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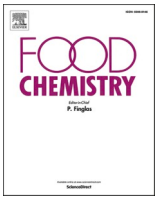

# Comparing the lipid self-assembly behaviour and fatty acid composition of plant-based drinks to bovine milk during digestion

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ABSTRACT

In this study, a static *in-vitro* digestion model was coupled with synchrotron small-angle X-ray scattering (SAXS) to compare the lipid self-assembly behaviour of plant-based drinks and bovine milk during digestion. The diffraction profiles were combined with principal component analysis (PCA) of the fatty acid (FA) composition during digestion. Half of the plant-based drinks were found to form an inverse micellar cubic phase which is substantially different from the inverse hexagonal and bicontinuous cubic phases determined in bovine milk during digestion. The PCA inferred that the plant-based drinks all had similar FA compositions with slight changes in oleic and linoleic acid ratios. The polyunsaturated long-chain fatty acids of the plant-based drinks forming inverse micellar structures are in agreement with the critical packing parameter theory. These findings increase the understanding of the behaviour of plant-based drinks and aid further development of new and existing plant-based dairy substitution products.

## 1. Introduction

Milk is an important source of macronutrients, vitamins and minerals such as calcium, important for bone growth and maintenance in young children, adults and the elderly alike (Valenze, 2011). Bovine milk is a complex oil-in-water emulsion consisting of fat droplets with tri-layer phospholipid membranes containing various glycoproteins. The core of the fat droplets contains lipids in the form of triglycerides with varying chain lengths (Hayes & Kelly, 2003; MacGibbon, 2020).

Many individuals cannot include bovine milk in their diet due to personally held ethical beliefs or lactose intolerance and hence turn to plant-based alternatives. Consequently, plant-based drinks are becoming increasingly popular as a substitute for bovine milk. The plant-based drinks are marketed as a healthy, sustainable and vegan alternative to bovine milk and are available with various plant bases, however there is little understanding of their relationship relative to bovine milk, especially during digestion (McClements et al., 2019; Sethi et al., 2016). In contrast to the structure of the fat droplets in bovine milk, the fat globule membranes of droplets in plant drinks consist of a single phospholipid layer and the triglyceride composition in the core of the fat globule is vastly different from that of bovine milk, with a greater proportion of longer unsaturated fatty acids (FA) in the triglycerides (Maurer et al., 2013; McClements et al., 2019; Sen et al., 2024).

During digestion of both animal and plant-based emulsions, FA chains are cleaved from the glycerol backbone of the triglycerides by lipase enzymes (gastric or pancreatic) releasing diglycerides, monoglycerides and FAs, with chain lengths depending on the initial triglyceride composition. At complete digestion the lipase enzymes favour the release of the *sn*-3 and *sn*-1 position FAs yielding one *sn*-2 monoglyceride and two FAs (*sn*-1 and 3). In some cases, it is possible to have the release of all three FAs and a glycerol backbone (Binte Abu Bakar et al., 2022; Clulow et al., 2018; McClements & Li, 2010).

Amphiphilic lipids such as monoglycerides and FAs can self-assemble to form a range of self-assembled 'mesophase' structures, due to the

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hydrophobic effect, where the structures formed depend on the composition, length of lipid chains and prevailing conditions such as temperature, pH, lipid concentration and ionic strength. The self-assembly behaviour can be described qualitatively by the critical packing parameter (CPP) equation shown below.

$$CPP = \frac{v}{a_o \times l_c} \quad (1)$$

where CPP is the critical packing parameter, $v$ is the volume of the lipophilic tail, $a_o$ is the area of the hydrophilic head group, and $l_c$ is the length of the lipophilic tail.

This parameter can be used to understand which mesophases are likely to form due to the curvature at the oil/water interface and changes in the lipid composition. A CPP below 1 gives rise to micellar ($L_1$) phases, a CPP of 1 gives rise to lamellar ($L_\alpha$) phases and a CPP above 1 gives rise to inverse phases, such as the inverse micellar cubic ($I_2$) phase, which in turn can exhibit different space groups (Israelachvili et al., 1976; Kumar, 1991).

Lipid digestion products are well known to have potential to self-assemble into colloidal structures. The release of FAs from triglycerides under the action of lipase, and consequent self-assembly into ordered mesophase structures that follows, was first observed in 1979 by Patton and Carey utilizing light microscopy. The fat droplets of an olive oil emulsion could be seen to rupture and form viscous $L_\alpha$ phases after the addition of pancreatin extract. The $L_\alpha$ phase was extracted by centrifugation and the calcium-45 activity was used to determine the calcium content of the $L_\alpha$ phase and showed a 1:2.1 mol ratio of calcium to FAs, indicating the formation of calcium oleate soaps during digestion of the fat droplets. Additionally, Patton and Carey discovered other viscous mesophases containing mixtures of FAs and monoglycerides and postulated the existence of several different liquid crystalline mesophases in addition to calcium soaps, which is now known to be the case (Patton & Carey, 1979).

The mesophase structures formed by bovine milk and other dairy-related systems including infant formula during digestion have been determined in previous studies and are illustrated in Fig. 1. The space group of the mesophase is usually determined using small-angle X-ray scattering (SAXS) (Demurtas et al., 2015; Pham et al., 2020; Salentinig et al., 2013; Salentinig et al., 2015; Salim et al., 2022). Mesophase structures have been shown to upregulate the uptake of nutrients, vitamins and pharmaceuticals through increased solubilisation and diffusion through water channels present in certain liquid crystal structures (Martiel et al., 2015; Sagalowicz et al., 2006). However, the potential for mesophase formation by the many commercially available plant-based alternatives during digestion has not been studied to date. As these products are marketed as a direct substitution for bovine milk, understanding the behaviour of these systems during digestion is important from a nutritional and quality perspective.

Therefore, in this study, the mesophase structures formed during digestion of bovine milk and different commercially available plant drinks were determined using *in situ* SAXS and the free FA composition was determined using gas chromatography fitted with a flame ionization detector (GC-FID) as illustrated in the schematic in Fig. 1. Correlation of the lipid composition with mesophase structures formed during digestion could lead to ease of product development and to better understand the behaviour of the new and popular plant drinks.

## 2. Experimental

### 2.1. Materials

Commercially available homogenised bovine milk, oat drink, soy drink, rice drink, almond drink, oat cream and soy cream were purchased from a local supermarket (Føtex, Copenhagen, Denmark). Compositional and nutritional information for the products studied are summarised in Table 1. n-pentane (≥99 %), methanol (≥99 %), dichloromethane (≥99 %), tromethamine maleate salt (≥99 %), calcium chloride dihydrate (≥99 %), sodium chloride (≥99 %), sodium hydroxide pellets (≥99 %), 4-bromophenylboronic acid (≥95 %), pancreatin from porcine pancreas (4 x USP specifications) were purchased from Sigma-Aldrich (Søborg, Denmark), water was obtained from a PureLab flex system from ELGA LabWater (High Wycombe, United Kingdom), with a resistance of 18.2 MΩ.

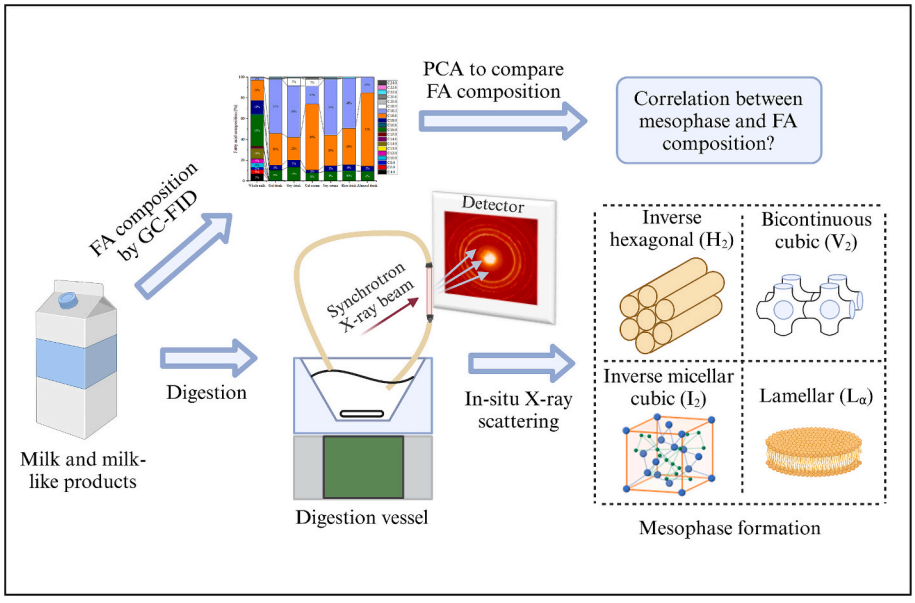


**Fig. 1.** Overview of the experimental design used in this study. To determine the FA composition during digestion, samples were taken at different times and analysed using GC-FID. The FA compositions were compared using principal component analysis to investigate similarities of the different milk and milk-like products. The mesophase formation during digestion of the milk and milk-like products were determined using *in-situ* synchrotron SAXS. The mesophases are then labelled after the principal component analysis to investigate potential correlations of FA composition to mesophase behaviour. Created with BioRender.com.

**Table 1**
Nutritional information on the plant-based drinks and bovine milk used in this study. The creams were diluted in a 1:4 ratio in Tris buffer, to normalise the fat content to that of the drinks. Original contents, before dilution, is given in the table and after dilution in brackets where applicable. Data is taken directly from publicly available ingredients and nutrition lists on the respective company websites.

| Product/ Content | Fat % (g/100 mL) | Carbohydrates % | Protein % | Calcium (mg/100 mL) | Added oil |
|---|---|---|---|---|---|
| Bovine milk | 3.5 | 4.6 | 3.4 | 119 | None |
| Oat drink | 3.5 | 6.4 | 0.7 | 120 | Sunflower oil |
| Soy drink | 2.1 | 0.6 | 3.7 | – | None |
| Rice drink | 1.1 | 10 | 0.1 | – | Sunflower oil |
| Almond drink | 0.9 | 2.7 | 0.4 | – | None |
| Oat cream | 13 [3.25] | 5.8 [1.4] | 1 [0.2] | – | Canola oil |
| Soy cream | 14 [3.50] | 1.2 [0.3] | 2 [0.5] | – | Sunflower oil |

## 2.2. In vitro lipolysis of milk

### 2.2.1. Preparation of lipase

Immediately prior to the *in vitro* lipolysis experiments 2 mL of porcine pancreatin suspension was prepared. Porcine pancreatic powder (2 g, 4 x USP specifications) was hydrated by addition of 5 mL of Tris buffer with a pH of 6.5 and vortexed for 5 min. The hydrated porcine pancreatin solution was transferred to two 2 mL polypropylene tubes and centrifuged at 12,100 x*g* for 15 min. The supernatant of each tube (1 mL) was then transferred to a new 2 mL polypropylene tube and stored at 5 °C until use. The lipase activity of the porcine pancreas powder was determined by a tributyrin assay and found to be approximately 10 tributyrin units (TBU)/mg. The total volume of the digest used in the digestion experiments was 20 mL. The amount of porcine pancreatin used in each digestion was 2 g, giving approximately 1000 TBU/mL of digest.

### 2.2.2. Digestion of milk and plant emulsions

A Stat Titrando 902 unit from Metrohm (Herisau, Switzerland) with a jacketed vessel and a pH electrode containing a temperature sensor was used for all lipolysis experiments. NaOH (1 M) was used as the titration solution. A water circulator was connected to the jacketed vessel to keep the contents of the vessel isothermal at 37 °C. Bovine milk or plant emulsion (18 mL) was added to the jacketed vessel and left under magnetic stirring until the temperature was constant at 37 °C. This was controlled by insertion of the pH electrode containing a temperature sensor into the solution. Afterwards, the burette tip was inserted into the vessel and the solution was adjusted to pH 6.5 ± 0.005. To initiate the lipolysis 2 mL of porcine pancreatin supernatant was added, with a lipase activity of approximately 1000 (TBU)/mL of digest. The FAs released during hydrolysis of the triglycerides lowers the pH of the solution triggering the addition of the 1 M NaOH titration solution. To keep the pH static at 6.5, the volume of the titration can be correlated to the amount of released FAs. The duration of lipolysis was approximately 1 h, after which the pH was increased to 9 to conduct a back-titration that enables determination of any unionized FAs which are not detected in the forward titration.

### 2.2.3. Determining extent of digestion from titration of NaOH during digestion

The volume of titrant (1 M NaOH) added during digestion and to bring the pH of the final digested sample to pH 9 was used to calculate the total amount of released free FAs during digestion. In this calculation, "blank" digestions for the samples are needed to subtract from the total volume of titrant discussed above. The blank samples were produced by ultracentrifugation of the corresponding sample at 40,000 x *g* for 45 min using a Sorvall Discovery 90SE ultracentrifuge fitted with a Sorvall T-865 rotor (Thermo Fisher Scientific, Waltham, MA, USA). The ultracentrifugation separates the lipids from the aqueous phase, which was extracted and functions as a blank sample. This blank sample was then digested using the same digestion protocol as was used in the original sample digestion. The theoretical maximum amount of FAs in the sample was determined by calculating the average molecular weight of the triglyceride side chains present in the sample. The triglyceride side-chain composition of the sample was determined by the GC-FID analysis. The average molecular weight is then used to calculate the theoretical total FA content based on the fat percentage given by the manufacturer. Eq. 2 gives an overview on the calculation of the extent of digestion.

$$\left(\text{Extent of digestion (\%)} = \left((\text{V\_}\mathit{NaOH}\ (\mathit{sample}) - \text{V\_}\mathit{NaOH}\ (\text{blank})\right) + \left(\text{V\_}\mathit{NaOH}\ (\mathit{sample}\ \text{pH9}) - \text{V\_}\mathit{NaOH}\ (\text{blank pH9})\right)\ [\mathit{mL}] \times \mathit{M\_NaOH}\ [\mathit{mol/L}]\right) / (\text{Theoretical total amount of FAs in the sample}\ [\mathit{mmol}]\ ) \times 100\%\ ) \quad (2)$$

### 2.2.4. In situ small-angle X-ray scattering

The *in vitro* lipolysis apparatus and its coupling to a synchrotron SAXS beamline has been previously described in detail (Salentinig et al., 2013). The samples were placed in a digestion vessel connected to an open-end capillary, to afford a constant flow at approximately 10 mL/min with a peristatic pump. After stirring and acquiring the SAXS patterns of non-digested milk and plant-based drinks for approximately 5 min, 2 mL of the pancreatin suspension was remotely injected into the vessel to initiate lipolysis. SAXS measurements were carried out at the SWING beamline of SOLEIL (Gif-sur-Yvette, France), using a fixed energy of 12.0 keV ($\lambda = 1.033$ Å). A PCCD170170 (AVIEX) 2D detector was placed with a sample-to-detector distance of 1.2 m, covering a scattering vector ($q$) range between ~0.006 and 0.9 $Å^{-1}$, defined by

$$q = \left(4\pi/\lambda\right) sin(\theta) \quad (3)$$

where $2\theta$ is the scattering angle. The suspended samples with a constant flow were exposed to the beam for 2 s with a 13 s interval between the data acquisition, typically for 1 h in total. All of the data was reduced (without background subtraction) using the FOXTROT software (V3.4.1, Xenocs). The scattering vector of the Bragg peaks was used to define the atomic plane spacing of the mesophase structures, also called d-spacing, by using the following equation.

$$d = 2\pi/q \quad (4)$$

Through the d-spacing, the lattice parameter ($\alpha$) of the mesophases was calculated using the following equations depending on the type of mesophase structure.

$$\alpha = d \tag{5}$$

$$\alpha = d\sqrt{h^2 + k^2 + l^2} \tag{6}$$

$$\alpha = d\sqrt{3/4\,(h^2 + k^2 + hk)} \tag{7}$$

where $\alpha$ is the lattice parameter, $h$, $k$ and $l$ are the Miller indices corresponding to the atomic planes found within the specific mesophase structure. The lattice parameter for $L_\alpha$ phases can be defined using Eq. 5, for cubic phases using Eq. 6 and for hexagonal phases using Eq. 7.

### 2.3. Fatty acid composition analysis using GC-FID

#### 2.3.1. Extraction and methyl esterification of lipids for GC-FID analysis

Samples were taken at set intervals prior to and during digestion of the milk and plant emulsions after the addition of the pancreatin supernatant. Samples were taken at 0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min. The lipolysis process was halted by adding 50 mM 4-BPBA in methanol, stopping enzyme activity. The composition of the 200 μL samples were 180 μL of milk or plant emulsion with 20 μL of 50 mM 4-BPBA in methanol. The samples were stored at −20 °C until further sample preparation.

To extract the lipids from the sample, 1.5 mL of dichloromethane (DCM) in methanol in an 8:2 ratio was added along with two steel balls with a diameter of 2 mm to the 2 mL polypropylene tube containing the sample. The polypropylene tube was inserted in a polypropylene tube adaptor for a Tissuelyser from Qiagen GmbH (Hilden, Germany). The samples are shaken at 20 Hz for 2 min, followed by centrifugation for 8 min at 10,621 x *g*. The DCM in methanol supernatant was then transferred to a Pyrex glass culture tube with a lid. The samples were washed with 1 mL of DCM in methanol and centrifuged as before after which the supernatant was collected and added to the corresponding culture tube, this step was repeated twice. The culture tubes were then set to evaporate until dry using a nitrogen evaporator. Once dry, the sample was dissolved in 200 μL of n-pentane containing 1 mg/mL of the internal standard methyl heptadecanoate. Then 1.5 mL of anhydrous methanol was added followed by 300 μL of 1.33 % methanolic HCl. The samples were then vortexed for 30 s and put into a 60 °C water bath for 1 h. In the final step 1 mL of n-pentane was added along with 2 mL of saturated NaCl in water solution and the sample was vortexed for 10 s and left to separate. The top layer of n-pentane was then extracted into 2 mL GC analytical vials and stored at −20 °C until analysis.

#### 2.3.2. GC-FID column and temperature program

All samples included in this study were run on an Agilent 7820 A GC with a FID detector module from Agilent Technologies (Glostrup, Denmark) on an Omegawax 320 column with dimensions of 30 m (l) x 0.32 mm (I.D.) x 0.25 μm (film) from Sigma-Aldrich (Søborg, Denmark). The injection volume was 1 μL with a split ratio of 1:8. Column flow was kept constant at 0.4 mL/min of dry nitrogen gas. The injector temperature was set to 250 °C. A ramped oven temperature program was used with a starting temperature of 50 °C (held for 1 min) increased by 15 °C/min to 180 °C (held for 0 min) then increased by 5 °C/min to 220 °C. The FID was set to 300 °C with an airflow of 350 mL/min, hydrogen flow of 35 mL/min and makeup gas flow of 40 mL/min. An n-pentane blank was injected in between each sample to prevent leaking of prior samples into subsequent runs.

## 3. Results

### 3.1. Digestion kinetics of bovine milk and plant-based drinks

In Fig. 2, the extent of digestion *versus* time is shown for all milk and milk-like systems included in the study. The calculation from Eq. 2 assumes that each triglyceride in the milk or plant drink yields 2 FAs and 1 monoglyceride when digested by pancreatic lipase enzymes. Bovine milk reached 95.5 % extent of digestion within 1 h of digestion. The oat, soy and rice drink showed an extent of digestion of 65.9 %, 79.8 % and 84.6 % respectively after 1 h of digestion. The almond drink showed an extent of digestion of 85.0 % after 50 min of digestion. The oat and soy cream showed an extent of digestion of 39.3 % and 43.1 % respectively after 1 h of digestion. The oat, soy and rice drinks were digested for an extended period during the synchrotron SAXS measurements and reached a total extent of digestion of 70.5 %, 88.4 % and 90.6 % respectively after 90 min of digestion (data not shown).

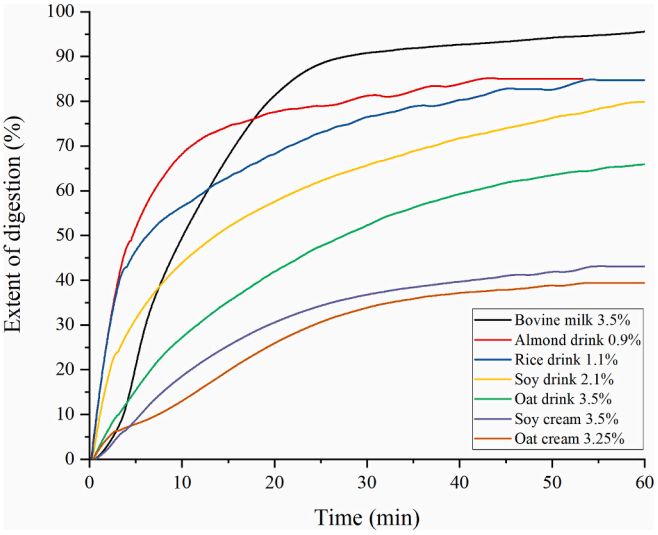


**Fig. 2.** Extent of digestion calculation using Eq. 2 against time of digestion for the milk and plant-based drinks.

The digestion kinetics of the milk and milk-like systems differ not only in final extent of digestion but also in the digestion dynamics to reach the full extent of digestion. It can be seen in Fig. 2 that bovine milk reaches 90 % extent of digestion within 25 min of digestion, whereas the plant-based creams generally digest to a lesser extent and at a decreased rate The plant drinks with low total fat content (almond and rice drink) were the fastest digesting drinks reaching an extent of digestion of approximately 60 % and 70 % respectively within 10 min. The difference in digestion kinetics between the milk and plant-based products will have a direct effect on the compositional relationship between the released FAs, di- and monoglycerides in solution. This change to the mixture of lipids present at specific time points should influence the self-assembly behaviour of the plant-based creams.

### 3.2. Mesophase structures formed during digestion of commercially available plant-based drinks compared with bovine milk

To investigate whether the plant-based drinks form mesophase structures due to lipid self-assembly, lipolysis of the various milk-like products were carried out coupled to an *in situ* synchrotron SAXS instrument. The diffraction profiles of the plant-based products can be seen in Fig. 3 with an overview of the changes in phase behaviour across all the systems during digestion presented in Fig. 4. Bovine milk is considered as a pseudo control system, as the mesophase formation of milk during digestion has been well studied and is the product that the plant-based drinks are supposed to substitute for in a marketing context. The sequence of structures formed during digestion due to self-assembly of lipid digestion products for milk from previous studies is characterised by the formation of calcium soaps, evident from the formation of a persistent $L_\alpha$ phase, followed by a transient inverse micellar cubic ($I_2$) phase, an inverse hexagonal phase ($H_2$) and concluding with the formation of a bicontinuous cubic phase ($V_2$) (Clulow et al., 2021; Eason et al., 2022; Pham et al., 2021). As expected, this behaviour was

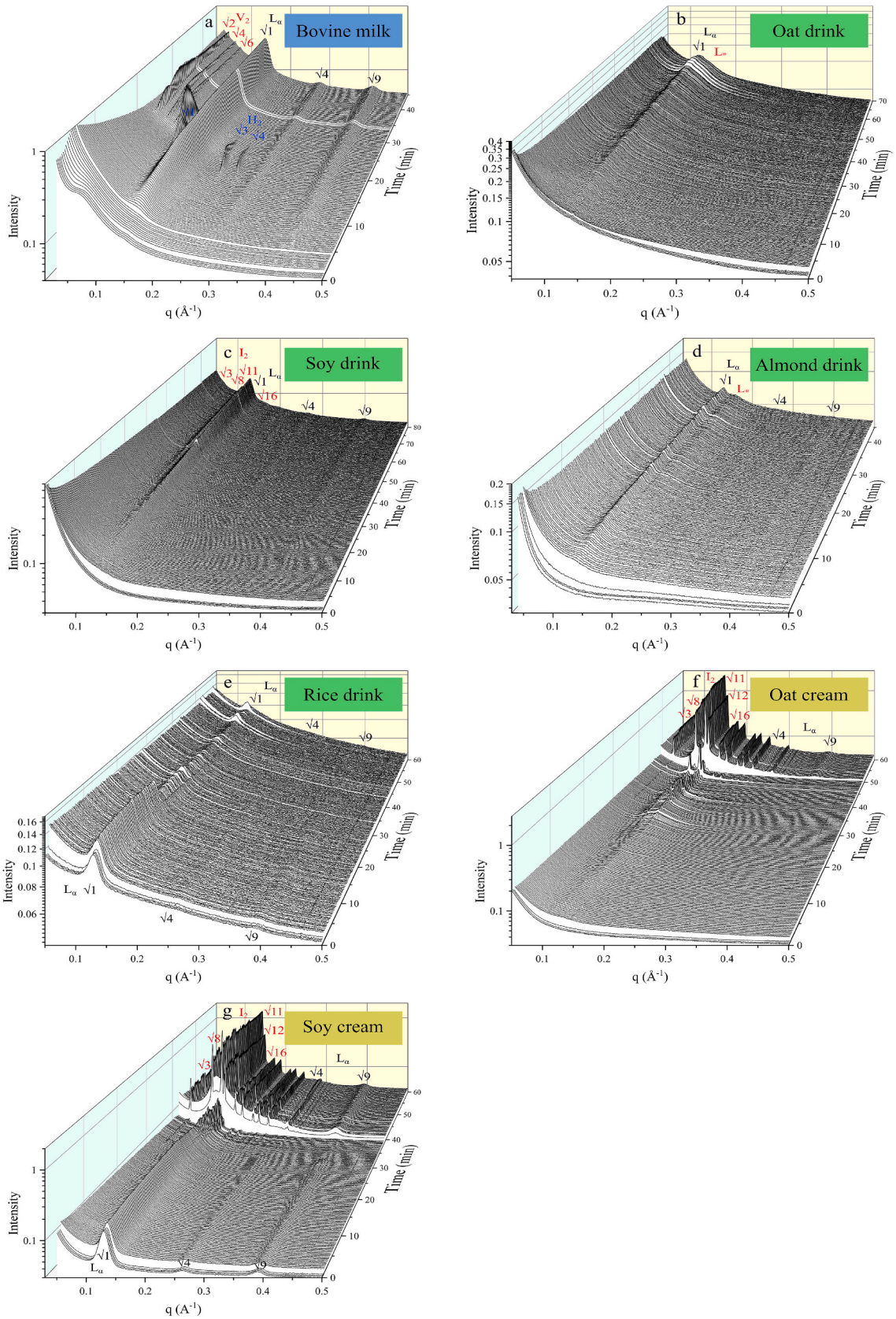


**Fig. 3.** Time-resolved diffraction patterns of bovine milk and plant drinks during digestion utilizing *in-situ* synchrotron SAXS. Waterfall plots of the diffractograms for bovine milk (a), oat drink (b), soy drink (c), almond drink (d), rice drink (e), oat cream (f) and soy cream (g) during digestion with notation of spacing ratios for the Bragg peaks corresponding to specific mesophase structures.

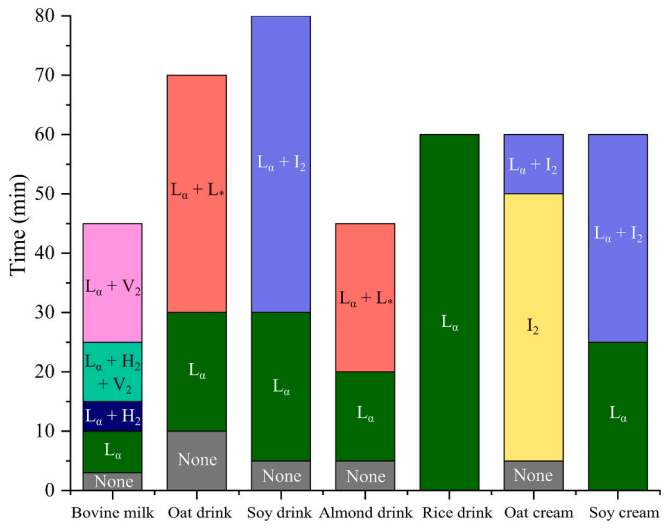


**Fig. 4.** Column bar plot depicting the mesophase behaviour of bovine milk, oat drink, soy drink, oat cream, soy cream, rice drink and almond drink against time of digestion. Each bar is partitioned into sections showing which mesophases are seen within the assigned time space. The labelling of mesophases shown are as follows: $L_\alpha$ (lamellar phase), $H_2$ (inverse hexagonal phase), $V_2$ (bicontinuous cubic phase), $I_2$ (inverse micellar cubic phase) and $L_*$ (unknown lamellar, inverse micellar or sponge phase).

confirmed by the sequence of structures formed, evident from the diffraction profiles obtained during the digestion of bovine milk in Fig. 3a. The transient $I_2$ phase with the Fd3m space group was not observed in the scattering profile for the bovine milk used in this study, however every other mesophase was observed as seen in previous studies. The $L_\alpha$ phase appeared several min after the start of digestion, the transient $H_2$ phase formed at approximately 10 min and disappeared at roughly 25 min of digestion and the $V_2$ phase was evident from approximately 15 min of digestion and persisted until end of digestion. The absence of the transient $I_2$ mesophase in bovine milk, seen in previous studies, might be due to differences in lipid composition of milk from different sources.

The oat drink (Fig. 3b) formed a $L_\alpha$ phase at approximately 10 min of digestion with a lattice parameter of 4.8 nm. A second phase can be seen to appear as a shoulder to the lamellar phase at around 30 min of digestion with a d-spacing of 4 nm (Fig. S2). It is unclear whether this phase is a second $L_\alpha$ phase, an inverse micellar phase ($L_2$) or a sponge phase ($L_3$) and is therefore denoted as an unknown $L_*$ phase.

The soy drink formed a $L_\alpha$ phase after 5 min of digestion and an $I_2$ phase after 30 min of digestion with a lattice parameter of 4.75 nm and 16 nm respectively. A single scan showing the peak alignment for the $I_2$ and the $L_\alpha$ phase for the soy drink can be seen in Fig. S1. Bragg peaks indicative of the $I_2$ mesophase are less clear in the soy drink compared to the $I_2$ phase in the plant-based cream products in Fig. 3f and g. It is however clear by aligning the peak spacing for the $L_\alpha$ phase, that the two most pronounced peaks can be assigned to the $I_2$ phase with the Fd3m spacing and that the first $L_\alpha$ peak can be seen as a shoulder to the third peak of the $I_2$ mesophase.

The rice drink showed a $L_\alpha$ phase with a lattice parameter of 4.8 nm present before the digestion was started and the $L_\alpha$ phase can be seen to decrease in intensity during digestion. The $L_\alpha$ phase in the rice drink cannot be due to calcium soaps as the rice drink does not contain calcium before the start of digestion according to the label. Several artefacts were observed during the digestion experiment of the rice drink. The pH would slowly drift towards basic conditions after the initial titration to pH 6.5 and the solution would become increasingly turbid during digestion. This made it difficult to work with, as the larger aggregates were not able to enter the flow-through loop, which might explain the decrease of the intensity of the $L_\alpha$ phase.

The almond drink formed a $L_\alpha$ phase after 5 min of digestion with a lattice parameter of 4.8 nm followed by an unknown $L_*$ phase after 20 min of digestion with a d-spacing of 3.8 nm. The almond drink contained the lowest amount of lipids with a concentration of 0.9 %.

The oat cream slowly formed an $I_2$ phase starting after 5 min of digestion, increasing in intensity until the end of digestion, while a $L_\alpha$ phase was observed after 50 min of digestion with lattice parameters of 14 nm and 4.8 nm respectively. The $L_\alpha$ phase became distinguishable after the increase in signal around 50 min. Whether the $L_\alpha$ phase was already present but undetectable before the increase in signal is unknown. However, since a strong $L_\alpha$ phase forms almost instantaneously at 50 min, it seems likely that the $L_\alpha$ phase simply was not identifiable before this point.

The soy cream displayed a $L_\alpha$ phase before digestion was initiated, with a lattice parameter of 4.8 nm, similar to the rice drink. At 30 min of digestion, the soy cream formed an intense $I_2$ phase with a lattice parameter of 14.5 nm similar to the oat cream. In this case, the $L_\alpha$ phase could be due to calcium soap formation as the soy cream is diluted with a Tris buffer containing calcium before digestion. The $L_\alpha$ phase transformed into the $I_2$ mesophase at around the 30 min mark – the sticky mesophase material induced some fluctuation in intensity, but overall the formation of the strongly diffracting $I_2$ mesophase was complete by around 40 min.

### 3.3. Fatty acid composition after digestion of the milk and plant-based systems

To investigate whether the mesophase structure of the bovine milk and plant-based drinks could be correlated with the release of a specific composition of FAs during digestion, the FA content at different time intervals of digestion was analysed using GC-FID. The FA distribution did not change significantly for the plant-based drinks during digestion, which can be seen in Fig. S3-S9. The bovine milk showed some compositional changes in FAs during digestion in the first 30 min, after which the composition remained constant. In Fig. 5, an overview of the FA composition at 60 min of digestion is presented for all systems.

The FA composition showed a remarkable difference between the bovine milk and the plant-based drinks. More than 20 % of the FAs released during digestion of the bovine milk were short to medium chain FAs (C12:0 and below). The plant-based drinks did not contain any significant amount of short or medium chain FAs, the “shortest” significant chain length detected being that of palmitic acid (C16:0). The plant-based drinks were composed of long chain FAs (LCFAs), the vast majority (around 80 % in all systems) being oleic and linoleic acid (C18:1 and C18:2 respectively). Small differences between the plant-based drinks were observed, such as the ratio between oleic and linoleic acid in oat cream and almond drink compared to the other four plant-based drinks. Furthermore, soy drink and oat cream were found to contain linolenic acid (C18:3). The plant-based drinks showed a FA composition of around 80–90 % unsaturated LCFAs compared to the 22 % found in bovine milk.

### 3.4. Principal component analysis of the fatty acid composition and correlation with mesophase behaviour

To investigate whether there is a relationship between FA composition and mesophase behaviour during digestion, principal component analysis (PCA) was applied. By using PCA, the FA composition of the systems at different times of digestion can be correlated to each other and described by loading plots. Three clusters can be seen in the PC1 against PC2 plot in Fig. 6a. The first cluster only contains the digestion time interval of bovine milk, the second contains the digestion intervals of soy drink, oat drink, rice drink and soy cream and the third contains the digestion intervals for almond drink and oat cream. Taking a closer look at the loading plots of PC1 (c) and PC2 (d), it can be observed that PC1 is positive if the ratio between oleic (C18:1) and linoleic acid

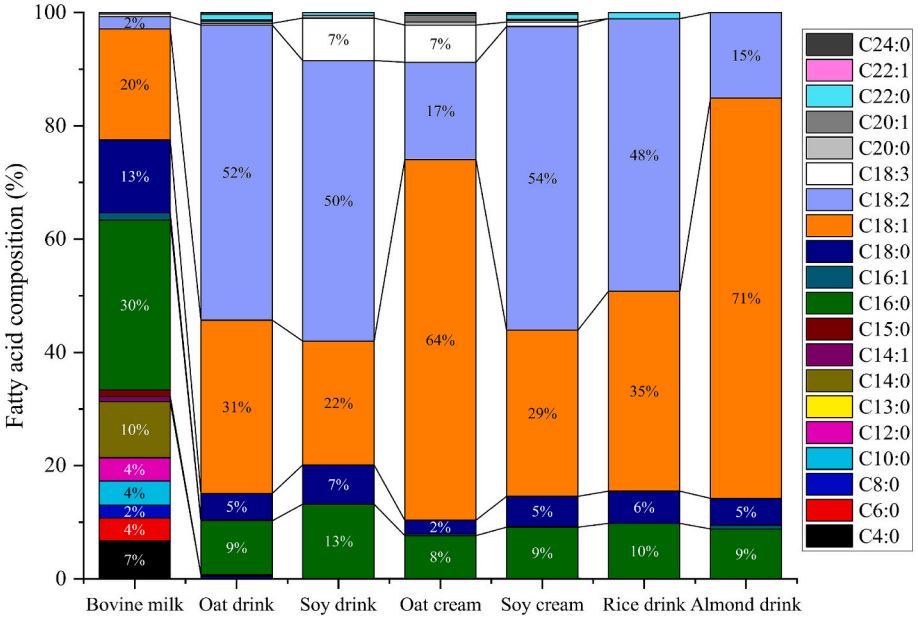


**Fig. 5.** FA composition after 60 min of digestion for bovine milk, oat drink, soy drink, oat cream, soy cream, rice drink and almond drink measured using GC-FID.

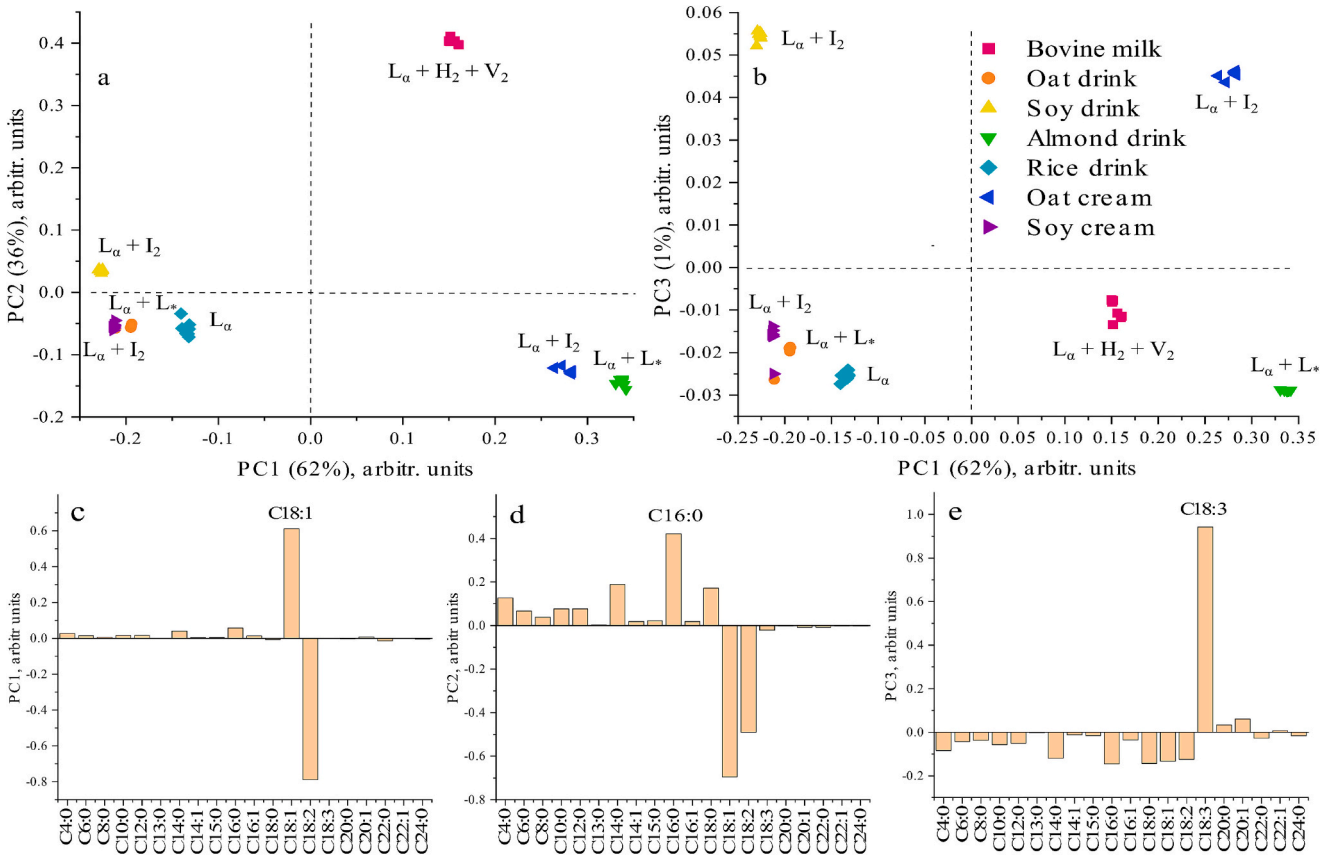


**Fig. 6.** Correlation of FA composition to mesophase behaviour of bovine milk and plant-based drinks at different digestion time intervals using PCA. More than 99 % of the variation was described by the three principal components included in the PCA. In this figure the PC1 *vs* PC2 (a), PC1 *vs* PC3 (b) and the loading plots for the three principal components (c, d and e) are shown.

(C18:2) favours the oleic acid and negative if the ratio favours linoleic acid. No other FAs are seen to give any significant contributions to the PC1 score.

The PC2 loading plot shows varying positive scores for all saturated FAs with a chain length of C18:0 and below and large negative scores for the unsaturated FAs, oleic and linoleic acid (C18:1 and C18:2). This translates to the separation of the bovine milk and plant-based drinks in the PC1 against PC2 plot. The PC3 loading plot shows a large positive score for the poly-unsaturated linolenic acid and mildly negative for most other FAs. This separates the oat cream and soy drink from the

other systems in the PC1 against PC3 plot.

The PCA makes it possible to contextualise the extent of similarities between the bovine milk and the plant-based drinks. Afterwards, the mesophases, at the given digestion intervals, are assigned manually to investigate the potential correlation of FAs and lipid self-assembly behaviour. The PCA shows that the plant-based drinks have a tendency to form the $I_2$ phase regardless of the plant-based PCA cluster association. Both the soy drink and the oat cream which contain 7 % linolenic acid (C18:3) formed the $I_2$ phase, indicating the linolenic acid shows strong correlation with this specific mesophase structure. However, the soy cream did not contain significant amounts of linolenic acid (C18:3) and still showed formation of the $I_2$ phase. Linolenic acid (C18:3) concentration can therefore not be said to be the only factor in the formation of the $I_2$ phase, but is likely to contribute to an increase in the CPP pushing the lipid self-assembly towards the inverse micellar mesophase structure.

## 4. Discussion

### 4.1. Lipid self-assembly of bovine milk and plant-based drinks during digestion

The formation of mesophase structures due to lipid self-assembly in bovine milk has been linked to increased nutritional uptake and potential pharmaceutical interactions, the formation of insoluble calcium soaps cause a decreased absorption of FAs and have importance especially in the design of infant formulas (Clulow et al., 2018; Mezzenga et al., 2019; Sagalowicz & Leser, 2010). To develop competent substitution products from plant matter, it is therefore important to determine which, if any, mesophase structures form during digestion. The plant-based drinks all showed mesophase formation due to lipid self-assembly, the $L_\alpha$ phase was formed during the digestion of all systems, and the $I_2$ phase was observed for both creams and in the soy drink. The $L_\alpha$ phase observed is likely due to a mixture of calcium oleate and calcium palmitate soaps formed during digestion, however other bilayer structures could contribute to $L_\alpha$ structures with similar lattice parameters. The calcium soaps of longer and unsaturated FAs have been documented to have less intense scattering properties (Clulow et al., 2018). This might be the reason why the intensity of the $L_\alpha$ phase in the bovine milk is larger than in the plant-based drinks, as the FA composition of the plant-based drinks primarily contain unsaturated LCFAs. Similarly, the intensity of the $I_2$ phase in the soy drink is lower than in the plant-based creams. However, the FA composition of these systems are almost identical and the difference in intensity might be due to the lower lipid content in the soy drink.

The plant-based drinks formed $I_2$ phases during digestion as the only non-$L_\alpha$ mesophase. Other milk and milk-like systems, which are known to form the $I_2$ mesophase during digestion, are certain infant formulas and human breast milk (Pham et al., 2020; Pham et al., 2021; Salim et al., 2020). The FA composition of various infant formulas have been determined in the literature and show remarkably similar FA composition to the plant-based drinks. This is not surprising as the lipid content of infant formulas is typically made by mixing different types of vegetable oils.

The lipid self-assembly of the plant-based drinks was different to bovine milk, with only calcium soap formation being a common factor in terms of mesophase behaviour. This correlates well with the different FA composition between the bovine milk and the plant-based drinks shown in Fig. 5. The FA composition of the plant-based drinks consisted of 80–90 % unsaturated LCFAs compared to the more diverse FA composition of bovine milk containing only 22 % unsaturated LCFAs, the remainder being short or medium chain saturated FAs. It therefore follows that from both a lipid nutrition and structure formation perspective the plant-based drinks are not similar to bovine milk.

### 4.2. Prediction of mesophase behaviour based on fatty acid composition

It is apparent that the large quantity of unsaturated LCFAs, particularly linolenic acid, drives the formation of the $I_2$ phase during digestion. This follows the general theory of the critical packing parameter. As the triglyceride fat droplets are digested by lipase enzymes, fatty acids are released along with di and monoglycerides. This reaction shifts the ratio of tri-, di-, monoglycerides and fatty acids in solution, which in turn will affect the self-assembly behaviour of the lipids. At the start of digestion the ratio will heavily favour triglycerides, towards the end of digestion the ratio will favour fatty acids and mono glycerides. The unsaturated LCFAs should have a relatively high lipophilicity, thereby increasing the CPP compared to bicontinuous cubic phases or inverse hexagonal phase, which would favour the formation of inverse mesophase structures. Several studies have tried to theoretically determine the CPP of different lipids at equilibrium (Khalil & Zarari, 2014; Kobierski et al., 2022){Khalil & Zarari, 2014 }. These studies focus mostly on phospholipids, cholesterols and long chain alcohols in a monolayer or aggregation formation. However, the CPP remains a qualitative method to predict potential mesophase behaviour as it is difficult to quantitatively define the local and global CPPs of a complex solution during dynamic changes in composition such as during digestion.

It should be noted that the *in vitro* simulated intestinal digestion model used in these studies has some limitations – firstly, no gastric step was involved – the impact of gastric digestion on subsequent intestinal processing is not yet known but as the distribution of FAs liberated during digestion, resulting from the starting triglyceride composition, is the primary driver of mesophase formation the lack of a gastric step is unlikely to change the conclusions with respect to mesophase formation by specific lipid compositions. *In vivo,* the absorption of lipids may not be uniform in terms of rates of absorption as monoglycerides have been suggested to be absorbed faster than FAs due to the interaction of FAs with calcium to form insoluble calcium soaps (Aoyama et al., 1996). Additionally, the surface chemistry of plant-based products compared to bovine milk should be investigated as this could affect both digestion dynamics and lipid self-assembly. This may result in changes in self-assembly compared to the closed *in vitro* model for a specific starting oil composition, but correlation with *in vivo* absorption and nutritional outcomes is beyond the discriminating studies presented here.

### 4.3. Implications of differences in lipid composition between plant-based drinks and bovine milk

The subject of plant-based drinks is controversial, in part due to their marketing as direct milk-substitutes as discussed above, but also to a lack of transparency about their composition for the consumer. Consumers could expect that a product marketed like milk with a specific plant name should consist primarily of components from that plant.

Controversially, the European Court of Justice ruled that plant-based drinks were **not** allowed to be labelled as milk, as it was found to be misleading to consumers *Verband Sozialer Wettbewerb eV v TofuTown.com GmbH,* 2017). On the contrary, the US Food and Drug Administration ruled that the plant-based drinks **could** be marketed as milks (*Labeling of Plant-Based Milk Alternatives and Voluntary Nutrient Statements: Guidance for Industry. (FDA-2023-D-0451-0002), 2023*). These conflicting rulings are examples of the uncertainty of whether these new plant-based drinks should in fact be viewed as substitutes for traditional dairy products. Only two of the products studied here, the soy and the almond drink, contain no added alternate vegetable oils. In contrast, the FA composition of the oat and rice drink, as well as the soy cream suggests that they are primarily or exclusively derived from sunflower oil. It could be argued that these liquids should be called sunflower drinks or creams as sunflower oil is the primary source of lipids in these systems. A similar lack of transparency is apparent for the oat cream used in this study as the FA distribution indicates that canola oil is the

primary source of lipids. This becomes apparent when comparing the FA composition of the plant drinks and creams in Fig. 5 with the FA composition of pure sunflower and canola oil in literature (Orsavova et al., 2015). The definition of what constitutes a specific plant-based drink is an interesting question and this study has shown that these drinks display differing structural behaviour during digestion, which could present a more relevant defining characteristic and may ultimately be shown to have a link to nutritional delivery.

Unsaturated LCFAs are in general considered as being healthy fats (Sokola-Wysoczanska et al., 2018), so whether this change in lipid composition from milk to plant-based drinks has a positive impact is up for debate. When looking at protein, fat and carbohydrate contents across the products (Table 1), bovine milk shows a more balanced distribution compared to the plant-based drinks. This is yet another factor to be aware of when substituting bovine milk with plant-based products.

Milk and infant formula are also being explored increasingly as lipid-based drug delivery systems as digestion of the lipid components can drive solubilisation of poorly water soluble drugs (Boyd et al., 2018; Gontsarik et al., 2021; Salim et al., 2022), presenting them in an absorbable form in the gastrointestinal tract. Plant-based drinks are directly analogous, and while they may be less likely to be utilised as an actual drug delivery system, the digestion of the lipids, the composition of liberated FAs and their self-assembly all are likely to play a role in interaction with drug molecules if consumed with medicines. An interesting topic, which deserves further exploration in the pharmaceutical research field.

## 5. Conclusion

This study investigated three research questions. Do the commercially available plant-based drinks form mesophase structures due to lipid self-assembly during digestion? If they do, are the mesophase behaviours of the plant-based drinks similar to bovine milk, to which they are marketed to be the substitutes? Moreover, is it possible to correlate and predict the FA compositions driving formation of the mesophase structure during digestion?

The mesophase structures formed during digestion of the plant-based drinks were determined and it was discovered that the $I_2$ phase was the only non-lamellar mesophase present during the digestion of the plant-based drinks. This limited variety of mesophases formed contrasts with the rich mesomorphism of bovine milk that has been observed to also form transient $H_2$ phase and a $V_2$ phase during digestion. Therefore, the first research question was answered in the positive while for the second the mesophase behaviours of the plant-based drinks during digestion cannot be said to be similar to that of bovine milk.

To address the third question, PCA was applied to the FA composition at different times of digestion and the correlation to mesophase formation was examined. The PCA showed three clusters clearly separating the bovine milk from the two different plant-based drink clusters. The mesophase behaviour correlated relatively well with the FA composition and the qualitative predictions based on the packing of lipids liberated on digestion of the milk and plant-based systems. However, the FA composition did not explain all mesophase behaviour and can therefore not be said to directly predict the lipid self-assembly during digestion.

Overall, the studies shed new light on the physical-chemical behaviour of plant-based drinks and how they compare to bovine milk. While a link between mesophase formation and nutritional delivery is still to be established in the field, the studies also illustrate the lack of clarity around what should actually constitute milk or plant drinks, and even highlighted significant differences in composition and behaviour of the plant-based drinks themselves. Future studies on implication for nutrient and possible drug delivery will enlighten opportunities or reservations of consumption of these systems.

## CRediT authorship contribution statement

**Peter Meiland:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Anas Aljabbari:** Methodology, Investigation, Conceptualization. **Shinji Kihara:** Writing – review & editing, Methodology, Formal analysis. **Kārlis Bērziņš:** Writing – review & editing, Methodology, Formal analysis. **Ulf Andersen:** Writing – review & editing, Supervision, Conceptualization. **Jacob J.K. Kirkensgaard:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Ben J. Boyd:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

## Declaration of competing interest

PM's PhD stipend is partly supported by Arla Foods through the Arla-KU PhD program. UA is an employee of Arla Foods. Arla Foods operates in the dairy industry. The authors declare no further conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.142031.

## Data availability

Data will be made available on request.

## References

Aoyama, T., Fukui, K., Taniguchi, K., Nagaoka, S., Yamaoto, T., & Hashimoto, Y. (1996). Absorption and metabolism of lipids in rats depend on fatty acid isomeric position. *The Journal of Nutrition, 126*(1), 225–231. https://doi.org/10.1093/jn/126.1.225

Binte Abu Bakar, S. Y., Salim, M., Clulow, A. J., Hawley, A., Pelle, J., Geddes, D. T., … Boyd, B. J. (2022). Impact of pasteurization on the self-assembly of human milk lipids during digestion. *Journal of Lipid Research, 63*(5), Article 100183. https://doi.org/10.1016/j.jlr.2022.100183

Boyd, B. J., Salim, M., Clulow, A. J., Ramirez, G., Pham, A. C., & Hawley, A. (2018). The impact of digestion is essential to the understanding of milk as a drug delivery system for poorly water soluble drugs. *Journal of Controlled Release, 292*, 13–17. https://doi.org/10.1016/j.jconrel.2018.10.027

Clulow, A. J., Salim, M., Hawley, A., & Boyd, B. J. (2018). A closer look at the behaviour of milk lipids during digestion. *Chemistry and Physics of Lipids, 211*, 107–116. https://doi.org/10.1016/j.chemphyslip.2017.10.009

Clulow, A. J., Salim, M., Hawley, A., & Boyd, B. J. (2021). Milk mimicry – Triglyceride mixtures that mimic lipid structuring during the digestion of bovine and human milk. *Food Hydrocolloids, 110*. https://doi.org/10.1016/j.foodhyd.2020.106126

Demurtas, D., Guichard, P., Martiel, I., Mezzenga, R., Hebert, C., & Sagalowicz, L. (2015). Direct visualization of dispersed lipid bicontinuous cubic phases by cryo-electron tomography. *Nature Communications, 6*, 8915. https://doi.org/10.1038/ncomms9915

Eason, T., Ramirez, G., Clulow, A. J., Salim, M., & Boyd, B. J. (2022). Revisiting the dissolution of Praziquantel in biorelevant media and the impact of digestion of Milk on drug dissolution. *Pharmaceutics, 14*(10). https://doi.org/10.3390/pharmaceutics14102228

Gontsarik, M., Yaghmur, A., & Salentinig, S. (2021). Dispersed liquid crystals as pH-adjustable antimicrobial peptide nanocarriers. *Journal of Colloid and Interface Science, 583*, 672–682. https://doi.org/10.1016/j.jcis.2020.09.081

Hayes, M. G., & Kelly, A. L. (2003). High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. *The Journal of Dairy Research, 70*(3), 297–305. https://doi.org/10.1017/s0022029903006320

Israelachvili, J. N., Mitchell, D. J., & Ninham, B. W. (1976). Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers [10.1039/F29767201525]. *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics, 72*(0), 1525–1568. https://doi.org/10.1039/F29767201525

Khalil, R. A., & Zarari, A.-H. A. (2014). Theoretical estimation of the critical packing parameter of amphiphilic self-assembled aggregates. *Applied Surface Science, 318*, 85–89. https://doi.org/10.1016/j.apsusc.2014.01.046

Kobierski, J., Wnetrzak, A., Chachaj-Brekiesz, A., & Dynarowicz-Latka, P. (2022). Predicting the packing parameter for lipids in monolayers with the use of molecular dynamics. *Colloids and Surfaces. B, Biointerfaces, 211*, Article 112298. https://doi.org/10.1016/j.colsurfb.2021.112298

Kumar, V. V. (1991). Complementary molecular shapes and additivity of the packing parameter of lipids. *Proceedings of the National Academy of Sciences, 88*(2), 444–448. https://doi.org/10.1073/pnas.88.2.444

*Labeling of Plant-Based Milk Alternatives and Voluntary Nutrient Statements: Guidance for Industry*. (FDA-2023-D-0451-0002). Retrieved from https://www.regulations.gov/comment/FDA-2023-D-0451-0472, (2023).

MacGibbon, A. K. H. (2020). Composition and structure of bovine Milk lipids. In P. L. H. McSweeney, P. F. Fox, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry, volume 2: Lipids* (pp. 1–32). Springer International Publishing. https://doi.org/10.1007/978-3-030-48686-0_1.

Martiel, I., Baumann, N., Vallooran, J. J., Bergfreund, J., Sagalowicz, L., & Mezzenga, R. (2015). Oil and drug control the release rate from lyotropic liquid crystals. *Journal of Controlled Release, 204*, 78–84. https://doi.org/10.1016/j.jconrel.2015.02.034

Maurer, S., Waschatko, G., Schach, D., Zielbauer, B. I., Dahl, J., Weidner, T., … Vilgis, T. A. (2013). The role of intact Oleosin for stabilization and function of Oleosomes. *The Journal of Physical Chemistry B, 117*(44), 13872–13883. https://doi.org/10.1021/jp403893n

McClements, D. J., & Li, Y. (2010). Review of in vitro digestion models for rapid screening of emulsion-based systems. *Food & Function, 1*(1), 32–59. https://doi.org/10.1039/c0fo00111b

McClements, D. J., Newman, E., & McClements, I. F. (2019). Plant-based milks: A review of the science underpinning their design, fabrication, and performance. *Comprehensive Reviews in Food Science and Food Safety, 18*(6), 2047–2067. https://doi.org/10.1111/1541-4337.12505

Mezzenga, R., Seddon, J. M., Drummond, C. J., Boyd, B. J., Schroder-Turk, G. E., & Sagalowicz, L. (2019). Nature-inspired design and application of Lipidic Lyotropic liquid crystals. *Advanced Materials, 31*(35), Article e1900818. https://doi.org/10.1002/adma.201900818

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences, 16*(6), 12871–12890. https://doi.org/10.3390/ijms160612871

Patton, J. S., & Carey, M. C. (1979). Watching Fat Digestion. *Science, 204*(4389), 145–148.

Pham, A. C., Clulow, A. J., & Boyd, B. J. (2021). Formation of self-assembled Mesophases during lipid digestion. *Frontiers in Cell and Development Biology, 9*, Article 657886. https://doi.org/10.3389/fcell.2021.657886

Pham, A. C., Peng, K. Y., Salim, M., Ramirez, G., Hawley, A., Clulow, A. J., & Boyd, B. J. (2020). Correlating digestion-driven self-assembly in Milk and infant formulas with changes in lipid composition. *ACS Applied Bio Materials, 3*(5), 3087–3098. https://doi.org/10.1021/acsabm.0c00131

Sagalowicz, L., & Leser, M. E. (2010). Delivery systems for liquid food products. *Current Opinion in Colloid & Interface Science, 15*(1–2), 61–72. https://doi.org/10.1016/j.cocis.2009.12.003

Sagalowicz, L., Leser, M. E., Watzke, H. J., & Michel, M. (2006). Monoglyceride self-assembly structures as delivery vehicles. *Trends in Food Science & Technology, 17*(5), 204–214. https://doi.org/10.1016/j.tifs.2005.12.012

Salentinig, S., Phan, S., Hawley, A., & Boyd, B. J. (2015). Self-assembly structure formation during the digestion of human breast milk. *Angewandte Chemie (International Ed. in English), 54*(5), 1600–1603. https://doi.org/10.1002/anie.201408320

Salentinig, S., Phan, S., Khan, J., Hawley, A., & Boyd, B. J. (2013). Formation of highly organized nanostructures during the digestion of Milk. *ACS Nano, 7*(12), 10904–10911. https://doi.org/10.1021/nn405123j

Salim, M., Eason, T., & Boyd, B. J. (2022). Opportunities for milk and milk-related systems as "new" low-cost excipient drug delivery materials. *Advanced Drug Delivery Reviews, 183*, Article 114139. https://doi.org/10.1016/j.addr.2022.114139

Salim, M., Ramirez, G., Peng, K. Y., Clulow, A. J., Hawley, A., Ramachandruni, H., … Boyd, B. J. (2020). Lipid compositions in infant formulas affect the Solubilization of antimalarial drugs Artefenomel (OZ439) and Ferroquine during digestion. *Molecular Pharmaceutics, 17*(7), 2749–2759. https://doi.org/10.1021/acs.molpharmaceut.0c00475

Sen, A., Acevedo-Fani, A., Dave, A., Ye, A., Husny, J., & Singh, H. (2024). Plant oil bodies and their membrane components: New natural materials for food applications. *Critical Reviews in Food Science and Nutrition, 64*(2), 256–279. https://doi.org/10.1080/10408398.2022.2105808

Sethi, S., Tyagi, S. K., & Anurag, R. K. (2016). Plant-based milk alternatives an emerging segment of functional beverages: a review. *Journal of Food Science and Technology, 53*(9), 3408–3423. https://doi.org/10.1007/s13197-016-2328-3

Sokola-Wysoczanska, E., Wysoczanski, T., Wagner, J., Czyz, K., Bodkowski, R., Lochynski, S., & Patkowska-Sokola, B. (2018). Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders-A review. *Nutrients, 10*(10). https://doi.org/10.3390/nu10101561

Valenze, D. (2011). *Milk: A local and global history*. Yale University Press.

*Verband Sozialer Wettbewerb eV v TofuTown.com GmbH*. (2017). Court of Justice of the European Union.