RESEARCH ARTICLE

European Journal of
Lipid Science and Technology
www.ejlst.com

Polar Lipids Reduce In Vitro Duodenal Lipolysis Rate of Oat Oil and Liquid Oat Base Products

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Alternative ways for increased appetite control are today widely sought for due to the growing global health issues connected to obesity. In in vivo studies, oat has been proven an attractive candidate for inducing satiety. Oat is rich in polar lipids, of which the galactolipids are especially interesting, and a hypothesis is that these lipids play an important role for the ileal brake mechanism. In this study, the aim is to investigate the role of polar oat lipids on pancreatic lipolysis rate, using a pH-stat based in vitro digestion model of the duodenum. Lipolysis of oat oil, a mix of oat oil/rapeseed oil (RSO), as well as a liquid oat base (OB) simulating an oat drink with different polar lipid content are investigated, and compared with RSO as control. Increasing the polar lipid content of the product digested leads to a significantly decreased lipolysis rate, and this effect is even observed when mixing RSO with a low amount of oat oil (10%). The results support the hypothesis that polar lipids can delay lipolysis also in a complex, natural system like the liquid OB, and even a minor amount of oat lipids can have large effect on lipolysis rates.
***Practical applications*: The number of studies connecting galactolipids with a decreasing effect on duodenal lipolysis is growing; however, the mechanism behind this phenomenon is still not clarified. Here, the same effect is seen in a complex, natural food system. These findings open up for interesting future food products, where inclusion of oat oil, even at low concentrations, can have a prolonging effect on satiety. Oat for human consumption is an increasing market, thanks to the positive health benefits oat has been connected to, in combination with the current trend toward climate-friendly plant-based options for meat and dairy products. It is believed that oat oil can be attractive as an ingredient in various food products, for example, protein bars and spreads. More studies are needed to confirm the results in vivo. However, a great potential is seen for the use of oat oil to enhance appetite control.**

1. Introduction

Overweight and obesity are increasing health problems in large parts of the world. An attractive way to combat these problems is the use of dietary components inducing satiety, thereby decreasing the further consumption of food. A promising approach is based on reduced lipolysis in the gastrointestinal tract so that some lipids remain undigested all the way to the ileum. There, the lipids can cause the release of hormones signaling satiety, in a mechanism called the ileal brake.[1] Considerable efforts have been made to design food for appetite control based on this mechanism.[2]

One type of product, suggested to work according to the ileal brake mechanism, is based on biological membranes, for example thylakoid membranes extracted from spinach leaves. The role of thylakoids on satiety has been studied thoroughly both in vitro,[3] and in vivo.[4–8] A hypothesis is that the reduction of lipolysis rate is mediated by the membrane proteins. These will cover the lipid droplets, thereby restricting the access of lipase to its lipid substrate. Alternatively, the membrane proteins could interact directly with the digestive enzymes, decreasing their catalytic activity, e.g. by binding to the pancreatic lipase/co-lipase complex.[9] This leads to an increased amount of free fatty acids (FFA) in the ileum, which in turn stimulates hormone signals of satiety.[2] For treatment of, for example, diabetes and obesity, orlistat (also known as Xenical) is commonly used. It is a lipase inhibitor acting by blocking the catalytic site of pancreatic lipase by binding to the serine residue.[10] However, an important difference between the action of orlistat and thylakoid membranes is that the use of orlistat leads to incomplete lipolysis, that is, leaving approximately 30% of the triacylglycerides (TAG) intact after digestion and reducing the level of released FFA.[9,10] This means that the natural release of satiety hormones is not activated when using orlistat, and that the use of the drug might lead to steatorrhea,[9] which in turn could lead to a loss of, and subsequent reduction of uptake of lipophilic micronutrients.[11] Using products targeting the ileal brake mechanism may help surpass these side effects.

A crop with reported native ability to increase satiety after intake is oat, a cereal that has drawn public attention lately due to

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/ejlt.202000317

DOI: 10.1002/ejlt.202000317

its related positive health effects. These effects are ascribed to its high levels of water-soluble β-glucan,[12] and antioxidants, such as tocopherols and avenanthramides.[13] The ability to prolong satiety has previously been credited the β-glucans,[14] however there is increasing evidence that the oat lipids also play an important role. Oat differs from other cereals in lipid profile, with a high total lipid content (5–8% oil of dry weight (w/w) measured in de-hulled grains, with lipid-rich varieties containing up to 18% oil), and a high level of polar lipids, that is, galactolipids and glycerophospholipids.[15] These polar lipids are interesting from a health perspective but are also surface-active agents, which makes them highly interesting as natural food additives. The polar lipids can prevent interaction between the enzyme and the neutral lipids by adsorbing to the oil–water interface and sterically hinder the adsorption and penetration of colipase and lipase, thereby slowing down the lipolysis rate of pancreatic lipase,[16] a mechanism which has specifically been ascribed to the galactolipid digalactosyl diacylglycerol (DGDG).[17,18] An oat oil-rich product on the market designed for increasing satiety is Olibra (now known as Fabuless), a 42% o/w emulsion containing palm oil and oat oil. This product has been shown to decrease energy and macronutrient intake up to 36 h post-consumption.[19] Olibra has also been shown effective in increasing satiety over short time (1–3h after intake),[20] and for weight maintenance followed over 18 weeks after weight loss.[16] Other promising results with oat based (OB) products were obtained using liposomes prepared from oat oil fractionates rich in polar lipids, evaluated in a pair of human studies.[21] The studies indicated that this product prolonged lipid digestion and had an influence on satiety in healthy, non-obese participants. Plasma concentrations of the intestinal hormones CCK, GLP-1, GLP-2, and PYY increased significantly. The liquid OB products used in our study are used in an ongoing human study as well.[22] Preliminary results from this study indicate increased concentration of intestinal hormones and reduced postprandial glucose and insulin responses after intake of liquid OB products.

Human studies constitute the best way to evaluate new functional food products, but these studies are expensive and time-consuming, which results in a limited number of tested products. In order to select products for such studies, it is attractive to have relevant, rapid, and inexpensive screening methods. To simulate lipolysis in the human gastrointestinal tract, various in vitro systems have been developed. Considerable efforts involving numerous researchers in the network Infogest have resulted in useful protocols and guidelines.[23,24] In the more comprehensive protocols, oral, gastric, and intestinal phase digestion is simulated. Alternative, simplified protocols focus on the intestinal phase, during which the main part of lipolysis occurs. These can be carried out using pH-stat methodology to monitor addition of base to continuously titrate fatty acids (FA) set free from the emulsified lipid substrate.[25]

The aim of the present study was to investigate the effect of oat oil with different polar lipid content on duodenal lipolysis rate, using a simplified and rapid pH-stat based in vitro digestion model. Lipolysis of oat oil was compared with lipolysis of rapeseed oil (RSO), and the effect of mixing the two oils in different ratios was followed. The hypothesis was that changes in polar lipid amount of the oil would affect the duodenal lipolysis rate. Furthermore, a liquid OB product supplemented with oat oil with increasing polar lipid amount was used as a starting food in the model, to simulate digestion of a commercial oat drink, following its lipolysis kinetics.

2. Experimental Section

2.1. Materials

SWEOAT Oil PL4, SWEOAT Oil PL15, and SWEOAT Oil PL40 (oat oil with 4%, 15%, and 40% polar lipids, respectively) were kindly provided by Swedish Oat Fiber AB (Bua, Sweden). Refined RSO was purchased from a local supermarket. Liquid OB with and without supplementation of 6% rapeseed oil, SWEOAT Oil PL4 or SWEOAT Oil PL40 was provided by Oatly AB (Landskrona, Sweden). The nutritional composition of the OB products can be found in **Table 1**, data provided by the manufacturer. Pancreatin from porcine pancreas (#P7545) and sodium taurodeoxycholate hydrate (#T0875) were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Lipid standards were purchased from Larodan AB (Solna, Sweden), and for fatty acid methyl esters (FAME) analysis, the GLC 426 mix from Nu-Check-Prep (Elysian, MN, USA) was used as external standard. Standards for the hydroxy FA C16:0 7-OH and C18:2 15-OH described by Leonova et al.[26] (C16:0 7-OH) and Hamberg et al.[27] (C18:2 15-OH), were kindly provided by Mats Hamberg (Karolinska Institutet, Stockholm, Sweden). All other chemicals used were of analytical grade.

2.2. Methods

2.2.1. Pancreatin Activity Assay

The lipase activity of pancreatin was measured in a pH-stat automatic titration unit (TitroLine7000, SI Analytics) according to Carriere, Barrowman,[28] adapted from Borgström and Erlansson.[29] The assay buffer contained 0.3 mM tris(hydroxymethyl)aminomethane, 150 mM sodium chloride, 1.7 mM calcium chloride, and 4 mM sodium taurodeoxycholate. Substrate (0.5 mL OB product or 50 mg oil) was added to the assay buffer (total volume 15 mL) and pH was adjusted to pH7. After mixing with an Ultra Turrax (IKA T25 digital ULTRA TURRAX) at 19 000 rpm for 2 min (oil samples), or by vortexing 30 s (OB samples), the emulsion formed was immediately transferred to a reaction vessel and the lipolysis reaction was started by addition of porcine pancreatin (0.1–1.2 mg depending on substrate). The titration was performed with 0.1 M NaOH at 37 °C and pH7 for 10 min. The volume of NaOH added was recorded and used to calculate the specific enzyme activity as µmol FFA released per minute and mg pancreatin during the initial, linear period of the lipolysis reaction. After the lipolysis reaction, aliquots of 1 mL were taken from the reaction mixture and put in −80 °C until further analysis.

2.2.2. Lipid Extraction and Analysis

Lipids were extracted from the 1 mL aliquots according to Bligh and Dyer,[30] by addition of 1.25 mL 0.15 M acetic acid, 3.75 mL

Table 1. Nutrient composition (g per 100g) and particle size distribution (PSD) of liquid oat base (OB) product in pH-stat assay solution, with or without added oil. RSO, rapeseed oil; PL4, and PL40, oat oil with 4%, and 40% polar lipids, respectively. Nutrient data received from manufacturer, PSD data representing mean ± std ($n = 3$).

Nutrient [g per100g]	OB + 6% H2O	OB + 6% RSO	OB + 6% PL4	OB + 6% PL40
Total fat	0.6	6.6	6.6	6.6
Polar lipids	0.0	0.0	0.2	2.4
Total carbohydrates	8.4	8.4	8.4	8.4
Sugars	5.1	5.1	5.1	5.1
Fiber	1.0	1.0	1.0	1.0
β-glucan	0.5	0.5	0.5	0.5
Protein	1.3	1.3	1.3	1.3
PSD				
$d_{4,3}$ [µm]	22.77 ± 1.94	13.61 ± 1.02	11.91 ± 1.62	5.4 ± 0.8
$d_{3,2}$ [µm]	0.56 ± 0.01	8.22 ± 0.19	5.74 ± 1.04	0.25 ± 0.01

methanol/chloroform (2:1 v/v) and 1.25 mL chloroform. The chloroform phases were recovered, dried under N_2 (g), and dissolved in chloroform. Lipids were separated by thin layer chromatography (TLC; Silica gel 60, Merck) in heptane:diethyl ether:acetic acid (70:30:1, v/v/v) or chloroform:methanol:water 95:20:2.5 (v/v/v) and visualized under UV-light after staining with primulin. Identification of lipid classes was done by comparing with lipid standards.

Methylated FA was prepared from the oils and lipid extracts using the 2 h methanolic-HCl transesterification method described by Cavonius, Carlsson[31] and analyzed on a Trace 1300 GC-FID with autosampler (Thermo Scientific). The GC was equipped with a flame ionization detector (FID) set at 270 °C, and hydrogen used as carrier gas (35 mL min^{-1}). To separate FAME, a Supelco Analytical Nukol fused silica capillary column was used (15 m × 0.25 mm i.d. × 0.25 µm film thickness). The oven was set at 80 °C as starting temperature with a temperature program ramping at 7 °C min^{-1} to 150 °C, followed by 10 °C min^{-1} to 215 °C and then kept until the end of the program (total runtime 40 min). Data was analyzed using the Chromeleon 7.2.10 Chromatography Data System (CDS) software (Thermo Scientific). All FA identified were confirmed and quantified using external standards and C17:0 as internal standard.

2.2.3. Particle Size Distribution

The particle size distribution (PSD) of the droplets formed by the liquid oat products in the pH-stat assay solution was analyzed to investigate the role of the droplet size on lipolysis rate. A laser diffraction particle analyser (Mastersizer 2000, Malvern) was used for collecting the PSD data and data was further processed using the software Mastersizer 2000 (Malvern). Obscuration was set to be 10–20% and samples were added dropwise to the dispersion unit (100 mL MilliQ water) during constant stirring (2000 rpm). From this, the PSD and the volume weighted mean ($d_{4,3}$), and surface weighted mean ($d_{3,2}$) were calculated.

2.2.4. Statistical Analysis

Results are shown as mean values, and error bars represent standard deviation. Replicate number is ≥3 for all data analyzed. Specific replicate number is described in figure or table text, respectively. Groups were compared by a one-way analysis of variance (ANOVA; Microsoft Office Excel Professional Plus 2016) and specific differences between groups were statistically analyzed by Student´s two-tailed, unpaired *t*-test, assuming unequal variances. Differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Fatty Acid Composition

The FA compositions (% of total FA detected) of the different substrates used in the pancreatin activity assays are presented in **Table 2**. C18:2 15-OH (avenoleic acid) is a hydroxy FA which is unique for oat and primarily found in the galactolipid fraction.[32] The largest differences between oat and rapeseed FA profile are observed for the C16:0, which is low in rapeseed and high in oat, and C18:1 and C18:3 which are both higher in rapeseed compared to oat. In the oat products, the dominating FA are C18:2 and C18:1, followed by C16:0. In RSO, C18:1 was the major FA followed by C18:2 and C18:3. The lower content of C18:1 in PL40 compared to PL4 and PL15 reflects well the expected changes in FA profile that comes with a higher polar lipid content.[26] The appearance of avenoleic acid is also significantly higher in the polar lipid enriched oil (PL40) compared to PL 4 and PL15 ($p \leq 0.001$), which is also according to the literature.[26]

3.2. Lipolysis of Oat Oil

The rate of in vitro lipolysis of oat oils with different amounts of polar lipids was compared with the rate of in vitro lipolysis of RSO. Under standard conditions, an initial lipolysis rate of about 6.7 µmol $(min)^{-1}$ $(mg\ pancreatin)^{-1}$ was observed for the RSO emulsion (**Figure 1**). This lipolysis rate is approximately tenfold lower compared to values obtained when using tributyrin as a substrate.[33] This might be due to differences in pancreatic lipase activity toward TAG with long-chain FA.[34] Substantially lower lipolysis rates were obtained for emulsions of oat oils ($p \leq 0.001$) and the rate decreased with increasing concentration

Table 2. Fatty acid (FA) composition of tested products from analysis of total fatty acid methyl esters (FAME), expressed as FA% of total FA. OB, liquid oat base; RSO, rapeseed oil; PL4, PL15, and PL40, oat oil with 4%, 15%, and 40% polar lipids, respectively; n.d., not detected; $n = 3$.

Compound	OB	RSO	PL4	PL15	PL40
C14:0	0.23 ± 0.14	0.05 ± 0.005	0.11 ± 0.02	0.11 ± 0.02	0.17 ± 0.03
C16:0	15.29 ± 0.56	4.20 ± 0.03	13.96 ± 0.31	14.22 ± 0.17	15.44 ± 0.06
C16:1	n.d	n.d.	0.19 ± 0.02	0.11 ± 0.06	0.16 ± 0.005
C18:0	1.42 ± 0.14	1.96 ± 0.02	1.33 ± 0.01	1.31 ± 0.01	1.12 ± 0.03
C18:1	35.13 ± 0.41	51.67 ± 0.11	42.71 ± 0.25	41.29 ± 0.23	35.00 ± 0.12
C18:2	44.00 ± 0.61	36.29 ± 0.10	39.34 ± 0.10	40.31 ± 0.09	44.06 ± 0.12
C18:3	1.85 ± 0.07	4.73 ± 0.03	1.08 ± 0.02	1.15 ± 0.01	1.35 ± 0.01
C20:0	n.d.	0.33 ± 0.01	0.10 ± 0.002	0.09 ± 0.01	0.07 ± 0.002
C20:1	0.80 ± 0.05	0.67 ± 0.01	0.64 ± 0.004	0.61 ± 0.02	0.53 ± 0.02
C16:0 7-OH	0.15 ± 0.19	n.d.	0.23 ± 0.07	0.18 ± 0.01	0.32 ± 0.04
C18:2 15-OH	1.14 ± 0.08	n.d.	0.28 ± 0.003	0.63 ± 0.02	1.79 ± 0.03

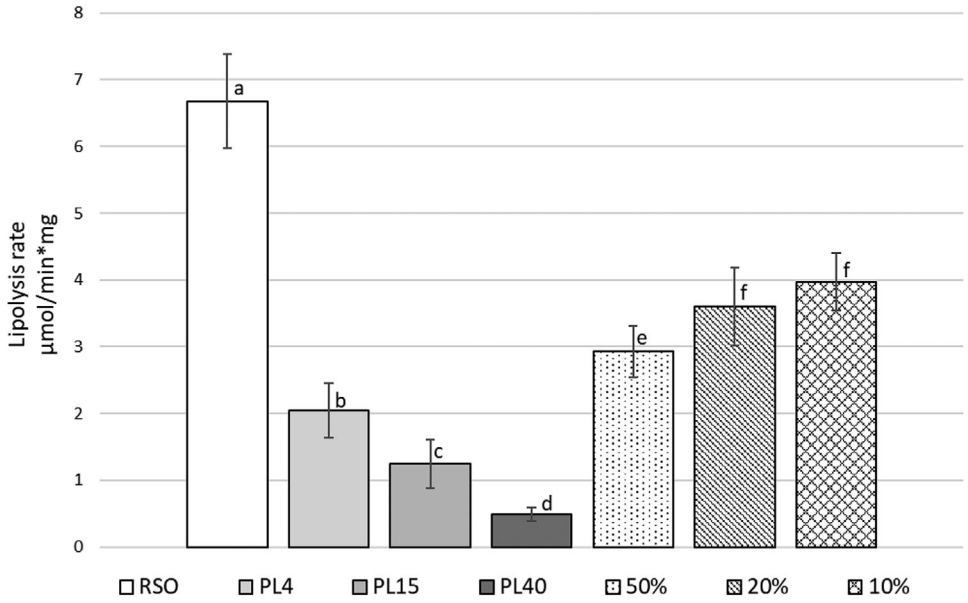

Figure 1. Initial lipolysis rates of porcine pancreatin with rapeseed oil (RSO), oat oils or rapeseed oil containing oat oil as substrates. PL4, oat oil with 4% polar lipids; PL15, oat oil with 15% polar lipids; PL40, oat oil with 40% polar lipids; 50%, RSO containing 50% PL40; 20%, RSO containing 20% PL40; 10%, RSO containing 10% PL40. Different letters indicate significant differences at a level of $p \leq 0.001$, with the only exception when comparing e–f) $p \leq 0.05$. $n = 7$ or 8. Standard deviation is shown as error bars.

of polar lipids in the oat oil. In the oat oil having 40% polar lipids, the initial lipolysis rate was only 7% of that of RSO. Interestingly, the lipolysis rate of the RSO decreased when oat oil with high levels of polar lipids was blended into the emulsion. Additions of 50%, 20%, and 10% oat oil (containing 40% polar lipids) were tested. The lipolysis rate decreased with increasing content of oat oil (Figure 1), but even an addition of 10% of oat oil reduced the lipolysis rate to 59% of that of pure RSO.

3.3. Lipolysis of Liquid Oat Products

The initial lipolysis rates of a series of more complex test products, based on oat, were measured. OB without extra addition of oil was digested with an initial rate of 7 µmol $(min)^{-1}$ (mg pancreatin)$^{-1}$ and the product containing an addition of RSO was digested with a similar rate (**Figure 2**). Products containing oat oil in addition to the OB were digested with considerably lower rates ($p \leq 0.01$). As in the previous tests, PL40 caused a larger rate reduction than PL4 ($p \leq 0.05$). The rate reductions were not as large as in the pure oat oil system (Figure 1), but still the addition of oat oil having 40% polar lipids resulted in a lipolysis rate of only 33% of that observed for OB with RSO.

When the lipolysis was followed over time, additional findings regarding the reaction were made. The lipolysis reaction with the pure OB, containing a low amount of lipids, was almost completed already after about 2 min, while the lipolysis of the OB containing addition of RSO continued much further, albeit at a lower rate (**Figure 3**). In contrast to the other liquid oat products, the lipolysis of the one containing PL40 did not slow down after

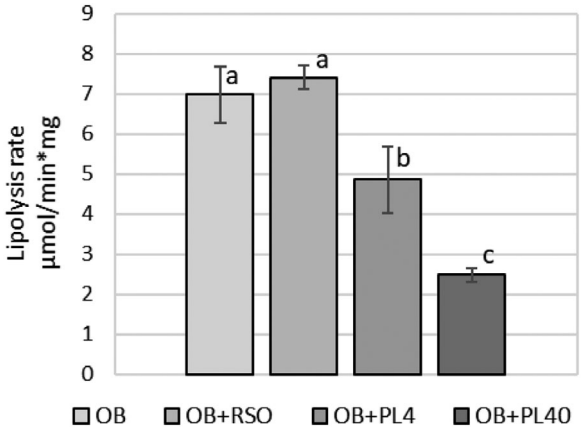

Figure 2. Initial lipolysis rates of porcine pancreatin with liquid oat base (OB) products as substrates. OB, OB without added oil; OB + RSO, OB with 6% RSO; OB + PL4, OB with 6% PL4; OB + PL15, OB with 6% PL15; OB + PL40, OB with 6% PL40. Different letters indicate significant differences at a level of $p \leq 0.01$, with the exception when comparing a with c ($p \leq 0.001$), and b with c ($p \leq 0.05$). $n = 4$. Standard deviation is shown as error bars.

the initial 2–3 min, but continued at a steady, low rate for more than 8 min.

TLC was used to visualize the presence of various lipid classes after digestion of the OB products. The TAG and the DAG of the pure OB product were completely hydrolyzed after 10 min with 0.5 mg pancreatin (**Figure 4**). OB with added RSO or oat oil had proportionally more TAG initially than the pure OB and some of the TAG remained at the end of the reaction. Both FFA and diacylglycerols (DAG) were detected as reaction products. In the OB products with added oat oil, more TAG remained after 10 min incubation, even with 0.5 mg pancreatin. Another TLC system was used to analyze polar lipids in the OB products before and after digestion. In OB supplemented with PL40, DGDG, monogalactosyl diacylglycerol (MGDG), and phosphatidylcholine (PC) were identified using lipid standards (figure not shown). When comparing the amount of lipids before and after lipolysis, no difference could be seen for these lipid classes or other polar lipids, and no additional bands originating from digestion products such as digalactosyl monoacylglycerol (DGMG) and monogalactosyl monoacylglycerol (MGMG) were found on the TLC plate. This indicates that the digestion of polar lipids was minor during the lipolysis reaction in the pH-stat.

PSD data of the liquid OB shows that the smallest droplet sizes were obtained for the OB with added PL40 followed by the pure OB (Table 1 and **Figure 5**). The mean particle size for the emulsion droplets was increasing in the order of OB+PL40 (0.24 µm) < OB (5.1 µm) < OB+PL4 (7.9 µm) < OB+RSO (11.0 µm). Pure OB showed however the broadest overall distribution, contributing to the highest $d_{4,3}$ value. OB with added PL4 and RSO showed similar PSD pattern with one dominating peak with a majority of the droplets of a similar size.

4. Discussion

Oat products have previously been shown to have positive effects on appetite control. The underlying mechanism is so far not fully clarified, but the unique lipid composition in oat, with a large proportion of polar lipids, seems to be an important factor. In this study, we examined the effect of polar lipid content in oat oil on lipolysis rate, in bulk oils but also in a more complex matrix; the OB product. The results when using oat oil products were compared with results when using the corresponding products with RSO.

All products containing oat oil were hydrolyzed with reduced rate, compared to the RSO. Grundy et al. recently reported that oat extracts can act inhibitory on digestion, decreasing the total FFA release level.[35] Oat phytosterols have been reported to reduce the extent of maximal lipolysis, but not the rate,[36] which is also true for oat β-glucans.[37] Instead, it is likely that the polar oat lipids caused the reduction in lipolysis rate in this study. The rate decreased with increasing concentration of polar lipids to a very low level in the substrate containing 40% polar lipids. One surprising observation was that even a minor addition of oat oil (10%) to RSO greatly decreased the lipolysis rate, something that could be very useful when designing food products to enhance satiety. We have focused on following the initial lipolysis rate, since there are many factors in a static in vitro digestion model that can limit the maximal extent of lipolysis. Since the lipase is active at the o/w interface of the oil droplets, the packing of enzyme at the surface, and hence, for example, the lipase amount in the reaction vessel, is very important. The space at the o/w interface needs to be large enough for the lipase to act, but at the same time, the concentration of the lipase in solution needs to be high enough to compete with bile and other surface-active agents at the droplet interface.[38] The competition of surfactants at the o/w interface makes both the lipase, calcium, and bile concentration important for maximal lipolysis, but also the presence of, for example, other emulsifiers in the reaction vessel. In vivo, there would be a continuous addition of enzymes and removal of products, factors influencing the maximal lipolysis dramatically. Since the limiting factors for reaching a steady-state in our digestion system are unknown, we have in this study chosen to focus on the initial linear lipolysis rate, which we believe reflects differences in pancreatic lipolysis that would be true in vivo as well.

When enriching the OB product with polar lipids, the reduction in lipolysis rate was substantial, with the most pronounced decrease after addition of PL40. It is possible that both the initial polar lipids present in these OB products, and the lipolysis products of their corresponding lipids contribute to this effect. It has long been known that PC inhibits lipolysis catalyzed by pancreatic lipase,[39] and it is known from the literature that approximately 50% of the glycerophospholipids in oat consists of PC.[40] One major effect that could be responsible for the decreased lipolysis rate is that the polar lipids, such as PC, compete with the lipase for binding at the water/substrate interface.[41] In fact, many surface-active substances behave in similar ways.[42] The oat oil contains several types of polar lipids, with glycerophospholipids and galactolipids as the dominating groups. It has been reported that the galactolipid DGDG is especially effective in preventing lipolysis.[17] A possible mechanism behind this effect is that the relatively large digalactosyl head groups prevent the lipase from contact with the surface of the lipid substrate. It has also been suggested that the FA composition of the DGDG fraction could contribute to the effect,[17] and it is known that the chain-length

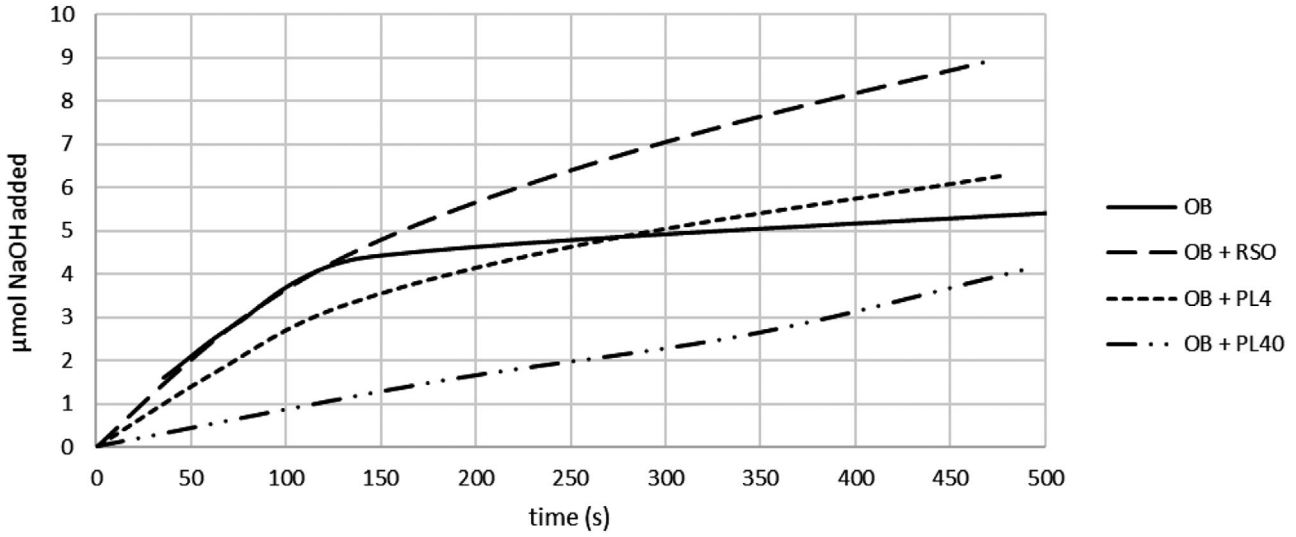

Figure 3. Lipolysis reactions of porcine pancreatin with liquid oat base (OB) products as substrates. The amount of NaOH added during the assay is shown as a function of the reaction time for one representative measurement. OB, OB without added oil; OB + RSO, OB with 6% RSO; OB + PL4, OB with 6% PL4; OB + PL15, OB with 6% PL15; OB + PL40, OB with 6% PL40.

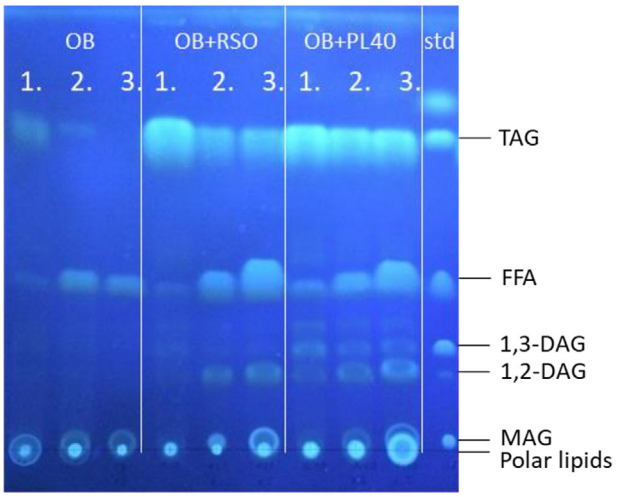

Figure 4. TLC separation of neutral lipids in liquid oat base (OB) products before and after lipolysis. OB, OB without added oil; OB + RSO, OB with 6% RSO; OB + PL40, OB with 6% PL40. Lanes: 1) before the lipolysis reaction, 2) after 10 min of lipolysis using 0.2 mg porcine pancreatin, 3) after 10 min of lipolysis using 0.5 mg porcine pancreatin, 4) lipid standard (std).

of FA effects the lipolysis rate, with longer chain-length leading to a decreased rate.[34] Hence, it is more likely that the hypothesis regarding sterical hindrance is the main driving effect in the case of oat oil on lipase inhibition. Another possibility is that the oat specific FA which are present in the start material has an inhibitory effect on the pancreatic lipase. One such interesting FA is the avenoleic acid, which in this study was detected at the highest levels in the PL40 oat oil. The possibility that this hydroxy-FA has an inhibitory effect on the lipolytic activity should be investigated further. Furthermore, it should be noted that galactolipids are poorly digested by pancreatic lipase, but degraded by the pancreatic enzymes HPLRP2 and CEH.[43] It has also been shown that both DGDG and MGDG are rapidly digested in the intestinal tract of rats.[44] CEH is present in porcine pancreatin, while PLRP2 has until today only been detected in porcine pancreatic juice, never in porcine pancreatic extract.[33,45] However, activity toward galactolipids has been recorded (0.3 µmol (min)$^{-1}$ (mg porcine pancreatic extract)$^{-1}$), which is about 25-fold lower activity compared to what has been detected with human pancreatic juice[33] and could explain differences found on digestion of PL-rich food between in vitro and in vivo studies. In this study, no significant difference in polar lipid content or profile before and after lipolysis of OB products could be detected with TLC analysis. Even if some digestion of polar lipids takes place, it seems to be minor compared to the digestion of TAG during the relatively short period of the in vitro assay. The results obtained here are consistent with the results presented in the study by Ohlsson et al.,[21] where healthy normal weight participants given oat oil were found to have increased levels of the intestinal appetite regulating hormones. This is in line with delayed intestinal lipolysis – probably due to the emulsifying effect of the polar lipids, creating sterical hindering and thereby delaying the binding of the pancreatic lipase to the oil water interface. Yet another hypothesis is that the delaying effect of the polar lipids on the lipase activity could be due to the emulsions created having a different PSD, which could affect the possibility of the lipase to reach the oil/water interface of the droplets. It is well known that emulsion droplet size plays a role for lipolysis, with smaller droplets being beneficial for increased lipolysis rate.[46] Smaller droplets unlock a larger surface area, hence decreasing the risk of limited surface capacity. The results from the PSD data show that the emulsions created with PL4 and RSO added to liquid OB are similar to each other, while addition of PL40 gives rise to a PSD pattern with smaller droplets. This is expected, since an increasing amount of polar lipids should facilitate emulsification and help forming smaller and more stable droplets. However, this should according to the literature increase the lipolysis rate,[46] and hence does not explain the delaying effect on lipolysis seen in this study.

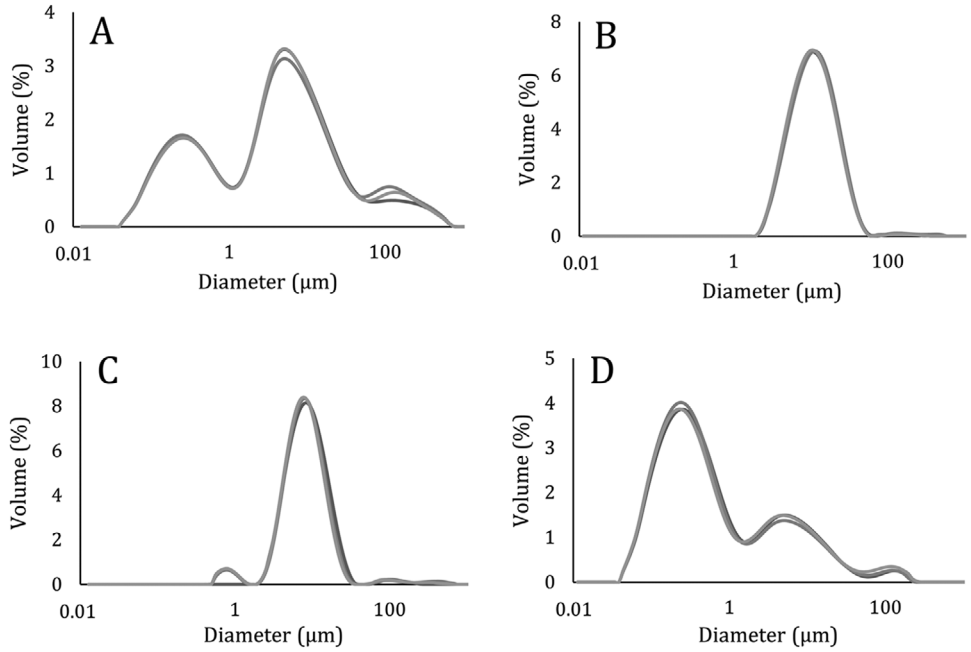

Figure 5. Particle size distribution (PSD) of liquid oat base (OB) products in pH-stat assay solution. A, OB without added oil; B, OB with 6% RSO; C, OB with 6% PL4; D, OB with 6% PL40; n = 3.

The initial lipolysis rate for the OB product without added oil was almost as high as the rate for OB with added RSO. However, these substrates differ substantially in lipid content and the lipolysis rates can therefore not be directly compared. It is seen from both the lipolysis assay and the TLC analysis after the reaction that the substrate is almost completely consumed during the first minutes of digestion of the OB product. The results indicate that the OB contains some lipids (according to the manufacturer 0.6%) which are hydrolyzed as rapidly as those in OB + RSO, but not enough of inhibitory compounds such as polar lipids to reduce the lipolysis rate to any large extent.

One should be aware that the model system set up in this study is a very simplified model of the lipolysis in the human intestinal tract. More sophisticated assay systems can be constructed to come closer to the physiological situation.[24] Possible improvements could be to introduce oral and gastric phases in the protocol. Humans do not have significant lipase activity in the saliva, but digestion of other food components can have an indirect effect on lipolysis. We do however have a gastric lipase, which is responsible for 10–25% of the total lipolysis.[45] The effect of the human gastric lipase on digestion of polar lipids has not been well studied, and the inclusion of gastric lipase in the model could affect the subsequent duodenal lipolysis. The role of gastric lipase on polar lipid digestion should therefore be investigated further. Part of the aim of this study was to find out if the simple and rapid pH-stat method is suitable as a screening method when investigating duodenal lipolysis in vitro, and our results indicate that the simple assay used in this study fulfills the requirements to show similar trends as in human studies. This study thus emphasizes the interesting effects of polar oat lipids, giving hints on the mechanism behind these effects.

The cultivation of oat has steadily been decreasing the last 50 years. This since oat predominantly has been used as horse feed, and its use has subsequently been strongly affected by the industrial revolution and changes in the agricultural sector.[47] However, the cereal is well adapted to the Nordic cold and humid climate, with a low need for fertilizers and with a high nutritional value, and the crop is therefore an optimal candidate to meet the global demands for future sustainable food production.[15] Oat oil has been raised as an interesting future food ingredient both from a nutritional and food processing point of view,[13] and various types of plant-based dairy alternatives, such as OB drinks are gaining market shares.[48] The possibility that is raised with the results from the current study; that addition of polar lipid enriched oat oil has a decreasing effect on duodenal lipolysis, is highly interesting from a food production perspective, especially as a way to address the globally increasing health problems connected to obesity. Potential applications are among others to include oat oil in energy bars, spreads, and nutritional drinks. Addition of oat oil to complex food matrices as a way to control appetite should therefore be further investigated in vivo.

5. Conclusions

We have used a pH-stat based digestion method to examine the duodenal lipolysis of oat oil emulsions in vitro. The method worked well for both simple o/w emulsions and complex relevant food samples containing proteins, carbohydrates, and more. The lipolysis rate was found to be lower for the oat oils compared to rapeseed oil, and the higher the polar lipid content, the lower the lipolysis rate. The results from this study are thus in agreement with previous findings that polar lipids reduce lipolysis rate, and this was found to be true both in simple systems containing o/w emulsions and more complex oat base containing systems. In addition, when rapeseed oil was supplemented with oat oil with high polar lipid content, the lipolysis rate was reduced. This

result is promising for the use of oat oil in food products with the aim to increase satiety.

Acknowledgements

The authors thank Swedish Oat Fiber and Oatly for providing the oat raw material. This study was performed with the help of funding from the Swedish Foundation for Strategic Research (the Industrial Research Center ScanOats).

Abbreviations

CEH, Carboxyl Ester Hydrolase; DAG, Diacylglycerol; DGDG, Digalactosyl Diacylglycerol; DGMG, Digalactosyl Monoacylglycerol; FA, Fatty Acids; FFA, Free Fatty Acids; FID, Flame Ionization Detector; GC, Gas Chromatography; HPLRP2, Human Pancreatic Lipase-Related Protein 2; MGDG, Monogalactosyl Diacylglycerol; MGMG, Monogalactosyl Monoacylglycerol; OB, Oat Base; PC, Phosphatidylcholine; PL, Polar Lipids; PLRP2, Pancreatic Lipase-Related Protein 2; RSO, Rapeseed oil; TAG, Triacylglycerol; TLC, Thin Layer Chromatography

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

J.L.Y.: Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Writing-original draft. P.A.: Conceptualization; Funding acquisition; Project administration; Writing-original draft. C.T.: Formal analysis; Investigation; Methodology; Project administration; Validation; Writing-original draft

Keywords

in vitro digestion, lipolysis, oats, polar lipids, satiety

Received: October 14, 2020
Revised: November 25, 2020
Published online:

[1] R. C. Spiller, I. F. Trotman, B. E. Higgins, M. A. Ghatei, G. K. Grimble, Y. C. Lee, S. R. Bloom, J. J. Misiewicz, D. B. Silk, *Gut* **1984**, *25*, 365.
[2] P. W. Maljaars, H. P. Peters, D. J. Mela, A. A. Masclee, I. brake, *Physiol. Behav.* **2008**, *95*, 271.
[3] P.-A. Albertsson, R. Köhnke, S. C. Emek, J. Mei, J. F. Rehfeld, H.-E. Akerlund, C. Erlanson-Albertsson, *Biochem. J.* **2007**, *401*, 727.
[4] R. Köhnke, A. Lindbo, T. Larsson, A. Lindqvist, M. Rayner, S. C. Emek, P. A. Albertsson, J. F. Rehfeld, M. Landin-Olsson, C. Erlanson-Albertsson, *Scand. J. Gastroenterol.* **2009**, *44*, 712.
[5] E. L. Stenblom, C. Montelius, K. Östbring, M. Håkansson, S. Nilsson, J. F. Rehfeld, C. Erlanson-Albertsson, *Appetite* **2013**, *68*, 118.
[6] C. J. Rebello, J. Chu, R. Beyl, D. Edwall, C. Erlanson-Albertsson, F. L. Greenway, *J. Am. Coll. Nutr.* **2015**, *34*, 470.
[7] C. Montelius, D. Erlandsson, E. Vitija, E. L. Stenblom, E. Egecioglu, C. Erlanson-Albertsson, *Appetite* **2014**, *81*, 295.
[8] E. L. Stenblom, E. Egecioglu, M. Landin-Olsson, C. Erlanson-Albertsson, *Appetite* **2015**, *91*, 209.
[9] C. Erlanson-Albertsson, P.-Å. Albertsson, *Plant Foods Hum. Nutr.* **2015**, *70*, 281.
[10] K. H. Lucas, B. Kaplan-Machlis, *Ann. Pharmacother.* **2001**, *35*, 314.
[11] A. T. Melia, S. G. Koss-Twardy, J. Zhi, *J. Clin. Pharmacol.* **1996**, *36*, 647.
[12] R. Singh, S. De, A. Belkheir, *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 126.
[13] P. Lehtinen, A. Kaukovirta-Norja, in *Oats: Chemistry and Technology* (Eds: F.H. Webster, P.J. Wood), AACC International Press, Devon, U.K. **2011**, pp. 143–156.
[14] C. J. Rebello, C. E. O'Neil, F. L. Greenway, *Nutr. Rev.* **2016**, *74*, 131.
[15] S. Leonova, *Sveriges Lantbruksuniversitet*, Alnarp, Sweden **2013**, p. 60.
[16] K. Diepvens, S. Soenen, J. Steijns, M. Arnold, M. Westerterp-Plantenga, *Int. J. Obes.* **2007**, *31*, 942.
[17] B. S. Chu, G. T. Rich, M. J. Ridout, R. M. Faulks, M. S. Wickham, P. J. Wilde, *Langmuir* **2009**, *25*, 9352.
[18] B. S. Chu, A. P. Gunning, G. T. Rich, M. J. Ridout, R. M. Faulks, M. S. Wickham, V. J. Morris, P. J. Wilde, *Langmuir* **2010**, *26*, 9782.
[19] A. A. Burns, M. B. E. Livingstone, R. W. Welch, A. Dunne, I. R. Rowland, *Eur. J. Clin. Nutr.* **2002**, *56*, 368.
[20] K. Diepvens, J. Steijns, P. Zuurendonk, M. S. Westerterp-Plantenga, *Appetite* **2007**, *49*, 287.
[21] L. Ohlsson, A. Rosenquist, J. F. Rehfeld, M. Härröd, *Food Nutr. Res.* **2014**, *58*.
[22] M. Hossain, J. Tovar, L. Cloetens, K. Pettersson, F. Prothon, A. Nilsson, *Obes. Rev.* **2020**, *21*, EP-322).
[23] M. Minekus, M. Alminger, P. Alvito, S. Ballance, T. Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig, D. Dupont, C. Dufour, L. Egger, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. Mackie, S. Marze, D. J. McClements, O. Ménard, I. Recio, C. N. Santos, R. P. Singh, G. E. Vegarud, M. S. J. Wickham, W. Weitschies, A. Brodkorb, *Food Funct.* **2014**, *5*, 1113.
[24] A. Brodkorb, L. Egger, M. Alminger, P. Alvito, R. Assunção, S. Ballance, T. Bohn, C. Bourlieu-Lacanal, R. Boutrou, F. Carrière, A. Clemente, M. Corredig, D. Dupont, C. Dufour, C. Edwards, M. Golding, S. Karakaya, B. Kirkhus, S. Le Feunteun, U. Lesmes, A. Macierzanka, A. R. Mackie, C. Martins, S. Marze, D. J. McClements, O. Ménard, M. Minekus, R. Portmann, C. N. Santos, I. Souchon, et al., *Nat. Protoc.* **2019**, *14*, 991.
[25] D. J. McClements, Y. Li, *Food Funct.* **2010**, *1*, 32.
[26] S. Leonova, T. Shelenga, M. Hamberg, A. V. Konarev, I. Loskutov, A. S. Carlsson, *J. Agric. Food Chem.* **2008**, *56*, 7983.
[27] M. Hamberg, E. Liepinsh, G. Otting, W. Griffiths, *Lipids* **1998**, *33*, 355.
[28] F. Carriere, J. A. Barrowman, R. Verger, R. Laugier, *Gastroenterology* **1993**, *105*, 876.
[29] C. Erlanson, B. Borgström, *Scand. J. Gastroenterol.* **1970**, *5*, 293.
[30] E. G. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol.* **1959**, *37*, 911.
[31] L. R. Cavonius, N. G. Carlsson, I. Undeland, *Anal. Bioanal. Chem.* **2014**, *406*, 7313.
[32] M. Hamberg, *Recent Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds: S.B. Sinzinger H., Vane J.R., Paoletti R., Ramwell P., Wong P.YK.), Springer, Boston, MA **1997**.
[33] A. Salhi, S. Amara, P. Mansuelle, R. Puppo, R. Lebrun, B. Gontero, A. Aloulou, F. Carrière, *Biochimie* **2020**, *169*, 106.
[34] X. Zhu, A. Ye, T. Verrier, H. Singh, *Food Chem.* **2013**, *139*, 398.
[35] M. M. L. Grundy, D. J. McClements, S. Ballance, P. J. Wilde, *Food Hydrocolloids* **2018**, *83*, 253.
[36] P. J. Wilde, G. Garcia-Llatas, M. J. Lagarda, R. P. Haslam, M. M. L. Grundy, *Food Chem.* **2019**, *278*, 683.
[37] M. M. L. Grundy, J. Quint, A. Rieder, S. Ballance, C. A. Dreiss, K. L. Cross, R. Gray, B. H. Bajka, P. J. Butterworth, P. R. Ellis, P. J. Wilde, *J. Funct. Foods* **2017**, *38*, 378.
[38] Y. Li, M. Hu, D. J. McClements, *Food Chem.* **2011**, *126*, 498.
[39] A. Larsson, C. Erlanson-Albertsson, *Biochim. Biophys. Acta* **1986**, *876*, 543.
[40] M. Zhou, K. Robards, M. Glennie-Holmes, S. Helliwell, *Oat Lipids* **1999**, *76*, 159.

[41] M. Dahim, H. Brockman, *Biochemistry* **1998**, *37*, 8369.
[42] Y. Gargouri, R. Julien, A. G. Bois, R. Verger, L. Sarda, *J. Lipid Res.* **1983**, *24*, 1336.
[43] J. Wattanakul, M. Sahaka, S. Amara, S. Mansor, B. Gontero, F. Carrière, D. Gray, *Food Funct.* **2019**, *10*, 7806.
[44] T. Sugawara, T. Miyazawa, *J. Nutr. Biochem.* **2000**, *11*, 147.
[45] J. C. Bakala N'Goma, S. Amara, K. Dridi, V. Jannin, F. Carrière, *Ther. Delivery* **2012**, *3*, 105.
[46] A. Gomes, G.d.F. Furtado, R. L. Cunha, *J. Agric. Food Chem.* **2019**, *67*, 13.
[47] Boczkowska, M., Podyma, W., Łapiński, B., in *Genetic and Genomic Resources for Grain Cereals Improvement* (Eds.: M. Singh and H.D. Upadhyaya), Academic Press, San Diego, CA **2016**, pp. 159–225.
[48] Markets and Markets, Dairy Alternatives Market by Source (Soy, Almond, Coconut, Rice, Oats, Hemp), Application (Milk, Cheese, Yogurt, Ice Creams, Creamers), Distribution Channel (Supermarkets, Health Stores, Pharmacies), Formulation and Region – Global Forecast to 2025, https://www.marketsandmarkets.com/Market-Reports/dairy-alternative-plant-milk-beverages-market-677.html (accessed: September 2020).

