid are as described in the flow diagram (Figure 1). As previously reported the infant stomach pH is between 4 -5, however, in this study the pH in stomach was brought to 1.5 with hydrochloric acid. Prior to intestinal digestion the pH was adjusted to 7 with sodium hydroxide solution.

Rheological measurement: The rheological properties of the samples were characterized at 37 °C using a Discovery HR1 rheometer (TA instruments UH Ltd., U.K.), fitted with 40mm cone plate and a gap of 50μm. Flow behaviour of the infant formula samples was determined by shear rate sweep (0.05-500 1/s). For the flow behaviour of the infant formula during digestion samples were collected every 15 minutes from the digestion bath and the rheological property was measured (peak hold for 300 s duration at a shear rate of 50 1/s). It is assumed that the shear rate in the digestive tract is within this range, although there will be variability of shear rate in mouth, stomach and intestine during the movement of food. For the consistency of the measurement and relative comparison, a single shear rate (50 1/s) was chosen (Cichero, Nicholson, & Dodrill, 2011). All measurements were run in triplicate for different infant formula and digested samples.

Particle size measurement: A Malvern MasterSizer MSE was used to determine the average particle size distribution of infant formula and digested samples. The samples were diluted in water in the measurement cell of the equipment until 11% obscuration was reached. The refractive index was set to 1.4. Mean particle diameters were calculated as the average of duplicate measurements and the measurements were run in triplicate on different milk samples and digested milk samples. The volume fraction representing the particle size range is presented as cumulative data.

3. Results and Discussions

3.1. Rheological behaviour of infant formula

A plot of the shear stress over shear rate (Figure 2) shows a decreasing curve slope that represents shear thinning behaviour for the four reconstituted infant formulas at 14.5% solids concentration.

The data was fit in to Ostwald/de Waele or Power law $\tau = K \times \gamma^n$ where τ = shear stress in Pa; γ = shear rate in s^{-1}; K = consistency index in Pa-s; n = power-law index or flow behaviour index (dimensionless). Power law model was able to represent the experimental data reasonably well with a R^2 value of 0.99, which is a measure of the goodness of fit. The model parameters are as shown in Table 2. The anti-reflux formula shows Yield stress (degree of force required to initiate flow).

The flow behaviour index (n) for all the infant formulas is close to 1 that suggests Newtonian behaviour (Chhabra, 2010) although at low shear rate non Newtonian behaviour is observed. The other constant, K (consistency index), which is a measure of the consistency of the fluids shows that lactose free, new born and soy infant formulas are of similar consistency while antireflux infant formula has a different consistency.

For all infant formulas, the viscosity decreased with increasing shear load (Figure 3) at 37°C. The different infant formulas exhibited non-Newtonian shear thinning behaviour as in Table 1. Therefore, the apparent viscosity must decrease with the shear rate. At higher shear rate (>1 1/s) all the infant formulas displayed Newtonian behaviour. The apparent viscosity of Anti-reflux infant formula was higher compared to the other infant formulas (newborn, lactose free and soy) and was non-Newtonian even above 100 (1/s) shear rate as seen in Figure 3.

Infant formulas are essentially blends of whey, casein or soy as a protein source, a blend of vegetable oils as a fat source, lactose or corn syrup as a carbohydrate source, a vitamin-mineral mix, and other ingredients depending on the manufacturer. The

ingredients contribute to the characteristic viscosity of the infant formulas depending upon type of their dispersion, concentration and hydration property.

Other factors that can influence the viscosity are pH, temperature, shear rate and heat treatments of the mixture during manufacturing process (McCarthy & Singh, 2009). When dispersed in water, proteins swell, unfold to some degree, and may increase in effective hydrodynamic volume, thereby increasing resistance to flow (Kinsella & Morr, 1984). Previous reports (Kinsella, 1979; Kinsella & Morr, 1984; McCarthy & Singh, 2009) stated that infant formulas exhibit pseudoplastic flow behaviour in which the fluid exhibits shear thinning over a wide range of shear rates which is characteristic of fluid milk and protein dispersions containing casein, whey protein, soy protein which was also observed in our study. The viscosities of the soy, newborn and lactose-free infant formulas were in the range (1.3- 5.1 mPas at a shear rate 50 1/s and 37 °C). The increased viscosity of anti-reflux formula containing corn starch as a thickener does not come as a surprise as previous studies have reported viscosity of reconstituted powder infant formulas as 2 mPas, increasing to 320 mPas by the addition of thickener (maltodextrin, starch, and carob bean gum) using a viscometer at shear rate of 50 1/s at 37^{o}C (Cichero, Nicholson, & September, 2013).

In vitro rheology study considered in this work consisted of three main stages: (a) flow behaviour in the mouth, (ii) flow behaviour in the stomach (cumulative to the mouth), and (iii) flow behaviour in the duodenum (cumulative of mouth and stomach). It should be noted that no adsorption of the digested ingredients or water takes place during the *in vitro* study, however in an infant body there is simultaneous digestion of ingredients and adsorption of the digested ingredients. Thus, the viscosity is contributed by both enzyme hydrolysed and residual contents.

3.2. *In vitro* mouth incubation of infant formula and rheology

Rheology plays a significant role in oral processing which is associated with

swallowing. Saliva is a dilute, viscous solute including water, protein (α-amylase) and electrolytes (sodium chloride and sodium bicarbonate) that will instantly mix with the infant formula before being swallowed. The holding time of milk in mouth is for a few seconds hence the flow behaviour of samples were determined immediately post addition of artificial saliva and apparent viscosity of the infant formulas is as shown in Figure 4. The mixing of saliva dilutes the infant formula however this does not have a drastic influence on the apparent viscosity of the formulas (newborn 1.38 and 1.32, lactose free 2.1 and 2.0 mPa.s, soy 1.45 and 1.42 and 5.07 mPa.s and 5.01 mPa.s before and after saliva addition respectively). The anti-reflux formula was the most viscous and new born the least viscosity similar to Figure 3. α-Amylase present in saliva initiates the digestion of starch and may result in a decrease in the perceived thickness of the fluid (de Wijk, Prinz, Engelen, & Weenen, 2004). However in our study there was no significant difference in the viscosity of the formulas before and after the addition of saliva. Saliva plays an important role, particularly in relation to its lubricating effect for swallowing and its enzymatic effect. Salivary amylase is the first enzyme to act on carbohydrates during digestion. However, short residence time of the fluid in the mouth (Vliet, 2002) was unable to influence the viscosity of the formulas.

3.3. *In vitro* stomach incubation of infant formula and rheology

Digestion is the first step necessary for the proper utilisation of ingested food. The infant formula when gets to the stomach, the acidic gastric juice containing pepsin aids in the breakdown of protein. Figure 5 shows a reduction in the viscosity of the three infant formulas in the stomach. Anti-reflux formula which had relatively higher initial viscosity showed bigger drops in viscosity compared to the other formulas. The viscosity of lactose free milk remained unchanged, probably the corn syrup solids which was a lactose replacement in the formulation was not contributing to the viscosity change in the stomach during its digestion or due to the low level of hydrolysis of shorter chain oligosaccharides in the stomach

In a relatively short time, the infant formula is carried by oesophageal peristalsis into the stomach. One of the key cells in the stomach for starch digestion is the parietal cell, which secretes HCl. The extremely acidic (pH 1-2) gastric environment retards the action of α-amylase salivary amylase (Lebenthal, 1987) but increases the acid hydrolysis of starch. Hence the anti-reflux infant formula (which contained corn starch as one of the viscosity raising agents) has a rapid reduction in viscosity as it passes through the gastric simulated conditions for 60 minutes. The viscosity change is induced by the changes in the size of starch granules and the number of chains of amylose and amylopectin (glucose chains) induced by pH change (Hirashima, Takahashi, & Nishinari, 2005). Enzymatic hydrolysis also reduces the apparent viscosity of soy protein and increases the flow behaviour as reported by Lamsal et al., (2007). Pepsin and rennin in the stomach also enzymatically hydrolyse proteins (Tunçtürk & Zorba, 2006) and should therefore influence the viscosity as suggested by Lamsal et al., 2007. Thus enzymatic hydrolysis has a major role to play in the reduction of viscosity of the four infant formulas in the stomach (Figure 5).

3.4. In *vitro* intestine incubation of infant formula and rheology

The viscosity of the infant formulas in the intestine first dropped within the first 15 to 30 minutes and then was steady until 2 minutes (Figure 6).

From the stomach, the ingested infant formula proceeds to the duodenum where it encounters the pancreatic secretion that contains two important components for starch digestion. Sodium hydrogencarbonate (bicarbonate) neutralises the acidity of the fluid arriving from the stomach to a pH of 8. Pancreatic fluid also contains α-amylase that continues the hydrolysis of starch into glucose and oligosaccharides (Dona, Pages, Gilbert, & Kuchel, 2010). Thus the additional breakdown of corn starch occurs in the intestine with amylase secreted from the pancreas thereby reducing the viscosity further to 1.25 mPas. The presence of other food components like proteins, lipids, anti-nutrients/inhibitors also affect starch digestibility to a significant extent (J. Singh,

Dartois, & Kaur, 2010). In humans, gastrointestinal lipid digestion takes place both in the stomach and in the small intestine. Lipid digestion in the stomach is due to hydrolysis of lipids by gastric lipase, whereas in the small intestine it is due to hydrolysis of lipids by pancreatic lipase (Mun, Decker, & McClements, 2007). The duodenal juice further hydrolyses the casein and α-lactalbumin or β-lactoglobulin with whey proteins relatively slowly digested in vitro in comparison to caseins by duodenal juice (Jakobsson, Lindberg, & Benediktsson, 1982; Lindberg, Engberg, Sjöberg, & Lönnerdal, 1998).

3.5. Particle Size distribution of infant formulas in *vitro*

The particle size distribution of the four reconstituted infant formula during digestion in mouth, stomach and intestine when compared with control is as shown in Figure 7 a-d. The four infant formulas show bimodal or multimodal distribution in control, mouth and during gastrointestinal digestion. The four different infant formulas show different trend in PSD, possibly due to the differences in the formulations.

The size distributions showed that the lactose free and soy protein infant formula in control, mouth and stomach were below 15 μm and 21 μm respectively (Fig. 7a and b). In contrast, after 2 hours of intestinal digestion, the size distribution became wider and was in the range 15–100 μm.

Figures 8 a-d, shows the plot of volume mean and surface mean diameters of the four infant formulas in control, mouth and during gastrointestinal digestion. As per Mun et al. (2007), the Sauter average diameter (d_{32}) is more sensitive to small particles and the volume mean diameter (d_{43}) is more sensitive to large particles. The plots shows a drop in d_{43} during stomach digestion (S60) followed by an increase in d_{43} towards the end of the intestine (I120) in all the four infant formulas. Similar results were observed by Gallier et al. (2012) in their work with in *vitro* digestion of bovine milk fat globules. The particles breakdown into smaller size as digestion progresses in the stomach. The

larger particle size distribution is assumed to be a result of fat and proteins undergoing disruption, flocculation, dissociation and coalescence during digestion as reported elsewhere (Berton et al., 2012; Ye, Cui, & Singh, 2010).

An increase in particle size distribution in the intestine was ensued with decrease in viscosity as reported in Figure 6. This confirms with the observations of Afoakwa, Paterson, Fowler, and Vieira (2008) that an increase in size distribution inversely influences viscosity.

The particle size distribution should be carefully dealt with as this will depend on the level of agitation and adsorption of nutrients in the real GI tract.

Conclusions

Feeding of infant formulas has been in practice as a substitute or supplement of breast feeding. However we require a good understanding to ensure that the ingested formula behaves on the same way physically and chemically in the digestive tract, as that of mother's milk. Understanding the rheological characteristics of infant milk formula both before and during the various stages of digestion is also necessary in designing formulas that cater to infants with different medical conditions e.g. dysphagia, gastroesophageal reflux that's causing worldwide parental distress. From this study, it can be seen that flow characteristics of the infant formula decreases as they pass from the mouth through to the gastrointestinal tract. Of all the infant formulas studied, only the anti-reflux infant formula was viscous in the stomach and possibly responsible for the preventing refluxes.

It is difficult to isolate the exact mechanism occurring in each component in a commercial infant formula product. Possibly, the further digestion of the various components and later their aggregation is responsible for the flow behaviour of the infant formulas during the intestinal digestion. It is worth noting that the adsorption and

digestion take place simultaneously in the intestine thus the actual rheology measured *in vitro* provides just an indication of the behaviour of the product in the digestive tract. Further work is needed to understand exactly how each component in the infant formula affect the rheology of the ingested food which can be done by considering a model formula system.

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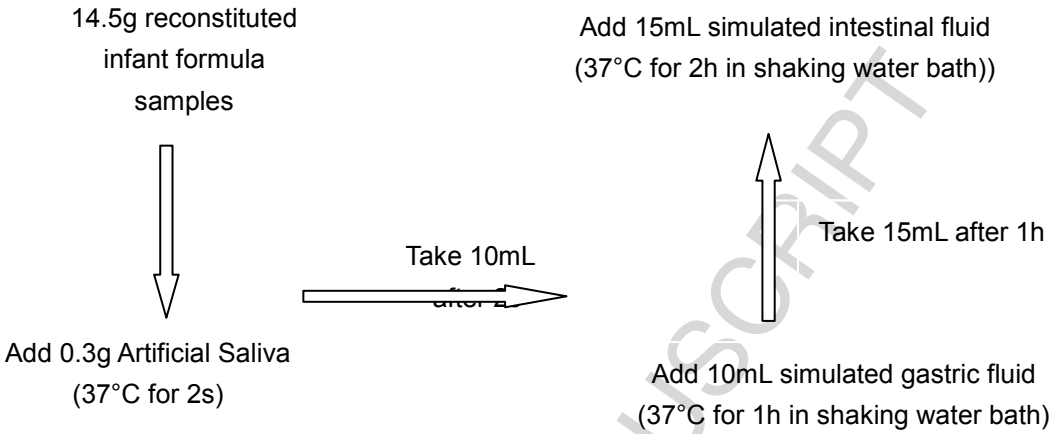


Figure 1

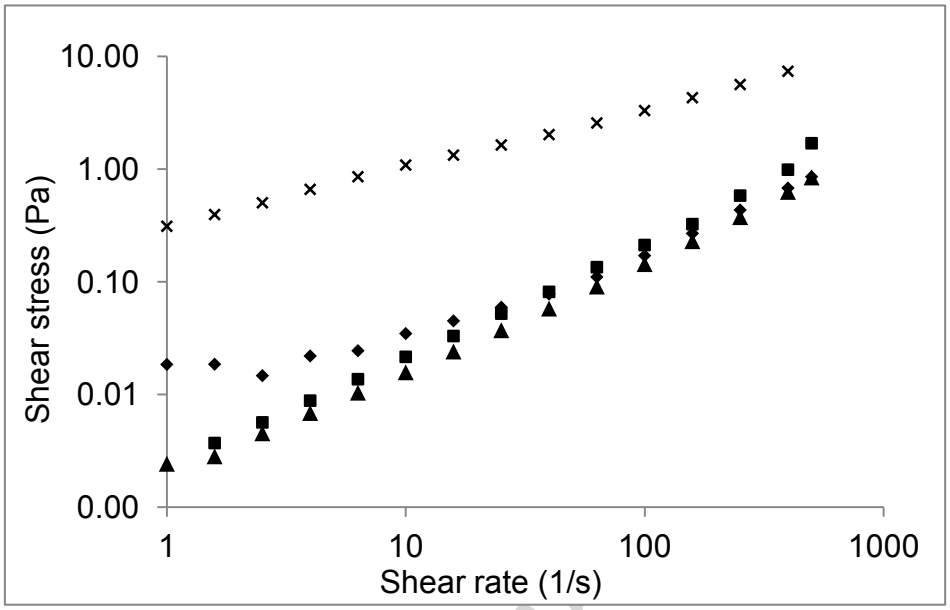


Figure 2

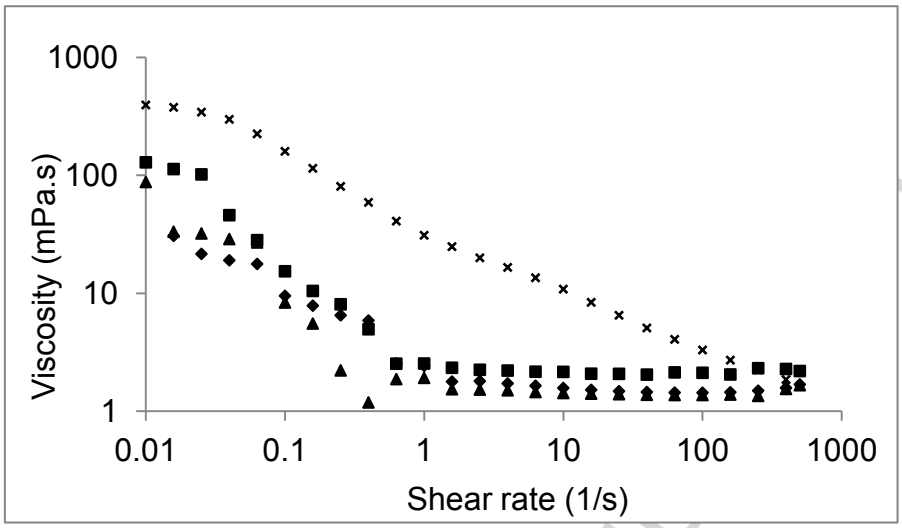


Figure 3

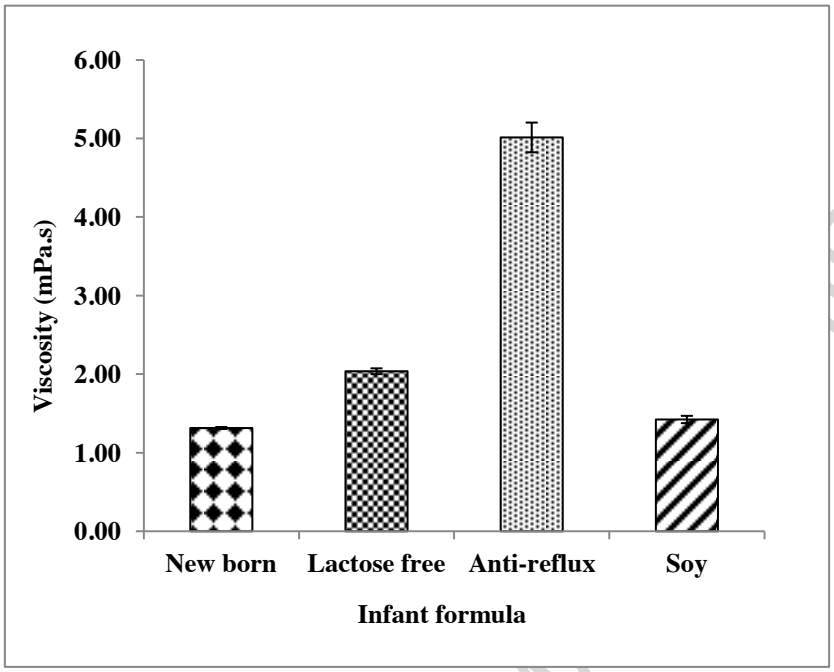


Figure 4

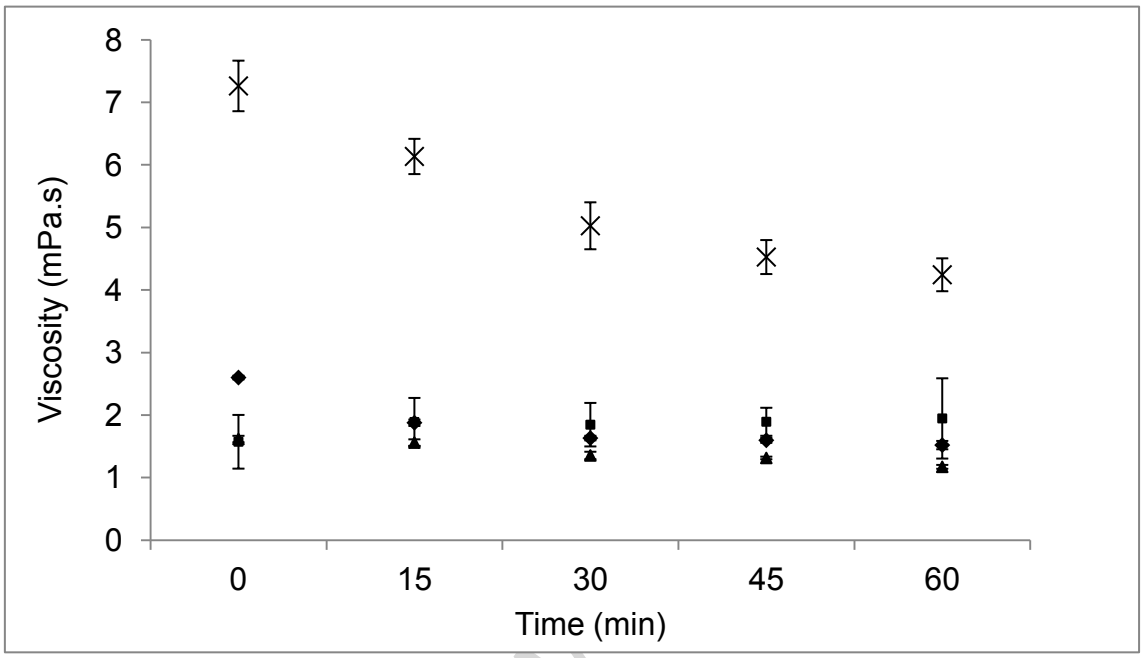


Figure 5

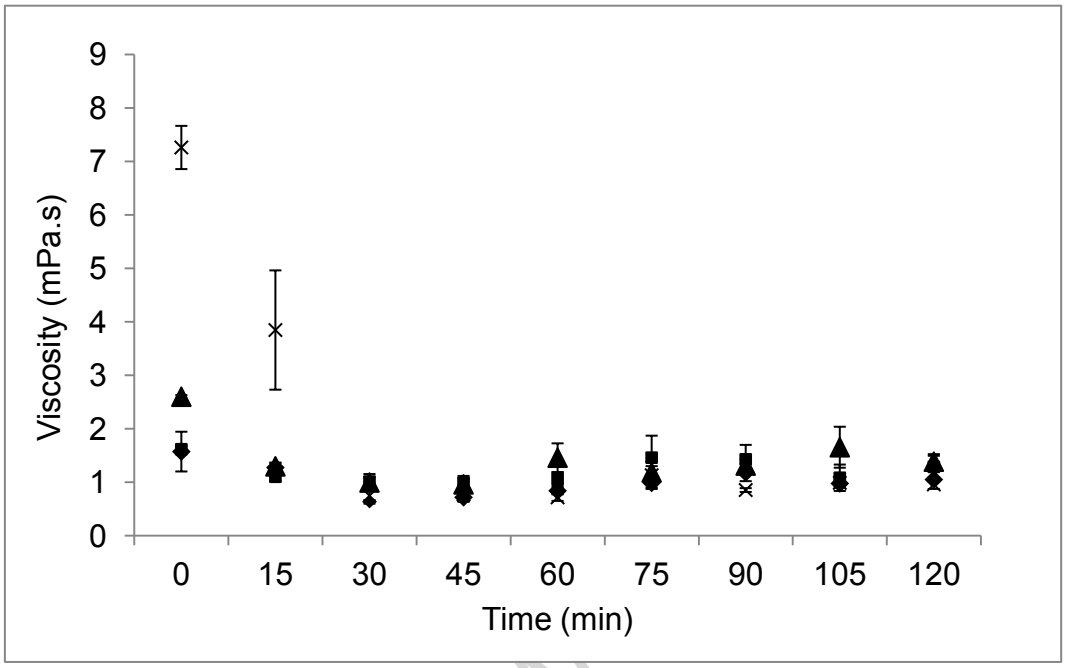
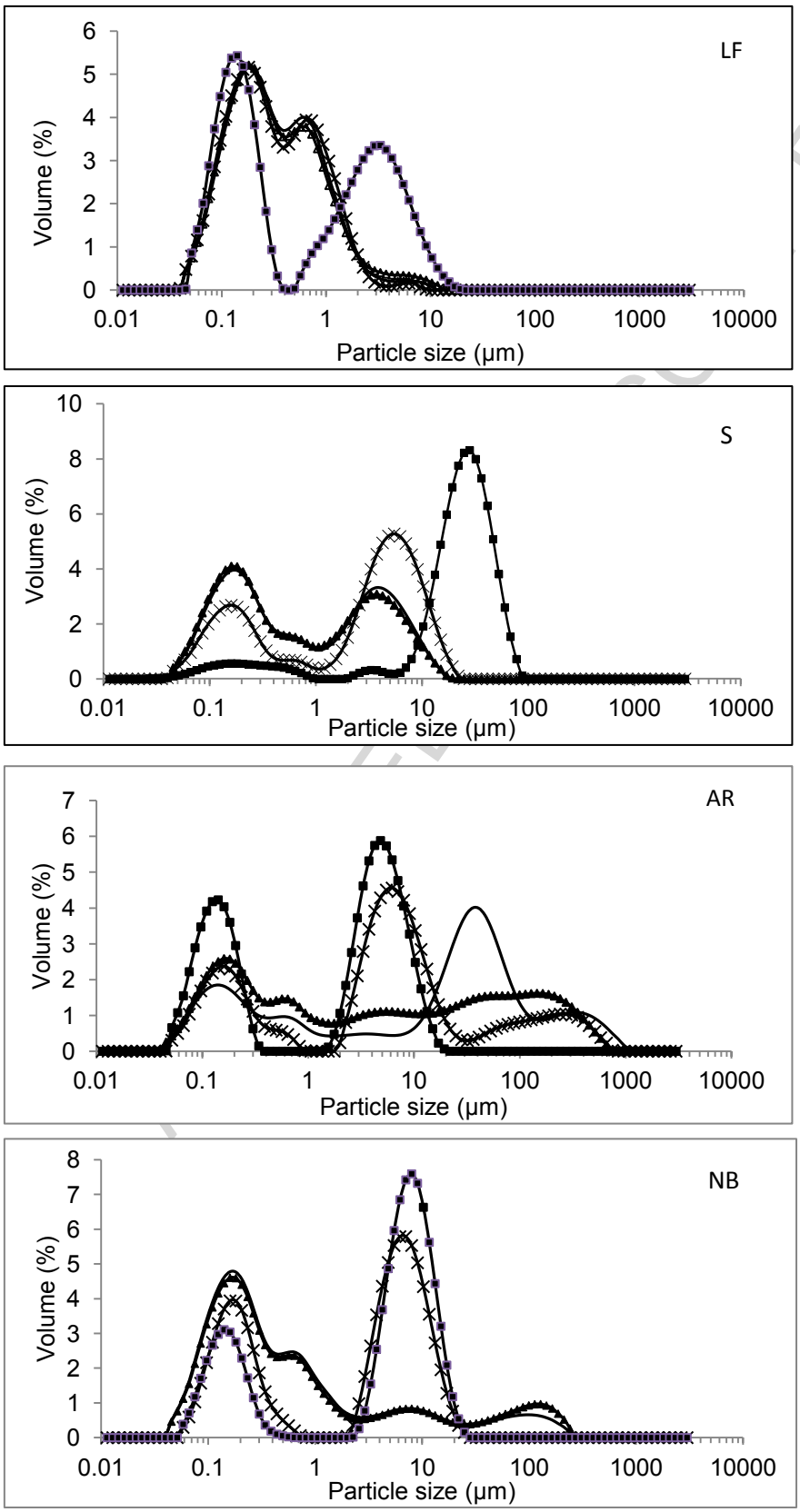


Figure 6



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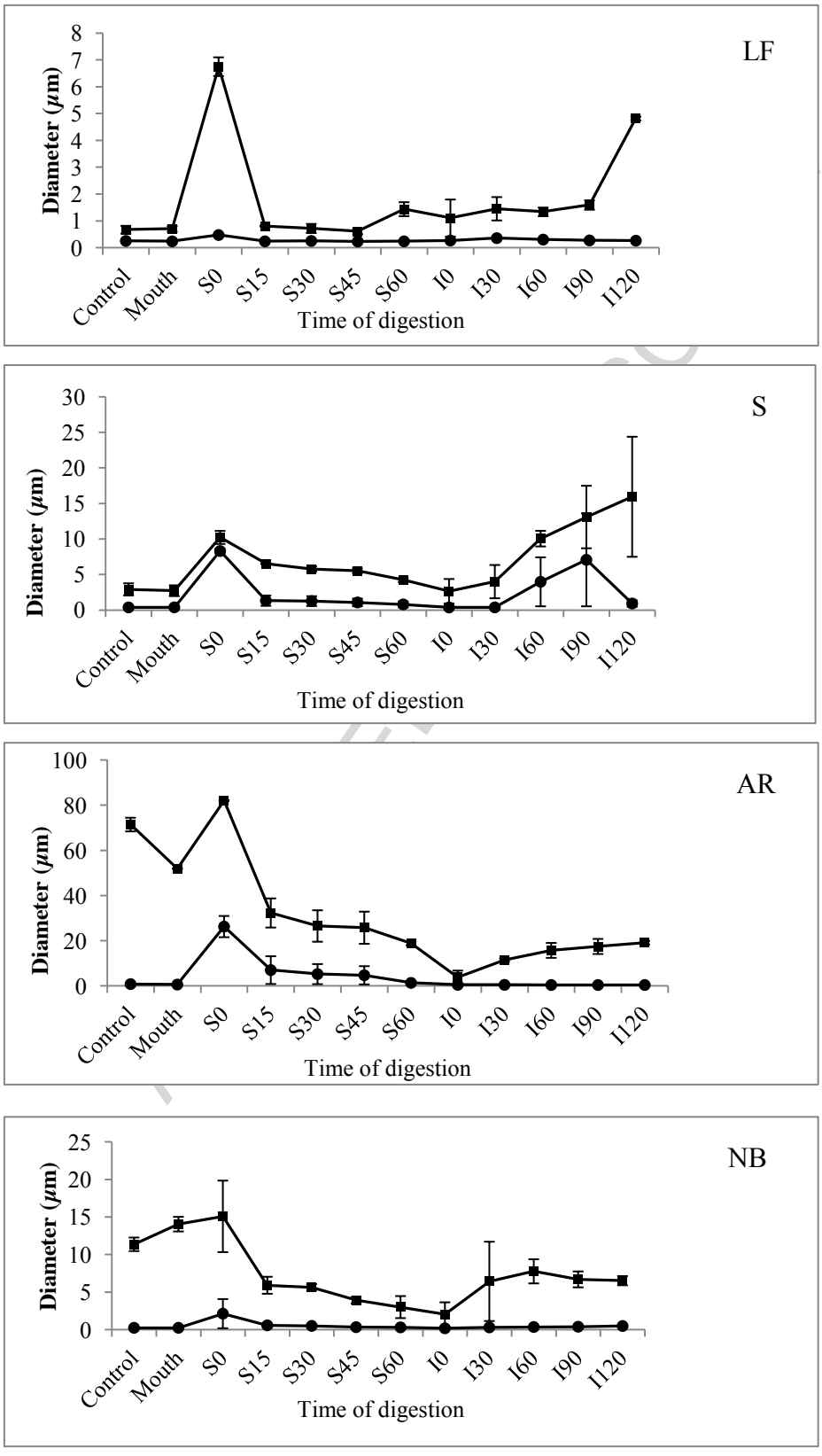

Figure 8

Table 1: Composition (as labelled on the container) of the different infant formulas used in the study

Name of infant formula	Main ingredients	Nutrition (per 100 mL reconstituted formula)
Anti-reflux	nonfat milk powder, vegetable oils, lactose, precooked corn starch, corn syrup solids, soy lecithin, minerals and vitamins	protein (1.5g), fat (3.6g), carbohydrates (7.0g)
Soy	corn syrup solids, vegetable oils, soy protein isolate, soy lecithin, minerals and vitamins	protein (1.8g), fat (3.6g), carbohydrate (6.9g)
Newborn	reduced mineral whey, vegetable oils, nonfat milk powder, lactose, soy lecithin, minerals and vitamins	protein (1.5g), fat (3.6g), carbohydrate (7.2g)
Lactose free	corn syrup solids, vegetable oils, whey protein concentrate, milk protein isolate, soy lecithin, minerals, vitamins	protein (1.5g), fat (3.6g), carbohydrate (7.2g)

Table 2: Power law parameters

Infant formula		Parameters
Antireflux	n	0.55
	K	0.28
	R^2	0.99
Soy	n	0.97
	K	0.002
	R^2	0.99
New born	n	0.93
	K	0.003
	R^2	0.99
Lactose free	n	0.95
	K	0.003
	R^2	0.99