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# Gastric viscosity and sugar bioaccessibility of instant and steel cut oat/milk protein blends

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ABSTRACT

Milk protein concentrate (MPC; 0 g, 5 g, and 10 g) was added to two commercially available oat products (instant oats and steel cut oats) to examine how MPC addition, and consequent changes in meal formulation, manipulates both gastric lumen viscosity and intestinal carbohydrate-digestion kinetics, *in vitro*. We used the TNO Intestinal Model-1 (TIM-1) to simulate gastrointestinal digestion of the oats-based meals. Meals containing 5 g or 10 g MPC yielded significantly less total bioaccessible sugar compared with those containing 0 g MPC, while the rate of starch digestion was significantly higher in meals containing 5 g or 10 g MPC. The TIM-1 was coupled with fluorescence spectroscopy and a luminescent molecular rotor to report changes in gastric viscosity *in situ*, showing that the gastric viscosity was higher in the meals containing MPC. Those findings suggest that MPC in oats-based meals significantly modifies the kinetics of carbohydrate digestion and increases gastric viscosity.



## 1. Introduction

Designing foods by modifying their form and formulation is becoming a plausible strategy to control how foods behave and are digested in the gastrointestinal tract (GIT). The resultant changes in the physiological impact of modified foods may serve as a dietary intervention to combat diet-related chronic diseases. Starch-rich foods are of special concern, given the persistence of the type II diabetes pandemic (Prevention, 2015; Whiting, Guariguata, Weil, & Shaw, 2011). Therefore, there is a great need for dietary interventions that target postprandial glucose levels. Oats are a staple starchy food that is typically consumed after exposure to hydrothermal processing (cooking); which involves gelatinization of the starch and the formation of a biphasic paste with an aqueous, continuous phase and a dispersed phase of swollen starch granules (Tecante & Doublier, 1999). We previously investigated the effect of differences in oat form, which are ultimately a consequence of different commercial processing techniques, on the digested gastric chyme viscosity and the biophysics of digestion of their carbohydrate composition (AlHasawi et al., 2017). Similarly, manipulating meal formulation facilitates control of the physical properties of the starch-paste matrix and the consequent physiological responses. For example, hydrocolloids have a high water retention capacity that was found to contribute to an increase in digesta viscosity throughout the GIT (Norton, Fyer, & Moore, 2006). Increased gastric digesta viscosity impedes motion and access of enzymes to the substrate, consequently reducing nutrient hydrolysis (Endress & Fisher, 2001; Montagne, Pluske, & Hampson, 2003; Tharakan, Norton, Fryer, & Bakalis, 2010), as well as hinders diffusion of the hydrolysis by-products to the luminal brush border and renders absorption less effective (Endress & Fisher, 2001; Montagne et al., 2003; Tharakan et al., 2010). The subsequent reduction in rate and extent of starch digestion, attributed to increased digesta viscosity (Cummings & Stephen, 2007) has been associated with controlling postprandial glycemia (Brennan, 2005; Brennan & Cleary, 2005; Malkki, 2004; McKeown et al., 2004; Sahyoun, Jacques, Zhang, Juan, & McKeown, 2006; Trepel, 2004). β-glucan is a soluble fiber found in oats that is capable of entrapping water in its network (Battilana et al., 2001; Dikeman & Fahey, 2006) and increasing chyme viscosity in the GIT. These properties of β-glucan

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are hypothesized to be central in the plasma glucose-lowering health claims associated with oats (Food and Drug Administration, 1997; Gidley, 2013).

Milk protein, especially milk protein concentrate (MPC), is another hydrocolloid that has received attention as a food additive. MPC has a casein-to-whey protein ratio very similar to that of milk (Martin, Williams, & Dunstan, 2010). The high protein content of MPC makes MPC an ideal thickening agent, capable of binding water and increasing the food-matrix viscosity. The rheological and physiological aspects of blends of MPC and β-glucan are largely unknown. The viscosity of starch paste prepared in milk is greater than that of pastes containing the same amount of starch but prepared in water (Abu-Jdayil, Mohameed, & Eassa, 2004; Bradley, 1993). Milk proteins are presumed to modify the properties of the dispersed phase of the starch paste and thus increase the overall viscosity of the paste (Tarrega, Velez-Ruiz, & Costell, 2005). Accordingly, we hypothesize that the addition of MPC to oat-based meals may increase the gastric viscosity during luminal transit and consequently influence the rate and extent of starch digestion. Albeit, the highly hygroscopic nature of MPC necessitates substantial increases in the aqueous composition of any given meal formulation to which MPC would be added. Such correlations between the final meal formulation and both the physico-chemical properties and physiological impacts upon digestion of the final meal matrix are vital for altering glucose bioaccessibility (Minekus, 2015) and, potentially, the glycemic index of oats-based meals. Glucose bioaccessibility is of distinct importance because it is the rate-limiting step to glucose bioavailability. Accordingly, the primary aim of this work was to characterize the physiological response (*in vitro)* to a commercially-relevant formulation of an "oat-based meal + MPC" matrix.

We evaluated the effects of the addition of two doses of MPC to instant oat-based and steel cut oat-based meals, and consequent changes to meal formulation, on gastric viscosity and carbohydrate-digestion kinetics in the intestinal lumen. We used simulated digestion in the TNO Intestinal Model-1 (TIM-1) paired with molecular rotors (optical chromophores) to assess real-time gastric viscosity throughout a simulated GIT environment. The TIM-1 is an advanced artificial digestive system that mimics the human stomach and upper small intestine. In comparison with other *in vitro* techniques, this dynamic, computer-controlled system is unique in its ability to regulate pH, temperature, gastric and intestinal emptying, transit time, and GIT secretions (Villemejane, Wahl, Aymard, Denis, & Michon, 2015). Real-time luminal viscosity (viscosity in both the gastric and the small intestinal compartments) in the TIM-1 was previously monitored using molecular rotors as luminescent probes to assess viscosity (AlHasawi et al., 2017). Molecular rotors are molecules that consist of two or more segments that are capable of rotating relative to one another (Kottas, Clarke, Horinek, & Michl, 2005). Photoexcitation of MR causes intramolecular twisting of the two segments at a rate that is dependent on the free-volume (or molecular crowding) of the surrounding environment. Less viscous environments facilitate a non-planar (twisted) configuration of the molecular rotor in the excited state, thus favoring twisted intramolecular charge transfer (TICT) state. For single band MR, relaxation from TICT to the ground state is predominantly in the form of non-radiative decay (without photon emission). Rigid or more viscous environments hinder the rotation of the two MR segments relative to one another, yielding a planar configuration in the excited state, and favoring the local excited (LE) state. Relaxation from LE to the ground state involves photon emission (radiative decay). The rate of formation of the TICT state is slower in highly viscous materials, and the two competing decay pathways determine the sensitivity of the probe to the micro-viscosity of the surrounding medium (Haiddekker & Theodorakis, 2010). We analyzed digestates from the TIM-1 to obtain total sugar-release profiles for each of the tested mixtures of oats and MPC. We used a shifted-logistic model to estimate the rate and extent of sugar release as a function of digestion time. The luminescence emitted by the molecular rotors during *in vitro* digestion suggests that mixtures of oats and MPC have a higher gastric viscosity than oats-only meals.

## 2. Methods

### 2.1. Materials and sample preparation

Quaker Oats (PepsiCo, Inc., IL, USA) supplied the oat products (instant oats and steel cut oats) and MPC80 (MPC, 80% protein) tested in our experiments. Oats were pooled from numerous production batches. We used standard methods to measure the moisture, ash, protein, fat, and soluble and insoluble (AOAC 991.43) dietary fiber compositions of the oat products (Table 1). We then subtracted those values from the total weight to estimate the total carbohydrate content [Total Carbohydrates (%) − Table 1]. We used AOAC methods 995.16 and 996.11 to quantify the β-glucan and starch compositions.

We prepared a 45-g serving of each type of oatmeal sample immediately prior to analysis. We mixed the instant oats with various amounts of water and MPC80 (Table 2) in a 1-quart Pyrex® measuring cup and heated them in a microwave oven (NN-SD987SA, 1250 W, Panasonic Corp., Osaka, Japan) on high power (Table 2). The steel cut oats were mixed with various amounts of MPC80, stirred into boiling water in a medium saucepan on a stovetop at high heat, and allowed to simmer on low heat for 25–30 min (Table 2).

### 2.2. Experimental meals

After the meals were allowed to cool for 2 min, a single volunteer masticated the prepared meals using the "10 chew-and-spit" method. Only one sample was chewed per day, the volunteer was fasted (except water) prior to performing the chewing procedure. Chewing was continued until 100 g of the samples was available for feeding into TIM-1. The chewing allowed the oats to be masticated and exposed to salivary α-amylase. To mimic the initial gastric conditions, we mixed 100 g of the chewed samples with 5 g gastric-secretion fluid, 95 g gastric-electrolyte solution, and 50 g water. We then fed the samples into the TIM-1 (TNO Triskelion, Zeist, The Netherlands) gastric compartment along with 50 g water for a total experimental meal weight of 300 g. Available total carbohydrates, starch and β-glucan of the meals (Table 3) were calculated based on the total carbohydrate content of each type of oat (Table 1) and the total weight of the meal (accounting for water loss during cooking) fed into the TIM-1 (Table 2). Calculating the available total carbohydrates required a 10/9 conversion factor to account for the cleavage of the glycosidic linkages of starch to yield glucose or maltose (K. Englyst, Englyst, Hudson, Cole, & Cummings, 1999).

### 2.3. TIM-1 simulated digestion

The TIM-1 mimics the digestive process of a healthy adult and replicates the stomach, duodenum, jejunum, and ileum. The ileal-secretion fluid consisted of small intestinal electrolyte solution (SIES; 5 g/l NaCl, 0.6 g/l KCl, and 0.25 g/l $CaCl_2$). The jejunal fluid consisted of SIES and 10% fresh porcine bile. A 7% pancreatin solution, prepared with Pancrex V powder (α-amylase activity = 25,000 units/g, lipase activity = 25,000 units/g, protease activity = 1400 units/g), was obtained from Paines & Byrne, UK. The gastric-secretion fluid consisted of 600 U/ml pepsin (P7012, Sigma-