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Impact of Dietary Fiber on the *In Vitro* Glucose Release: the Role of Physicochemical Properties and Food Processing

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Contents

Preliminary Remarks

The work presented in this thesis is a selection of papers published in international peer-reviewed journals listed below.

Full papers

1. Miehle, E., Bader-Mittermaier, S., Schweiggert-Weisz, U., Hauner, H., & Eisner, P. (2021). Effect of Physicochemical Properties of Carboxymethyl Cellulose on Diffusion of Glucose. Nutrients, 13(5), 1398. doi:https://doi.org/10.3390/nu13051398
   (Special Issue: Dietary Carbohydrate and Human Health)

2. Miehle, E., Haas, M., Bader-Mittermaier, S., & Eisner, P. (2022). The role of hydration properties of soluble dietary fibers on glucose diffusion. Food Hydrocolloids, 107822. doi:https://doi.org/10.1016/j.foodhyd.2022.107822

3. Miehle, E., Eisner, P., & Bader-Mittermaier, S. (2024). Effects of food processing on *in vitro* glucose release of high methylester pectin-enriched doughs. Food Chemistry, 138331. doi:https://doi.org/10.1016/j.foodchem.2023.138331
   (Special Issue: Molecular characterization, technology, and strategies for designing novel functional foods in the food industry)

4. Miehle, E., Pietrynik, K., Bader-Mittermaier, S., Skurk, T., Eisner, P. & Hauner, H. (2024) Impact of food processing on the *in vitro* and *in vivo* glycemic response to citrus fiber-enriched dough products. Journal of Functional Foods, 106230. doi:https://doi.org/10.1016/j.jff.2024.106230

Oral presentations

1. Miehle, E., Pietrynik, K., Bader-Mittermaier, S., Skurk, T., Eisner, P., Hauner, H., (2021): Dietary fiber and postprandial glycaemia. Cluster Conference – Competence Cluster Nutrition Research, ‘Innovative Research: Optimizing nutrition for all ages’, 17–19 May 2021, Online event

Poster presentations

1. Miehle, E., Bader-Mittermaier, S., Eisner, P., (2021): Development and validation of an *in-vitro* glucose release model for dietary fibres and fibre-rich food matrices. Cluster Conference – Competence Cluster Nutrition Research, 'Innovative Research: Optimizing nutrition for all ages', 17–19 May 2021, Online event

2. Miehle, E., Bader-Mittermaier, S., Eisner, P., (2021): Effect of physicochemical properties of carboxymethyl cellulose on glucose release. 35th EFFoST International Conference, 'Healthy Individuals, Resilient Communities, and Global Food Security', 02–04 November 2021, Lausanne, Switzerland

3. Miehle, E., Bader-Mittermaier, S., Eisner, P., (2022): The role of hydration properties of dietary fibers and food processing on *in vitro* glucose release. 5th Food structure and functionality symposium, 'Structuring Foods for a Sustainable World', 18–21 September 2022, Cork, Ireland

4. Miehle, E., Bader-Mittermaier, S., Eisner, P., (2022): The role of physicochemical properties of dietary fibers and food processing on *in vitro* glycemic response. 8th International Dietary Fibre Conference, 'Towards Unlocking the Full Potential of Fibre for Food, Function, and Global Health', 16–18 October 2022, Leuven, Belgium

Summary

The worldwide prevalence of type 2 diabetes is increasing significantly. The main risk factors for developing type 2 diabetes are a lack of exercise, obesity, and increased consumption of food with high glycemic indices. These factors can cause permanent metabolic stress, leading to reduced insulin sensitivity, ultimately disturbing insulin secretion, and significantly elevated post-prandial plasma glucose levels. Several epidemiological studies have shown that consuming dietary fibers (DF) slows the increase in plasma glucose levels after a meal rich in carbohydrates. This positively affects human health, including a reduced risk of type 2 diabetes. Various mechanisms have been proposed to explain the positive effect of DF on flattening the plasma blood glucose peak. These mechanisms include slowing gastric emptying, influencing enterohormones, reducing amylolysis, and delaying sugar absorption from the small intestinal epithelium. However, the impact of each mechanism on glucose release and the contribution of additional mechanisms remain unclear. Additionally, it is reported that the physiological behavior in the gastrointestinal tract of different types of DF might be influenced by different physicochemical characteristics such as molecular weight, water binding, or viscosity. In particular, soluble DF is believed to have increased viscosity properties, which slows down the mass transfer and can help reduce postprandial blood glucose levels. However, the physiological effect of other molecular mechanisms of DF on blood glucose levels remains unclear.

To close those gaps and contribute to a better understanding of the impact of different physicochemical characteristics of DF, *in vitro* methods are well-suited for investigating the structure-function relationships of different types of DF. Most published *in vitro* studies have focused on the viscosity-forming properties of DF and their effect on sugar release. The viscosity formed is influenced by both the concentration and the molecular weight of the DF molecules. However, the influence of each individual parameter on sugar release has only been a minor subject of past studies. Therefore, this study aims to investigate the function of DF in the chyme using a specific experimental setup to obtain a deeper understanding of the molecular structure-function relationship. For this, the transport of glucose as a low-molecular weight amylolytic product from the chyme to the small intestinal epithelium with the unstirred water layer was mimicked in the experiments using a two-cell system with a dialysis membrane.

In the first part of this work (CHAPTER 1), the impact of concentration, molecular weight, and the resulting viscosity on *in vitro* glucose release by diffusion and convection were investigated systematically in solutions containing different types of carboxymethyl celluloses (CMC). The diffusion of glucose was significantly slowed down above the critical concentration c^*, which is the

overlap concentration of molecules in solution. The molecular weight of soluble DF also significantly impacted glucose diffusion. However, a deviation from Stokes-Einstein behavior was observed, indicating the presence of additional influencing factors beyond molecular weight and viscosity since a high concentration of low molecular weight CMC reduced the diffusion, even with a lower viscosity present.

In the second part of the work (CHAPTER 2), the causes of the deviation from Stokes-Einstein behavior were further examined. Therefore, the hydration properties of DF, such as the water holding capacity, osmotic pressure, and water mobility, were investigated and evaluated concerning their effect on glucose diffusion. Five soluble DF – namely low and high methylester pectin, xanthan gum, locust bean gum, and carboxymethyl cellulose – were evaluated for their hydration properties and compared with citrus fiber, which consists of 16 % soluble and 72 % insoluble fibers. Glucose diffusion was studied in solutions with concentrations above c^*. The obtained results showed that the hydration properties of DF play a crucial role in an osmotically active system, such as for glucose release. This is due to the fact that the DF exhibited increased water holding capacities as well as increased osmotic pressures in the applied *in vitro* digestion and diffusion model. The results suggest that the hydration properties of DF, such as water's amount and mobility, besides viscosity, molecular weight, and the concentration used, are essential in lowering *in vitro* glucose release.

The physicochemical properties of DF, such as viscosity, molecular weight, and hydration properties, can be modified by food processing and by adding food ingredients. As a result, studies based on aqueous solutions with added isolated DF may differ from those with DF incorporated into foods. Therefore, the third study of this thesis (CHAPTER 3) investigates the influence of food processing on the *in vitro* glucose release of fiber-enriched foods. Soluble high methylester pectin was incorporated into a dough comprising a high amount of glucose and processed differently by baking (180 °C) and extrusion cooking (150 °C, 180 °C). Pectin lowered the starch digestibility, altered the food matrix, and increased the viscosity of the *in vitro*-digested chyme. However, food processing through baking and extrusion increased starch digestibility and the amount of resistant starch to different extents. The results of the mass transfer in this study revealed a complex interplay between altered starch digestibility, viscosity, and the food matrix, as well as the addition of DF.

Processed, matrix-based products are significantly altered by human digestion compared to solutions, making it challenging to transfer from *in vitro* to *in vivo* for such products. Therefore, the last study of the thesis (CHAPTER 4) investigated the previous study's high glucose control bakery

products (CHAPTER 3) enriched with citrus fiber. The glycemic behavior was analyzed *in vitro* based on starch digestibility and glucose release. Additionally, a short-term human study investigated glycemia and insulinemia. *In vitro*, citrus fiber incorporated in the food products significantly lowered the glucose release with a simultaneous lower starch digestibility. *In vivo*, the fiber enrichment and the different processed food products did not significantly influence the study participants' postprandial glucose and insulin concentrations. Thus, the *in vivo* results could not confirm the *in vitro* findings to the same extent.

In conclusion, this work contributes significantly to understanding the structure-function relationship between DF and postprandial glucose response. The study demonstrates that the use of DF with varying concentrations, molecular weights, and hydration properties affects *in vitro* glucose release. Furthermore, the study demonstrated that variations in food processing have complex effects on fiber-enriched products, impacting both *in vitro* starch digestibility and glucose release. However, these effects could not be confirmed *in vivo,* indicating the limitations of the static *in vitro* model. It is important to note that in addition to glucose release from the chyme, many other factors contribute to regulate blood glucose levels *in vivo*. In order to predict blood glucose levels accurately, it is essential to consider a holistic view that includes, e.g., gastric emptying, active glucose absorption, insulin response and clearance, and feedback mechanisms. Therefore, when developing new foods in the future, it will be essential to take a holistic approach in order to predict blood glucose levels in addition to glucose release.

Zusammenfassung

Die Prävalenz von Typ-2-Diabetes nimmt weltweit stark zu. Zu den Hauptrisikofaktoren für die Entwicklung dieser Erkrankung gehören Bewegungsmangel und Übergewicht in Verbindung mit dem zunehmenden Verzehr von Lebensmitteln mit einem hohen glykämischen Index. Der durch diese Risiken bedingte andauernde Stress des Stoffwechsels kann zu einer verminderten Insulinsensitivität führen. Dies wiederum stört langfristig die Insulinsekretion und führt zu deutlich erhöhten postprandialen Plasmaglukosespiegeln. In mehreren epidemiologischen Studien konnte gezeigt werden, dass der Verzehr von Ballaststoffen (BS) den Anstieg des Plasmaglukosespiegels nach einer kohlenhydratreichen Mahlzeit verlangsamt. Dies hat positive Auswirkungen auf die menschliche Gesundheit, unter anderem durch ein verringertes Risiko für Typ-2-Diabetes. In der Fachwelt werden verschiedene Mechanismen genannt, um die positive Wirkung von BS auf eine Abflachung des Blutzuckerpeaks im Plasma zu erklären. Dazu gehören die Verlangsamung der Magenentleerung, die Beeinflussung von Enterohormonen, die Verringerung der Amylolyse und die Verzögerung der Zuckerabsorption durch das Dünndarmepithel. Die Auswirkungen der einzelnen Mechanismen auf die Glukosefreisetzung und der Einfluss weiterer Mechanismen sind bisher noch unklar. Darüber hinaus ist bekannt, dass die physiologische Wirkung von BS im Gastrointestinaltrakt durch ihre unterschiedlichen physikochemischen Eigenschaften wie Molekulargewicht, Wasserbindung und Viskosität beeinflusst wird. Insbesondere wird der Viskositätsausbildung der löslichen BS mit der einhergehenden Verlangsamung des Stoffübergangs eine Abflachung des postprandialen Blutzuckerspiegels zugeschrieben. Allerdings ist wenig über die physiologischen Auswirkungen weiterer molekularer Mechanismen von BS auf den Blutzuckerspiegel bekannt.

Um diese Lücken zu schließen und zu einem besseren Verständnis der Auswirkungen verschiedener physiko-chemischer Eigenschaften von BS beizutragen, sind *in-vitro*-Methoden gut geeignet, Struktur-Funktions-Beziehungen verschiedener Arten von BS zu untersuchen. Ein Großteil der *in-vitro*-Studien befasst sich mit den viskositätsausbildenden Eigenschaften von BS und deren Wirkung auf die Zuckerfreisetzung. Die ausgebildete Viskosität wird dabei sowohl von der Konzentration als auch dem Molekulargewicht der Ballaststoffmoleküle beeinflusst, wobei der jeweilige Einfluss auf die Zuckerfreisetzung nur in geringem Maße untersucht wurde. In der vorliegenden Arbeit wurde daher die Funktion von BS im Speisebrei in einem speziellen Versuchsaufbau untersucht, um ein tieferes Verständnis der molekularen Struktur-Funktions-Beziehung zu erhalten. Der Stofftransport von Glukose als niedermolekulares amylolytisches Produkt aus dem Speisebrei zu dem Dünndarmepithel, welches das sogenannte *unstirred water*

layer umgibt, wurde in den Versuchen mittels Zweizellensystem mit einer Dialysemembran simuliert.

Im ersten Teil dieser Arbeit (KAPITEL 1) wurden die Auswirkungen der Konzentration, des Molekulargewichts und der daraus resultierenden Viskosität in Lösungen, die verschiedene Arten von Carboxymethylcellulosen (CMC) enthalten, auf den *in-vitro*-Glukosetransport mittels Diffusion und Konvektion systematisch untersucht. Oberhalb der kritischen Konzentration *c**, welche ein Maß für die Überlappungskonzentration von Molekülen in Lösung ist, zeigte sich eine signifikante Abschwächung der Diffusion von Glucose. Das Molekulargewicht der löslichen BS hatte ebenfalls einen erheblichen Einfluss auf die Glukosediffusion. Zudem wurde eine Abweichung vom Stokes-Einstein-Verhalten beobachtet, was auf weitere Einflussfaktoren neben dem Molekulargewicht und der Viskosität hindeutet. So verringerte eine hohe Konzentration an niedermolekularen CMC die Diffusion, auch wenn eine niedrigere Viskosität vorlag.

Im fortführenden zweiten Teil der Arbeit (KAPITEL 2) wurden die Ursachen für die Abweichung vom Stokes-Einstein-Verhalten tiefergehend betrachtet. Dazu wurden die Hydratationseigenschaften von BS, wie das Wasserhaltevermögen, der osmotische Druck und die Wassermobilität untersucht und hinsichtlich ihrer Wirkung auf die Glukosediffusion evaluiert. Es wurden fünf lösliche BS – nieder- und hochverestertes Pektin, Xanthan, Johannisbrotkernmehl und Carboxymethylcellulose – in ihren Hydratationseigenschaften bewertet und mit Citrusfaser verglichen, die aus 16 % löslichen und 72 % unlöslichen Fasern besteht. Die Glukosediffusion wurde in Lösungen mit Konzentrationen oberhalb *c** untersucht. Dabei konnte gezeigt werden, dass die Hydratationseigenschaften der BS in einem osmotisch aktiven System wie der Glukosefreisetzung eine wichtige Rolle spielen, da die BS in dem eingesetzten *in-vitro*-Verdauungs- und Diffusionsmodell eine erhöhte Wasserhaltekapazität und einen erhöhten osmotischen Druck aufwiesen. Die Ergebnisse deuten darauf hin, dass die Hydratationseigenschaften von Ballaststoffen, wie z.B. die Wassermenge und -mobilität, neben der Viskosität, dem Molekulargewicht und der verwendeten Konzentration eine wesentliche Rolle bei der Verringerung der *in-vitro*-Glukosefreisetzung spielen.

Die erwähnten physiko-chemischen Eigenschaften wie Viskosität, Molekulargewicht und Hydratationseigenschaften von Ballaststoffen können durch die Verarbeitung von Lebensmitteln und den Zusatz von Lebensmittelzutaten verändert werden. Daher können sich die Ergebnisse von Studien, die auf wässrigen Lösungen mit zugesetzten isolierten Ballaststoffen basieren, von denen mit in Lebensmitteln eingearbeiteten Ballaststoffen unterscheiden. In der dritten Studie dieser Arbeit (KAPITEL 3) wurde deshalb der Einfluss der Lebensmittelverarbeitung auf die *in-*

vitro-Glukosefreisetzung von ballaststoffangereicherten Lebensmitteln untersucht. Lösliches hochverestertes Pektin wurde in einen Teig mit einem hohen Anteil an Glucose eingearbeitet und durch Backen (180 °C) und Extrusion (150 °C, 180 °C) unterschiedlich prozessiert. Pektin senkte die Stärkeverdaubarkeit, veränderte die Lebensmittelmatrix und erhöhte die Viskosität des *in-vitro* verdauten Speisebreis, wohingegen die Lebensmittelverarbeitung durch Backen und Extrusion die Stärkeverdaubarkeit und die Menge der resistenten Stärke in unterschiedlichem Maße erhöhte. Die Ergebnisse zum Stoffübergang in dieser Studie zeigten ein komplexes Zusammenspiel zwischen veränderter Stärkeverdaubarkeit, Viskosität und der Lebensmittelmatrix sowie dem Zusatz von Ballaststoffen.

Durch die Verdauung des Menschen werden insbesondere verarbeitete, matrixbasierte Produkte im Vergleich zu Lösungen stark verändert, was eine Übertragung von *in-vitro* auf *in-vivo* bei matrixbasierten Produkten erschwert. Daher wurden in der letzten Studie dieser Arbeit (KAPITEL 4) die in der vorhergehenden Studie untersuchten Backwaren mit hohem Glukoseanteil (KAPITEL 3) mit Citrusfasern angereichert. Das glykämische Verhalten wurde *in-vitro* anhand der Stärkeverdaulichkeit und die Glukosefreisetzung analysiert. Außerdem wurden Glykämie und Insulinämie *in-vivo* in einer Kurzzeithumanstudie untersucht. *In-vitro* verringerte die eingearbeitete Citrusfaser die Glukosefreisetzung bei gleichzeitiger geringerer Stärkeverdaulichkeit erheblich. *In-vivo* hatten die Faseranreicherung und die unterschiedlich verarbeiteten Lebensmittel keinen signifikanten Einfluss auf die postprandiale Glukose- und Insulinkonzentration der Studienteilnehmer. Somit konnten die *in-vivo*-Ergebnisse die *in-vitro*-Ergebnisse nicht in gleichem Maße bestätigen.

Zusammenfassend lässt sich festhalten, dass die Ergebnisse dieser Arbeit einen entscheidenden Beitrag zur Aufklärung der Struktur-Funktions-Beziehung von Ballaststoffen auf die postprandiale Glukoseantwort leisten. Es konnte gezeigt werden, dass der Einsatz von Ballaststoffen mit unterschiedlicher Konzentration, Molekulargewicht und Hydratationseigenschaften einen Einfluss auf die *in-vitro*-Glukosefreisetzung ausübt. Des Weiteren zeigte die Studie, dass Variationen in der Lebensmittelverarbeitung komplexe Auswirkungen auf ballaststoffangereicherte Produkte haben, welche sowohl die *in-vitro* Stärkeverdaulichkeit als auch die Glukosefreisetzung betreffen. Diese Auswirkung konnten *in-vivo* nicht bestätigt werden, was auf die Grenzen des statischen *in-vitro*-Modells hinweist. Neben der Freisetzung von Glukose aus dem Speisebrei tragen viele andere Faktoren zur Regulierung des Blutzuckerspiegels *in-vivo* bei. Ein ganzheitlicher Ansatz ist erforderlich, um den Blutzuckerspiegel genau vorhersagen zu können. Dazu gehören unter anderem die Magenentleerung, die aktive Glukoseabsorption, die

Insulinreaktion sowie Clearance- und Feedback-Mechanismen. Daher ist bei der Entwicklung neuer Lebensmittel in Zukunft neben der Glukosefreisetzung eine ganzheitliche Betrachtung zur Vorhersage des Blutzuckerspiegels unerlässlich.

General Introduction

1. Glucose Dysregulation: Epidemiology, Causes and Dietary Prevention

In Germany, there are currently 11 million people affected by diabetes, with approximately 90-95 percent suffering from type 2 diabetes (Tönnies & Rathmann, 2022). Healthcare expenditures for diabetes amount to 37 billion euros per year in Germany, and the healthcare costs for people diagnosed with diabetes are about twice as high as for comparable people without diabetes (Brüne, 2022). Worldwide, the number of people with diabetes is estimated to be 537 million and is predicted to increase significantly to 783 million by 2045 (Tönnies, Rathmann, Hoyer, Brinks & Kuss, 2021). Those numbers indicate the heavy burden on our health care system and, thus, also on our society.

Type 2 diabetes is predominantly caused by a disturbance in insulin action, with low glucose tolerance causing chronic early elevation and late depression of postprandial glucose concentrations (Bhupathiraju et al., 2014). An adverse lifestyle, such as lack of exercise, poor dietary habits, obesity, and a genetic predisposition, are considered the most crucial causes of the development of type 2 diabetes (Kabisch, 2022; Ledochowski, 2010). Excessive consumption of foods with a high glycemic response, also known as the high glycemic index (GI) (Jenkins et al., 1981), such as sweetened beverages or fine pastries is particularly conducive to the development of type 2 diabetes (Malik et al., 2010; Schulze et al., 2004; Villegas et al., 2007). Nevertheless, dietary modification and weight reduction can significantly reduce the risk of developing this disease (Heidemann & Scheidt-Nave, 2017; T. Tönnies et al., 2019). In parallel, the reduction of high intake levels of carbohydrates can more often lead to both weight reduction and diabetes remission with an actual effect on insulin resistance and glucose tolerance (Jacobs, Tönnies, Rathmann, Brinks & Hoyer, 2019; Kivimäki et al., 2018). Thereby, the inclusion of high levels of dietary fibers is reported to be protective in epidemiologic studies (Tamayo, Brinks, Hoyer, Kuß & Rathmann, 2016; Tittel et al., 2020) and the impact of dietary fiber intake on insulin sensitivity and glucose tolerance has been increasingly studied since the 1960s (Dhingra, Michael, Rajput & Patil, 2012; Erbersdobler, Gusko & Meyer, 1999). In this regard, soluble dietary fibers, in particular, are thought to contribute to increased insulin sensitivity and glucose tolerance by reducing the rapid rise in postprandial plasma glucose levels (Brennan, 2005; Jenkins, Jenkins, Zdravkovic, Wursch & Vuksan, 2002; Weickert & Pfeiffer, 2008). Insoluble fibers, on the other hand, have a negligible effect on the post-meal glucose rise (Papathanasopoulos & Camilleri,

2010), but overall, a reduction in the risk of developing type 2 diabetes by increasing insulin sensitivity (Jenkins, Kendall, Axelsen, Augustin & Vuksan, 2000; Weickert & Pfeiffer, 2008). However, the exact relationships between dietary fiber consumption and the resulting higher glucose tolerance and lower risk of developing type 2 diabetes are not fully understood.

2. Dietary Fiber

The definition of DF was subject to a variety of discussions, and it changed over the years. The definition of DF varies depending on either focusing on differences in the chemical structure or differences in the physiological effects (Prosky, 2001). One of the most recent definitions is summarized in the Codex Alimentarius, which is adopted as a default in many countries, as a general global agreement, and comprises both physiological and chemical structure discriminations as follows (WHO/FAO, 2009):

> *'Dietary fiber is defined as carbohydrate polymers with ten or more monomeric units (MU), which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following categories:*
>
> *(i) Edible carbohydrate polymers naturally occurring in the food as consumed,*
>
> *(ii) Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic, or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,*
>
> *(iii) Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.'*
>
> *Further remarks:*
>
> *'When derived from a plant origin, dietary fiber may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fiber. However, such compounds are not included in the definition of dietary fiber if extracted and re-introduced into a food' and 'Decision on whether to include carbohydrates of 3 to 9 MU should be left up to national authorities*'.

The components included in the definition for dietary fibers related to the Codex Alimentarius are non-starch polysaccharides (NSP), resistant starch (RS), and resistant oligo-saccharides (RO) – if MU number 3-9 is locally approved – and lignin if it is present in dietary fiber preparations obtained from plant origin by extraction or fractionation, but not as a single compound. The main differences between the most recent definitions are related to 'associated substances' (mainly lignin), the minimum number of MU (mostly ≥3 or ≥10), the inclusion of RO, and the requirement of showing health benefits (mainly for extracted, isolated, modified of synthetic carbohydrate polymers) (Stephen et al., 2017). As two examples, the European Food Safety Agency (EFSA) defines dietary fiber as non-digestible carbohydrates composed of NSP, RO, RS, and lignin, if associated with DF polysaccharides, with a minimum of MU by three or higher (EFSA Panel on Dietetic Products, 2010). The European Community agrees with the EFSA definition, except for the inclusion of lignin (Commission, 2008).

2.1. Classification

DF are often classified according to their chemical structure or their water solubility. Chemically, DF are carbohydrate polymers, which include non-digestible RO (MU 3 – 9) ,e.g., inulin, fructo-oligosaccharides, galacto-oligosaccharides, resistant dextrins, NSP (MU ≥10), e.g., cellulose, hemicellulose, pectin, hydrocolloids as gums, mucilages, and resistant starches (RS), which are physically trapped, resistant granules or retrograded starches (Stephen et al., 2017). Each carbohydrate polymer is built of individual MU, which can be of the same monomer type (homoglycans) or different types (heteroglycan). The MU can be linked linearly (e.g., cellulose) or branched (e.g., alginates). DF serve plants as supporting and structural elements (e.g., cellulose, hemicellulose, pectin, lignin), reserve substances (e.g., dextrins, fructans), and water-binding compounds (e.g., agar, pectin, alginate) (Belitz, Grosch & Schieberle, 2001).

From the analytical side, it is more suitable to distinguish the DF according to their water solubility – classified as soluble and insoluble DF – than by the chemical structures, as those are very diverse (McCleary et al., 2012). The methods for DF determination improved over time and can be divided into three categories (i) non-enzymatic-gravimetric, (ii) enzymatic-gravimetric, and (iii) enzymatic-chemical (including GLC/HPLC methods). The enzymatic-gravimetric method is the most commonly used method in literature for determining soluble and insoluble DF fractions. It provides an almost complete quantification of the DF by enzymatic digestion of protein and non-resistant starch, followed by precipitation of soluble fiber components using aqueous ethanol, filtration, and weighing of the DF residue and correction for protein and ash contents in the precipitate and the insoluble residue (Prosky, Asp, Schweizer, Devries & Furda, 1988).

Modifications of this method for fostering the determination of specific types of DF, such as oligosaccharides or certain types of RS, were developed to overcome the limitations of the basic determination method. The most common quantification methods are the AOAC 985.29, 991.43, 2009.01, 2011.25, 2017.16 methods, with the most recent method of 2022.01 (McCleary & McLoughlin, 2023). The solubility of a fiber depends on its chemical structure. Perfect linear polysaccharides are poorly soluble or insoluble in water, as the regular conformation leads to a partially crystalline state with intermolecular interactions and is, therefore, more energetically stable in the solid state than in the solution. Irregularities in the structure, like branching, elevate the solubility because intermolecular interactions are reduced. Also, substitution with acidic groups, such as carboxyl, sulfate, phosphate groups and neutral groups, such as methyl, ethyl, as well as hydroxypropyl groups increase the solubility of polysaccharides (Belitz et al., 2001). Table 1 shows the most common DF subgroups classified by their chemical composition and their water solubility.

Table 1 Classification of dietary fiber according to their chemical structure and water solubility (adapted according to Stephen et al. (2017))

Subgroup	Dietary fiber class	Chemical composition	Water solubility
NSP (MU ≥10)	Cellulose	Backbone: (1→4) β-Glucose Linearly 500 – 10,000 MU	Insoluble
	Hemicellulose (e.g., heteroxylans, xyloglucans)	Backbone: (1→4) and/or (1→3) β-D-xylan or β-D-glucan Side-Branched/substituted by units or short side chains containing: Pentoses of xylose and/or arabinose Hexoses of mannose, galactose and/or glucose Uronic acids of galacturonic acid and/or glucuronic acid 150–200 MU	Insoluble Soluble
	Pectin (e.g. Homogalacturonan, rhamnogalacturonan)	Backbone: (1→4) β-D-galacturonic acid, interrupted by L-rhamnose Substituted with arabinose, xylose, fructose galactose Differentially methylated & acetylated	Soluble
	Heteromannans (e.g., guar gum, locust bean gum)	Backbone: 1→4) β-D-mannose Substituted with galactose (Galactomannans) Glucose residues in the backbone (Glucomannan) or mixtures (Galactoglucomannan)	Soluble
	Mucilages; gums (e.g., Xanthan gum, carrageenan, alginates, agar-agar)	Highly branched polysaccharide structure consisting of galactose, uronic acid, rhamnose, arabinose, mannose, xylose, fructose	Soluble
	Inulin and fructans	Backbone: (1→2) β-fructan Partly α-D-glucosyl-unit at the reducing end MU 3 to >30	Soluble
Resistant oligosaccharides (MU <10)	E.g., Resistant dextrins, polydextrose, FOS, GOS, TOS, XOS	Poly-D-glucose with α and β (1→2), (1→3), (1→4), (1→6) being predominant	Soluble
Resistant starch (MU ≥10)	RS 1 – entrapped starch RS 2 – granular starches RS 3 – retrograded starches RS 4 – chemically modified RS 5 – Amylose-lipid complexes	Cross-linked starches with amylose and amylopectin units (high degree of reticulation)	Insoluble Crystalline
Associated substances (non-carbohydrates)	E.g., Lignin	Complex polymer from aromatic compounds	Insoluble

NSP, non-starch polysaccharides; MU, monomer units; FOS, ß-fructo-oligosaccharides; GOS, α-galacto-oligosaccharides; TOS, β-galacto-oligosaccharides, XOS, xylo-oligosaccharides;

2.2. Dietary Fiber in Food

Dietary fibers supplementation in food products can impart various benefits, such as calorie reduction, shelf life improvement, texture, sensory characteristics, functionality, and freeze-thaw stability. The technological properties of DF derive from their physicochemical properties (refer to Section 3.3). DF, for example, with a high water holding capacity can be used to avoid synaeresis, improve shelf-life, and modify the viscosity and texture of the food, or DF with high oil binding capacity allows the stabilization of high-fat food products and emulsions (Grigelmo-Miguel, Carreras-Boladeras & Martín-Belloso, 1999). DF are most commonly added to bakery products to prolong freshness or modify the volume, springiness, and firmness of the loaf or the softness of the crumb (Sangnark & Noomhorm, 2004).

In addition to their beneficial impact on food products, DF have shown physiological efficacy and a modification of disease risk in clinical and experimental data, which were accumulated for the past 30 years due to their various physicochemical properties. Despite the health effects, foods high in DF, based on vegetables, fruits, legumes, whole grains, and unprocessed foods, find low acceptance in many highly civilized countries such as Germany. In contrast, sensory qualities of highly refined ingredients such as white flour, sugar, and fat, which are low in DF, are preferred (Meuser, 2008). The National Nutrition Survey from the years 2005-2006 shows an average total fiber intake of 24 g per day for male and female adults (19 – 64 years) in Germany (Nationale Verzehrsstudie, 2015). Therefore, the average total DF intake is lower than the recommended amount for fiber of ≥30 g per day (Deutsche Gesellschaft für Ernährung (DGE), 2008). Most nutritionists recommend that around 20–30 % of the daily fiber intake should derive from soluble DF (Elleuch et al., 2011).

To reach higher contents of DF in foods, supplementing the food with DF would be one approach. However, DF supplementation is challenging since the physicochemical properties of DF affect the food matrix and the processing of the food and can also cause undesirable changes in color, texture, and palatability (Tosh & Yada, 2010). The supplemented DF can also be changed by additional food components and processing techniques, which are discussed more closely in CHAPTER 4. Therefore, it is essential to monitor changes in DF properties and the impact of the changes on its associated functionality for health – for instance, the impact on postprandial glucose (Cassidy, McSorley & Allsopp, 2018).

Supplementing food with DF can lead to fitness-promoting ‘low calories’ and ‘low fat’ foods and health-promoting products (Elleuch et al., 2011). The amount of DF in foods determines whether

a claim regarding the DF content can be made in the European Union (EU), which can be a ‘source of fiber’ or ‘high in fiber’ (Parliament, 2006). There are also authorized health claims related to DF in food in the EU, which are very specific about the condition of use, such as the specific source of DF and its recommended intake. For the health effect ‘*contributes to the reduction of postprandial glycaemic response*’, five claims exist with the DF α-cyclodextrin, arabinoxylan (from wheat endosperm), beta-glucan (from oats or barley), pectin and resistant starch in different recommended intakes (Commission, 2012, 2013). For example, a beneficial effect on the postprandial glycemic response for pectins can be obtained with an intake of 10 g as part of the meal (Commission, 2012; EFSA Panel on Dietetic Products, 2010). This high amount is challenging to process in a meal because pectin, as a polysaccharide, builds a highly viscous structure in food.

3. Dietary Fiber and Glucose Regulation

One of the many health effects of DF consumption is the regulation of glycemic and insulinemic responses after consuming a meal rich in glycemic carbohydrates. Thereby, different mechanisms are discussed in literature for being responsible for regulating the glycemic and insulinemic responses including delay of gastric emptying, retarded sugar absorption, and reduction in starch hydrolysis.

3.1. Carbohydrate Metabolism and Blood Glucose Regulation

The physiological value of available dietary glucose in food is quantified in terms of the glycemic index (GI), which presents the potential of the food to increase the blood glucose level compared with the potential of a reference food material, such as white bread or pure glucose. Foods can be classified as low GI (≤55), medium GI (56 – 69), or high GI (≥70) (Wolever et al., 2019). Another physiologically essential and closely related term is the glycemic load (GL), which additionally considers the carbohydrate amount in a food portion (Livesey et al., 2019). Carbohydrates can be physiologically classified as glycemic and non-glycemic. Glycemic carbohydrates, such as starch, are chemically, mechanically, and enzymatically hydrolyzed in the upper gastrointestinal tract (oral cavity, stomach, duodenum of the small intestine) and absorbed at the end of the small intestine as monosaccharides into the hepatic portal vein. Therefore, they directly contribute to the glycemic blood response and insulin release. The glycemic carbohydrates can be further classified into rapidly and slowly digestible carbohydrates related to their contribution to glucose homeostasis. Non-glycemic carbohydrates, such as DF, are not hydrolyzed in the small intestine and are further transported within the colon (Gropper & Smith, 2017).

In the oral cavity – the first phase of carbohydrate breakdown – glycemic carbohydrates are degraded mechanically by the teeth and wetted with saliva containing α-amylase, which cleaves α-1-4-glycosidic bonds. The process takes several few seconds at a pH between 6.5 – 7 (Grimble, 2017). A short number of chews results in larger particle sizes and less hydrolyzed starch, which leads to a lower glycemic response (Tan et al., 2016). Contrarily, not well-chewed food can increase food intake and lead to rapid consumption, which results in increased glycemic response (Kavitha, Alphonse & Reddy, 2019). In the stomach – the second phase – hydrolysis of the carbohydrates continues until the α-amylase is inactivated by the low acidity (pH <3.5) (Fried, Abramson & Meyer, 1987). The pH changes to 1.5 – 3.5 with a remaining time of 1 – 2 h on average (Grimble, 2017). Starch hydrates and soluble carbohydrates are dispersed with liquids during the mixing process by antral contraction waves (Gopirajah, Raichurkar, Wadhwa & Anandharamakrishnan, 2016). Only particles smaller than 2 mm pass to the small intestine for further digestion, while larger particles stay in the stomach for further degradation (Goyal, Guo & Mashimo, 2019). The gastric emptying (GE) rate is determined by the volume, acidity, osmolarity, and fat emulsions, whereby delayed GE lowers the glycemic response (A. R. Mackie et al., 2017). In the duodenum of the small intestine – the last digestion step of the carbohydrates – gastrointestinal hormones, such as cholecystokinin (CCK), are released to regulate digestion processes further. Pancreatic secretions of bicarbonate and α-amylase elevate the pH of the chyme back to neutral and continue starch hydrolysis, while intestinal contractions cause sufficient mixing of the chyme with the secretions. The intestinal motility consists of segmentation motion – for mixing and chopping the chyme – and peristalsis – for transporting the chyme towards the large intestine (Y. Zhang et al., 2020). During the intestinal motility, amylolytic products, get in contact with the intestinal brush border on the epithelium, where di- and oligosaccharides are further hydrolyzed to glucose by glucosidases, such as maltase (Gropper & Smith, 2017; G. Zhang, Hasek, Lee & Hamaker, 2015). The increase of glucose in the small intestine activates peptide hormones (glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP)), which lead to the release of insulin from the beta cells in the pancreas in the bloodstream. Released monosaccharides get absorbed from the small intestine across the brush border and through enterocytes by transport proteins, such as sodium-dependent glucose cotransporter 1 (SGLT 1), and are transported in the portal vein directly to the liver, where they get further metabolized and released to the bloodstream (Gropper & Smith, 2017; G. Zhang et al., 2015).

One of many health benefits of DF is postprandial blood glucose control following ingestion of meals rich in rapidly digestible carbohydrates, which is an essential dietary strategy for people

with type 2 diabetes or impaired glucose tolerance or preventing these diseases. The mechanisms of glycemic control by DF are described in the following sections.

3.2. Potential Mechanisms of Glucose Regulation after Dietary Fiber Consumption

The most postulated potential mechanisms of action by DF for glycemic control are (i) slowed gastric emptying (GE), (ii) modulation of the release of digestion-related gut hormones, (iii) reduction in enzymatic digestion of carbohydrates, and (iv) delay of sugar absorption at the mucosa (refer to Figure 1) (Brownlee, 2011; Goff, Repin, Fabek, El Khoury & Gidley, 2018; I. Johnson & Gee, 1981). Thereby, either one or more of the postulated mechanisms can influence glycemic control at the same time. It is also discussed that the fermentation products of DF produced in the colon can influence glycemic control through the second meal effect (Furio Brighenti et al., 2006). Besides the beneficial effects of lowered sugar absorption, high loads of DF addition can also reduce the bioavailability of minerals, vitamins, and phytochemicals during digestion (Kim, 1998; Kim, Atallah, Amarasiriwardena & Barnes, 1996). The unique mechanisms of action by DF are explained in more detail in the following sections.

Delay of Gastric Emptying

The delay in GE due to DF supplementation can lead to a lowered glycemic response and is attributed to the structuring of gastric digesta (Brownlee, 2011). Structuring can lead to (a) an elevated luminal viscosity, followed by a higher dilution by gastric secretion (Marciani et al., 2001), (b) the formation of gels (Wanders et al., 2014), and/or (c) a delay in demixing of solids and liquids in the bolus, with liquids having a higher GE rate than solids. (Camilleri, Malagelada, Brown, Becker & Zinsmeister, 1985). Additionally, lower GE rates can be caused by an increase in the energy load of a meal (Wilmshurst & Crawley, 1980).

Modulation of Hormone Segregation

DF can also lead to an increase in the postprandial gut hormones levels and peaks of cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY) (Chater, Wilcox, Pearson & Brownlee, 2015; Sánchez, Miguel & Aleixandre, 2012), which leads to a decreasing GE rate (Näslund et al., 1999), and to a stimulation of insulin secretion, directly impacting plasma blood glucose levels (Drucker, 2006; Herzberg-Schäfer, Heni, Stefan, Häring & Fritsche, 2012). However, a slower GE rate is also related to a delayed stimulation of peptide release from the intestine and, therefore, to a lower postprandial increase in GLP-1, CCK, and PYY (Juvonen et al., 2009). Therefore, it seems that the modulation of gut hormone release after

DF consumption can either partly explain the slower GE rate or result from the slower GE rate due to DF consumption. Hence, a clear context between DF consumption and postprandial gut hormones release is difficult to phrase.

Reduction in Starch Hydrolysis

DF reduces amylolysis through various pathways: (a) by forming a starch-DF complex that creates a physical barrier between the enzyme and substrate, (b) through non-competitive inhibition of enzymes caused by interactions such as DF adsorption to enzymes, and (c) by binding water, which reduces its availability for starch gelatinization and hydrolysis. (d) The frequency of interaction between the enzyme and starch is reduced due to the slowed diffusion of both, mainly caused by the increased viscosity of the digesta. (e) The diffusion of the amylolytic products is decreased, leading to increased concentration and inhibition of the amylase in a feedback reaction. This is also mainly attributed to the increased viscosity (Dhital, Gidley & Warren, 2015; Sasaki, Sotome & Okadome, 2015; Shelat et al., 2010; Slaughter, Ellis, Jackson & Butterworth, 2002).

Ionic interactions of soluble DF also can attenuate the viscosity of the digesta and the process of starch hydrolysis. Thereby, strong electrostatic interactions between anionic soluble DF and cationic starch can result in aggregation. On the contrary, non-ionic soluble DF loosely wrap around the starch granules, hindering hydrolysis (Chaisawang & Suphantharika, 2005). Potential inhibition of starch hydrolyses could also originate from the attenuation of enzyme activity by non-specific binding of the enzyme with insoluble, low viscous DF as cellulose (Dhital et al., 2015). However, the pre-digestion of starch by salivary α-amylase during the oral phase is assumed to minimize the effect of DF on starch amylolysis in the small intestine (Goff et al., 2018).

Delay of Sugar Absorption

The delay of sugar absorption by DF is mainly attributed to an increased viscosity of the digesta. This could originate from (a) the lowered transport/diffusion of amylolytic products from the lumen to the small intestinal brush-border, as well as reduced mixing of the intestinal content and the mucus layer (Edwards, Johnson & Read, 1988; Gouseti et al., 2014; DJA Jenkins, Jenkins, Wolever, Taylor & Ghafari, 1986; Ou, Kwok, Li & Fu, 2001; Srichamroen & Chavasit, 2011). (b) The formation of a barrier layer due to mucosal interactions increases the apparent thickness of the unstirred layer, leading to a decrease in the diffusion and transport of monosaccharides across the intestinal membrane (Brownlee, Havler, Dettmar, Allen & Pearson, 2003; Hino et al., 2013; I. Johnson & Gee, 1981; Leclère et al., 1994; A. Mackie, Rigby, Harvey & Bajka, 2016). Also

(c) the activity of glucose transporters is downregulated by retaining high glucose content to the apical surface of epithelial cells via limited diffusion (Abbasi, Purslow, Tosh & Bakovic, 2016).

The slower absorption of carbohydrates in the small intestine can undesirably push the carbohydrates to the large intestine, where they are fermented or excreted (Lužnik, Polak, Demšar, Gašperlin & Polak, 2019). It is still not fully elucidated to what extent each of the described mechanisms of action by DF addition affects the attenuation of blood glucose levels and if there are still unknown mechanisms involved.

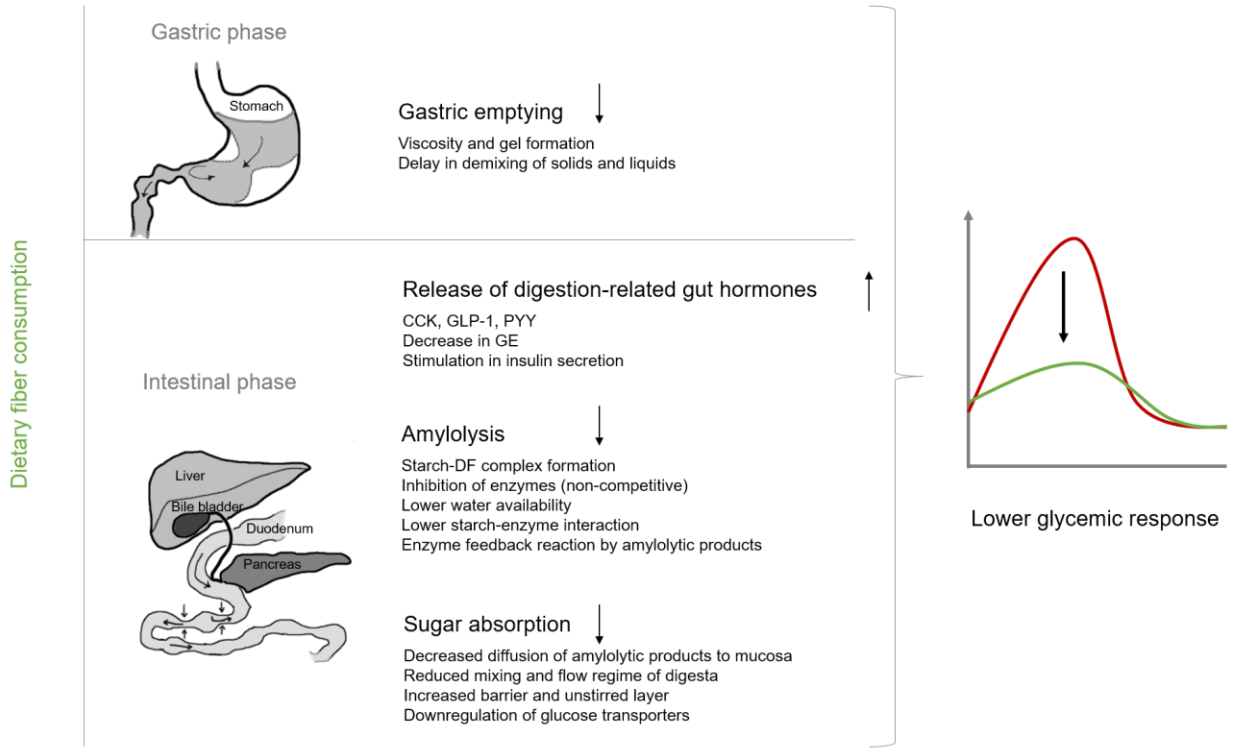

Figure 1 Potential mechanisms in glucose regulation by dietary fiber consumption (adapted from *Goff et al. (2018)*)

3.3. Physicochemical Properties of Dietary Fibers and Glycemic Response

Dietary fiber (DF) can exert different properties, including (i) molecular characteristics, such as the chemical structure and the degree of polymerization, (ii) structural attributes, such as molecular interactions, cell and tissue structures, and (iii) physicochemical properties, such as viscosity, water binding, and solubility. The physicochemical properties of DF have been extensively studied regarding their impact on:

- the food quality, for example, sensory properties, textural characteristics and storage stability,
- digestion-related physiology like the intestinal transit time of digesta and nutrient absorption in the small intestine and
- on health, such as the modulation of cardiovascular disease, cancer protection, and sugar metabolism (Poutanen, 2008).

The characteristic physicochemical properties of DF, which are essential for gastrointestinal functions, are known as physiochemical properties, such as water solubility, viscosity, hydration properties like WHC and bulk volume, which depends on the porosity and particle size of mostly insoluble fiber (Guillon & Champ, 2000). Those properties regulate the rate and site of digestion and absorption, leading to the disease prevention and health promoting effects of DF (I. T. Johnson, 2012; Schneeman, 1999). Viscosity is seen as a critical factor in understanding the glycemic response (GR). Viscosity highly depends on the solubility, the concentration and the molecular weight (M_W) of the fiber (E. R. Morris, 2001). In parallel, other physiochemical properties, such as WHC, contribute to viscous polysaccharides' effect on GR. However, other properties besides viscosity are not seen to be sufficient to modify the GR solely (Schneeman, 2008). The most prominent physicochemical properties of DF impacting the GR are shown in Figure 2.

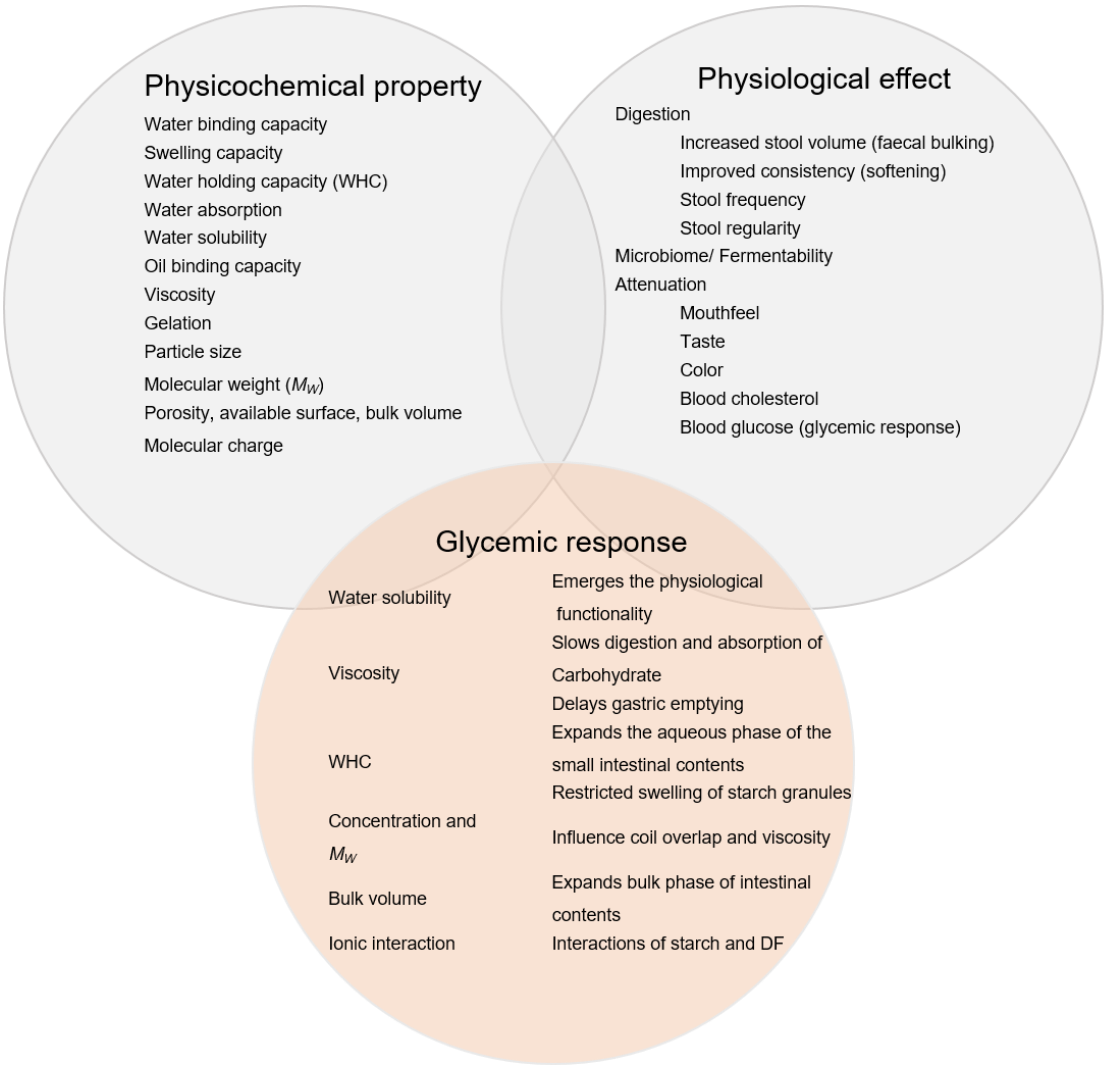

Figure 2 Physicochemical properties and physiological functionality of dietary fibers and the physicochemical properties that impact glycemic response (adapted from Meuser (2008) and Schneeman (2008))

Solubility

The water-solubility of DF has been used to classify DF in terms of their functionality and physiological effects (Jiménez-Escrig & Sánchez-Muniz, 2000; Roehrig, 1988). Soluble DF are characterized by their capacity to increase viscosity and hydration capacity, whereas porosity and low density characterize insoluble DF (Elleuch et al., 2011). The solubility of DF is usually determined after digestion of the food component under conditions related to the gastrointestinal tract (McCleary et al., 2012), but sometimes the solubility is evaluated prior to *in vitro* digestion. The soluble part can be separated from the insoluble fiber by filtration or centrifugation. The soluble and insoluble parts of the DF are attributed to various functionalities. Soluble DF are often

attributed to increasing both viscosity and hydration properties (refer to Figure 2). Besides the structure of the polymer that affects the solubility, as discussed in the previous section, solubility can also be affected by temperature and ionic strength (Bertin, Rouau & Thibault, 1988; Fleury & Lahaye, 1991; Manas, Bravo & Saura-Calixto, 1994). For example, pectin, containing charged groups, is highly soluble in saline solutions due to electrostatic repulsion, which inhibits an ordered form but is insoluble in an acid or high ionic strength solution. In addition, most locust bean gums are insoluble in cold water and dissolve in high temperatures, as heat removes the bonding, leading to a disordered and more soluble form (Guillon & Champ, 2000).

In glycemic control, the properties of soluble DF are known to be particularly important by blunting the postprandial blood glucose and insulin rise. Thereby, the ability of soluble fibers to increase viscosity and bind high amounts of water is highly important for their ability to lower GR by reduction of GE and slowing nutrient absorption in the small intestine (Adiotomre, Eastwood, Edwards & Brydon, 1990; Cameron-Smith, Collier & O'dea, 1994; Schneeman, 1987). However, insoluble DF do not affect the postprandial GR, but show a high association with the reduction of the risk of diabetes due to an increase in insulin sensitivity, although the mechanisms involved are mostly unclear (Jenkins et al., 2000; Liu et al., 2000; Pereira et al., 2002; Weickert & Pfeiffer, 2008). The classification by water solubility is method-dependent and does not always imply physiological effects (Stephen et al., 2017). Thereby, the solubility of DF in the upper gastrointestinal tract is important so that they can develop their effect.

Concentration, M_W, and Coil Overlap

Fiber content per se does not directly equate to a glycemic answer as the fiber matrix plays an essential role in efficiency (DJ Jenkins et al., 1988). The structuring ability of DF as given for alginates, guar gum, beta-glucans, and pectins is essential in the ability to blunt the postprandial glycemic and insulinemic response (Haber, Heaton, Murphy & Burroughs, 1977; Sierra et al., 2001; Torsdottir, Alpsten, Holm, Sandberg & Tölli, 1991; Tosh, Brummer, Wolever & Wood, 2008). Clinical studies have shown that the M_W of soluble DF (SDF) plays an essential role in glycemic control besides concentration. The M_W and the concentration of SDF influence the ability to build a network and structure foods. Thereby, there is a critical M_W (M_C) at a given concentration and a critical concentration (c^*) at a given M_W, above which entanglement and coil overlap of the polysaccharides occurs, and a network is formed, accompanied by a substantial viscosity increase. The c^* is a specific parameter for each polymer. This increase in viscosity is, therefore, an interplay of M_W and c, also termed the coil overlap parameter ($c^* \times M_W$).

Food products containing SDF with low M_W are less efficient in lowering the postprandial glycemic and insulinemic response than SDF with medium or high M_W (Tosh et al., 2008; Y. Wang et al., 2015; Wolever et al., 2010; Wood, 1990), probably because the combination of M_W and c are below the occurrence of coil overlap. This effect was shown for whole food products, such as muffins (Tosh et al., 2008), bread (E. Östman, Rossi, Larsson, Brighenti & Björck, 2006), and bars (Regand, Chowdhury, Tosh, Wolever & Wood, 2011). Also, a standardized presentation of *in vivo* data on the glycemic response of beta-glucan-containing food by Rieder, Knutsen and Ballance (2017) pointed toward the critical role of coil overlap regarding the efficiency in reducing GR. Coil overlap has similar postulated mechanisms of action in glycemic control as viscosity. However, it is unclear how c and M_W individually affect glycemic control.

Viscosity

Viscosity is related to other physicochemical properties, including solubility, molecular weight, linearity, and degree of branching, where water-soluble and high molecular DF and high concentrations of DF can form high viscosities. The viscosity (η) of solutions refers to the resistance to flow, also described by the ratio of shear stress (τ) to shear rate ($\dot{\gamma}$). In polymer solutions, a rise in viscosity is caused by physical interactions (entanglements) between the polymer chains. Therefore, the viscosity is mainly influenced by the concentration and intrinsic characteristics of the polymers, which are described by the M_W, and the structure of the molecules, affecting the amount of space occupied by the polymer ('effective volume') within the solution. Further influencing parameters are the applied solvent and temperature. At low concentrations, the polymers in the solution are well separated and independent from the shear rate (Newtonian). As concentration rises, the polymers get entangled, forming a network. Below c^*, viscosity increases almost directly proportional to concentration, while above c^*, the increase of viscosity with concentration gets much steeper. At this concentration range, the viscosity of the solution decreases with an increase in shear rate exhibiting shear thinning behavior (E. Morris, Cutler, Ross-Murphy, Rees & Price, 1981). The concept of c^* in context to viscosity has been studied in detail for many high molecular soluble DF, such as guar gum, alginate, carboxymethyl cellulose, carrageen, guar gum, locust bean gum, beta-glucan, gum arabic, and pectins (Doublier & Wood, 1995; Kpodo et al., 2017; E. Morris et al., 1981; Mothe & Rao, 1999). It was stated that linear polysaccharides obtain higher viscosities than branched polysaccharides as the 'effective volume' of the molecule rises with the lengths of the polymer (E. R. Morris, 2001). Therefore, polysaccharides such as pectins and beta-glucans accelerate higher viscosities than oligosaccharides such as inulin and dextrin. Soluble DF significantly determines the overall viscosity in the digesta from the stomach and small intestine, even if solid particles can also modify

the flow behavior (Guillon & Champ, 2000). E. Morris (1992) recommended that the viscosity at an infinitive low shear rate – known as 'zero shear' viscosity (η_0) – should be determined to receive a meaningful value for comparison of different polysaccharide solutions, as most of them exhibit shear thinning behavior. Furthermore, Guillon and Champ (2000) suggested monitoring at least the polymer's concentration, structure, and molecular weight ('effective volume') for interpreting the data of digestion trials.

For the postulated mechanisms of action in glycemic control, the literature mainly emphasizes the influence of viscosity, which has been well-demonstrated by human intervention studies (Brownlee, 2011; Goff et al., 2018). The studies of viscosity on GE rate are ambivalent. Some studies show a delayed GE after the consumption of viscous fibers (H. Lin et al., 1992; Marciani et al., 2000; Schwartz, Levine, Singh, Scheidecker & Track, 1982), while others show no effect (Hoad et al., 2004; van Nieuwenhoven, Kovacs, Brummer, Westerterp-Plantenga & Brouns, 2001). The high viscosity of the gastric content leads to a dilution in the stomach, which expands the volume to be emptied and delays the GE. Thereby, viscous DF reduce more strongly the GE than gel-forming DF or DF with high bulk volume (Wanders et al., 2014). A high mixing rate of the digesta ensures a rapid transport and absorption of sugars. Increasing the viscosity of the digesta reduces the mixing and the flow regime within the lumen. Thereby, the flow regime can decrease from a turbulent to a laminar flow and lead to a retarded transit through the small intestine. A lower flow regime and mixing of the digesta increase the thickness of the unstirred water layer at the enterocyte surface. A thick, unstirred water layer builds a barrier for sugars, which must diffuse to get absorbed, leading to a lowered absorption of sugars (I. Johnson & Gee, 1981; Lentle & Janssen, 2008; Macagno, Christensen & Lee, 1982). The lower mixing of the digesta also leads to less frequent interactions between carbohydrates and enzymes for amylolysis (Singh, Dartois & Kaur, 2010), and the continuous, highly viscous network of DF could also barrier the access of enzymes to carbohydrates (Koh, Kasapis, Lim & Foo, 2009). The high viscosity of the small intestinal content also lowers the diffusion of amylolytic products from the lumen to the brush border (Fabek & Goff, 2015; Fabek, Messerschmidt, Brulport & Goff, 2014; Lund, Gee, Brown, Wood & Johnson, 1989). To induce the flow and mixing of the high viscous digesta, additional pressure is required to disrupt the entanglements of the DF.

Slowing the rate of sugar absorption at the upper areas of the intestine leads to a higher concentration and delayed absorption of sugars in more distal areas (Schneeman & Richter, 1993). The nutrient exposure in the distal areas causes a feedback mechanism, which slows intestinal transit and enhances CCK release, which also slows GE (H. C. Lin, Zhao, Chu, Lin & Wang, 1997). It is essential to mention that the viscosity of foods may not necessarily reflect the

physiological viscosity. The viscosity of the intestinal content will change due to shearing, gastrointestinal secretions, and the presence of other nutrients (Brownlee, 2011; Marciani et al., 2000), and, thus, evaluating the actual viscosity of the digesta, which is physiologically relevant, is a very challenging task (Guillon & Champ, 2000). Therefore, foods with DF forming high viscosity digesta cannot retard the GR in the expected way if the digesta is strongly shear-thinning by intestinal contractions. Despite viscosity, also the M_W and c of the DF individually can play a separate role retarding the GR, which is not well understood to date (Rieder et al., 2017; Tosh et al., 2008).

Hydration Properties

The hydration properties can be determined regarding swelling, water retention capacity, and water absorption, for which clear definitions were made within the EU concerted action group, Profibre (Guillon & Champ, 2000). Swelling describes '*the volume occupied by a known weight of fibre under the condition used',* water retention capacity is '*the amount of water retained by a known weight of fibre under the condition used',* and water absorption describes '*the kinetics of water movement under defined conditions*' (Robertson, 1998). The water retention capacity is further distinguished by water binding capacity (WBC), where the 'used condition' is centrifugation, and water holding capacity (WHC), where the 'used condition' is applied suction pressure. The latter is similar to physiological digestion and suitable for soluble DF, as no centrifugation or filtration step is included (Robertson & Eastwood, 1981). Water absorption provides specific information about the pore volume of the fiber. It is, therefore, suitable for the characterization of insoluble fibers, whereas swelling and water retention provide more general information about fiber hydration (Guillon & Champ, 2000). In addition, information on the hydration properties of DF can be obtained using ^{1}H NMR relaxometry, which determines the water conditions in terms of mobility and amount of bound water (Kerr & Wicker, 2000; Ruan & Chen, 1998). The hydration ability is strongly related to the chemical composition, anatomy, and physical characteristics of the fibers and, therefore, to the source of DF. In general, higher solubility of DF elevates the hydration properties (Elleuch et al., 2011). Also, the processing of the fibers, such as grinding, heating, or extrusion, and environmental conditions, such as pH, ionic strength, and type of ions, influence the hydration properties (Renard, Crépeau & Thibault, 1994; Thibault, Lahaye & Guillon, 1992).

In terms of glycemic control, the WHC is most likely to have a physiological meaning, as the solubility and viscosity of DF impact the WHC. Further, the WHC helps to understand the behavior of the fibers during gut transit (Guillon & Champ, 2000). DF with high viscosity typically show a high water holding capacity (WHC). The high WHC of the DF increases the aqueous phase of the

intestinal content and, therefore, the total volume in the intestine (Schneeman, 1999). Subsequently, the concentration of nutrients is diluted, and the absorption is slowed down. Water-soluble and hydrophilic nutrients as sugars can also diffuse through the aqueous phase in the fiber matrix or get entrapped between the fibers, and are released in a later stage of the intestine with a retarded availability for absorption (Karim, Raji, Karam & Khalloufi, 2023; Schneeman, 2008). In the Colon, DF with a high WHC can get degraded more easily by microbes (Bourquin, Titgemeyer, Fahey & Garleb, 1993; McBurney, 1991; McBurney, Horvath & Jeraci, 1985), where the fermentation products can also influence glucose regulation. The formation of short-chain fatty acids (SCFAs) by fermentation is known to improve postprandial responses, especially to a second meal, known as the second meal effect (Furio Brighenti et al., 2006). The potential of fermentable carbohydrates in the colon in terms of improved glucose metabolism still requires further investigation.

Bulk Volume

The definition of intestinal bulk volume is the volume of particles occupying the total mass of the chyme. The bulk volume is primarily increased by low-density, insoluble DF, which are not degraded in the small intestine (Schneeman, 1982). Besides the concentrations, the bulk density, which among others depends on the particle size and the porosity of the DF, influence the bulk volume (Huang, Liao, Cheng & Chan, 2009; López Córdoba & Goyanes, 2017). *In vitro*, lower particle sizes and, therefore, higher bulk volumes of mostly insoluble fibers led to a lower glucose diffusion and a lowered amylolysis of starch (Huang et al., 2009). As the concentration of DF increases, the contribution to particle-particle interactions, the degree of friction, and ease of entanglement also increases. A higher bulk is postulated to decrease the efficiency of mixing the content with digestive secretions and, therefore, the digesta's transit is slowed down to supply sufficient mixing (Lentle & Janssen, 2008; Macagno et al., 1982). In contrast, in the large intestine, an increased bulk volume of insoluble DF generally accelerates the overall gut transit time (Goff et al., 2018)

In general, it should also be noted that digestion alters the physicochemical properties of DF depending on the site of the gut and digestion time by, e.g., variation in pH, dilution effects, and progress in hydrolysis (Dhital, Dolan, Stokes & Gidley, 2014; Marciani et al., 2001; Takahashi & Sakata, 2002). Therefore, digestion affects the impact of physicochemical properties on glucose regulation, which should be considered by investigating DF in terms of improved glucose metabolism.

4. Composition and Processing of Foods, and Glycemic Response

There are several factors of food affecting GR, whereby the composition of the food plays a significant role. In particular, a nutrient content high in carbohydrates containing free starch, a low amylose to amylopectin ratio, a high amount of rapidly digestible starch, and a high glucose content have a high impact on the GR. Additionally, foods having high acidity increase the GR. In contrast, a food containing high amounts of protein, fat, resistant starch, DF, and polyphenols has a low impact on the GR (Fardet, Leenhardt, Lioger, Scalbert & Rémésy, 2006; Priyadarshini, Moses & Anandharamakrishnan, 2022). Furthermore, the structure and viscosity of a food item can influence the GR, whereby a porous structure, fluffy texture, fine particles, and low viscosity can increase the GR and a regular structure with large particle size and high viscosity can, in turn, decrease the GR (Priyadarshini et al., 2022).

Food processing conditions can influence the GR due to affecting both the composition and structure of the food. Thereby, parboiling, freezing, toasting, steaming, frying, shallow frying, and cold extrusion is reported to decrease the GR, whereas de-hulling, flaking, grinding, boiling, soaking, popping, extrusion cooking, autoclaving, baking, microwave heating, and roasting tend to increase the GR (Priyadarshini et al., 2022; Singh et al., 2010; S. Wang & Copeland, 2013). An increase in GR due to processing can result from an increase in the susceptibility of starch, a reduction of anti-nutrients, and a decrease in particle size distribution, which increases the surface area (Anguita, Gasa, Martín-Orúe & Pérez, 2006; Rehman & Shah, 2005). The susceptibility of starch to enzymatic breakdown is influenced by the temperature, rate and duration of heating, water availability, and shear forces (S. Wang & Copeland, 2013). Thermal treatment with high water availability leads to high water absorption and swelling of starch granules and disruption of the highly ordered crystalline structure through gelatinization, by which starch granules get easier enzymatically degraded (Blazek & Copeland, 2010; S. Wang & Copeland, 2013). Thereby, the plasma glucose response (Holm, Lundquist, Björck, Eliasson & Asp, 1988; Parada & Aguilera, 2009) and insulin response (Holm et al., 1988) are strongly positively correlated with the degree of starch gelatinization. With increasing temperature, the rapidly digestible starch (RDS) increases, whereas the slowly digestible starch (SDS) and resistant starch (RS) decrease gradually (Chung, Lim & Lim, 2006; Miao, Zhang, Mu & Jiang, 2010). Limited water conditions in food systems, such as in baked products or due to the presence of other food ingredients like protein or DF with high WHC, restrict the swelling and lead to incomplete gelatinization of starch (Delcour & Hoseney, 2010), whereby the ease and extend of hydrolysis with amylases during digestion is reported to be lowered (Kaur, Singh, Singh & McCarthy, 2008; Tester & Sommerville,

2001). Furthermore, the presence of organic acids during gelatinization could limit the enzymic accessibility due to stronger interactions between starch and proteins (E. M. Östman, Nilsson, Elmståhl, Molin & Björck, 2002). Extrusion cooking can cause partial gelatinization of starch under controlled moisture conditions and can increase the starch digestibility and glycemic index of foods (Alonso, Aguirre & Marzo, 2000; Fredriksson, Silverio, Andersson, Eliasson & Åman, 1998). However, a lower starch digestibility due to extrusion cooking was also observed, probably caused by the formation of amylose-lipid complexation, starch-protein interaction, and/or limited water availability (Guha, Ali & Bhattacharya, 1997). Liquid starchy food that turns due to processing into solid starchy food tends to have a more sustained GR, whereas the overall glycemic area under the curve stays the same (Shafaeizadeh, Muhardi, Henry, Van de Heijning & Van der Beek, 2018).

Upon cooling and storage, gelatinized starch experiences a process called retrogradation, where the amylose and amylopectin molecules realign into more ordered semi-crystalline structures. This results in decreased digestibility and RS formation, consequently leading to a lowered GR. (Abd Karim, Norziah & Seow, 2000; Singh et al., 2010). The extent of retrogradation varies depending on the time and temperature conditions (S. Wang & Copeland, 2013). For example, amylopectin crystallization occurs at later stages during refrigerated storage compared to amylose crystallization (Singh et al., 2010).

Processing can also modify dietary fibers contained in food. Thereby, the amount and quality of DF may be changed during processing, whereby the technological and physiological functionality of DF can be influenced (refer to Section 3.3.). Changes in DF during processing can include molecular, structural, and functional properties of DF, which are based on hydrolytic enzymatic reactions, chemical degradation, or crafting reactions (Poutanen, 2008). The processes that change the DF can be chemical, enzymatic, mechanical, thermal, or thermo-mechanical treatments, whereby water availability plays an important role during processing. Mechanical processing, such as milling or flaking, can alter the particle size and hydration properties, resulting in varied fractions of DF. This can lead to an increase in soluble DF. (Glitsø & Knudsen, 1999; Plaami & Kumpulainen, 1996; D. Zhang & Moore, 1999). Shear forces generated, e.g., by a decanter, can cause depolymerization of the DF and lower viscosity (Wood, Weisz, Fedec & Burrows, 1989). Thermal processing such as steaming, baking, or microwave treatment can increase or decrease the total DF content and the solubilization of the DF (McDougall, Morrison, Stewart & Hillman, 1996). Solubilization of DF by thermal treatment, resulting in a decrease in viscosity, is achieved by degradation of DF, which leads to a lower M_W (S. M. Svanberg, Gustafsson, Suortti & Nyman, 1995), as observed by baking (Autio et al., 1996; Sundberg et al., 1996) and microwave treatment (T. S. Svanberg, Margareta Nyman, Maria, 1999). Further, heat

processing as baking can activate endogenous enzymes, such as beta-glucanases, and can cause depolymerization and solubilization of DF (Jaskari et al., 1995; Poutanen, 1997). Extrusion combines thermal and mechanical treatment, and the effects on the changes in DF can be controlled by the main extrusion parameters such as water content, temperature, extruder geometry, and screw speed. Extrusion has been shown to increase the fiber content and extensively degrade the DF, which results in increased solubility and possibly reduced viscosity (Camire, Violette, Dougherty & McLaughlin, 1997; Ralet-Renard, Thibault & Della Valle, 1991). In this context, extrusion processing is also known to convert insoluble fiber to soluble fiber (Bader Ul Ain et al., 2019; Naumann, Schweiggert-Weisz, Martin, Schuster & Eisner, 2021). The main challenge of the food industry is to maintain or enhance the positive effect of the physiochemical properties of DF on glycemic control during food processing.

5. Analysis of the Glycemic Response of Dietary Fiber

In this chapter, different approaches with focus on relevant methods for this thesis, including their merits and limitations, in order to measure or predict the glycemic response are discussed. A recent review by Priyadarshini et al. (2022) offers an additional, comprehensive insight into the topic.

5.1. *In Vivo* and *Ex Vivo* Approaches to Study Glycemic Response

The foremost essential and ‘gold standard’ technique for implementing glycemic studies are *in vivo* human methods. The most common rate to express the glycemic response (GR) of a meal is the glycemic index (GI). The benchmark method to measure GI is via *in vivo* human studies (Wolever et al., 2019). Numerous *in vivo* studies were conducted on the development of low-GI foods. To determine the GI of a food, 50 g of available carbohydrates are given to a human subject within 15 min, along with 250 to 300 mL of water, and the blood glucose is assessed during a specific time (usually 2 h). The same proband receives the test food, and again, blood glucose is assessed over time (Livesey et al., 2019). Human blood glucose can be monitored incrementally by collecting blood samples or continuously by a monitoring device inserted underneath subcutaneous tissue (Heinemann et al., 2018). The GI is calculated by the total area under the blood glucose curve (AUC) of the test food and the pure glucose solutions, most widely based on the trapezoid rule (Brouns et al., 2005), which is as follows.

$$\text{GI (\%)} = 100 \cdot \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{pure glucose}}} \quad (1)$$

The majority of the human studies examining the effects of dietary fiber on postprandial glycemia, GI and insulinemia investigate foods containing 50 g of available carbohydrate (Chillo, Ranawana, Pratt & Henry, 2011; A. L. Jenkins et al., 2008; Matsuoka et al., 2020; Robert, Ismail & Rosli, 2016; Pariyarath S Thondre & Henry, 2009), with fewer studies exploring higher amounts (Panahi et al., 2014; Papakonstantinou et al., 2022; Pariyarath Sangeetha Thondre & Henry, 2011; Willis et al., 2011). Thereby, foods with 50 g of available carbohydrate are more likely to benefit from the addition of dietary fiber compared to those with higher amounts of available carbohydrate. The advantages of the *in vivo* sampling method include considering the effects of gastric emptying, osmolarity, volume, acidity, disease, and sex and age factors. There is no equivalent method that can consider these factors. The significant concerns by a method involving human subjects are the variability in each individual's metabolism and the ethical constraints (Peyser, Balo, Buckingham, Hirsch & Garcia, 2018). Therefore, close screening of the individuals, such as monitoring fasting conditions and meals provided before and during the study, following specific guidelines, and leading to an enormous effort, is required (Haldar et al., 2020). Animal-based *in vivo* studies may be a proper predictive method before a human study, revealing reduced costs, labor, and human discomfort. The GI in the animal *in vivo* studies can be calculated with the same AUC method as in human *in vivo* studies, and the correlation between these studies is very good (Falsafi, Maghsoudlou, Aalami, Jafari & Raeisi, 2019). A study by Nielsen et al. (2014) found similar metabolic responses in pigs and humans when different contents and compositions of DF were used in bread. In contrast, Hasselwander et al. (2017) observed significant differences in AUC between mice and human trials when using soluble fiber blends containing dextrose. The selection of the animal on gene similarity to humans, for instance, must be considered carefully. Despite the various advantages of animal models, ethical issues and disadvantages exist, such as needing professional personnel, time-consuming procedures, and housing costs (Doke & Dhawale, 2015). Therefore, *ex vivo* experiments represent a less invasive alternative to *in vivo* methods. *Ex vivo* models are developed outside an organism and include isolated or cultured tissues in adequate growth medium and appropriate environment. The advantages of the isolated intestinal segments are the natural structure of multiple cell types with hormones, microbial load, and cell-to-cell communications (Pearce et al., 2018).

The *ex vivo* method with isolated tissue considers the apparent permeability and absorption kinetics of glucose through the gut tissue. Different suitable apparatus uses *ex vivo* intestinal

tissue to measure glucose permeability, such as the gut sac apparatus, the Ussing chamber, and the InTESTine™ developed by TIM (Pearce et al., 2018; Ripken & Hendriks, 2015). Similar to *in vivo* animal studies, *ex vivo* animal intestine selection should be carefully considered and closely correlated with the human intestine's permeability (Nunes, Silva & Chaves, 2016). The isolation of the tissue without damage and integrity maintenance is challenging. The biological variation and small changes in harvesting time, age, sex, diet, stress level, and method of killing can influence the study's outcome (Alam, Al-Jenoobi & Al-Mohizea, 2012). Therefore, multiple replications have to be performed. Cultured tissue, such as self-assembling 3D aggregates or monolayers of 2D cultures, is a reasonable alternative to isolated tissue with the advantage of a less invasive method and fewer biological variations (Yi et al., 2017). The cell lines must constantly be screened for cross-contamination, and the precision and repeatability of the method are very time-consuming. Therefore, *in vitro* approaches address these limitations as being less time-consuming, ethically harmless, and producing readily analyzable outcomes.

5.2. *In Vitro* and *In Silico* Approaches to Study Glycemic Response

In vitro assays are models that mimic a sequence of physiological digestion and are valuable methods to test modifications, associations, and bioavailability of nutrients in a time-, labor-, and cost-saving way. Furthermore, the lower variance, fewer trials, and the chance to measure the effect of one variation at a time and with constant conditions make *in vitro* trials attractive (Minekus et al., 2014). Therefore, *in vitro* methods are useful for precisely characterizing ingredients or foods, which could not be achieved through *in vivo* tests. However, it is important to note that the results of *in vitro* tests should not be equated with *in vivo* results. *In vitro* methods were already used at an early stage to determine the availability of carbohydrates (Southgate, 1969), and to date, numerous *in vitro* models have been developed.

In vitro methods can be distinguished by static and dynamic approaches. The static *in vitro* model contains the oral, gastric, and intestinal phases in separate steps. In contrast, the dynamic *in vitro* model automatically interconnects the individual phases based on the human digestive system and, therefore, can be a more accurate simulation of *in vivo* conditions. In the dynamic system, for instance, the particles' size and size distribution after oral processing, the gastric emptying rate, or the absorption level at intestinal villi can be calculated and set (Bornhorst & Singh, 2012). To date, different dynamic *in vitro* digestion methods are available, each specialized in a particular part of the digestion or type of food. The range encompasses mimicking the dynamic human *in vivo* system, including the colonic microbiota (Barroso, Cueva, Peláez, Martínez-Cuesta & Requena, 2015), simulating duodenum segmentation motion (Wright, Kong, Williams & Fortner,

2016), and digesting starch in infants (Passannanti et al., 2017). Further, Wu et al. (2017) simulated digestion in rats with automated enzyme secretion, Parthasarathi, Bhushani and Anandharamakrishnan (2018) mimicked passive diffusion at the villus level, Qing et al. (2019) imitated different degrees of satiety and Li, Zhu, Zhang, Zhan and Gao (2020) simulated the folding of stomach and villus structures.

Both static and dynamic models can be based on restricted methods with dialysis bags and unrestricted methods without dialysis bags. Using a dialysis bag – in which the *in vitro* digesta is filled – represents the time-delayed mass transfer in the chyme and is a boundary for diffusion. It further mimics the rate-limiting process of starch hydrolysis, and changes in the viscosity of the incubated dialysate can be represented (Granfeldt & Björck, 1991; Naumann, Schweiggert-Weisz, Bader-Mittermaier, Haller & Eisner, 2018). D. J. Jenkins et al. (1984) started to determine the *in vitro* glycemic response (GR) with a restricted method, where released glucose from digested carbohydrates was measured after placing the *in vitro* digesta in a dialysis bag, and a solid correlation to *in vivo* data was found (R >0.86). The restricted method was further used to test the impact of various dietary fibers on sugar release. The findings indicated that viscous fibers specifically result in a slower release (D. J. Jenkins & Jenkins, 1985; I. Johnson & Gee, 1981). Subsequently, *in vitro* studies were used to better understand the effect of DF on sugar release by mimicking the physiological digestion more closely (Berry, 1986; Granfeldt & Björck, 1991) by including separate digestion steps (Englyst, Kingman & Cummings, 1992), and by introducing glass balls within the digestion step for the simulation of peristaltic movement (F Brighenti, Pellegrini, Casiraghi & Testolin, 1995). A harmonized static *in vitro* protocol for food was developed by the COST INFOGEST network (Minekus et al., 2014) to simplify the comparability of data from *in vitro* digestion.

The diffusion of sugar out of the dialysis bag mimics the diffusion process of a sugar molecule, which can move freely in the intestinal lumen. This process is termed by the diffusion coefficient (D), which can be calculated based on the random walk approach, based on the average square of molecular displacement (Einstein-Smoluchowski-Equation), or based on the hydrodynamic approach in solutions applying the Stokes-Einstein-Equation as (Crank, 1979)

$$D = \frac{k_B T}{\pi R \eta} \tag{2}$$

where k_B is the Boltzmann constant, T is the absolute temperature, and R is the hydrodynamic radius of the sphere.

In order to investigate starch digestibility using *in vitro* methods, Englyst et al. (1992) introduced an unrestricted method to classify the fractions of starches as rapidly digestible (RDS), slowly digestible (SDS), and resistant starch (RS), which could explain even more closely the physiological GR (R =0.91) (Goñi, Garcia-Alonso & Saura-Calixto, 1997). The characterization of the rheological properties of *in vitro* digesta should be assessed alongside starch digestion, as viscosity plays a crucial role in GR (Wood, 2004; Woolnough, Monro, Brennan & Bird, 2008). The released glucose over time, which can, for example, be based on glucose release by starch hydrolysis, follows a first-order non-linear kinetic (Goñi et al., 1997):

$$c = c_{\infty} \left(1 - e^{-kt}\right) \tag{3}$$

with *k* as the rate constant and *t* as the time of the reaction. Equation (3) can be used to calculate the area under the curve, which can be applied to determine the hydrolysis index (Goñi et al., 1997; Granfeldt, Björck, Drews & Tovar, 1992). This index provides an indication of the *in vivo* glycemic index.

Some *in vivo* parameters concerning GR, such as hormonal effects, active transporters, the movement of the intestine, the mass distribution throughout the intestine, or diseases, are challenging to mimic by *in vitro* methods (Navale & Paranjape, 2016). These factors can be substituted with the inclusion of *in silico* approaches in addition to *in vitro* (and *ex vivo*) models. *In silico* models are computer-generated models based on experimental data and can be used to predict glycemic effects. The motility of the intestine is an essential part of the computer simulation to understand glucose absorption from the gut better. Computational fluid dynamic models clarify, for example, the effect of longitudinal und axial compressions on mixing and fluid movement properties (Lentle & De Loubens, 2015; Lim, de Loubens, Love, Lentle & Janssen, 2015) as well as the effect of visco-elastic properties of the digesta on the fluid-dynamic in the intestine (Lentle, Sequeira, Hardacre & Reynolds, 2016; Sinnott, Cleary & Harrison, 2017). The transport of glucose in the small intestine can be estimated using the mass transfer process and the change in concentration by the second law of Fick (Moxon, Gouseti & Bakalis, 2016). Further mathematical models based on *in vivo* data of the postprandial kinetic constants of the blood glucose response curve (Rozendaal et al., 2018) or on insulin release (Gyuk, Vassányi & Kósa, 2019) can be utilized to forecast the GR by DF intake.

The TIMCarbo digestion model is an example, which successfully combines *in vitro* and *in silico* methods to closely predict the GR (R = 0.94) by simulating *in silico* the insulin component of glucose homeostasis and *in vitro* the digestion steps and starch hydrolysis (Bellmann, Minekus,

Sanders, Bosgra & Havenaar, 2018; Dupont et al., 2019). However, further studies taking into account additional hormones involved in GR, such as glucagon, are needed for a more accurate *in silico* prediction of GR after DF intake. In summary, no single method can replace accurate *in vivo* glycemic response. Each method has advantages and limitations from the *in vivo* animal models to the *ex vivo*, *in vitro*, and *in silico* methods. By combining the methods, they can support each other to better understand and predict the glycemic response.

6. Aims of the Study

The incidence of diet-related diseases is progressively increasing in western countries. High postprandial plasma glucose levels contribute to the development of these diseases, particularly type 2 diabetes. Consuming meals with high DF content can help to control blood glucose levels after consuming a high-carbohydrate meal. Different potential physiological mechanisms are proposed in the literature for modulating blood glucose levels upon ingestion of DF. Previous investigations have shown that soluble DF (SDF) can reduce the postprandial absorption rate of glucose, mainly due to viscosity formation. However, the impact of other relevant physicochemical properties of SDF on glucose release still remains unclear and requires further investigation. In addition, the impact of various food processing conditions on glucose release, with a main focus on the alteration of physicochemical properties of DF and structural changes affecting food or starch digestibility, has been poorly investigated.

Therefore, the aim of the thesis was to elucidate the factors that affect *in vitro* glucose release with a focus on DF and the alteration of DF and food by processing.

The first part of the study (CHAPTER 1) aimed to comprehensively examine the effects of physicochemical properties related to SDF on glucose release in the chyme. To achieve this goal, a diffusion-based *in vitro* model should be developed to simulate the transfer of amylolytic substances from the intestinal content to the small intestinal mucosa. The study aimed to systematically investigate the glucose diffusion coefficient (D) and the influence of convection on glucose release in carboxymethyl cellulose (CMC) solutions, considering the CMC concentration, M_W, and viscosity. Optical density measurement and cryo-scanning electron microscopy (SEM) images should be utilized to clarify and reinforce the research findings.

The subsequent second study (CHAPTER 2) endeavors to clarify the factors influencing glucose diffusion in DF solutions, going beyond viscosity and considering hydration properties. The study aimed to dive deeper into the hydration properties, including water holding capacity, the amount of bound water, and osmotic pressure influencing glucose diffusion in DF solutions. The study should explore the impact of various types of soluble DF (low and high methylester pectin, xanthan gum, locust bean gum, and carboxymethyl cellulose) and partly insoluble DF (citrus fiber) at concentrations above the c^*. Further, the effect of an osmotic gradient on glucose release should be worked out using the *in vitro* release model.

The third study (CHAPTER 3) should focus on the application potential of high hydration SDF, specifically high methylester pectin (HMP), in food matrices to evaluate their impact on glucose

release. The study aimed to examine the influences of HMP enrichment and processing of cake dough on composition, microstructure, starch digestibility, and glucose release by baking and extrusion cooking. To comprehensively understand the outcomes, various analytical techniques such as TD-NMR (Time-Domain Nuclear Magnetic Resonance), SEM imaging, rheology, and DF composition analysis should be utilized.

The fourth and final study (CHAPTER 4) aimed to understand the impact of a partly insoluble DF, which exhibits distinct hydration properties compared to SDF, on glycemic responses in different food matrices – both *in vitro* and *in vivo*. The composition, microstructure, viscosity, and starch digestibility of baked and extruded bakery products containing citrus fiber should be analyzed and their impact on *in vitro* glucose release should be investigated. The *in vitro* findings will be compared to the findings of an *in vivo* human study in order to evaluate the transferability of *in vitro* data to human metabolism.

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Chapter 1: Effect of Physicochemical Properties of Carboxymethyl Cellulose on Diffusion of Glucose

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Soluble dietary fibers (SDF), such as sodium carboxymethyl cellulose (CMC), support glycemic control by reducing the post-prandial plasma glucose level. Previous investigations indicate that this effect is associated with rising viscosities in the gut. However, the effects of individual physicochemical properties on glucose release in *in vitro* digestion models and the human gut still remain unclear. This study aims to systematically explore the impact of physicochemical properties related to SDF on glucose release using a side-by-side cell system. Three CMCs were used to investigate the correlations between molecular weight (M_W), viscosity, and concentration on the release of glucose by diffusion and convection in CMC solutions. Correlations between individual parameters of CMC solutions, such as the critical concentration c^*, and the diffusion of glucose, were demonstrated. To enhance and substantiate the study's outcomes, optical density measurements and cryo scanning electron microscopy (SEM) images were employed.

Solutions with concentrations above c^* significantly lowered the diffusivity of glucose *in vitro,* whereas CMC solutions below c^* influenced the glucose diffusivity only marginally. All CMC solutions showed a systematic positive deviation from Stokes-Einstein behavior, indicating a more pronounced increase in viscosity than a reduction in diffusion. Furthermore, low M_W solutions with lower viscosity were more effective in retarding glucose diffusion than higher M_W solutions with higher viscosity, likely due to a tighter entangled and stronger branched network. These findings propose a new method for evaluating glucose diffusion in SDF solutions. This offers insights that could contribute to a better understanding of the mechanisms responsible for reducing post-prandial plasma glucose levels.

Author Contributions:

Miehle, E.: Conceptualization, Data curation, Methodology, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. Bader-Mittermaier, S.: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. Schweiggert-Weisz, U.: Conceptualization, Writing – review & editing. Hauner, H.: Conceptualization, Funding acquisition, Writing – review & editing. Eisner, P.: Conceptualization, Funding acquisition, Supervision, Writing – review & editing

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Effect of Physicochemical Properties of Carboxymethyl Cellulose on Diffusion of Glucose

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Abstract: Soluble dietary fibers (SDF) are known to reduce the post-prandial plasma glucose levels. However, the detailed mechanisms of this reduced glucose release in the human gut still remain unclear. The aim of our study was to systematically investigate the effect of different types of SDF on glucose release in an in vitro model as a prerequisite for the selection of fibers suitable for application in humans. Three types of carboxymethyl cellulose (CMC) were used to investigate the correlations between fiber concentration, molecular weight (M_W), and viscosity on diffusion of glucose using a side-by-side system. CMC solutions below the coil overlap (c^*) influenced the glucose diffusivity only marginally, whereas at concentrations above c^* the diffusion of glucose was significantly decreased. Solutions of lower M_W exhibited a lower viscosity with lower glucose diffusion compared to solutions with higher M_W CMC, attributed to the higher density of the solutions. All CMC solutions showed a systematic positive deviation from Stokes-Einstein behavior indicating a greater rise in viscosity than reduction in diffusion. Therefore, our results pave the way for a new approach for assessing glucose diffusion in solutions comprising dietary fibers and may contribute to further elucidating the mechanisms of post-prandial plasma glucose level reduction.

Keywords: in vitro glucose release; sodium carboxymethyl cellulose; soluble dietary fiber; critical concentration; viscosity; diffusion coefficient; molecular weight

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1. Introduction

One of many health benefits of soluble dietary fibers (SDF) includes the reduction of post-prandial blood glucose level [1–3], which is of significance for people with impaired glucose tolerance or overt Type 2 diabetes mellitus. Several studies indicated that some types of SDF—like carboxymethyl cellulose (CMC)—reduce sugar absorption by altering the viscosity of the gut content [1,4–8].

The transfer of low molecular weight (M_W) substances like glucose within the gut can be attributed to two different mechanisms, i.e., namely diffusion and convection [9]. It is well known that viscosity influences both diffusion and convection. However, the extent of such effects remains unclear. Higher viscosity of the chyme is considered to lead to decreased diffusion of low molecular substances from the gut lumen to the enterocytes and to widen the unstirred mucosal layer [10,11]. Besides, convection driven by intestinal

contractions is also reduced by enhanced viscosity leading to a lower absorption of low M_W substances [9,12,13] and resulting in reduced post-prandial plasma glucose levels in the case of glucose absorption [1]. Viscosity of the chyme is affected by the viscosity of the consumed food product, but also by dilution and/or electrolytes mediated by gastrointestinal secretion [3,14,15]. Viscosity of food products can be easily modified by incorporating dietary fiber ingredients in different concentrations and M_W [16].

Thus, when considering dietary fiber solutions, the viscosity of a solution depends on the one hand on the volume occupied by each of the polymer coils, also known as the intrinsic viscosity, which is mainly influenced by the conformation of dietary fiber molecules, the M_W and the type of solvent used for solubilization. On the other hand, it depends on the number of coils present in the solution, equating to the concentration [17].

Solutions of soluble dietary fibers with concentrations below the critical concentration (c^*) show Newtonian and above shear thinning flow behavior. According to Morris, Cutler, Ross-Murphy, Rees and Priceet al. [17] the flow behavior can be explained by the entanglement model for "random coil" polysaccharide solutions, whereby the formation of an entangled network characterized by coil overlap leads to a strong increase in viscosity. The onset of coil overlap can be induced by the critical concentration c^* at a given M_W of a polysaccharide [17]. As solutions with concentrations above c^* show shear thinning behavior, viscosity has to be extrapolated to zero shear rate for a better comparison.

The zero-shear viscosity (η) and the diffusion coefficient (D) of the continuous phase in a solution are indirectly related according to the Stokes-Einstein (SE) relation:

$$D = \frac{k_B T}{6\pi R \eta} \quad (1)$$

where by k_B is the Boltzmann constant, T is the absolute temperature and R is the hydrodynamic radius of the sphere.

Deviations from SE relation regarding the diffusivity in viscous polymer solutions have been observed in the past [18–21]. The entanglements and differences in viscosity occurring in the polysaccharide solutions could be a possible reason for the deviations [20,21].

Despite the findings that the presence of SDF in the gut content increases viscosity and leads to altered glucose absorption, it is not well understood to what extent individual parameters of SDF like M_W, concentration and viscosity have an effect on glucose release [22,23].

Therefore, the main objective of this work was to investigate the impact of M_W, concentration, particularly c^*, and viscosity of CMC, which distinguish solely in their M_W, on glucose diffusion using an in vitro side-by-side system. To examine the impact of c^* on the diffusivity of glucose, we conducted experiments with solutions in concentrations below and above c^*. We used three types of food grade sodium CMC differing in their M_W, in consistent quality and well-characterized due to the non-natural occurrence in comparison to natural fibers like beta-glucan, which are subject to natural fluctuations for example in branching or molecular weight. CMC is used in a wide range of food products in various concentrations to achieve particular effects as water binding or thickening according to their physicochemical properties [24].

Medium and highly substituted CMC—induced by a high content of carboxymethyl groups—have an excellent solubility over a wide temperature range (0 °C–100 °C). They also form clear and smooth solutions, with a high electrolyte, temperature and pH stability [24] and exhibit shear thinning flow behavior at higher concentrations [25–27].

The flow behavior of the three different types of CMC in aqueous solutions were investigated by steady-state rheological measurements, and the c^* were determined using the zero-shear viscosities according to the model of Cross [28]. Both diffusion coefficients of glucose in the CMC solutions under static conditions as well as convection release behavior under stirred conditions were determined.

2. Materials and Methods

2.1. Materials

Three types of food grade sodium carboxymethyl celluloses (CMC) were purchased from Ashland Industries Europe GmbH (Schaffhausen, Switzerland) and were denoted as CMC-L (Blanose™ 9LCF, weight-average molecular weight (M_W) was 100 kDa), CMC-M (Blanose™ 9M31 F, M_W of 395 kDa) and CMC-H (Blanose™ 9H4F, M_W of 725 kDa). The degree of methylation (i.e., the amount of substituted OH-groups) of each sample ranged from 0.80 to 0.95, which corresponds to an average of 30% substituted OH-groups. All other reagents and chemicals used were of analytical grade and were supplied by VWR (Darmstadt, Germany) or Chemsolute (Th. Geyer GmbH & Co. KG, Renningen, Germany).

2.2. CMC Solution Preparation

2.2.1. Solutions for Determining the Critical Concentration c*

CMC solutions with concentrations ranging from 0.03 to 7 g/100 g were prepared as described by Kpodo et al. [29] with some modifications. Sodium phosphate buffer (0.1 mol/L, pH = 7.2) with 0.1 g/100 g sodium azide to prevent microbial growth was mixed with the appropriate amount of CMC. For complete solubilization, the solutions were gently stirred using a magnetic stirrer at room temperature overnight for 16 h. Prior to the rheological determination of the zero-shear viscosity, the solutions were equilibrated to 37 °C in a water bath for 20 min and degassed by ultrasonication at 37 °C for 3 min. Each solution was prepared at least in duplicate without any further storage between production and equilibration.

2.2.2. Solutions for Determining the Glucose Diffusion

The determined critical concentration (c^*) of each type of CMC was used to calculate the concentrations for determining in vitro glucose release (Section 2.3), using the listed factors shown in Table 1 (three concentrations below and three concentrations above c^*). The obtained CMC concentrations were 0.28 to 4.52 g/100 g for CMC-L, 0.09 to 1.50 g/100 g for CMC-M and 0.05 to 0.80 g/100 g for CMC-H.

Table 1. Fiber concentrations for the in vitro glucose release test.

Calculation	Concentration [g/100 g]		
	CMC-L	**CMC-M**	**CMC-H**
$c_0 = 0$	0	0	0
$c_1 = 0.22 \times c^*$	0.28	0.09	0.05
$c_2 = 0.43 \times c^*$	0.57	0.19	0.10
c^*	1.30	0.43	0.23
$c_4 = 1.30 \times c^*$	1.70	0.56	0.30
$c_5 = 2.17 \times c^*$	2.83	0.93	0.50
$c_6 = 3.48 \times c^*$	4.52	1.50	0.80

CMC-L: low-M_W CMC; CMC-M: medium-M_W CMC; CMC-H: high-M_W CMC. c^* presents the critical concentration.

The respective amount of the different types of CMC was hydrated using distilled water in half of the final weight and stirred at 180 rpm and room temperature for two hours. Electrolytes were added in the final electrolyte concentration of the digestion mixture according to Minekus et al. [30] along with 0.1 mol/L glucose and 0.1 g/100 g NaN_3 and distilled water added to reach the final concentration. Stirring was continued under the same conditions for 14 h to ensure complete dissolution of the CMC. The concentrations of electrolytes in the final CMC solutions were 7.27 mmol/L KCl, 1.12 mmol/L KH_2PO_4, 51.3 mmol/L $NaHCO_3$, 32.6 mmol/L NaCl, 0.21 mmol/L $MgCl_2 * 6\ H_2O$, 0.15 mmol/L $(NH_4)_2CO_3$, 0.53 mmol/L $CaCl_2$. For performing the in vitro glucose release measurements, the pH of the samples were adjusted to 7.0 ± 0.1 with 6 mol/L HCl or 3 mol/L NaOH. A sample without CMC comprising distilled water, electrolytes in the same concentration as described before, 0.1 mol/L glucose and 0.1 g/100 g NaN_3 was used as blank.

2.3. *In Vitro* Glucose Release (IVGR) Measurement and Glucose Determination

2.3.1. IVGR

The IVGR was determined for all concentrations and types of CMC displayed in Table 1 without stirring to determine diffusion coefficients. The highest concentration (c_6) of each CMC was further used to evaluate the glucose release under convection. For the IVGR, a side-by-side diffusion system (SES GmbH, Bechenheim, Germany) (Figure 1) was used. A dialysis membrane (regenerated cellulose) with a molecular weight cut-off of 12–14 kDa (SERVA Electrophoresis GmbH, Heidelberg, Germany) and an effective area of mass transfer (A) of 1 cm^2 (diameter = 1.128 cm) was applied.

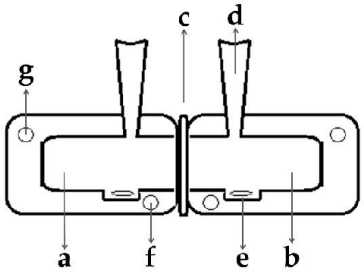


Figure 1. Schematic of the side-by-side diffusion system. Heatable double wall donor cell with a nominal volume of 4 mL (**a**), heatable double wall receptor cell with a nominal volume of 4 mL (**b**), position of the semipermeable cellulose membrane (**c**), sampling port (**d**), magnetic stir bar (**e**), water inlet (**f**) and water outlet (**g**).

The side-by-side system was heated to 37.0 ± 0.1 °C by a thermostatic circulating water bath and kept constant during IVGR. The membrane was placed between two Teflon foam gaskets for fixation between the donor and the receptor cell. 4 mL of phosphate buffer (174.6 mmol/L, with 0.1 g/100 g NaN_3, adjusted to pH 7) was used as receptor fluid to receive the same molarity as the blank sample. After addition of the receptor fluid, the two sampling ports were closed and equilibration at 37.0 °C ± 0.1 °C was carried out for at least 10 min under constant stirring (300 rpm), which was pursued during IVGR.

An aliquot of 15 mL of the sample (donor fluid, pH 7) was equilibrated at 37 °C for 10 min in a water bath. 4 mL of the heated test sample was transferred to the donor cell. Aliquots of 100 µL of the receptor fluid were taken at 1, 5, 10, 20, 30, 40, 60, 90, 120, 180, 240, 360, 480 and 1440 min, respectively, and immediately replaced by 100 µL of phosphate buffer. After 24 h, also 100 µL of the donor fluid was sampled. The amount of glucose, which was reduced by replacing the dissolved glucose solution by phosphate buffer after sampling, was taken into account by the following calculation for the next sampling period:

$$c_{n^*} = c_n + \sum_{i=1}^{n-1} c_i \times 0.025 \quad (2)$$

where c_n is the glucose concentration of the sample at a certain sampling time, in total 14 samples (c_1–c_{14}), and c_{n^*} is the calculated concentration of the sample c_n.

The IVGR of each CMC concentration and type of CMC was conducted at least in duplicate with freshly prepared solutions using two different side-by-side diffusion systems. For convection experiments, the donor fluid was stirred at 150 rpm throughout IVGR.

2.3.2. Glucose Determination

The glucose concentration of the samples was determined using an enzymatic test kit for D-glucose (D-Glucose, Food & Feed Analysis, R-Biopharm AG, Darmstadt, Germany)

following the manufacturer's instructions with slight modifications for application using a 96-well plate. A volume of 200 µL of the diluted sample was mixed with 100 µL of the assay reagent and 2 µL of mixed enzyme solution. The reaction mixture was incubated at room temperature under shaking conditions until the reaction has stopped (no change in absorption in a time range of 15 min). Absorbance was measured at a wavelength of 340 nm using a microplate reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany). The glucose concentration of the samples was calculated based on a standard curve with four measurement points (10, 30, 50, 100 mg/L) in each measurement. From each sample, the glucose concentration was determined in duplicate.

2.4. Glucose Release Kinetics and Determination of the Diffusion Coefficient

2.4.1. Glucose Release Kinetics

The glucose release kinetics were fitted using a non-linear first-order kinetic described in Equation (3) according to Macheras et al. [31] and Naumann et al. [32]:

$$c_t = c_f\,[1 - \exp(-kt)] \quad (3)$$

where c_f is the concentration of glucose after reaching equilibrium, t is the time in minutes and k is the apparent permeability rate constant.

With the determined c_f the glucose transfer index (GTI)—the glucose concentration after reaching equilibrium proportional to the blank—was calculated using the equation of Espinal-Ruiz et al. [33] as follows:

$$\mathrm{GTI} = 100\,(c_f / c_{f,\mathrm{blank}}) \quad (4)$$

2.4.2. Determination of the Diffusion Coefficient

The diffusion coefficient of glucose was determined by fitting to Higuchi equation [34]:

$$Q/c_0 = 2\sqrt{Dt/\pi} \quad (5)$$

where Q is the areal cumulative released amount [mg/cm^2], c_0 is the initial glucose concentration in the sample [mg/mL], D the diffusion coefficient [cm^2/min] and t is the time [min].

The areal cumulative released amount of glucose was normalized using the initial glucose concentration (Q/c_0) and plotted against the square root of time. The D value was then calculated from the slope of the linear regression k_H [cm/min$^{1/2}$] using the following equation:

$$k_H = 2\sqrt{D/\pi} \quad (6)$$

2.5. Rheological Investigation

2.5.1. Zero-Shear Viscosity

Rheological investigations were performed using a rotational rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) equipped with Rheoplus software version 3.40 (Anton Paar GmbH). Determinations were performed in at least duplicate using a concentric cylinder system (diameter: 27 mm, shear gap: 1.14 mm) (CC27-SN24807, Anton Paar GmbH) at a constant temperature of 37.0 ± 0.1 °C. The samples were pre-sheared at 2 s^{-1} for 30 s and allowed to rest for 90 s before starting the measurement.

A hysteresis curve was recorded by using a steady shear mode in logarithmic scale ranging from 0.5 to 500 s^{-1} with 10 measuring points per decade and a measurement point duration from 20 to 10 s during the forward ramp and 10 to 20 s during the backward ramp. One determination contained a forward and a backward ramp.The solutions were prepared in duplicate (Section 2.2) and analyzed twice, resulting in four separate determinations for evaluating the zero-shear viscosity as described below.

2.5.2. Determination of Viscosity of CMC Solutions for Correlation to IVGR

CMC solutions for IVGR investigations (Section 2.2) were analyzed before and after performing the IVGR using a parallel plate geometry (diameter: 50 mm, shear gap: 1.00 mm) (PP50-SN23165; Anton Paar GmbH) for determining the zero shear viscosities of those solutions.

The measurements were conducted at 37.0 ± 0.1 °C and the samples were pre-sheared at 2 s^{-1} for 10 s and allowed to rest for 60 s. Viscosity flow curves were obtained in duplicate with the operating shear rate in logarithmic scale ranging from 0.5 to 500 s^{-1} with 10 measuring points per decade and a measurement point duration from 20 to 10 s.

2.5.3. Curve Fitting and Determination of the Critical Concentration c*

Zero-shear viscosities were determined by fitting the data of the forward ramp to the Cross model (Equation (7)) [28] using the Rheoplus software (Anton Paar GmbH).

$$\eta_{\dot{\gamma}} = \eta_{\infty} + \left[\eta_0 - \eta_{\infty}\right] / \left[1 + \left(C\dot{\gamma}\right)^P\right] \quad (7)$$

where η_0 is the zero-shear viscosity at the lower Newtonian plateau, η_{∞} is the viscosity at infinite high shear rate, $\dot{\gamma}$ is the shear rate and C is the Cross time constant or Consistency of a solution and P is the (Cross) rate constant. The zero shear viscosity of each solution was calculated using data from the forward ramp. Low viscous samples displaying ideal Newtonian behavior were evaluated using the Newtonian model of the Rheoplus software (Anton Paar GmbH):

$$\eta = \tau / \dot{\gamma} \quad (8)$$

where η and $\dot{\gamma}$ are the dynamic viscosity and the shear rate and τ is the shear stress.

The viscosity is expressed as the specific viscosity (η_{sp}), which is defined as the ratio of the viscosity of the dissolved polymer (η) and the solvent viscosity (η_0) [17]:

$$\eta_{sp} = (\eta - \eta_s) / \eta_s \quad (9)$$

To obtain the critical concentration c^*, a double logarithmic plot of the zero-shear specific viscosity ($\eta_{sp,0}$) against concentration [g/100 g] was used and data in the diluted region and the concentrated region were both fitted using a power law equation. The interception of the two fits is the critical concentration c^*.

A power Law model (Equation (10)) was used to analyze the flow curves using Rheoplus software (Anton Paar GmbH):

$$\dot{\gamma} = K \eta^{-n} \quad (10)$$

where $\dot{\gamma}$ is the shear rate, η is the viscosity, K is the flow consistency index and n is the flow behavior index.

2.5.4. Determination of the Reynolds Number

The Reynolds numbers (Re)—a ratio of inertial and viscous forces—of the solutions of the stirred release experiments have been calculated:

$$Re = \rho N D^2 / \eta_0 \quad (11)$$

where ρ is the density of the solution, N is the rotational speed of the stirrer (150 rpm), D is the diameter of the stirrer (0.5 cm) and η_0 is the zero shear viscosity of the solution.

2.6. Optical Density

The optical density of CMC solutions for IVGR investigations (preparation Section 2.2) was determined using a spectrophotometer (Specord 210 plus, Analytik Jena AG, Jena, Germany). Spectral analysis of wavelengths ranging from 190 nm to 900 nm was conducted

at 37 °C. The wavelength at 285 nm was chosen for the analysis, as the optical density showed the greatest deviation compared to the blank.

2.7. Absolute Density

The absolute densities of the CMC solutions were measured by a Gay-Lussac pycnometer (50 mL, Blaubrand®, Brand GmbH & Co. KG, Wertheim, Germany) at 37 ± 0.1 °C. The pycnometer was filled with sample solution, covered and left over night to ensure enclosed air bubbles to ascend. Before weighing, the filled pycnometer was equilibrated at 37 °C in a water bath for 20 min and degassed by ultrasonication at 37 °C for 3 min. The pycnometer was closed with the lid enabling excess solution to exit through a capillary and immediately weighed.

The density of the solution was calculated according to the following equation [35]:

$$\rho_S = \frac{m_S - m_0}{V} \quad (12)$$

where ρ_S is the density of the sample, m_S is the mass of pycnometer filled with sample, m_0 is the mass of the empty pycnometer and V is the volume of the pycnometer. The volume of the pycnometer was determined using distilled water at 37 °C.

2.8. Cryo Scanning Electron Microscopy of CMC Solutions

A droplet of the highest concentrated CMC solution (3.5 × c^*) (Section 2.2) was placed in between two rivet eyelets in a sample carrier and plunged into slush nitrogen at atmospheric pressure at −205 °C. A very small droplet of the solution was used for fast freezing to avoid structural change caused by expansion. To study the microstructure, the frozen specimen was transferred to a cryo preparation unit (PolarPrep 2000, Cryo Transfer System, Quorum Technologies Ltd., Lewes, UK) and freeze-fractured at −150 °C within high vacuum ($p = 10^{-3}$−10^{-4} Pa). The surface water was slightly sublimated at −80 °C for 20 to 30 min and the fractured sublimated sample was then sputter coated at −150 °C ($p = 1.5 \times 10^1$ Pa) with 3–5 nm of Pt/Pa. The coated sample was transferred into a scanning electron microscope (JSM F7200, JEOL Ltd., Tokyo, Japan) using the SE detector and the SEM mode. The images were taken with 1–5 kV accelerating voltage, probe current 5–10 and maintaining the sample below −140 °C.

2.9. Statistical Analysis

Results are expressed as mean ± SD. Statistical analysis was performed using SigmaPlot 12.0 for Windows (Systat Software GmbH, Erkrath, Germany). After testing for homogeneity of variance (Levene's test) and normal distribution (Shapiro-Wilk test), one-way analysis of variances (ANOVA) was applied, and Tukey's honestly significant difference post hoc test was used to determine the significance of differences between samples for $p \leq 0.01$. Regressions were calculated using OriginPro 2018 for Windows (Origin Lab Corporation, Northampton, MA, USA).

3. Results and Discussion

*3.1. Flow Behavior and Determination of Critical Overlap Concentration c**

3.1.1. Flow Behavior of the CMC Solutions

Flow curves of the carboxymethyl cellulose (CMC) solutions in a wide range of concentrations (c) from 0.03 to 7.00 g/100 g were determined. Diluted solutions at low concentrations showed Newtonian behavior, exemplarily shown for CMC-H at varying concentrations from 0.03 to 0.1 g/100 g in Figure 2. At higher concentrations, a shear thinning behavior was observed, as seen for CMC-H at concentrations of 0.3 g/100 g or higher (Figure 2).

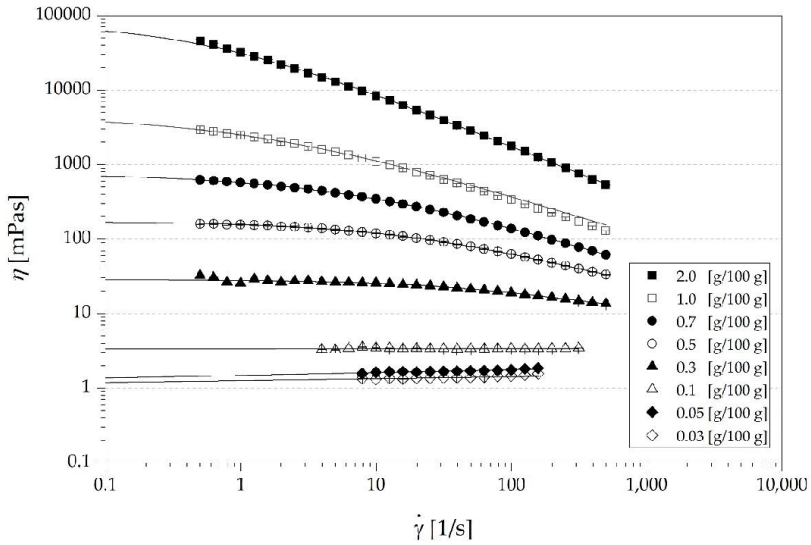


Figure 2. Flow behavior exemplarily shown for the high M_W carboxymethyl cellulose solutions (CMC-H) at different concentrations [g/100 g]. Shear rate dependent viscosity appeared for solutions in concentrations above c^* (0.3 g/100 g–2.0 g/100 g). Points correspond to experimental data and lines to fitted data according to Cross equation (Equation (7)) for concentrations of 0.3–2.0 g/100 g and Newtonian equation (Equation (8)) for concentrations of 0.03–0.1 g/100 g ($R^2 \geq 0.99$).

Those curves displayed a horizontal "Newtonian plateau" at low shear rates, whereas the shear thinning behavior became obvious as shear rates increased. With increasing CMC concentrations, the onset of shear thinning shifted to lower shear rates. Shear thinning behavior appears above the onset of coil overlap, that is commonly referred to as c^* [17], which was determined between 0.1 g/100 g and 0.3 g/100 g for CMC-H (Figure 2). Furthermore, the viscosity of all solutions rose with increasing concentration of CMC as expected. The data of the forward and backward ramp overlaid for all measurements implying shear stability (data not shown). For determining zero-shear viscosities (η_0), the shear thinning flow curves of the different types and concentrations of CMC were fitted to the Cross equation (Equation (7)). The pseudoplastic flow behavior of the CMC solutions agree with current data of other random coil polysaccharide flow curves [20,27,29], which also exhibit shear thinning behavior above c^*.

3.1.2. Determination of c^* of the CMC Solutions

Figure 3a shows a double logarithmic plot of specific viscosity at zero shear ($\eta_{sp,0}$) against the solution concentration of the three different types of CMC. The viscosity of the solvent (phosphate buffer, 37 °C) was 0.80 ± 0.01 mPa s, being close to the dynamic viscosity of water at 37 °C with 0.69 mPa s. The calculated critical coil overlap concentration c^* of CMC-L, CMC-M and CMC-H were 1.3 g/100 g, 0.43 g/100 g and 0.23 g/100 g linked to a η_0 of 6.4, 6.3 and 6.0 mPas at the c^*, respectively. At $c < c^*$ the individual coils are free to move independently and at $c > c^*$ the individual coils begin to touch, overlap and interpenetrate [17], represented by a strong rise of $\eta_{sp,0}$ with increasing concentration (Figure 3a). Most likely due to the polydispersity of CMC the increase of $\eta_{sp,0}$ with concentration was more gradual and did not represent sharp c^* [17]. Benchabane and Bekkour [25] and Shelat et al. [21] also reported similar findings of a non-sharp c^* for CMC and arabinoxylan solutions. Wagoner et al. [27] reported a c^* of 0.67 g/100 g for a CMC with a slightly lower molecular weight (M_W) of 250 kDa (DS 0.7) compared to CMC-M and

with a higher η_0 of 22 mPas. In contrast to those findings, Charpentier et al. [26] found a lower c^* of 1.5 g/L of a CMC with a similar molecular weight of 300 kDa (DS 0.9) compared to CMC-M in our investigation. However, the determined c^* is strongly dependent on molecular weight and substitution degree and thus direct comparisons are difficult.

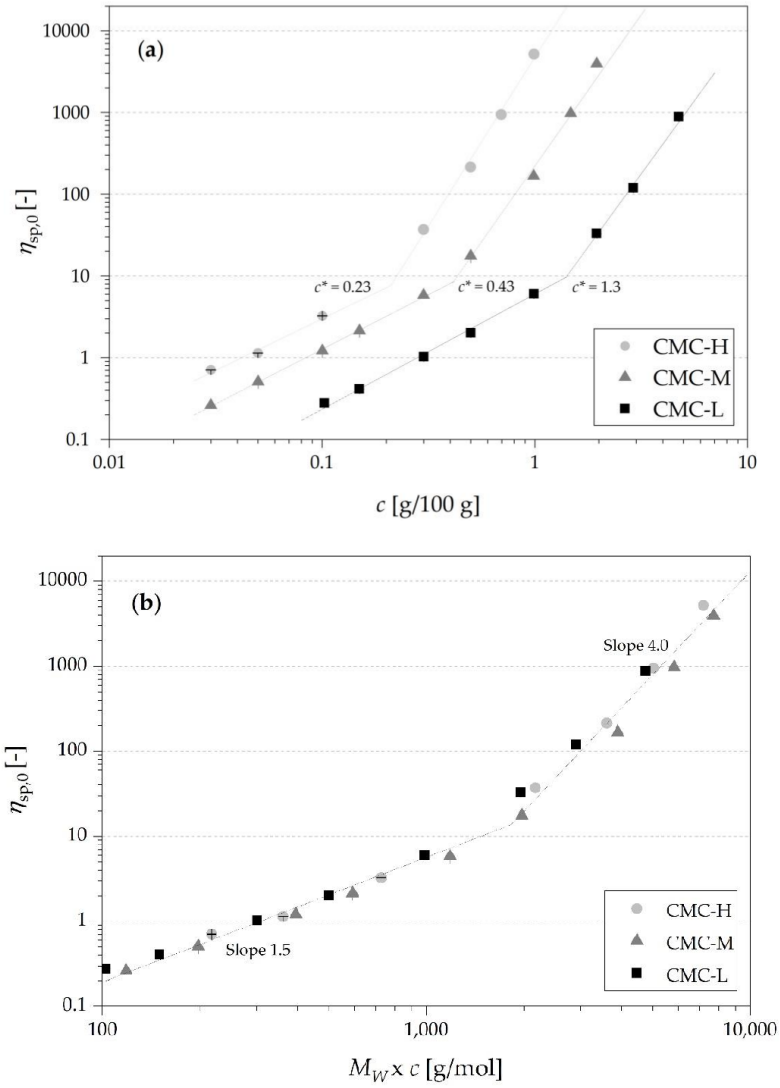


Figure 3. Double logarithmic plot of zero shear specific viscosity against concentration c (**a**) and against the product of c and M_W (**b**) of three types of CMCs with different M_W: CMC-L (100 kDa), CMC-M (395 kDa), CMC-H (725 kDa). Data in the diluted regions and the concentrated regions were both fitted using a power law equation ($R^2 \geq 0.95$).

Figure 3b shows a plot of $\eta_{sp,0}$ against $M_W \times c$. The product of c and M_W led to a close overlap in the double logarithmic plots of the three types of CMC.

The slope values for the fitted regions (one for $c \leq c^*$ and the second one for $c \geq c^*$) were 1.5 and 4.0, meaning a power law dependency of viscosity on concentration in the dilute region with $c^{1.5}$ and above coil overlap with $c^{4.0}$, respectively. As the second slope is approx. 2.5 times higher than the first slope, the solutions show a strong hyperentanglement and a high dependency of viscosity on concentration above c^* [17].

The product of $M_W \times c$ describes the total degree of space occupied by the CMC in the solution, as the concentration is proportional to the number of coils present and the M_W to the volume occupied by each of the CMC coils [36].

The observed slope values correspond quite well with the slope values of 1.4 and 3.3 for different disordered polysaccharides such as alginate, carrageenan or carboxymethyl amylose reported by Morris et al. [17]. Similar slopes were also reported for guar gum solutions with 1.5 and 4.2 [37], for beta glucan solutions of 1.3 and 4.1 [23] and for xyloglucan solutions of 1.3 and 4.0 [38]. Recently published works of Wagoner et al. [27] and Benchabane and Bekkour [25] also reported two slopes of CMC solutions with a slope of 2.7 in the concentrated region above c^* [27].

3.2. *Concentration-Dependent Diffusion of Glucose*

The glucose release over a time of 24 h is exemplarily shown for the CMC-L solutions with concentrations of 0.28 to 4.52 g/100 g and the solvent blank in Figure 4. The release of glucose increased with time and a reduced glucose release of the solutions with $c \geq c^*$ was visible in the decelerated rise of the curves. The blank solution showed the fastest glucose release. The equilibrium of the blank (54.1 ± 0.2 mmol/L) and samples with lower concentrations < c^* was slightly higher than the expected equilibrium of around 50 mmol/L, likely caused by a slight evaporation of water over time and hence an increased concentration of the solution.

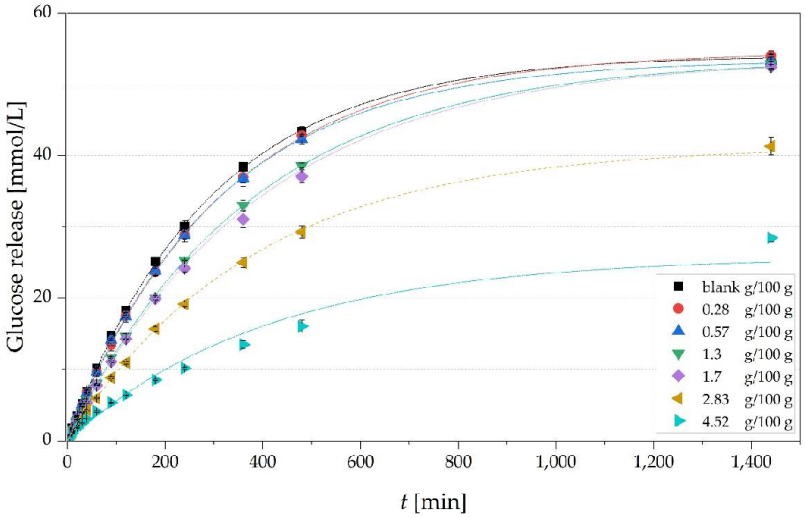


Figure 4. Time-dependent glucose release from solutions with different concentrations of CMC-L (0.28–4.52 g/100 g; blank = 0 g/100 g). 1.3 g/100 g is the calculated c^* concentration of CMC-L. Points correspond to experimental data and lines to fitted data according to equation 3 ($R^2 \geq 0.9998$).

The glucose release at equilibrium was significantly lower for the two highest concentrations of CMC-L of 2.83 g/100 g and 4.52 g/100 g with 41.4 ± 0.8 mmol/L and 25.8 ± 3.4 mmol/L, respectively, compared to the blank (54.1 ± 0.2 mmol/L). This results in a significantly lower maximum glucose transfer index (GTI) in equilibrium of

76.5 ± 1.5% and 47.8 ± 6.3% compared to the blank (100.0 ± 0.3%) (Table 2). This implies that 23.5% and 52.2% of the glucose was retarded. Consistent with the observations in Section 3.1, the rise in *c* led to an significant increase in viscosity of the solutions in a wide range from 0.8 ± 0 mPa s (blank) to 1355.3 ± 41.2 mPa s (0.8 g/100 g CMC-H) (Table 2).

Table 2. Zero-shear viscosity before and after 24 h release experiment, diffusion coefficient and optical density at different concentrations around the c^* of CMC-L, CMC-M, CMC-H.

Concentration	η_0 [mPa s]	η_0 after 24 h [mPa s]	Diffusion Coefficient (D) [10^{-8} m^2s^{-1}]	Maximum GTI [%]	Optical Density at 285 nm
0 × c^* (blank)	0.8 ± 0.0 ^{a}	0.8 ± 0.0 ^{a}	1.42 ± 0.04 ^{a}	100.0 ± 0.3 ^{a}	0.17 ± 0.0 ^{a}
CMC-L					
0.2 × c^* (0.28 g/100 g)	1.5 ± 0.0 ^{a}	1.6 ± 0.0 ^{a}	1.33 ± 0.06 ^{a,b}	100.9 ± 0.5 ^{a}	0.23 ± 0.0 ^{a}
0.4 × c^* (0.57 g/100 g)	2.6 ± 0.1 ^{a}	2.7 ± 0.0 ^{a}	1.29 ± 0.06 ^{a,b}	98.7 ± 0.6 ^{a}	0.28 ± 0.03 ^{a}
c^* (1.30 g/100 g)	8.3 ± 0.1 ^{a}	8.8 ± 0.2 ^{a}	1.03 ± 0.02 ^{c}	99.1 ± 1.0 ^{a}	0.48 ± 0.02 ^{b}
1.3 × c^* (1.70 g/100 g)	14.5 ± 0.2 ^{b}	15.3 ± 1.1 ^{a}	0.97 ± 0.04 ^{c}	99.3 ± 0.5 ^{a}	0.55 ± 0.07 ^{b}
2.2 × c^* (2.83 g/100 g)	78.7 ± 2.0 ^{c}	66.9 ± 5.4 ^{b}	0.61 ± 0.05 ^{d}	76.5 ± 1.5 ^{b}	0.88 ± 0.02 ^{c}
3.5 × c^* (4.52 g/100 g)	519 ± 9.1 ^{d}	559.5 ± 16.1 ^{c}	0.16 ± 0.02 ^{e}	47.8 ± 6.3 ^{c}	1.4 ± 0.04 ^{d}
CMC-M					
0.2 × c^* (0.09 g/100 g)	1.7 ± 0 ^{a}	1.8 ± 0.1 ^{a}	1.35 ± 0.08 ^{a,b}	101.5 ± 0.7 ^{a}	0.18 ± 0.0 ^{a}
0.4 × c^* (0.19 g/100 g)	3.4 ± 0.1 ^{a}	2.9 ± 0 ^{a}	1.3 ± 0.09 ^{a,b}	98.3 ± 0.9 ^{a}	0.2 ± 001 ^{a}
c^* (0.43 g/100 g)	11.3 ± 0.4 ^{a,b}	11 ± 0.2 ^{a}	1.18 ± 0.08 ^{b,c}	97.0 ± 1.8 ^{a}	0.25 ± 0.0 ^{b}
1.3 × c^* (0.56 g/100 g)	17.9 ± 0.1 ^{b}	19.5 ± 0.4 ^{a}	0.97 ± 0.06 ^{c}	89.6 ± 1.0 ^{b}	0.28 ± 0.01 ^{b}
2.2 × c^* (0.93 g/100 g)	99.8 ± 3.1 ^{c}	99.1 ± 6.3 ^{b}	066 ± 0.02 ^{d}	88.0 ± 1.2 ^{b}	0.35 ± 0.03 ^{c}
3.5 × c^* (1.50 g/100 g)	816.2 ± 12.6 ^{d}	1010.2 ± 18.0 ^{c}	0.56 ± 0.02 ^{d}	86.5 ± 1.3 ^{b}	0.51 ± 0.01 ^{d}
CMC-H					
0.2 × c^* (0.05 g/100 g)	1.9 ± 0.1 ^{a}	1.7 ± 0.0 ^{a}	1.43 ± 0.05 ^{a}	102.7 ± 3.3 ^{a}	0.18 ± 0.0 ^{a}
0.4 × c^* (0.10 g/100 g)	3.5 ± 0.5 ^{a}	3.7 ± 0.2 ^{a}	1.31 ± 0.02 ^{a}	102.5 ± 1.0 ^{a}	0.2 ± 0.0 ^{a}
c^* (0.23 g/100 g)	14.9 ± 1.2 ^{a}	15.2 ± 1 ^{a}	1.16 ± 0.05 ^{b}	98.6 ± 1.1 ^{a}	0.24 ± 0.0 ^{b}
1.3 × c^* (0.30 g/100 g)	15 ± 0.7 ^{a}	15.1 ± 0.3 ^{a}	1.08 ± 0.02 ^{b}	97.8 ± 1.8 ^{a}	0.26 ± 0.0 ^{b}
2.2 × c^* (0.50 g/100 g)	159.2 ± 4.1 ^{b}	165.1 ± 3.1 ^{b}	0.93 ± 0.04 ^{c}	94.7 ± 1.2 ^{b}	0.32 ± 0.01 ^{c}
3.5 × c^* (0.80 g/100 g)	1355.3 ± 41.2 ^{c}	1744.5 ± 30 ^{c}	0.53 ± 0.01 ^{d}	87.3 ± 2.0 ^{c}	0.46 ± 0.02 ^{d}

Different superscript letter (a–e) within a column indicate significant difference between means ($p < 0.01$).

We observed a slight rise in viscosity of the same solutions before and after the release experiments (Table 2), probably caused as well by a slight evaporation of water during the 24 h. This rise in viscosity during the 24 h might have led to a stronger retardation of glucose and hence to a slightly too low measured GTI. A reduced and slowed glucose transfer is most likely caused by the rise in viscosity with increasing *c* of the different types of CMC, which has already been described previously [10]. For example, a concentration increase from the blank to 4.52 g/100 g CMC-L in solution led to a significant rise in viscosity from 0.8 ± 0.0 to 519 ± 9.1 mPa s and a significant decrease in the maximum GTI from 100.0 ± 0.3 to 47.8 ± 6.3%, respectively (Table 2). This rise in viscosity also came along with a significant reduction in the diffusion coefficient (D) of glucose from 1.42 ± 0.04 × 10^{-8} to 0.16 ± 0.02 × 10^{-8} m^2s^{-1}, respectively (Table 2). Similar observations with retarded glucose diffusion and lowered amount of glucose transfer due to increased viscosity were reported by Espinal-Ruiz et al. [33] for pectin, by Ou et al. [4] for different soluble and insoluble fibers including CMC and by Srichamroen and Chavasit [5] for malva nut gum.

The reduced GTI can be caused by an entrapment of the glucose molecules by a highly concentrated and viscous fiber network and as well by a chemical bonding of

glucose molecules to the fibers [4,10]. However, as glucose is electrically neutral at pH 7.0, electrostatic interactions with the different dietary fibers were considered unlikely (Espinal-Ruiz et al., 2016), as chemical bonding—except weak hydrogen bonds—of glucose to fiber molecules is more likely to occur in insoluble fibers [4,39,40].

3.3. Correlations between Diffusion Coefficients, Concentrations and Molecular Weight

A linear dependency of D with both the concentration and M_W of the different types of CMC was observed (Figure 5). The solvent blank exhibited along with a concentration of 0.05 g/100g of CMC-H the highest D of $1.42 \pm 0.04 \times 10^{-8}$ m^2 s^{-1} (Table 2). No significant differences ($p \leq 0.01$) were observed between the D of the solutions below c^* and the D in the blank solution (Table 2). In solutions with concentrations $\geq c^*$ the D was significantly lower ($p \leq 0.01$) for all types of CMC compared to the D in the blank solution, which indicates that for impairing glucose release the knowledge of c^* is important and that only concentrations of CMC higher than c^* significantly influenced in vitro glucose release.

Moreover, there was a trend that the diffusion coefficients of CMC-H were highest, followed by CMC-M and CMC-L, respectively, when comparing the same proportional $c \times M_W$ (Figure 5). Contrary the viscosities were even higher for CMC-H and CMC-M compared to CMC-L at the given $c \times M_W$ (Table 2). For example, at the highest $c \times M_W$ of each sample, CMC-M and CMC-H showed higher D (0.56 ± 0.02 and $0.53 \pm 0.01 \times 10^{-8}$ m^2 s^{-1}, respectively) compared to CMC-L with the lowest D of $0.16 \pm 0.02 \times 10^{-8}$ m^2 s^{-1}. The viscosity at the given $c \times M_W$ of the solution (Table 2) was 1.6 and 2.6 times higher (Table 2) for CMC-M and CMC-H compared to CMC-L, respectively.

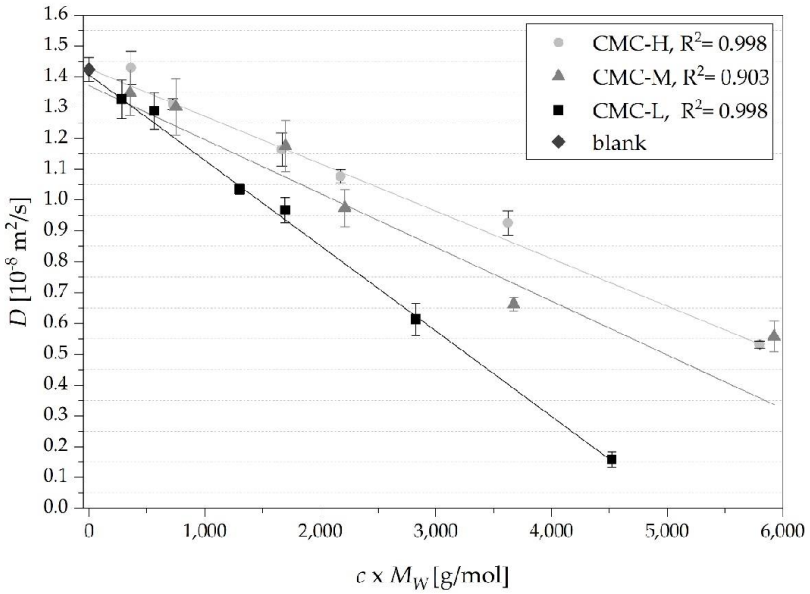


Figure 5. Correlation of diffusion coefficient of glucose in CMC solutions and increasing concentrations × M_W of the three different CMC types: CMC-L (100 kDa), CMC-M (395 kDa) and CMC-H (725 kDa). Points correspond to experimental data and lines to fitted data using linear regression ($R^2 \geq 0.903$).

These results indicate that soluble fibers with a low M_W in higher c are more effective in retarding glucose diffusion than high M_W soluble fibers in lower c. Similarly, Shelat et al. [21] observed as well at a given concentration of two types of arabinoxylans the solution with the arabinoxylan forming a higher viscosity showed a higher probe D compared

to the solution with the arabinoxylan forming a lower viscosity. Shelat et al. [20,21] and Liu et al. [40] observed also a decrease of D with an increase of polysaccharide concentration ranging from 0.01–1.00% (w/v) (arabinoxylan), from 0.5–1% (w/v) (β-glucan) and from 0.01–2% (w/w) (nano-fibrillated cellulose) respectively. Unlike our results, the authors observed an initial sharp decrease of D with an increase of c resulting in an exponential shaped curve. Han et al. [41] observed an initial increase in diffusion of aspartame in very low concentrated CMC solutions and consistent with our results a decrease in D with concentrations higher than c^* due to the formation of an entangled network. The positive influence on glucose diffusion of solutions with concentrations of CMC above the coil overlap ($>c^*$.) further supports the findings of Rieder et al. [23] of a significant in vivo effect of c^* on the post-prandial blood glucose rise as shown by in-depth meta-analysis on glucose responses to β-glucan samples.

To reach concentrations higher than the c^* in the small intestine, it has to be taken into account that the fiber containing meal might be strongly diluted depending on the composition of the more complex and heterogeneous meal. According to Minekus et al. [30] the dilution factor can be up to eight. Therefore, the concentration of fiber in the food has to be adopted in order to remain the impact of diffusion in the small intestine [3].

3.4. Correlations between Diffusion Coefficients and Viscosity—Deviation from Stokes-Einstein Equation

The $D\eta/D_0\eta_s$ ratio demonstrates the quantitative deviation from the Stokes-Einstein (SE) behavior (Equation (1)), whereby D and D_0 are the diffusivities of glucose in the sample and blank solution, respectively, and η and η_s are the viscosities of the sample and blank solution, respectively.

Figure 6 shows a positive deviation from Stokes-Einstein behavior with increasing $c \times M_W$ up to over 615 ± 166 for CMC-H and 394 ± 71 and 71 ± 1 for CMC-M and CMC-L, respectively. The positive deviation of $D\eta/D_0\eta_s > 1$ implies that the decrease in diffusivity is less strong compared to the increase in viscosity of the solution [19].

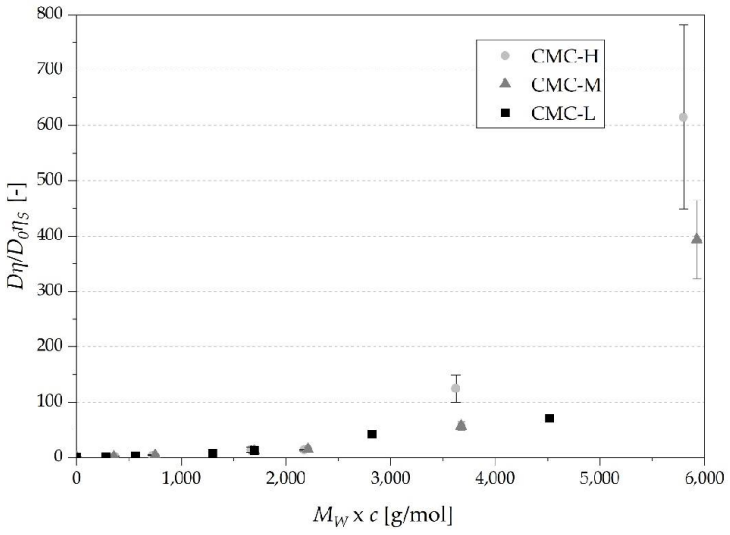


Figure 6. Deviation from Stokes-Einstein equation (Equation (1)) of the three different types of CMC. The calculated $M_W \times c^*$ for CMC-L, CMC-M and CMC-H were 130,000, 169,850 and 166,750 g/mol, respectively.

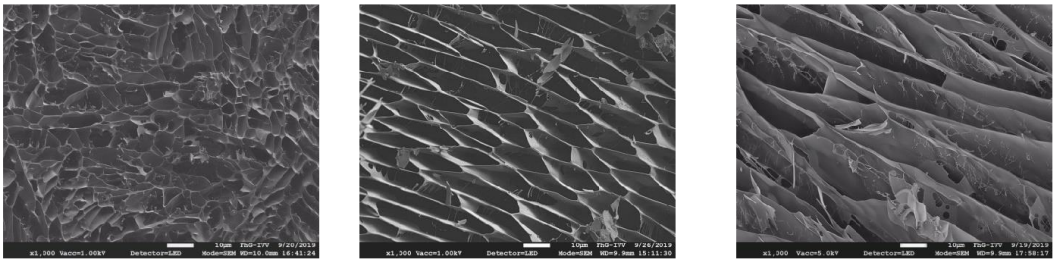
The sharp increase at the c^* with deviations of 15 ± 4, 12 ± 2 and 7 ± 1 for CMC-H, CMC-M and CMC-L, respectively, is visible and refers to the strong increase in viscosity due to the entanglement of the CMC polymers. Shelat et al. [20,21] measured the diffusivity of a dextran probe in arabinoxylan and β-glucan solutions at different concentrations ranging from 0.2–1% and 0.5–1%, respectively, and determined as well a positive deviation from Stokes-Einstein relation up to app. 50. For comparability, it has to be taken into account that the M_W of the probe of the authors is almost 400 times higher than the M_W of glucose used in our work.

For small spheres as glucose and large chains as present in our study, the positive deviations can be explained by an easier movement of the glucose through the solution even with the CMC chains still tensed in conformation and forming a high viscous network [19].

Another possible reason is an inhomogeneous formation of an aggregated network by forming regions with high and low concentrations of CMC, faster for glucose to move through [21].

In addition, the positive deviations could also originate from a decrease in the hydrodynamic radius of glucose with increasing concentration of CMCs. This is due to interactions between glucose and the CMC molecules [21]. However, as the total degree of space occupied by the CMC is proportional to $M_W \times c$ (Section 3.1) and consequently proportional to the amount of water bound to the CMC, the decrease in the hydrodynamic radius of the glucose molecules in the solutions with similar $M_W \times c$ should be effectively equal. Hence, the influence of a decreased hydrodynamic radius of glucose on the diffusivity in solutions with similar $M_W \times c$ should be negligible.

Figure 7 shows cryo scanning images of the three different types of CMC in solutions at their highest concentrations (4.5 g/100 g for CMC-L, 1.5 g/100 g for CMC-M, 0.8 g/100 g for CMC-H). It is visible that the shorter chains of CMC-L in a higher concentration formed a solution in a higher density with less free spaces compared to the longer chains of CMC-M and CMC-H in a lower concentration forming a solution with more free spaces. The higher concentration of the short chain molecules hamper the glucose molecules in their diffusion through the solution in a greater extent than a lower concentration of the long chain molecule at a similar given viscosity.



(**a**) (**b**) (**c**)

Figure 7. Cryo scanning electron microscope images of the three CMC solutions in their highest concentration of 3.5 × c^* at the same scale and a magnification of 1000: 4.5 g/100 g CMC-L (**a**), 1.5 g/100 g CMC-M (**b**) and 0.8 g/100 g CMC-H (**c**).

Fujita [42] implemented the exponential dependence of the diffusivity of drugs on the free volume in polymers, where a more difficult diffusion in the CMC-L solution is most likely. This corroborates our finding of a lower D of the CMC-L solutions compared to CMC-H and CMC-M solutions and further the observation of the more positive deviations from SE behavior for the CMC-H and CMC-M solutions compared to CMC-L solutions, leading to a high diffusivity despite high viscosity.

The optical densities of the three solutions at a wavelength of 285 nm also confirmed the observation of the SEM images with a rise in density of 0.46 ± 0.02, 0.51 ± 0.01 and 1.4 ± 0.04 for the highest concentrated solutions of CMC-H (0.8 g/100 g), CMC-M

(1.5 g/100 g) and CMC-L (4.5 g/100 g), respectively (Table 2). Liu et al. [43] detected CMC absorption in aqueous solutions at a similar wavelength, probably involving the stimulation of carboxylic acids [44].

Nsor-Atindana et al. [15] also observed differences in retarding glucose and diffusion depending on the size of nanocrystalline cellulose, whereby the smallest particles were the most effective in attenuating glucose diffusion.

The diffusivity of glucose and the viscosity—as the macroscopic behavior of the solution—are not necessarily correlated [21] and other microscopic properties are able to affect the diffusivity as the M_W of the fiber and/or the density of the entangled network in the solution. Therefore, diffusivity and viscosity of a solution should be determined independently.

3.5. Impact of Convection

In addition to the previously described diffusion experiments, we also investigated the impact of viscosity on glucose release under convection conditions by applying constant stirring (150 rpm) of the donor cell. The release experiments were conducted with the highest concentrated CMC solutions (4.5 g/100 g for CMC-L, 1.5 g/100 g for CMC-M, 0.8 g/100 g for CMC-H) and the results are given in Figure 8.

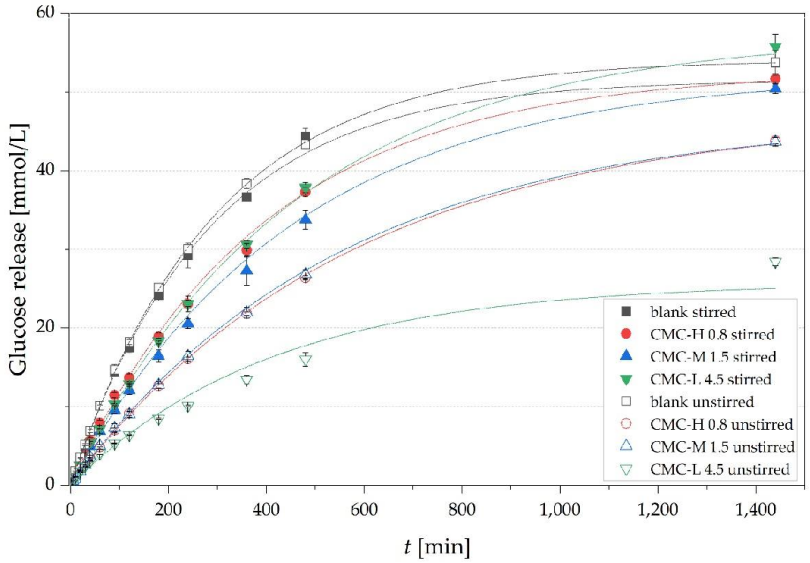


Figure 8. Time-dependent glucose release from CMC-H, CMC-M and CMC-L at their highest concentration of 0.8 g/100 g, 1.5 g/100 g and 4.5 g/100 g, respectively, and the blank with and without stirring (150 rpm). Points correspond to experimental data and lines to fitted data according to equation 3 ($R^2 \geq 0.9998$).

Stirring increased glucose release and the maximum GTI for all CMC solutions except the blank, indicating a limitation of glucose release by the membrane. The Reynolds numbers (*Re*) of the solutions (Table 3) were calculated according to Equation (11) using ρ and η_0 of the solutions listed in Table 3.

The highest acceleration of the released glucose by convection has occurred in the CMC-L solution (maximum GTI from 47.8 ± 6.3 to 111.2 ± 3.2%) with the highest *Re* of the three types of CMC in solutions, referring to the highest mixing of the solution (Table 3). The CMC-H solution had the strongest minimization of the *Re* by a factor of approx. 1500 compared to the blank leading to a more than 2.5 times lower *Re* compared to the CMC-L

solution. Nevertheless, the glucose release was comparable with the CMC-L solution and not lower as expected. One reason could be the lowest n of 0.66 ± 0.01 of CMC-H of the three solutions, indicating a strong dependence of viscosity on the shear rate, leading to an around 8-fold drop in viscosity by an increase of the shear rate from 0 to 158 s^{-1}. This decrease in viscosity could result in a faster release of glucose.

Table 3. Power Law parameters (obtained by Power Law model Equation (10); $R^2 \geq 0.941$), viscosities with shearing, Reynolds numbers (*Re*) and densities of the three different CMC solutions at the highest concentration (4.5 g/100 g for CMC-L, 1.5 g/100 g for CMC-M, 0.8 g/100 g for CMC-H); the calculated *Re* of the blank was 76.53 ± 1.69; viscosities at zero shear see Table 2.

Sample	Maximum GTI [%]	K [Pa s]	n [-]	$\eta(\dot{\gamma} = 158\ s^{-1})$ [mPa s]	*Re* [-]	Density [kg/m^3]
CMC-L 4.52 g/100 g	111.2 ± 3.2 ^{a}	0.5 ± 0.01 ^{a}	0.91 ± 0 ^{a}	320 ± 6.3 ^{a}	0.13 ± 0 ^{a}	1027 ± 0.8 ^{a}
CMC-M 1.5 g/100 g	101.6 ± 1.4 ^{a}	0.76± 0.02 ^{b}	0.77 ± 0 ^{b}	221.5 ± 4 ^{b}	0.08 ± 0.02 ^{a,b}	1011.5 ± 2.1 ^{b}
CMC-H 0.8 g/100 g	102.3 ± 1.6 ^{a}	1.09 ± 0.04 ^{c}	0.66 ± 0.01 ^{c}	175 ± 4.3 ^{c}	0.06 ± 0.02 ^{b}	1007.9 ± 0.7 ^{b}

Different superscript letter (a–c) within a column indicate significant difference between means ($p < 0.01$).

The high *Re* of CMC-L solution could be caused by a better mobility of the short CMC-L chains. This led to a high glucose acceleration with stirring because glucose can move without waiting for the CMC-L chains to relax their conformation. Since convection in the solution increased the maximum GTI of all types of CMC solutions, we assume that a lowered maximum GTI is more likely caused by entrapped glucose in the viscous matrix than chemical bonding.

The effects of convection on glucose release were also reported by Liu et al. [40]. The retardation of glucose by a 2% (w/w) nano-fibrillated cellulose solution dropped from 50% to 27.7% due to shaking. Dhital et al. [45] observed that mixing at different speeds (0, 200 and 750 rpm) significantly accelerated the mass transfer of glucose out of barley beta-glucan solutions. The results show that the ability of CMC to form high viscous solutions could inhibit turbulence, induced by peristalsis, by reducing the Reynolds numbers in the gut content and provide laminar flow, leading to a reduced rate of glucose absorption [9].

4. Conclusions

The main objective of this work was to investigate the impact of the parameters molecular weight, concentration—particularly the critical concentration (c^*)—and viscosity of CMC in solutions on glucose release using an in vitro side-by-side system. Our hypothesis of a positive influence on glucose diffusion of solutions with concentrations of CMC above the coil overlap ($\geq c^*$.) was confirmed and the results underline physiological outcomes of in vivo studies. Our results indicate that chyme in a higher concentration of lower molecular weight (M_W) fibers leads to a stronger decrease in diffusion of glucose from the gut lumen to the enterocytes then a lower concentration of higher M_W fibers, at a similar given viscosity. The outcome of our study provides an excellent basis to elucidate the occurrence of the strong deviation from Stokes-Einstein behavior in fiber solutions. The subject of future research should comprise further studies on additional influencing factors of dietary fibers on glucose diffusion in order to improve the overall understanding of the health effects of dietary fibers.

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Chapter 2: The Role of Hydration Properties of Soluble Dietary Fibers on Glucose Diffusion

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The presence of elevated levels of dietary fiber in meals has been associated with decreased glucose release in the human gut. However, the commonly considered viscous effects of soluble dietary fiber (SDF) are not sufficient to explain the improved glucose release, and additional influencing factors besides viscosity have so far been disregarded. Building on this, the present study sought to elucidate the factors that influence glucose diffusion in SDF solutions, going beyond viscosity and taking into account hydration properties. The study focused on investigating the influence of hydration properties – specifically water holding capacity (WHC), amount of bound water, and osmotic pressure – on *in vitro* glucose diffusion under physiological conditions using a side-by-side cell system. Five types of SDF were used in solution and dispersion at concentrations above the critical concentration *c**. These included low and high methylester pectin, xanthan gum, locust bean gum, carboxymethyl cellulose, and citrus fiber, which consists of 30 % SDF and 70 % insoluble DF.

The diffusion of glucose decreased significantly in all fiber solutions at low concentrations, which were above *c**. A strong correlation was observed between the hydration properties of SDF and the glucose diffusivity. The correlation coefficients ranged from $R^2 = 0.94$ for WHC to $R^2 = 0.81$ for the amount of bound water, and $R^2 = 0.79$ for water mobility. The citrus fiber, partly insoluble and swollen, exhibited a gel-like behavior. This also resulted in a reduction in glucose diffusivity. Therefore, it is essential to investigate the hydration properties, particularly the WHC, the amount of bound water, and water mobility, in addition to viscosity and molecular weight, for the characterization of SDF and their impact on glucose diffusion in an osmotic-driven system.

Author Contributions:

Miehle, E.: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. Haas, M.: Investigation, Validation, Writing – original draft. Bader-Mittermaier, S.: Conceptualization, Methodology, Project administration, Validation, Writing – review & editing. Eisner, P.: Conceptualization, Resources, Supervision, Writing – review & editing

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The role of hydration properties of soluble dietary fibers on glucose diffusion

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ABSTRACT

The inclusion of high levels of dietary fibers in meals is associated with a reduction of the glucose release in the human gut. However, the commonly considered viscous effects of soluble dietary fibers (SDF) are insufficient to explain the altered glucose release. In this study, we therefore, aimed to investigate the effect of hydration properties (i.e. water holding capacity (WHC), amount of bound water, osmotic pressure) on *in vitro* glucose diffusion along an osmotic gradient using a side-by-side cell system. For this, five types of SDF (low-and high-methylester pectin, xanthan gum, locust bean gum, carboxymethyl cellulose) and citrus fiber comprising 16% of soluble fibers and 72% of insoluble fibers were used.

The diffusivity of glucose decreased significantly in all fiber solutions at low concentrations, which were above the critical concentration for SDF. We observed a strong correlation between the hydration properties of SDF and the glucose diffusivity revealing correlation coefficients ranging from $R^2 = 0.94$ for WHC to $R^2 = 0.81$ for the amount of bound water and $R^2 = 0.79$ for water mobility. The partly insoluble swollen citrus fiber exhibited a gel-like behavior, which resulted in reduced glucose diffusivity. Therefore, we were able to show that – besides viscosity and molecular weight – the hydration properties, particularly WHC, the amount of bound water and water mobility should be investigated for the characterization of soluble dietary fibers and their impact on glucose diffusion in an osmotic-driven system.

1. Introduction

The incidence of diet-related diseases like diabetes is progressively increasing in western countries (Magrone et al., 2013). High energy intake, particularly due to sugar-rich foods such as sweetened beverages enhances the development of diabetes type 2, a disease of impaired blood glucose level (Erdman Jr, MacDonald, & Zeisel, 2012; Ruxton, Gardner, & McNulty, 2009; Schulze et al., 2004). Dietary fibers, in particular water-soluble dietary fibers (SDF), contribute to improving blood glucose metabolism by blunting postprandial glucose responses (Brownlee, 2011; Goff, Repin, Fabek, El Khoury, & Gidley, 2018). Low glycaemic load diets have been associated with reduced incidence of diabetes type 2 (D. J. Jenkins, Kendall, Axelsen, Augustin, & Vuksan, 2000; Schulze et al., 2007) and therefore, the consumption of SDF could reduce the risk of diabetes type 2.

The reduction in the glycemic response by SDF after consumption is often attributed to an increase in viscosity of the chyme due to fiber addition and therefore a reduced glucose diffusion (Nsor-Atindana, Goff, Liu, Chen, & Zhong, 2018; Ou, Kwok, Li, & Fu, 2001; Srichamroen & Chavasit, 2011). Concentrations above the critical concentration c^* – referred to the concentration of a specific type of soluble dietary fiber at which entanglement occurs – significantly lowered *in vitro* glucose diffusion in solutions of carboxymethyl cellulose (Miehle, Bader-Mittermaier, Schweiggert-Weisz, Hauner, & Eisner, 2021). The *in vitro* observations were also underlined by a significant *in vivo* effect of c^* on the postprandial blood glucose rise in various *in vivo* studies (Rieder, Knutsen, & Ballance, 2017). Nonetheless, strong deviations from Stokes-Einstein behavior regarding the diffusivity in fiber solutions have been observed (Alvarez-Manceñido et al., 2006; Miehle et al., 2021; Shelat, Vilaplana, Nicholson, Gidley, & Gilbert, 2011; Won,

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Onyenemezu, Miller, & Lodge, 1994) and therefore, the reduction in glucose diffusivity cannot only be related to viscosity, molecular weight (M_W) and c^* of the dietary fiber (Miehle et al., 2021; Shelat et al., 2011).

Besides viscosity, M_W, and the c^*, several researchers suggested an influence of hydration properties of SDF in the digestive tract, which could account for some of their physiological effects as the glycemic response (Adiotomre, Eastwood, Edwards, & Brydon, 1990; Elleuch et al., 2011; F; Guillon & Champ, 2000; J.; Robertson & Eastwood, 1981). Thereby, the hydration properties, which are the ability of a fiber to interact and accumulate, immobilize and/or bind water, is one of the main characteristics to be investigated (Kerr & Wicker, 2000).

Hydration properties are related to the chemical structure and the physical properties of the dietary fiber particles, environmental conditions in solution such as pH, temperature, ionic strength and type of ions, and mechanical processes applied for dissolution or fiber ingredient processing such as grinding or heating. The chemical structure includes, among others, the substitution patterns and the M_W (Bertin, Rouau, & Thibault, 1988; Elleuch et al., 2011; Fleury & Lahaye, 1991; F Guillon et al., 2000). However, the detailed effect of the hydration properties of dietary fibers on the glycemic response remains unclear and is investigated in the present study. Hydration properties comprise different properties such as water holding capacity (WHC), water mobility, or osmotic pressure.

WHC is defined as the ability of food ingredients or foods to hold water during the application of osmotic pressure or centrifugation (J. Robertson et al., 1981). To simulate the impact of dietary fibers during digestion, the application of osmotic pressure for determining WHC has previously been conducted (Stephen & Cummings, 1979). For this, polyethylene glycol (PEG) might be used as an osmotic agent to establish osmotic pressure, since it does not permeate through a semi-permeable dialysis membrane due to its availability in high M_W and its non-ionic effect (Lagerwerff, Ogata, & Eagle, 1961; J. A.; Robertson et al., 2000).

In addition, the amount of bound and free water, as well as water mobility in dietary fiber solutions, can be determined by means of ^{1}H NMR relaxometry with transverse (spin-spin) relaxation time (T_2) (Belton, 1990; Han et al., 2014; Kerr et al., 2000; Ruan & Chen, 1998). Decreased mobility of water protons leads to small T_2-values, which can be caused by strong interactions like hydrogen bonds or dipole-dipole interactions (Kerr et al., 2000).

Furthermore, the osmotic pressure determined with a membrane osmometer is used to characterize SDF in solutions (Kruk, Kaczmarczyk, Ptaszek, Goik, & Ptaszek, 2017; Liszka-Skoczylas, Ptaszek, & Żmudziński, 2014) The higher the concentration of the solute, the lower the chemical potential and the higher the osmotic pressure (Π) (Granik, Smith, Lee, & Ferrari, 2002; Kruk, Kaczmarczyk, et al., 2017; Wiegand & Köhler, 2008). Solutions of SDF can be categorized as non-ideal dilute solutions (Harding, Vårum, Stokke, & Smidsrød, 1991; Kruk, Kaczmarczyk, et al., 2017), which can be represented in a power virial equation that shows a correlation between Π, the number-average molecular weight (M_n), and the concentration (c) due to its colligative property. The higher the Π, the higher the c and/or lower the M_n (Harding et al., 1991; Kruk, Pancerz, & Ptaszek, 2017):

$$\frac{\Pi}{c} = \frac{RT}{Mn} + A_2 RTc \quad (1)$$

with R as the ideal gas constant, T as the temperature, and A_1 and A_2 as the first and second virial coefficient, respectively.

Therefore, our study aimed to investigate the impact of hydration properties (i.e. water holding capacity, water mobility, osmotic pressure) of certain soluble dietary fibers on the *in vitro* glucose diffusion under simulated physiological conditions.

To achieve this, we used low and high methylester pectin, xanthan gum, locust bean gum, and carboxymethyl cellulose, which are classified as hydrocolloids due to their high M_W and their richness in polar and ionic groups on their sidechains (Kerr et al., 2000). Citrus fiber – a partly insoluble fiber – was used for comparison. Hydration properties such as osmotic pressure, WHC, water mobility, and the resulting rheological behavior – dynamic viscosity and dynamic oscillatory measurements – of the fiber solutions were determined. Diffusion coefficients of glucose in fiber solutions in concentrations two times higher than the c^* and glucose retention indices were investigated using an *in vitro* side-by-side system. Furthermore, the effect of simulated physiological conditions (i.e. application of osmotic pressure in the acceptor cell, *in vitro* digestion) on the diffusion of glucose was determined. These findings will help to improve the overall understanding of the effect of dietary fibers on glucose diffusion in the matrix of the chyme mostly related to their hydration properties in the human gut, besides the well-known active transport of glucose through the SGLT1 transporters.

2. Materials and methods

2.1. Dietary fiber ingredients, chemicals, and enzyme preparations

Sodium carboxymethyl cellulose (CMC) (Blanose™ 9M31 F, weight-average molecular weight (M_W) of 395 kDa, degree of methylation 0.80–0.95) was provided from Ashland Industries Europe GmbH (Schaffhausen, Switzerland). High methylester pectin (HMP) (Classic CU 201, degree of esterification (DE) 69%, M_W 74 kDa) and low methylester pectin (LMP) (Classic CU-L 054, DE 34%, M_W 52.7 kDa) were supplied by Herbstreith & Fox KG (Neuenbürg/Württemberg, Germany). Xanthan (Xanthan Gum FNCSP-PC, M_W 1.08×10^3 kDa) was purchased from Jungbunzlauer Austria AG (Vienna, Austria), locust bean gum (LBG) (GENU ® Gum RL-200Z) from CP Kelco (Atlanta, U.S.) and citrus fiber (CF) (Herbacel AQ Plus Citrus – N, derived from citrus fruit pomace) from Herbafood Ingredients GmbH (Werder, Germany). All types of dietary fibers were food grade.

The determined critical concentrations (c^*) of the five soluble fibers were 0.43 g/100 g for CMC, 0.88 g/100 g for HMP, 0.92 g/100 g for LMP, 0.05 g/100 g for xanthan and 0.24 g/100 g for LBG.

The determined composition of CF was for insoluble dietary fiber (IDF) 71.6 ± 2.9 g/100 g, for soluble dietary fiber (SDF) 16.3 ± 3.2 g/100 g, for protein 5.8 ± 0.1 g/100 g, and for ash 1.3 ± 0.2 g/100 g. The dietary fiber contents (SDF and IDF) were determined using an enzymatic-gravimetric analysis according to AOAC 991.43 (Chemists., 2016). According to the manufacturer, all other fibers had a total soluble dietary fiber content of ≥97 g/100 g on dry matter.

For the preparation of the *in vitro* digestion fluids, alpha-amylase from human saliva (Type IX-A, 1,000–3,000 units/mg protein; A0521), pancreatin from porcine pancreas (8 x USP specifications; P7545), pepsin from porcine gastric mucosa (3200–4500 units/mg protein; P6887), and bile extract from porcine (B8631) were purchased from Merck KGaA (Darmstadt, Germany). Stock solutions of the simulated digestion fluids (simulated salivary fluid, simulated gastric fluid, and simulated intestinal fluid) were prepared as described by Minekus et al. (2014). Polyethylene glycol (PEG) (M_W 20 kDa) was used for adjusting the osmotic pressure and also purchased from Merck KGaA (Darmstadt, Germany). D-Glucose (anhydrous), sodium azide and all other reagents and chemicals used were of analytical grade and supplied by Chemsolute (Th. Geyer GmbH & Co. KG, Renningen, Germany).

2.2. Fiber solution preparation

2.2.1. Solutions for determining the glucose diffusion

To determine the glucose diffusion, solutions of the fibers comprising 0.93 g CMC/100 g, 1.91 g HMP/100 g, 2.00 g LMP/100 g, 0.11 g xanthan/100 g, 0.53 g LBG/100 g, and a suspension of CF with 0.87 g/100 g.were used. Those concentrations are related to the previously determined c^* and based on the intention to reach a value of 2.2 x c^* for the different soluble fiber ingredients and a value of 2.2 × 0.4 g/100 g for CF. For the determination of the c^* and the comparable c of CF, solutions and dispersions with concentrations ranging from 0.01 to 5 g/100 g

were prepared and viscosity measurements were performed (cf. Section 2.5.1). The plot of the zero shear specific viscosity ($\eta_{sp,0}$) against concentration (c) is shown in Fig. 6 (appendix), whereby the interception of the two fits of the data in the diluted region and the concentrated region forms the c^*.

The detailed procedure for the determination of c^* was previously published by Miehle et al. (2021). For the partly insoluble CF, we plotted the viscosities (η) at a shear rate of 0.5 s^{-1} of the suspensions against c (Fig. 7 appendix). The concentration c of CF, at which a high rise in viscosity appeared, was determined comparable to c^* related to the soluble fibers. This concentration was 0.40 g/100 g for CF.

Additionally, solutions of HMP and LMP in concentrations of 0.19 g HMP/100 g and 0.88 g HMP/100 g as well as 0.20 g LMP/100 g and 0.92 g LMP/100 g were prepared to reach values of 0.2 x c^* and c^*, respectively.

The respective amount of fiber ingredients were dissolved in distilled water under constant stirring for 2 h at room temperature (RT), except for LBG. LBG was stirred for 30 min at RT, then heated to 80 °C for 1 h to achieve complete dissolution, and cooled down to RT under constant stirring for another 30 min. Afterward, electrolytes in concentrations of the final digestion mixture according to Minekus et al. (2014) (7.27 mmol/L KCl, 1.12 mmol/L KH_2PO_4, 51.3 mmol/L $NaHCO_3$, 32.6 mmol/L NaCl, 0.21 mmol/L $MgCl_2$ * 6 H_2O, 0.15 mmol/L $(NH_4)_2CO_3$, 0.53 mmol/L $CaCl_2$) along with 0.1 mol/L glucose, 0.1 g/100 g NaN_3, and distilled water were added to reach the final fiber concentration and stirred for another 14 h at RT as previously described by Miehle at al. (2021). Before performing the *in vitro* glucose release experiments, the pH of the samples was adjusted to 7.0 ± 0.1. The sample blank contained only distilled water, electrolytes, 0.1 mol/L glucose, and 0.1 g/100 g NaN_3.

2.2.2. In vitro digested fiber solutions

The static *in vitro* digestion was performed with slight modifications to the harmonized INFOGEST protocol (Minekus et al., 2014). The *in vitro* digestion included an oral, gastric and intestinal phase. Powders of the fiber samples were prepared in Erlenmeyer flasks and diluted with demineralized water to obtain concentrations of 2.2 x c^* or 0.87 g/100 g for CF in the final digestion mixture (for the final concentrations, see Section 2.2.1). A sample blank was prepared with distilled water instead of the fiber sample. Glucose and NaN_3 were added to obtain concentrations in the final digestion mixture of 0.1 mol/L and 0.1 g/100 g, respectively. During the oral phase, the diluted fiber samples were mixed with tempered (37 °C) simulated salivary fluid containing α-amylase in a final concentration of 75 U mL^{-1} in the digestion mixture (ratio 50:50 *w/w*) and stirred at 37.0 ± 0.1 °C at 180 rpm for 2 min. For the gastric phase, the pH was lowered to 3.0 ± 0.1 with 6 mol/L HCl and mixed with tempered (37 °C) simulated gastric fluid (ratio 50:50 *w/w*) containing pepsin with an enzymatic activity of 2000 U mL^{-1} in the final digestion mixture. The mixture was incubated at 37.0 ± 0.1 °C for 2 h under constant stirring at 180 rpm. In the intestinal phase, the pH was adjusted to 7.0 ± 0.1 with 1 mol/L NaOH and tempered (37 °C) simulated intestinal fluid (ratio 50:50 *w/w*) containing pancreatin, and bile acid salts were added to reach an enzyme activity of 100 U mL^{-1} for trypsin (15.38 mg pancreatin/mL final digestion mixture) and 10 mmol/L of bile acid salts in the final digestion mixture, respectively. After incubating at 37.0 ± 0.1 °C for 2 h under constant stirring at 180 rpm, the samples were stored at 4 °C overnight to perform diffusion experiments the next day.

2.3. In vitro glucose diffusion and glucose determination

2.3.1. In vitro glucose diffusion

The determination of the *in vitro* glucose diffusion was conducted by a side-by-side diffusion system (SES GmbH, Bechenheim, Germany) with a donor and a receptor cell. The trial was performed as described previously by Miehle et al. (2021) with slight changes. As buffer, a carbonate buffer (193.0 mmol/L, 0.1 g/100 g NaN_3, pH 7.0) containing 0.15 mmol/L $(NH_4)_2CO_3$, 7.27 mM KCl, 1.12 mM KH_2PO_4, 151.8 mM $NaHCO_3$, 32.6 mM NaCl, and 0.21 mM $MgCl_2$*$6H_2O$ was added to the receptor cell. For the trials with external osmotic pressure, PEG in a concentration of 4 g/100 g was added to the carbonate buffer – generating an osmotic pressure of 46.6 hPa.

After transferring the digested or undigested fiber solutions to the donor cell, aliquots of 100 µL of the receptor fluid were taken after 1, 5, 10, 20, 30, 40, 60, 90, 120, 180, 240, 360, and 480 min, respectively, to analyze the glucose concentration. The missing volume in the receptor cell was immediately replaced by 100 µL of the carbonate buffer. During the diffusion experiment, the donor cell was left unstirred to allow diffusion, whereas the receptor cell was constantly stirred at 300 rpm. The experiments of each twice-prepared fiber sample were conducted at least in duplicate with freshly prepared solutions using two different side-by-side diffusion systems, leading to four separate values.

2.3.2. Glucose determination

The glucose concentration of the receptor fluid samples was determined by an enzymatic test kit for D-glucose (D-Glucose, Food & Feed Analysis, R-Biopharm AG, Darmstadt, Germany) and conducted as described previously by Miehle et al. (2021).

2.4. Glucose release kinetics and determination of the diffusion coefficient

2.4.1. Glucose release kinetics

The measurements of the fifty-two diffusion samples – 13 sampling times in fourfold determination – were fitted using a non-linear first-order kinetic according to Macheras, Koupparis, and Tsaprounis (1986) and Naumann, Schweiggert-Weisz, Bader-Mittermaier, Haller, and Eisner (2018) to describe the glucose diffusion kinetics:

$$c_t = c_f\,[1 - exp(-kt)] \tag{2}$$

where c_t is the concentration of glucose at the corresponding time, c_f is the concentration of glucose after reaching equilibrium, t is the time in minutes and k is the apparent permeability rate constant.

The effect of fiber on the glucose transfer was investigated with the determined c_f and the glucose transfer index (GTI) was calculated using the equation of Espinal-Ruiz, Restrepo-Sánchez, and Narváez-Cuenca (2016):

$$GTI = 100\,(c_f / c_{f,\,blank}) \tag{3}$$

Based on equation (3), the glucose retention index (GRI) was calculated using the equation of Adiotomre et al. (1990):

$$GRI = 100 - GTI \tag{4}$$

2.4.2. Determination of the diffusion coefficient

The diffusion coefficient of glucose was determined by fitting the data to the Higuchi equation (Higuchi, 1962) using equation (5):

$$Q \Big/ c_0 = 2\sqrt{Dt} \Big/ \pi \tag{5}$$

where Q is the cumulative areal amount of glucose released [mg/cm^2], c_0 is the initial glucose concentration in the sample [mg/mL], D the diffusion coefficient [cm^2/min] and t is the time [min].

The cumulative areal amount of glucose released was normalized using the initial glucose concentration (Q/c_0) and plotted against the square root of time. The D value was then calculated from the slope of the linear regression k_H [cm/$min^{1/2}$] of the curve using the following equation:

$$k_H = 2\sqrt{D/\pi} \tag{6}$$

2.5. Rheological investigation

2.5.1. Shear viscosity measurements

All rheological measurements were performed on a rotational rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) equipped with Rheoplus software version 3.40 (Anton Paar GmbH) in at least duplicate. Viscosity measurements of the fiber solutions at 2.2 x c^*, the suspension of CF with 0.87 g/100 g and the *in vitro* digested fiber solutions/suspension of the glucose diffusion experiments (cf. Section 2.2.1 and 2.2.2) were conducted using a parallel plate geometry (diameter: 50 mm, shear gap: 1.00 mm) (PP50-SN23165; Anton Paar GmbH). For the soluble fiber solutions, zero-shear viscosities were determined by fitting the data to the Cross model. Soluble dietary fibers show shear-thinning flow behavior above the critical concentration (c^*), at which "coil overlap" occurs. According to the entanglement model for "random coil" polysaccharide solutions, at the point of "coil overlap" the network of polysaccharides entangles and leads to a strong increase in viscosity (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981). For a better comparison, viscosities of solutions with shear-thinning flow behavior can be extrapolated to zero shear rate, resulting in the zero-shear viscosity. Insoluble dietary fiber suspensions such as the CF suspensions do not follow the entanglement model and therefore viscosities have to be compared at a selected shear rate, which was in our investigation a shear rate of 0.5 s^{-1}. The exact implementation of the measurements and the determination of the zero-shear viscosities are described in detail by Miehle et al. (2021).

2.5.2. Dynamic oscillatory rheology

Dynamic oscillatory measurements were conducted using a parallel plate geometry (diameter: 50 mm) (PP50-SN23165; Anton Paar GmbH). CF suspensions were measured with a shear gap of 0.5 mm; for all other fiber solutions, the shear gap was 0.1 mm.

The strain sweep test was used to determine the linear viscoelastic range and conducted at an angular frequency of 10 rad/s with a logarithmic strain ramp of 0.01%–10% at a constant temperature of 37.0 ± 0.1 °C. The viscoelastic behavior was subsequently determined by frequency sweep tests at a constant strain of 0.5% sample deformation, which was in the linear viscoelastic range. The frequency sweep tests were performed on a decreasing logarithmic scale from 300 to 0.08 s^{-1} at a constant temperature of 37.0 ± 0.1 °C; the storage (G') and the loss modulus (G'') were evaluated using the RheoPlus software (Anton Paar GmbH).

2.6. Optical density

The optical density of the solutions of the five soluble fibers – CMC, HMP, LMP, xanthan, LBG – for the glucose diffusion experiments (preparation, Section 2.2.1) was determined using a spectrophotometer (Specord 210 plus, Analytik Jena AG, Jena, Germany). The spectral analysis of the wavelength at 285 nm was conducted at 37 °C. The method was applied as previously published by Miehle et al. (2021).

2.7. Osmotic pressure

The osmotic pressure of our solutions was evaluated using a membrane osmometer (OSMOMAT 090, Gonotec, Berlin, Germany) according to Kruk, Kaczmarczyk, et al. (2017) with minor modifications. The measurements were conducted at a cell temperature of 37 °C using a semipermeable membrane (Gonotec, Berlin, Germany) with a cut-off of 10 kDa in at least duplicate. For high polymer concentrations (≥1.9 g/100 g), a direct measurement of the osmotic pressure (Π) was not possible due to high viscosity. Therefore, the low concentration data were fitted and the Π for high concentration was calculated according to Kruk, Kaczmarczyk, et al. (2017) and J. Robertson et al. (1981) using equation (6):

$$\Pi = ax2 + bx + c \quad (7)$$

where Π is the osmotic pressure in hPa, x is the concentration of the polymer [g/100 g] and a (10^4 hPa), b (10^2 hPa) and c (hPa) are the virial coefficients.

The obtained equations followed a polynomic regression of 2nd degree (J. Robertson et al., 1981) (eq. (7)), since the solutions behaved as non-ideal solutions and the Van't Hoff's law does not apply (Harding et al., 1991; Kruk, Pancerz, & Ptaszek, 2017).

The osmotic pressure of PEG solutions in water with 0.5 g/100 g NaN_3 was determined in concentrations of 2, 4, 8 g/100 g, whereas the osmotic pressure of 12, 16, and 20 g PEG/100 g was calculated using equation (7). Similarly, the osmotic pressure of PEG in the carbonate buffer used for the diffusion experiments was determined at 4 g/100 g.

The osmotic pressure of our soluble dietary fiber solutions of CMC, HMP, LMP, xanthan and LBG (preparation, cf. Section 2.2.1) was determined directly for all concentrations, except for the 2.0 g LMP/100 g solution and the 1.9 g HMP/100 g solution. For those two concentrations, four obtained values in the range of 0.2–1.2 g/100 g for LMP and 0.19 to 1.15°g/100°g for HMP were fitted (LMP: $R^2 = 0.9998$; HMP: $R^2 = 0.9987$) and Π was calculated according to equation (7) with 21.3, 4.4, and 1.2 for the virial coefficients a, b and c, respectively, for LMP and with 11.0, 0.4 and 0.7 for the virial coefficients a, b and c, respectively, for HMP. The determination of the Π of CF was not possible due to insoluble particles in the dispersion.

2.8. Water holding capacity

The water holding capacity (WHC) was measured according to J. Robertson et al. (1981) with applied suction pressure. 0.1 g of dietary fiber sample was pre-soaked in 10 mL distilled water at 4 °C overnight containing 0.5 g NaN_3/100 g solution to avoid microbial growth. PEG solutions in the concentrations of 2, 4, 8, 12, 16, 20 g/100 g with 0.5 g/100 g NaN_3 were prepared to simulate increasing suction pressure (cf. Section 2.7).

Ten mL of the pre-soaked samples were transferred into dialysis tubes (regenerated cellulose, diameter 1.6 cm, M_W cut-off of 12–14 kDa) (SERVA Electrophoresis GmbH, Heidelberg, Germany) and placed in a 120 mL tube containing 100 mL of PEG solution with a specific concentration. The tubes were tightly closed to prevent water evaporation and incubated at 37 °C for 72 h in a shaking water bath (100 rpm). Afterward, the content of the dialysis tube was weighed and dried overnight at 105 °C to weight constancy. The WHC (g/g) was calculated with the following equation:

$$WHC = (m_w - m_d)/m_d \quad (8)$$

where m_w is the mass of the wet sample and m_d is the mass of the dried sample.

For determining the WHC of fiber solutions (preparation, Section 2.2.1) and *in vitro* digested fiber solutions (preparation, Section 2.2.2), the sample amount and the PEG solution were halved. Thus, 5 mL of the aforementioned solutions were transferred into dialysis tubes and placed in a 50 mL tube containing 50 mL PEG solution in a concentration of 4 g/100 g with 0.1 g/100 g NaN_3.

2.9. Determination of water mobility by ^1H NMR

Relaxation measurements were performed with a low field ^{1}H NMR (mq 20 NMR analyzer, Bruker BioSpin GmbH, Rheinstetten, Germany). Using the Carr-Purcell-Meiboom-Gill (CPMG) measurements, the transverse relaxation time was assessed for different fiber solutions. For comparability reasons, the weight of the measured samples was standardized to 1 g, weighed into 8 mm closed bottom inlays for 10 mm NMR tubes. The solutions were tempered in a water bath to 40 °C before the measurement, which represented the probe head temperature as

well. The parameters for the CPMG measurement were tau (90°–180° pulse separation) of 1×10^{-4} s and a total amount of echos of 10,000. The signal quality was optimized by choosing a total number of 16 scans with a recycle delay of 1 s. The obtained data were processed using a two-phase exponential decay function with time constant parameters. Measurements were performed in triplicate.

2.10. Statistical analysis

Results are expressed as mean ± standard deviation. SigmaPlot 12.0 for Windows (Systat Software GmbH, Erkrath, Germany) was used for statistical analysis. A one-way analysis of variances (ANOVA) was used with Tukey's honestly significant difference post hoc test to determine significant differences between samples at $p \leq 0.05$. Before that, homogeneity of variance (Levene's test) and normal distribution (Shapiro-Wilk test) were applied. Regressions were calculated using OriginPro 2018 for Windows (Origin Lab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Glucose diffusion kinetics of dietary fiber solutions

The glucose release kinetics of dietary fiber solutions at different concentrations in 2.2 times higher than the critical concentration (2.2 x c^*) of carboxymethyl cellulose (CMC) (0.93 g/100 g), high methylester pectin (HMP) (1.91 g/100 g), low methylester pectin (LMP) (2 g/100 g), xanthan (0.11 g/100 g), locust bean gum (LBG) (0.53 g/100 g), citrus fiber (CF) (0.87 g/100 g) and the blank over a time of 8 h are shown in Fig. 1.

The blank showed the fastest glucose release, followed by LBG, xanthan, HMP, CMC, LMP, and CF. All fiber solutions retarded the glucose concentration in equilibrium (c_f, equation (2)) significantly, shown by the glucose retention indices (GRI) in Table 1. The highest GRI was determined for CF with 75.3 ± 5.8%, followed by LMP with 53.9 ± 5.4% and HMP with 38.6 ± 1.6%. All fiber solutions reduced the diffusion coefficients D_0 compared to the blank significantly in the concentration 2.2 x c^*. Similarly, the comparable c of 0.87 g/100 g for CF significantly reduced the diffusion coefficients.

Thereby, CF showed the lowest D_0 for glucose with 0.7×10^{-9} m^2s^{-1}, followed by LMP with 1.4×10^{-9} m^2s^{-1} and CMC with 1.8×10^{-9} m^2s^{-1} (Table 1). As expected, the addition of fibers to the solutions led to a rise in viscosity, with CF showing the highest viscosity of 3,147.5 ± 220.0 mPas at a shear rate of 0.5 s^{-1} of all investigated dietary fibers. LBG and HMP had zero shear viscosities of 369.0 ± 23.5 mPas and 253.0 ± 7.5 mPas, respectively, and exhibited the highest and second-highest viscosity of the investigated soluble dietary fibers (Table 1). *In vitro* digestion of the fibers led to an increase in GRI and D_0 compared to the pure fiber solutions, except for LBG, where D_0 decreased. Simultaneously, the viscosity of the samples increased or remained the same after *in vitro* digestion, particularly for the pectins HMP and LMP, except for CF, which exhibited a decrease in viscosity after *in vitro* digestion (Table 1). As dietary fibers in solution form an osmotic pressure (see Table 2), an osmotic gradient exists in the system and no pure diffusion takes place.

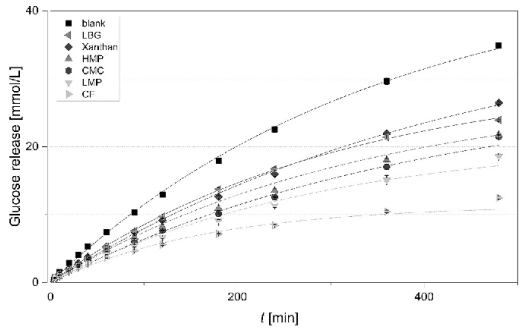

Fig. 1. Diffusion kinetics of glucose from solutions without (blank) and with the dietary fibers of locust bean gum (LBG), xanthan, high methylester pectin (HMP), carboxymethyl cellulose (CMC) and low methylester pectin (LMP) in the concentration of 2.2 x c^* or of 0.87 g/100 g for citrus fiber (CF). The symbols correspond to the experimental data and the lines to fitted data according to equation (2) ($R^2 \geq 0.9995$).

The determined frequency spectra of storage (G') and loss (G'') moduli of the solutions suggested a stable dispersion or solution with predominantly elastic properties for the partly insoluble CF and the soluble LBG, as for all frequencies G' was higher than G''. For all other solutions, a viscous behavior was determined with a higher G'' than G' at all frequencies (Fig. 5, appendix).

The reduced amount of released glucose with the highest GRI of 75.3 ± 5.8% and slowed glucose release indicated by the highest reduction in D_0 of 85.8 ± 1.1% compared to the blank can be attributed to an entrapment of the glucose molecules by the highly concentrated fiber network and/or by chemical bonding of the glucose molecules to the dietary fibers (Johnson & Gee, 1981; Ou et al., 2001). D. Jenkins et al. (1978) and Wood et al. (1994) showed that drinks containing 50 g glucose and different soluble fibers (guar, pectin, gum tragacanth and methylcellulose) in the same concentration (12 g/400 mL) or differently hydrolyzed oat gum, reduced blood glucose concentration in human subjects in correlation with the viscosity (7 mPas–1910 mPas). Furthermore, the results of Repin et al. (2017) indicated that the consumption of soluble dietary fibers in different concentrations at similar viscosity levels is able to attenuate postprandial glycemic and insulinemic responses.

Contrary to the previous findings our results showed that D_0 and GRI were mainly influenced by fiber concentrations for the soluble fibers. Thus, solutions with higher concentrations of soluble fibers (LMP > HMP > CMC > LBG > xanthan) exhibited lower D_0 ($R^2 = 0.69$) and higher GRI ($R^2 = 0.79$) values independently of the viscosity ($R^2 = 0.05$) of the solutions. For example, despite a higher viscosity of LBG (369.0 ± 23.5 mPas) at a lower concentration (0.53 g/100 g), the diffusion in LBG solution was higher (3.3×10^{-9} m^2s^{-1}) compared to LMP (1.4 x $10^{-9}m^2s^{-1}$), which showed a lower viscosity (149.3 ± 16.1 mPas) at a higher concentration (2 g/100 g).

Cameron-Smith, Collier, and O'dea (1994) also observed that meals containing the same concentration of different soluble dietary fibers (in this case, guar gum, xanthan, and methylcellulose were used) in a diet showed the same postprandial glycemic response in rats, despite a large difference in viscosities of the gastrointestinal content. This also corroborates our findings. In addition, several authors suggested that viscosity caused by SDF in solutions is not alone predictive of the *in vitro* or *in vivo* glycemic response (Kwong, Wolever, Brummer, & Tosh, 2013; Sasaki & Kohyama, 2012; Shelat et al., 2010; Shelat et al., 2011).

The rise in viscosity due to *in vitro* digestion could originate from the lower pH during the gastric phase (pH 3). Especially the nonionic association mechanism of the LMP (Cesaro, Ciana, Delben, Manzini, & Paoletti, 1982; Gilsenan, Richardson, & Morris, 2000). and the hydrophobic interactions and hydrogen bonds of the HMP (Oakenfull & Scott, 1984) become increasingly important as the pH decreases and may be only partially reversible as pH increases. In contrast, the higher temperature during *in vitro* digestion (37 °C) can lead to a degradation of pectins due to β-elimination (Pilnik, 1996; Sila et al., 2009), which might play a lower role compared to the viscosity rise due to pH shift. The optical density (OD) of our soluble fiber solutions also underlined a lower diffusion in a stronger entangled and higher concentrated network, where the OD of LMP (2.58 ± 0.07) was also significantly higher than that of LBG (0.50 ± 0.00) (Table 1). In a stronger entangled network of polymers, there is less free volume for glucose to diffuse

Table 1
Concentration, diffusion coefficient (D_0), viscosities (η), glucose retention index (GRI), and optical density (OD) of the solutions and D_0 and η of the *in vitro* digested (*i. v.* d.) solutions with locust bean gum (LBG), xanthan, high methylester pectin (HMP), carboxymethyl cellulose (CMC), low methylester pectin (LMP) and citrus fiber (CF) and the blank. The viscosities were determined at zero-shear except for citrus fiber, which was determined at a shear rate of 0.5 s^{-1}.

	c [g/100 g]]	D_0 [10^{-9} m^2s^{-1}]	Reduction of D_0 compared to the blank [%]	GRI [%]	OD at 285 nm [−]	η [mPas]	η (*i. v. d.*) [mPas]	D_0 (*i. v. d.*) [10^{-9} m^2s^{-1}]
blank	0	5.1 ± 0.1 ^{a}	0.0 ± 3.1 ^{a}	0.0 ± 0.0 ^{a}	0.16 ± 0.00 ^{a}	0.8 ± 0.0 ^{a}	1.1 ± 0.0 ^{a}	6.2 ± 0.4 ^{a}
CMC	0.93	1.8 ± 0.1 ^{e,d}	65.0 ± 3.5 ^{e,d}	36.9 ± 4.0 ^{c}	0.34 ± 0.01 ^{a,c}	101.0 ± 0.6 ^{a,b}	696.7 ± 108.7 ^{a}	2.6 ± 0.0 ^{c}
HMP	1.91	1.9 ± 0.1 ^{e}	62.3 ± 4.6 ^{e}	38.6 ± 1.6 ^{c}	2.57 ± 0.11 ^{b}	253.0 ± 7.5 ^{b,c}	18,391.7 ± 6521.1 ^{b}	2.7 ± 0.2 ^{c}
LMP	2.00	1.4 ± 0.1 ^{d}	73.3 ± 2.9 ^{d}	53.9 ± 5.4 ^{d}	2.58 ± 0.07 ^{b}	149.3 ± 16.1 $^{a, b}$	15,518.6 ± 3,901.9 ^{b}	1.8 ± 0.1 ^{c,d}
Xanthan	0.11	2.9 ± 0.2 ^{c}	43.0 ± 6.5 ^{c}	13.3 ± 0.8 ^{b}	0.30 ± 0.00 ^{a,c}	33.1 ± 28.4 ^{a}	31.2 ± 0.9 ^{a}	4.3 ± 0.6 ^{b}
LBG	0.53	3.3 ± 0.1 ^{b}	36.1 ± 5.7 ^{b}	33.5 ± 0.7 ^{c}	0.50 ± 0.00 ^{c}	369.0 ± 23.5 ^{c}	398.5 ± 79.1 ^{a}	0.9 ± 0.2 ^{d}
CF	0.87	0.7 ± 0.0 ^{f}	85.8 ± 1.1 ^{f}	75.3 ± 5.8 ^{e}	–	3147.5 ± 220.0 ^{d}	505.3 ± 31.5 ^{a}	0.8 ± 0.1 ^{d}

Different superscript letters (a-g) within a column indicate significant differences between means ($p < 0.05$).

Table 2
Water holding capacity (WHC), osmotic pressure (Π), transverse relaxation time ($T_{2,1}$) and relative intensity ($A_{2,1}$) of the solutions and WHC of the *in vitro* digested (*i. v.* d.) solutions with locust bean gum (LBG), xanthan, high methylester pectin (HMP), carboxymethyl cellulose (CMC), low methylester pectin (LMP) and citrus fiber (CF).

	WHC [g/g]	WHC (*i. v.* d.). [g/g]	Π [hPa]	$A_{2,1}$ [%]	$T_{2,1}$ [ms]
CMC	63.0 ± 1.0 ^{a}	45.0 ± 0.6 ^{b}	18.0 ± 0.2 ^{c}	0.52 ± 0.04 ^{b}	26.64 ± 1.65 ^{c}
HMP	48.3 ± 1.6 ^{c}	42.6 ± 1.3 ^{c}	41.6 ± 0.1 ^{ab}	0.53 ± 0.02 ^{b}	17.58 ± 0.88 ^{c,d}
LMP	56.8 ± 0.8 ^{b}	49.5 ± 0.4 ^{a}	77.2 ± 0.0 ^{a}	0.55 ± 0.01 ^{b}	10.00 ± 0.38 ^{d}
Xanthan	23.9 ± 0.4 ^{d}	13.8 ± 0.1 ^{d}	0.1 ± 0.0 ^{e}	0.17 ± 0.02 ^{d}	25.39 ± 4.10 ^{c}
LBG	16.0 ± 1.3 ^{e}	12.7 ± 1.4 ^{d}	1.0 ± 0.1 ^{d}	0.31 ± 0.01 ^{c}	55.29 ± 3.55 ^{b}
CF	13.8 ± 0.1 ^{e}	10.5 ± 0.4 ^{e}	0.6 ± 0.0 ^{d}	6.19 ± 0.02 ^{a}	163.40 ± 0.76 ^{a}

Different superscript letters (a-f) within a column indicate a significant difference between means ($p < 0.05$).
[a] Calculated value, cf. equation 7.

through, thus leading to lower D_0 (Fujita, 1961). Previously, we made the same observations in differently concentrated solutions of water-soluble CMC, where a higher number of lower molecular weight (M_W) polymers led to a stronger decrease in diffusion of glucose than a lower number of higher M_W polymers, at a similarly given viscosity (Miehle et al., 2021).

The partly insoluble CF showed the lowest D_0 and the highest GRI despite less than half of the concentration of the investigated pectins. The predominantly solid-like properties of the CF solutions (G' of 20.8 ± 8.4 > G'' of 2.4 ± 0.9 at a frequency of 1 s^{-1}) with high viscosity could lead to an entrapment of the glucose in the gel-like fiber network of soluble (SDF 16.3 ± 3.2%) and insoluble parts (IDF 71.6 ± 2.9%). Thereby, the glucose release can be strongly retarded and lowered. Srichamroen et al. (2011) also observed that a mixture of malva nut gum (mostly water-insoluble) and guar gum (water-soluble) had the greatest glucose retention compared to the fibers alone. Also, soluble and insoluble fibers tend to operate simultaneously in delaying or retarding glucose diffusion in foods (Nishimune et al., 1991).

3.2. Effect of the water holding capacity of dietary fibers and their relation to glucose diffusion

The amount of water held by the fibers at a specific osmotic pressure is shown in Fig. 2. Carboxymethyl cellulose (CMC) showed the highest WHC at all osmotic pressures followed by low methylester pectin (LMP), high methylester pectin (HMP), xanthan, locust bean gum (LBG), and citrus fiber (CF). At an osmotic pressure of 126 hPa (at 4 g/100 g PEG), CMC had a WHC of 47.8 ± 0.6 g/g, followed by LMP with 42.1 ± 0.5 g/g, HMP with 33.1 ± 0.4 g/g, xanthan with 16.7 0 ± 0.1 g/g, LBG with 14.7 ± 0.6 g/g, and CF with 9.8 ± 0.2 g/g.

An initial increase in osmotic pressure from 41 to 126 hPa corresponding to 2 and 4 g/100 g PEG led to a significant decrease of almost 50% in the water holding capacity (WHC) for the investigated dietary fibers (Fig. 2). At high osmotic pressures of >900 hPa corresponding to 12 g/100 g PEG, no significant differences in the WHC between the various dietary fibers were obtained and a further increase in osmotic pressure only led to a marginal decrease in WHC.

Similar results were also described by Adiotomre et al. (1990), Stephen, et al. (1979), and F Guillon et al. (2000) for a wide range of dietary fibers showing that soluble dietary fibers have higher WHC compared to partially or mainly insoluble dietary fibers. The water retained by the fibers is suggested to be linked hydrodynamically or trapped physically (López-Vargas, Fernández-López, Pérez-Álvarez, & Viuda-Martos, 2013). The high WHC of CMC might originate from the relatively high degree of methylation (0.89–0.95), which is attributed to increasing

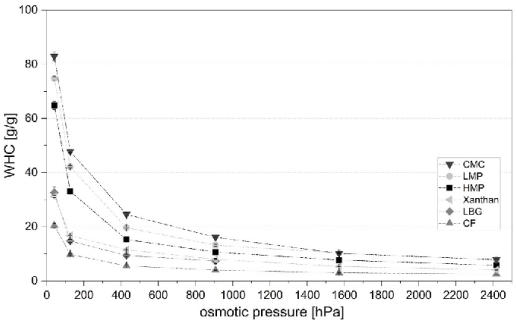

Fig. 2. Effect of osmotic pressure on the water holding capacity (WHC) of carboxymethyl cellulose (CMC), low methylester pectin (LMP), high methylester pectin (HMP), xanthan, locust bean gum (LBG), and citrus fiber (CF).

numbers of free carboxyl groups and thus greater interactions with water molecules can occur (Coffey, Bell, & Henderson, 2006). The difference in WHC between the two types of pectins may also occur according to the differences in the degree of esterification (DE). HMP is highly esterified with a DE of 69%, whereas LMP is low esterified with a DE of 34%. Thus, LMP obtains a higher number of free, not esterified hydroxyl groups, which are available for the formation of hydrogen bonds, consequently leading to a higher WHC (Oakenfull, 2001). One additional effect might be that LMP has a smaller molecular weight (M_W) (52.7 kDa) compared to HMP (74 kDa). A small M_w implicates a higher number of particles at the same concentration, leading to pronounced interactions between water molecules and dietary fiber molecules (Panchev, Kirtchev, Kratchanov, & Proichev, 1988). The partly insoluble CF showed the lowest WHC at all investigated osmotic pressures (Fig. 2)

A strong inverse correlation between the water holding capacity (WHC) of the soluble fibers and the diffusion coefficient of glucose (D_0) (Fig. 3, $R^2 = 0.94$) was observed. The highest WHC of CMC and LMP with 63.0 ± 1.0 g/g and 56.8 ± 0.8 g/g (Table 2) showed the lowest D_0 of 1.8×10^{-9} m^2s^{-1} and 1.4×10^{-9} m^2s^{-1} (Table 1), respectively, and on the other hand, LBG with the lowest WHC of 0.33 ± 0.01 g/g showed the highest D_0 of 3.3×10^{-9} m^2s^{-1}

The WHC is influenced by different properties like the M_W, the chemical structure, and osmotic pressure (F Guillon et al., 2000; J. Robertson et al., 1981). We also observed that a higher osmotic pressure (Π) of the solutions due to fiber addition led to a lower D_0 ($R^2 = 0.77$), where LMP had the highest Π (77.2 ± 0.0 hPa) (Table 2) and lowest D_0 (1.4×10^{-9} m^2s^{-1}) (Table 1). According to equation (1), higher concentrations of soluble fibers in the solution implied higher osmotic pressures (77.2 ± 0.0 hPa for 2.00%, 41.6 ± 0.0 hPa for 1.91%, 18.0 ± 0.2 hPa for 0.93%, 1.0 ± 0.1 hPa for 0.53%, 0.1 ± 0.0 hPa for 0.11%) (Table 2). As previously observed, a higher c of a lower M_W CMC led to a stronger decrease in diffusion of glucose than a lower c of higher M_W CMC (Miehle et al., 2021). Therefore, the inverse correlation of the D_0 of glucose and the Π underline the previous findings. In summary, SDF with a higher WHC, as hydrocolloids, are more likely to retain a higher amount of glucose in a system with an osmotic gradient and thus, reduced transport of glucose in the chyme might be expected. A study of glucose transport with an osmotic driving force applied on SDF solutions is discussed in detail in section 3.4.

The partly insoluble CF showed despite the lowest WHC (13.8 ± 0.1 g/g) and low osmotic pressure (0.6 ± 0.0 hPa) (Table 2) the lowest D_0 of

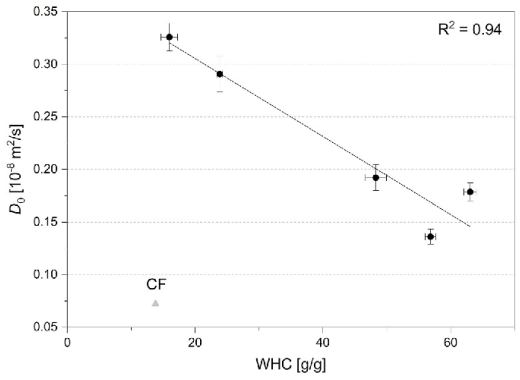

Fig. 3. Correlation of the diffusion coefficient of glucose (D_0) and the water holding capacity (WHC) of the different soluble fibers. Symbols correspond to the experimental data and lines to the fitted data using linear regression ($R^2 = 0.94$). Citrus fiber (CF) is shown in gray as a comparison for a partly insoluble fiber and is not integrated into the fit.

0.7×10^{-9} m^2s^{-1} (Table 1). The low D_0 can be caused by a chemical bonding of the glucose molecules to the CF, which is more likely to occur in insoluble fibers (Ou et al., 2001; Qi et al., 2016) and also by the entrapment of water and glucose molecules in the gel-like structure of the CF solution (Fig. 5, appendix).

To further investigate the effect of WHC on the D_0, we determined both the WHC and the D_0 after *in vitro* digestion of the fiber solutions. *In vitro* digestion of the fibers led to a decrease in WHC (Table 2) and a significant increase in D_0 (Table 1) compared to the pure fiber solutions, except for LBG, where the D_0 decreased simultaneously with decreasing WHC.

The decrease in WHC due to *in vitro* digestion might be attributed to structural changes in the dietary fibers. These structural changes can originate from the change in environmental conditions such as pH, electrolytes, and increased protein content due to digestion enzymes and pancreatin, which affects how water is held by dietary fibers (Auffret, Ralet, Guillon, Barry, & Thibault, 1994; Thibault, Lahaye, & Guillon, 1992). The structural changes could be influenced by electrostatic repulsion of the charged groups of the fibers, inhibition of hydrogen bonding due to ionic interaction, and the precipitation and degradation of the polymers at low pH due to pH shifts and electrolytes during *in vitro* digestion (Coffey et al., 2006; Diaz, Anthon, & Barrett, 2007; Guillon, Renard, Hospers, Thibault, & Barry, 1995). Those structural changes might favor an expansion of the fiber network, leading to a looser network with less hydrogen bonding and decreased WHC (Guillon et al., 1995), which could cause a faster diffusion of glucose.

3.3. Correlation between the bound water of the fibers and the diffusion of glucose

Additionally, we determined the amount of bound water, measured by the relative intensity ($A_{2,1}$) and the mobility of bound water, measured by the transverse relaxation time ($T_{2,1}$) of the five water-soluble fibers locust bean gum (LBG), xanthan, high methylester pectin (HMP), low methylester pectin (LMP), carboxymethyl cellulose (CMC) and the partly insoluble citrus fiber (CF) (Table 2).

Thereby, there was a good linear dependency of the amount ($A_{2,1}$) and mobility ($T_{2,1}$) of the water bound to the soluble fibers and the diffusion of glucose through the solved fiber-water network, where the diffusion coefficient (D_0) correlated inversely to $A_{2,1}$ (Fig. 4a, $R^2 = 0.81$) and directly to $T_{2,1}$ (Fig. 4b, $R^2 = 0.79$). LMP had the highest value of $A_{2,1}$ (0.55 ± 0.01%) and the lowest value of $T_{2,1}$ (10.00 ± 0.38 ms) with the lowest diffusion coefficient D_0 of 1.4×10^{-9} m^2s^{-1} (Table 1, Table 2). We could not observe a tendency of D_0 and the amount and mobility of free or very week-bound water ($A_{2,2}$, $T_{2,2}$) (data not shown).

Overall, higher concentrations of soluble fibers in the solution implied higher amounts of bound water ($A_{2,1}$) (0.55 ± 0.01% for 2.00%, 0.53 ± 0.02% for 1.91%, 0.52 ± 0.04% for 0.93%, 0.31 ± 0.01% for 0.53%, 0.17 ± 0.02% for 0.11%) (Table 2).

The partly insoluble CF behaved differently from the soluble dietary fibers. CF showed the lowest D_0 of 0.7×10^{-9} m^2s^{-1} (Table 1) and the highest amount of bound water ($A_{2,1}$: 6.19 ± 0.02%) (Table 2), but at the same time, a very weak bonding of the water molecules ($T_{2,1}$: 163.40 ± 0.76 ms) was observed. Therefore, CF exhibiting a gel-like structure (Fig. 5, appendix) more likely entraps water and glucose molecules in the gel network and a reduced D_0 is obtained.

In general, our measured values for the amount of bound water ($A_{2,1}$) were lower compared to the findings of other authors (Han et al., 2014; Kerr et al., 2000). The lower values could originate from the electrolytes as divalent cations in the dietary fiber solutions leading to the ionic interaction and inhibition of hydrogen bonding (Renard, Crépeau, & Thibault, 1994). However, as the population of $A_{2,1}$ is not equivalent to the true amount of water molecules (Belton, 1990), the values give a good tendency of the amount of bound water in our fiber solutions.

High $A_{2,1}$ could originate from free carboxyl and hydroxyl groups that lead to a higher interaction with water molecules – as dipolar and

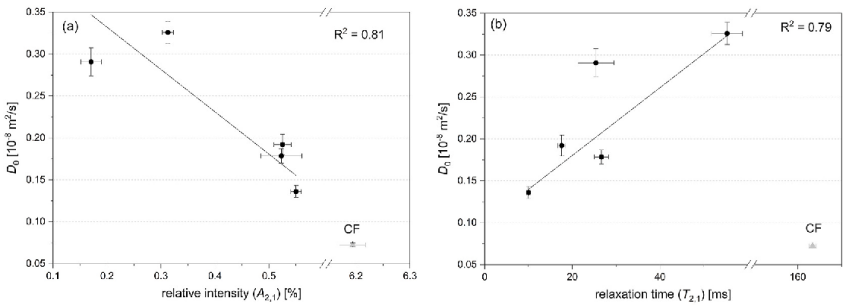

Fig. 4. Correlation of the diffusion coefficient (D_0) of glucose and the relative intensity ($A_{2,1}$) (a) and the transverse relaxation time ($T_{2,1}$)(b) of the different soluble fibers. Symbols correspond to the experimental data and the lines to the fitted data using linear regression (R2 ≥ 0.79). Citrus fiber (CF) is shown in gray as a comparison for a partly insoluble fiber and is not integrated into the fit.

hydrogen bonds – as seen for pectins and CMC (Table 2, Section 3.2) (Coffey et al., 2006; Kerr et al., 2000; Oakenfull, 2001).

Pectins decreased the mobility of water protons in a greater amount compared to the other fibers shown by small values of $T_{2,1}$ (Table 2). The higher degree of esterification of HMP – and therefore less free hydroxyl groups – led to higher $T_{2,1}$ (17.58 ± 0.88 ms) compared to the LMP (10.00 ± 0.38 ms) and therefore, higher mobility of the water molecules can be expected for HMP (Table 2). Kerr et al. (2000) also reported higher $T_{2,1}$ times for HMP than for LMP pectins at similar concentrations.

The proton exchange between the hydroxyl groups of dietary fibers and water greatly influences the $T_{2,1}$ and therefore the mobility of water surrounding them (Hills, Cano, & Belton, 1991). A high proton exchange leads to low mobility and the amount and accessibility of the hydroxyl protons of the fibers to exchange with water protons is essential (Hills et al., 1991).

The high amount of bound water and the low mobility of the bound water could contribute to a lower diffusion of low M_W molecules like glucose, and therefore to a decreased freedom of particles to move, also seen by a change of entropy (Han et al., 2014). Han et al. (2014) made this observation with a decreased diffusion of aspartame in solutions of CMC and sodium alginate at concentrations above c^*.

3.4. Impact of external osmotic pressure on glucose diffusion

Since the hydration properties of fibers strongly depend on the osmotic pressure of the surrounding media (J. Robertson et al., 1981), we examined the effect of a changed osmoticum on the diffusion coefficient of our pectin solutions to further simulate physiological conditions in the small intestine.

Table 3 shows the main results of the impact of external osmotic pressure (Π) on the glucose release of solutions containing the two types of pectins in three different concentrations (0.2 x c*, c* and 2.2 x c*) and the sample blank. The blank solution showed the fastest glucose release with a diffusion coefficient with external osmotic pressure (D_Π) of 5.6 × 10^{-9} m^2s^{-1}, which is an increase of approximately 10% compared to the diffusion coefficient without external osmotic pressure (D_0).

At all three concentrations, the D_0 of low methylester pectin (LMP) was lower (4.9 × 10^{-9} m^2s^{-1}, 1.9 × 10^{-9} m^2s^{-1}, and 1.4 × 10^{-9} m^2s^{-1}) compared to the D_0 of high methylester pectin (HMP) (5.0 × 10^{-9} m^2s^{-1}, 2.5 × 10^{-9} m^2s^{-1} and 1.9 × 10^{-9} m^2s^{-1}). Simultaneously, LMP exhibited significantly higher values in Π at concentrations of c* and 2.2 x c* with 15.1 ± 0.2 hPa and 77.2 ± 0.0 hPa, respectively, compared to HMP at c* and 2.2 x c* with 9.4 ± 0.1 hPa and 41.6 ± 0.1 hPa, respectively. With external osmotic pressure, D_Π increased compared to D_0 in each of the concentrations 0.2 x c* and c* in both HMP and LMP solutions, with the greatest significant increase of 44.0% at c* for HMP.

Table 3
Osmotic pressure (Π), zero shear viscosity (η_0) and diffusion coefficient with (D_Π) and without (D_0) external osmotic pressure (46.6 ± 0.0 hPa) of the solution with high methylester pectin (HMP) and low methylester pectin (LMP) in the concentrations of 0.2 x c*, c* and 2.2 x c* and 0 x c* (blank).

Concentration	D_0 [10^{-9} m^2s^{-1}]	D_Π [10^{-9} m^2s^{-1}]	Change in D [%]	Π [hPa]	η_0 [mPas]
0 × c* (blank)	5.1 ± 0.1 [a]	5.6 ± 0.2 [a]	+9.8 ± 2.5 [a]	0.0 ± 0.0[a]	0.8 ± 0 [a]
LMP					
0.2 × c* (0.20 g/100 g)	4.9 ± 0.5 [a]	5.8 ± 0.7 [a]	+18.4 ± 1.6 [a]	1.1 ± 0.1 [b]	2.2 ± 0.1 [a,b]
c* (0.92 g/100 g)	1.9 ± 0.2 [b,c]	2.2 ± 0.2 [b,c]	+15.8 ± 1.9 [a]	15.1 ± 0.2 [d]	19.1 ± 0.4 [b,c]
2.2 × c* (2.00 g/100 g)	1.4 ± 0.1 [c]	1.3 ± 0.1 [c]	−7.1 ± 2.7 [b]	77.2 ± 0.0 * [f]	149.3 ± 16.1 [d]
HMP					
0.2 × c* (0.19 g/100 g)	5.0 ± 0.3 [a]	5.3 ± 0.3 [a]	+6.0 ± 0.5 [a]	1.0 ± 0.0 [b]	1.7 ± 0.1 [a]
c* (0.88 g/100 g)	2.5 ± 0.1 [b]	3.6 ± 0.1 [b]	+44.0 ± 2.5 [d]	9.4 ± 0.1 [c]	35.6 ± 4.7 [c]
2.2 × c* (1.91 g/100 g)	1.9 ± 0.1 [b,c]	1.5 ± 0.0 [c]	−21.1 ± 2.0 [c]	41.6 ± 0.1 * [e]	253.0 ± 11.2 [e]

Different superscript letters (a-f) within a column indicate a significant difference between means ($p < 0.05$).
*Calculated value, cf. equation 7.

However, for the concentration of 2.2 x c* for both pectins (LMP and HMP), a slight decrease from D_0 to D_Π was observed (−7.1 ± 2.7% and −21.1 ± 2.0%, respectively), which was significant in the case of the HMP sample.

The results indicate a complex interaction between the SDF and the diffusion of glucose along an osmotic gradient.

LMP with higher Π at all concentrations showed smaller D_0 compared to HMP at each concentration, despite a lower viscosity (Table 3). Since Π depends on the chemical structure, Mw, and the number of fiber molecules (Harding et al., 1991; Müller & Frings, 2009), the differences in Π might be caused by the difference in esterified groups and therefore by the number of free hydroxyl groups between the highly esterified HMP and the lower esterified LMP. In addition, the M_W of LMP was lower (52.7 kDa) compared to the M_W of HMP (74.0 kDa), leading to a higher number of molecules at the same concentration and therefore to a higher Π (Granik et al., 2002). As well, the actual higher concentrations of LMP ($c^* = 0.92$) compared to HMP ($c^* = 0.88$) could contribute to a higher Π (Harding et al., 1991).

The results show for both pectins in the concentrations of 0.2 x c* and c* a significant increase from D_0 to D_Π by applying external osmotic pressure, which indicates higher glucose release (Table 3). As there was a difference in Π of ≥31.6 ± 0.4 hPa between the donor cell with the

solutions of 0.2 x c^* and c^* with LMP/HMP and the acceptor cell with buffer solution, it is a natural intent to balance the chemical potentials between the solutions of both cells due to osmosis (Kruk, Pancerz, & Ptaszek, 2017). This might lead to a solvent movement from the side with smaller Π (donor side) to the side with greater Π (acceptor side) until an equilibrium of the hydraulic pressure of water movement and the diffusion pressure of the solved molecules is reached (Müller et al., 2009). The movement in the system from the donor to the acceptor side could lead to an accelerated initial glucose increase in the acceptor side, leading to higher measured D_Π.

For the concentrations of 2.2 x c^*, D_Π slightly decreased (LMP: 7.1 ± 2.7%, HMP: 21.1 ± 2.0%), and the glucose transfer seemed to be restricted. The difference in Π between the acceptor (46.6 ± 0.0 hPa) and the donor cell (41.6 ± 0.1 hPa) was almost equal for HMP compared to the experiment without external Π (−41.6 ± 0.1 hPa), so we assume that the system was almost balanced. Consequently, pure diffusion without water movement could occur, which leads to lower D_Π compared to D_0.

For LMP at a concentration of 2.2 x c^*, the difference in Π between the acceptor (46.6 ± 0.0 hPa) and the donor cell (77.2 ± 0.0 hPa) was diminished (−30.6 ± 0.0 hPa) compared to the experiment without external Π (−77.2 ± 0.0 hPa). This could lead as well to a more balanced system and reduced movement in the system, where consequently the measured value for D_Π was smaller compared to D_0.

These experiments show a high impact of the external Π on the diffusion of glucose. The external Π and the Π of the fiber solutions form the overall Π of the system and therefore the Π of the fibers also influence the diffusion of glucose. Higher Π of LMP compared to a lower Π of HMP led to lower D_Π and D_0. The higher Π of LMP could also affect the water holding capacity (WHC), which was higher for LMP compared to HMP (cf. Section 3.2).

External Π on the acceptor cell in our experiments represents the Π of the blood plasma, the hydrostatic pressure by water resorption from the small intestine to the mucosa, and/or high osmolality due to solved nutrients in the chyme (Rehner & Daniel, 2010). The degradation of starch during digestion, the active absorption of glucose and sodium via SGLT-1 and the osmotic-coupled resorption of water lead to a dynamic change in the osmotic conditions in the lumen (Rehner et al., 2010). In contrast, the osmotic potential of the fibers should stay balanced during the digestion process in the small intestine, as the dietary fibers are not getting absorbed, and they can therefore develop their effect. High Π of the fiber solutions leads to higher hydration and a higher amount of water held by the fibers (cf. Section 3.2) (Harding et al., 1991). As a higher amount of water is held by the dietary fibers compared to the sample blank, glucose diffusion time increases and thus a reduced transport of glucose in the chyme and a prolonged time until absorption in the small intestine might be expected. Furthermore, since the active transport of glucose and sodium generates an osmotic force, Pappenheimer and Reiss (1987) suggested that hydrophilic molecules such as glucose get entrained by fluid absorption through paracellular flow, leading to enlarged glucose absorption. Enhanced water holding capacity and osmotic pressure of the dietary fibers could slow down the paracellular flow and therefore glucose absorption.

Moreover, a higher osmotic pressure of the chyme, caused by fiber addition, could lead to increased diffusion of water from the intestinal mucosa into the small intestine to establish plasma isotonic conditions in the intestine, which are described to vary from 0.5 to 1 osmol/L (Müller et al., 2009). Consequentially the chyme gets diluted – possibly altering the glucose concentration and absorption (Barker, Cochrane, Corbett, Hunt, & Roberts, 1974; Biesalski & Grimm, 2011). As the osmotic conditions during digestion change dynamically over the digestion and resorption process, the impact of the osmotic pressure on glucose release is yet to be investigated in further detail.

Concluding, we determined that the selected dietary fibers are able to slow down the diffusion of glucose in the matrix of a simulated chyme caused by their hydration properties, concentration and the resulting altered rheological behavior. Similar results were also reported by Goff et al. (2018), Repin, et al. (2017), Adiotomre, et al. (1990) and Rieder et al. (2017). The altered transport of glucose along an osmotic gradient is caused by altered hydration properties. Thereby, the dissolved SDF exhibited increased osmotic pressure and elevated water holding capacities compared to the control, which influenced glucose diffusion. The partly insoluble CF is unable to occupy a large volume of space due to its insolubility and therefore behaved differently compared to the SDF, also observed by F Guillon et al. (2000). The hydration capacity of CF is mainly defined by its capacity of swelling and thus the physical restrictions of the cell wall matrix to occupy water (Elleuch et al., 2011).

The concentration of each SDF type was determined by its c^* and thus the ability of the SDF to alter the physical properties of the solutions, with even the small concentrations defined leading to a significant decrease in glucose diffusion. In order to obtain similar results when consuming a fiber-enriched food item, the concentration of the dietary fiber in the human gut should be equal to or higher than the determined c^*. Therefore, food items containing LMP, for example, with a c^* of 0.9 g/100 g should contain at least 2.8 g/100 g to account for at least threefold dilution by digestive fluids (Borgström, Dahlqvist, Lundh, & Sjövall, 1957).

The extent to which our findings on *in vitro* glucose diffusion influence *in vivo* glucose responses is investigated in the near future by conducting human intervention trials.

4. Conclusions

Our investigations confirmed the need for a detailed study of the physicochemical properties of dietary fibers, particularly the hydration properties, for gaining a deeper understanding of their role in modulating the diffusion of glucose beyond the well-known viscosity-related effect. High hydration properties of the five investigated soluble dietary fibers accompany a decreased diffusion of glucose in the presence of osmotic driving forces *in vitro* by forming osmotic pressure and elevated water holding capacities.

The results implicated that dietary fibers with high hydration properties could contribute to improving postprandial blood glucose response by incorporating them into the daily diet. However, in further studies, the obtained findings should be supplemented with other dietary fibers to show general correlations.

To complete the overall understanding of the health effects of dietary fibers in foods, the presented *in vitro* findings should be complemented by physiological outcomes of further studies comprising *in vivo* experiments.

Author statement

Elisabeth Miehle: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing - original draft.

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All authors have seen and approved the final version of the manuscript being submitted. The manuscript is the authors' original work, has not been published and is not under consideration for publication elsewhere.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

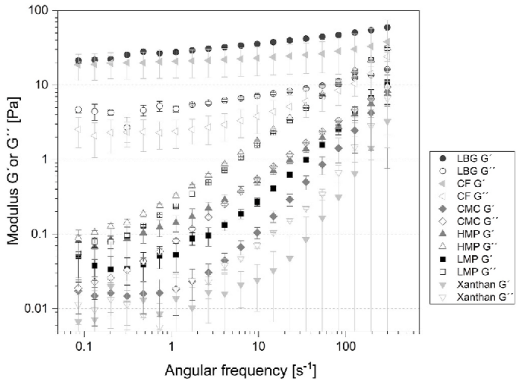

Fig. 5. Frequency spectra of storage (G') and loss (G″) moduli of the solutions with dietary fibers of locust bean gum (LBG), citrus fiber (CF), carboxymethyl cellulose (CMC), high methylester pectin (HMP), low methylester pectin (LMP) and xanthan in the concentration of 2.2 x c^*.

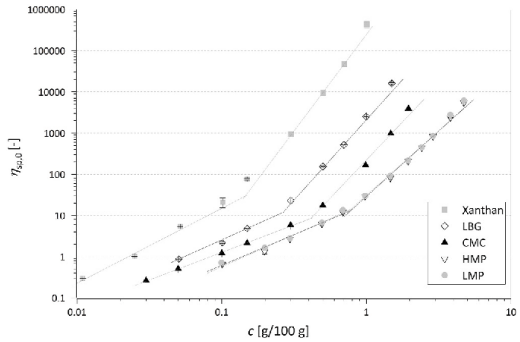

Fig. 6. Double logarithmic plot of zero shear specific viscosity against concentration of the five types of soluble fibers with xanthan, locust bean gum (LBG), carboxymethyl cellulose (CMC), high methylester pectin (HMP) and low methylester pectin (LMP). The determined critical concentration was 0.05 g/100 g for xanthan, 0.24 g/100 g for LBG, 0.43 g/100 g for CMC, 0.88 g/100 g for HMP and 0.92 g/100 g for LMP. Data in the diluted regions and the concentrated regions were both fitted using a power-law equation ($R2 \geq 0.96$). Points correspond to experimental data and lines to fitted data.

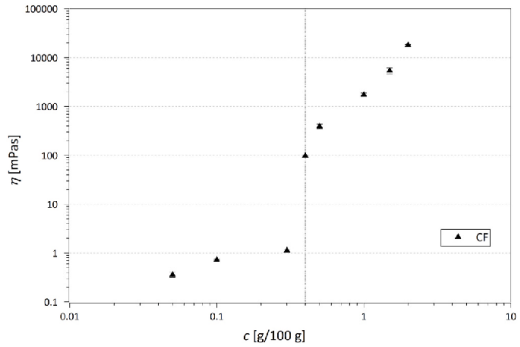

Fig. 7. Viscosities at a shear rate of 0.5 s^{-1} against different concentrations of the partly insoluble citrus fiber (CF). The concentration of 0.40 g/100 g was chosen as the comparable c to the c^* of the soluble fibers.

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Chapter 3: Effects of Food Processing on *In Vitro* Glucose Release of High Methylester Pectin-enriched Doughs

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The incidence of type 2 diabetes is increasing in Western countries, primarily due to the consumption of high-glycemic, highly processed foods that contain low amounts of dietary fiber. Soluble dietary fibers are known to improve blood glucose tolerance. This study aimed to investigate how food processing impacts the *in vitro* glucose release of fiber-rich, high-glycemic model foods. The impact of composition and microstructure on *in vitro* glucose release and starch digestibility was evaluated in untreated, baked (180 °C), and extruded (150 °C, 180 °C) cake doughs, with and without partial enrichment of high methylester pectin. Using TD-NMR, SEM-imaging, rheology, and dietary fiber composition analysis, we found that enriching pectin reduced starch digestibility, altered the food matrix, and increased *in vitro* chyme viscosity. This resulted in decreased *in vitro* glucose release in baked (180°C) and extruded (150°C) products.

However, baking or extrusion cooking increased starch digestibility on the one hand by converting slowly available starch into rapidly available starch and free glucose. On the other hand, the levels of resistant starch were increased by up to five times. Therefore, the differences in glucose release *in vitro* were attributed to a complex interplay between starch digestibility, viscosity, and the food matrix. This could impact the development of new food products that can attenuate the postprandial glycemic response.

Author Contributions:

Miehle, E.: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. Eisner, P.: Conceptualization, Supervision, Writing – review & editing. Bader-Mittermaier, S.: Conceptualization, Project administration, Validation, Funding acquisition, Writing – review & editing

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Effects of food processing on *in vitro* glucose release of high methylester pectin-enriched doughs

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ABSTRACT

The incidence of type 2 diabetes is linked to consuming processed, high-glycemic foods low in dietary fiber. Soluble dietary fibers are known to improve blood glucose tolerance. This study examined the impact of processing on the *in vitro* glucose release of fiber-rich, high-glycemic foods. The impact of composition and microstructure on *in vitro* glucose release and starch digestibility was evaluated in doughs – untreated, baked at 180 °C, and extruded at 150 °C and 180 °C – with partial enrichment of high-methylester pectin. Pectin enrichment decreased starch digestibility, altered the food matrix, and doubled *in vitro* chyme-viscosity resulting in reduced glucose release in baked (180 °C), and extruded (150 °C) products. Baking or extrusion cooking increased starch digestibility – converting slowly into rapidly available starch and free glucose. Additionally, resistant starch levels were enhanced by up to fivefold. The variations in glucose release originated from a complex interplay between starch digestibility, viscosity, and the food matrix.

1. Introduction

Type 2 diabetes is one of the most common lifestyle-related diseases, and the number of sufferers is increasing (Tönnies et al., 2021). High glycemic responses to carbohydrates in foods, also understood via the glycemic index concept (Foster-Powell et al., 2002), are associated with a greater risk of developing type 2 diabetes in the course of life (Al-Jawaldeh & Abbass, 2022). Carbohydrates in foods with high glycemic indices lead to a larger area under the plasma glucose curve during the postprandial period than an equivalent carbohydrate concentration in foods with a low glycemic response (Ludwig, 2002). Both the type of ingredients and the processing can influence the glycemic indices (Fardet et al., 2006). In detail, several *in vivo* studies have shown that the supplementation of starch-containing meals with dietary fibers (DF) can attenuate glycemic responses (Goff et al., 2018; Repin et al., 2017). Thereby, DF lead to a reduction of glucose release, mainly attributed to viscosity formation and interact in the small intestine in three postulated ways: (i) inhibition of amylase activity and thus slowed starch hydrolysis; (ii) decreased diffusion of amylolytic products such as mono-, di-, and oligosaccharides to the small intestinal membrane; and (iii) formation of a barrier layer through mucosal interactions (Brownlee, 2011; Goff et al., 2018; Singh et al., 2010). However, incorporating DF into traditional and commonly consumed products, like bread, cookies, and pasta, presents challenges in various ways, such as changing the properties of the food and the food processing (Cassidy et al., 2018). Thereby, the interaction of DF with water is of significance, as the availability of water impact the interaction between ingredients, the conformational state of biopolymers such as proteins or starches, or the dough structuring by changing its rheological behavior (Serial et al., 2016).

According to Englyst et al. (1992), starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) based on the rate of its digestibility. Elevated contents of SDS fractions are more desirable than RDS fractions as they are related to a retarded release and absorbance of sugars in the small intestine, as well as a gradual increase of postprandial plasma glucose and insulin levels (Lehmann & Robin, 2007). RS is indigestible by the enzymes in the small intestine and is recognized as an insoluble dietary fiber that offers several health benefits, e.g., lowering glycemic response – the basis of the EFSA health claim for RS (Agostoni et al., 2011) –, improved insulin sensitivity, and increased satiety (Lockyer & Nugent, 2017).

Processing of starch or starch-containing products using heat,

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moisture, and shear, such as in extrusion cooking, leads to a depolymerization and phase transition (known as gelatinization), and the highly ordered, crystalline structure of native starch becomes disrupted (Wang & Copeland, 2013). Thereby, the hydroxyl groups of amylose and amylopectin become more exposed and are more likely to form hydrogen bonds with water, thus increasing the susceptibility of starch to enzymatic breakdown. This leads to enhanced digestibility and, thus, a rising plasma glucose and insulin response, respectively (Holm et al., 1988; Parada & Aguilera, 2011; Ye et al., 2018). Limited water conditions in a food system lead to the incomplete swelling and gelatinization of starch granules and therefore restricted digestion (Tester & Sommerville, 2001). Therefore, there is an inverse relationship between the degree of starch gelatinization and the amount of SDS in food products (Englyst et al., 2003). During cooling, gelatinized starch realigns into a more ordered structure, also known as retrogradation (Wang & Copeland, 2013). Thereby, the long-branched chains of amylopectin form double helices that cannot be enzymatically hydrolyzed during digestion, and the retrograded starch counts as RS, more precisely, RS type 3 (Lockyer & Nugent, 2017).

Besides the composition, food processing can also affect the structure of the food and therefore influence the glycemic response (Priyadarshini et al., 2022). There are several studies on the effects of food processing as baking or extrusion cooking on glycemic response, where different processing methods decreased (e.g. Delcour & Hoseney, 2010; Guha et al., 1997; Papakonstantinou et al., 2022) or enhanced (e.g. Alonso et al., 2000; Fredriksson et al., 1998) the glycemic response.

However, to date, only a few studies have addressed the combined impact of food processing and DF addition on the glycemic response and starch digestibility (Cesbron-Lavau et al., 2021; Ferrer-Mairal et al., 2012; Fujiwara et al., 2017; Papakonstantinou et al., 2022). Thereby, the impact of DF addition on the glycemic response of different types of products, such as muffins, spaghetti, and bread, was compared, but only minor conclusions about the composition and structure of the food were drawn. In addition, the doughs of the products differed in composition, particularly in their glucose content, making it difficult to differentiate between the impacts of fiber addition or processing on glucose release.

To underline the positive impact of pectin enrichment in processed foods, pectin enriched doughs with high glycemic load were processed either by baking or by extrusion cooking at different temperatures in the present study. Therefore, our main objectives were to elucidate the complex interactions of pectin enrichment and processing on *in vitro* glucose release as well as on starch digestibility by recording the changes in composition, microstructure, and water mobility.

2. Materials and methods

2.1. Materials

High methylester pectin (HMP) (Classic CU 201) with an esterification degree of 71 % and a soluble dietary fiber content of ≥ 97 g/100 g in dry matter was supplied by Herbstreith & Fox KG (Neuenbürg/Württemberg, Germany). Alpha-amylase from human saliva (Type IX-A, 1,000–3,000 units/mg protein; A0521), pancreatin from porcine pancreas (8 × USP specifications; P7545), pepsin from porcine gastric mucosa (3200–4500 units/mg protein; P6887), bile extract from porcine (B8631), and polyethylene glycol (PEG) (M_W 20 kDa) were purchased from Merck KGaA (Darmstadt, Germany). Sodium azide (NaN_3) and all other reagents and chemicals used were of analytical grade and supplied by Chemsolute (Th. Geyer GmbH & Co. KG, Renningen, Germany). Carrez reagent I and Carrez reagent II were prepared by dissolving 1.06 g of $K_4[Fe(CN)_6]\cdot 3H_2O$ or 2.88 g of $ZnSO_4\cdot 7H_2O$ in 10 mL of distilled H_2O, respectively. The ingredients for the dough preparation such as glucose monohydrate (Roquette GmbH, Frankfurt, Germany), all-purpose wheat flour (Aurora Mühlen GmbH, Hamburg, Germany, starch content 73.7 ± 3.9 g/100 g), baking powder (diphosphate, sodium carbonate, starch) (Dr. August Oetker Nahrungsmittel KG, Bielefeld, Germany), sunflower oil (Nestlé Deutschland AG, Frankfurt, Germany), salt (Südwestdeutsche Salzwerke AG, Bad-Reichenhall, Germany), and pasteurized egg (EIPRO-Vermarktung GmbH & Co. KG, Lohne, Germany) were purchased locally.

2.2. Dough preparation and food processing

The dough, the basis for all products, was based on a commercial muffin recipe, whereby glucose replaced sucrose. All samples contained all-purpose wheat flour, glucose, baking powder, salt, sunflower oil, egg, and water. The pectin-enriched samples also contained pectin at a concentration two times higher than the critical concentration (c^*) (which amounted to 1.9 %, Table supplementary material) and adjusted water content. The c^* of 0.9 g/100 g was previously determined by Miehle et al. (2022), and the concentration 2 × c^* showed significant retardation in the *in vitro* glucose release of solutions (Miehle et al., 2022). The exact ingredient concentrations of the control (DoughC) and pectin-enriched doughs (DoughP) are provided in the table in the supplementary material. The wet and dry ingredients were mixed separately and whipped in a universal kitchen mixer (Hobart N 50, Illinois Tool Works, Illinois, USA) until the dry ingredients were wetted and a smooth dough was formed. The chemical composition of both doughs (DoughC and DoughP) was similar, with a protein content of 6.4 %, a fat content of 12 %, and an ash content of 1 %.

The dough was baked in a universal kitchen oven (EEBK 6400.8 MX-G, Küppersbusch Hausgeräte GmbH, Gelsenkirchen, Germany) at 180 °C for 30–40 min for the muffin samples. The dough for the extruded samples was processed using a co-rotating, intermeshing twin screw extruder (Haake Rheocord, Thermo Fisher Scientific Inc., Waltham, MA, USA). The extruder was equipped with a screw diameter of 16 mm, a smooth barrel, and a length-diameter ratio of 25:1. A long die with dimensions of 19 × 2 × 210 mm ($W \times H \times L$) was attached to the end of the extruder, which was kept at a constant temperature of 80 °C using water for cooling. The screw profile was built using various screw elements described previously by Osen et al. (2014). The dough was placed directly into the center of the feed port during operation, and each dough was extruded at two different barrel temperatures, which were 150 °C and 180 °C.

For extrusion cooking, the barrel was divided into five temperature-controlled zones. The first four zones increased stepwise from 30 °C to 50 °C, 70 °C, and then 100 °C, while the last zone (fifth) was tempered to the desired cooking temperature of 150 °C or 180 °C, respectively. The screw speed was set constantly at 250 rpm. In order to monitor the extrusion process, the pressure (bar) in front of the cooling die was recorded in-line using the Polylab software (Thermo Fisher Scientific Inc., Waltham, MA, USA). The processing pressures in the barrel increased with respect to dough viscosities (data not shown). Once the extruder reached steady-state conditions, samples were collected.

After extrusion cooking and baking, the samples were cooled to room temperature and lyophilized prior to analysis except for determining the water mobility by ^{1}H NMR. For lyophilization, the samples were frozen at −50 °C and lyophilized at 5–45 °C for 72 h at a pressure of 103 Pa (freeze dryer beta 1–8, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Subsequently, the freeze-dried samples were ground to pass through a 500 μm screen using a ZM-200 mill (Retsch GmbH, Haan, Germany), except for observation by SEM. The dry matter content (DM) of the lyophilized samples ranged between 93.3 % and 99.2 %.

The DM of the fresh samples was analyzed immediately after cooling to room temperature and ranged from 81.5 ± 0.1 % for MuffinC to 77.5 ± 0.1 % for MuffinP. In contrast, the extruded samples ranged from 79.3 ± 0.3 % for Extr150C to 77.8 ± 0.4 % for Extr150P, and 79.2 ± 0.7 % for Extr180C to 78.3 ± 0.7 % for Extr180P.

2.3. Dietary fiber composition

The soluble (SDF) and insoluble dietary fiber content (IDF) of the lyophilized and ground samples were determined using an enzymatic–gravimetric test kit (K-TDFR 04/17, Megazyme, Bray, Ireland) according to AOAC 991.43. The total dietary fiber content (TDF = IDF + SDF) and the insoluble proportion (IDF/TDF) were calculated. The dry matter and ash contents were analyzed on a thermogravimetric basis at 105 °C and 550 °C using a TGA 601 analyzer (Leco Instrumente GmbH, Mönchengladbach, Germany) following the official methods, L 18.00–12 and L 15.00–7, respectively. Nitrogen (N) (AOAC Official Method 968.06) was determined according to the Dumas combustion method using a Nitrogen Analyzer TruMac N (LECO Instrumente GmbH, Mönchengladbach, Germany) and converted to protein content at a conversion factor of N × 6.25 (Chemists, 2016).

2.4. Scanning electron microscopy (SEM)

The lyophilized sample (without milling) was incised with a razor blade and torn before SEM observation. A fracture of the sample was placed onto the surface of double-sided Cu adhesive tape, mounted on a sample carrier, and then sputter coated with gold from two directions for 1 min (U = 5 kV und I ~ 10–15 mA) in an argon atmosphere (17.3 Pa). The digital images were obtained at an accelerating voltage of 7.5 kV and a magnification of 500-fold using a scanning electron microscope (JSM F7200, JEOL Ltd., Tokyo, Japan) with a secondary electron detector (SED) and a backscattered electron detector (BED), to image the surface of the sample and below the surface (Reimer, 2000).

2.5. Time domain ^{1}NMR relaxation measurements

Proton relaxation measurements were performed in triplicate using a low field ^{1}H NMR (mq 20 NMR analyzer, Bruker BioSpin GmbH, Rheinstetten, Germany). The freshly prepared muffin and extruded samples were ground using a knife mill (600 rpm, 20–40 s), weight in 8 mm closed bottom inlays for 10 mm NMR tubes, and sealed to avoid further changes in moisture. The weight of the measured samples was standardized to 1 g for comparability purposes. The tubes were pre-heated to 40 °C before measurement in the instrument with the same probe head temperature. The spin–spin relaxation time (T_2) was measured based on the simultaneously recorded free induction decay (FID) and the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence. The measurement settings were adapted from Alpers et al. (2022). The sampling rate for acquiring the FID signal was one point per 1.36 μs. For the CPMG sequence, 15,000 echoes were acquired with an echo time of 175 μs between the 90° and 180° pulses. Sixteen scans were performed at a recycle delay time of 1 s. The data obtained were processed using an exponential decay function, as performed by Rondeau-Mouro et al. (2015), whereby four proton populations were detected according to their mobility in the dough, and the data were compared by their transverse relaxation times ($T_{2,\ i}$) and intensities ($A_{2,\ i}$). The first population (A, with low mobility) was obtained from the FID, and the three further populations (B, C, and D) were obtained with higher mobility from the CPMG measurement. The detected $T_{2,i}$ agreed with previous ^{1}H NMR data of biscuit doughs (Assifaoui et al., 2006; Serial et al., 2016).

2.6. Rheological investigation

The rheological characteristics were measured at a constant temperature of 37.0 ± 0.1 °C using a rotational rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria). Shear viscosity was determined at the beginning and the end of the small intestine *in vitro* digestion (see Section 2.9) using a parallel plate geometry (diameter: 25 mm, shear gap: 1 mm, PP25/TG-SN23582). The samples were pre-sheared at 2 s^{-1} for 10 s and then allowed to rest for 60 s. Viscosity was monitored using a steady shear mode in a logarithmic scale ranging from 0.1 to 500 s^{-1} (comprising 19 measurement points, with measurement point duration from 20 to 10 s). All measurements were conducted in at least one duplicate for each separately prepared sample.

2.7. In vitro digestion

In vitro digestion of the lyophilized samples was performed before the determination of shear viscosity (Section 2.6), the digestible and resistant starch determination (Section 2.8), and *in vitro* glucose release determination (Section 2.9). The static simulation of oral, gastric, and small intestinal digestion was performed with slight modifications to the harmonized INFOGEST protocol (Minekus et al., 2014). The *in vitro* digested control sample obtained a final dry matter concentration of 12.5 %, and the *in vitro* digested pectin-enriched sample of 12.8 % (factor of 1.024 higher) in order to achieve the same concentration of dough ingredients, especially glucose and starch (see table supplementary material). NaN_3 was added as an antimicrobial agent to reach a concentration of 0.1 g/100 g in the final digestion mixture. In the oral phase, lyophilized and ground samples were mixed with simulated salivary fluid (37 °C) containing α-amylase (75 U mL^{-1} in the final digestion mixture) (ratio 50:50 *w/w*) and kept for 2 min in a water shaking bath (37 ± 0.1 °C, 100 rpm). For the gastric phase, the pH level of the sample was lowered to 3.0 ± 0.1 (using 3 M HCl), and simulated gastric fluid (37 °C) (ratio 50:50 *w/w*) containing pepsin (2000 U mL^{-1} in the final digestion mixture) was added. The mixture was incubated (37.0 ± 0.1 °C, 100 rpm) for 2 h. For the intestinal phase, the pH was increased to 7.0 ± 0.1 using 1 M NaOH. Subsequently, the sample was mixed with simulated intestinal fluid (37 °C) (ratio 50:50 *w/w*) containing pancreatin and bile acid salts to reach an enzyme activity of 100 U mL^{-1} for trypsin, 250 UmL^{-1} for alpha-amylase (15.38 mg pancreatin/mL final digestion mixture) and 10 mmol/L of bile acid salts in the final digestion mixture, respectively. An aliquot of around 3 mL was taken for shear viscosity determination at the beginning of the intestinal digestion phase (Section 2.6). For the *in vitro* glucose release experiments (Section 2.9), 4 mL of the digesta was transferred into the donor cell of the side-by-side system (SES GmbH, Bechenheim, Germany) and incubated (37.0 ± 0.1 °C, 100 rpm) for 4 h. The rest of the sample was used to determine starch digestibility (Section 2.8). Shear viscosity was again determined at the end of the simulated small intestine *in vitro* digestion.

2.8. Digestible and resistant starch

The digestible and resistant starch contents were determined using an enzymatic test kit (K-DSTRS 11/19, Megazyme, Bray, Ireland), based on a method described by Englyst et al. (1992), with slight modifications. An aliquot of 100 μL of each sample was used to determine the digestible starch content (rapidly digestible starch (RDS), slowly digestible starch (SDS), as well as total starch (TS)) and the free glucose content. For this, the *in vitro* digested sample solution prepared according to the standardized INFOGEST digestion model (Section 2.7) was mixed with 900 μL of 0.05 M acetic acid after 0 min, 20 min, 120 min, and 240 min of *in vitro* digestion to stop further digestion. The levels of free glucose, RDS, SDS, and TS after the incubation times were determined, after centrifugation (13,000 rpm, 5 min at room temperature). The supernatant was used for analysis. Before analysis, a modified Carrez precipitation (Carrez, 1908) was performed to remove interfering substances from the sample. The supernatant (100 μL) was transferred into fresh reaction tubes (2 mL) containing distilled water (dH_2O) (1 mL). Carrez reagent I and Carrez reagent II (100 μL each), NaOH (200 μL, 100 mM), and dH_2O (500 μL) were added, and the sample was vortexed after the addition of each of the chemicals. Precipitation of the interfering substances was conducted during incubation (20 °C, 20 min), and the precipitate was removed by a centrifugation step (13,000 rpm, 5 min). The supernatant (50 μL) was analyzed for glucose content following the kit instructions based on the GOPOD (glucose oxidase/

peroxidase) assay by adding 50 μL amyloglucosidase (AMG) solution (110 U/mL – on soluble starch at pH 4.5 and 40 °C), in 0.1 M sodium acetate buffer at pH 4.5), which was incubated at 50 °C for 30 min while shaking at 300 rpm. 1.5 mL GOPOD reagent (glucose oxidase, peroxidase and 4-aminoantipyrine in reagent buffer with p-hydroxybenzoic acid and sodium azide (0.0045 % (w/v)) at pH 7.4) was added and incubated at 50 °C for 30 min while shaking at 300 rpm. A blank was prepared by adding 100 μL sodium acetate buffer (0.1 M, pH 4.5) with 1.5 mL GOPOD reagent. D-glucose standards were prepared in duplicate in concentrations of 0.05, 0.1, 0.5, and 1.0 g/L dissolved in 0.05 M acetic acid. 50 μL of the standard solution was mixed with 50 μL sodium acetate buffer (0.1 M, pH 4.5) and 1.5 mL GOPOD reagent. For resistant starch determination, a sample (1 mL) was taken after 240 min and washed with an equivalent volume of ethanol (95 % (v/v)). After centrifugation (4000 rpm, 10 min), the pellet was rewashed four times with 1 mL of aqueous ethanol (50 % (v/v)). After the last centrifugation step, the pellet was re-suspended with 500 μL of cold 1.7 M NaOH at 1 °C for 20 min at 300 rpm. 2 mL of buffer (1.0 M sodium acetate buffer, pH 3.8) was added and immediately after stirring 25 μL AMG solution (3300 U/mL – on soluble starch at pH 4.5 and 40 °C) was added and incubated at 50 °C for 30 min at 300 rpm. 2 mL of the dispersion was centrifuted (13,000 rpm, 5 min) and 50 μL of the supernatant was mixed with 1.5 mL of GOPOD reagent and incubated (20 min, 50 °C, 300 rpm). The color change of the reaction was determined at 510 nm using a microplate reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany), and glucose concentration was calculated based on a standard curve with four measurement points. All measurements were conducted in at least duplicate for each separately obtained sample.

2.9. In vitro glucose release

The *in vitro* glucose release measurements were conducted using a side-by-side cell system (SES GmbH, Bechenheim, Germany) using a donor and receptor cell separated by a dialysis membrane as previously described by Miehle et al. (2021), with slight modifications. Phosphate buffer (4 mL, 74.6 mmol/L, with 0.1 g/100 g NaN_3, pH 7.0) was filled into the receptor cell and stirred at 300 rpm. The glucose release experiment started immediately after transferring 4 mL of the dispersion at the beginning of the small intestinal *in vitro* phase (section 2.7) to the donor cell, which was stirred at 100 rpm. An aliquot of 100 μL samples of the receptor fluid was taken after 10 min, 20 min, 60 min, 120 min, 180 min, and 240 min, respectively, to determine the glucose concentration and immediately replaced by 100 μL of phosphate buffer solution. Each sample was digested at least twice, and the release experiments of each sample were conducted in duplicate using two different side-by-side cells, leading to a total of four separate determinations. The glucose concentration of the receptor fluid samples was analyzed utilizing an enzymatic test kit for D-glucose (D-Glucose-Food & Feed Analysis, R-Biopharm AG, Darmstadt, Germany) and conducted as described by Miehle et al. (2021).

2.10. In vitro glucose release kinetics

A first-order equation, according to Goñi et al. (1997), was applied to describe the kinetics of the glucose release over time, with slight modifications:

$$c_t\ (\mathrm{mmol/L}) = c_\infty[\,1-\exp(-kt)] \quad (1)$$

where c_t is the glucose concentration at the corresponding time t, c_∞ is the maximal glucose concentration at equilibrium, t is the time in hours, and k is the apparent permeability rate constant.

According to Eq. (2) the area under the release curve (AUC) was calculated according to Ferrer-Mairal et al. (2012); and Goñi et al. (1997):

$$\mathrm{AUC\ (mmol*h/L)} = c_f(t_f - t_0) - (c_f/k)\,[\,1-\exp(-k(t_f-t_0)\,] \quad (2)$$

where c_f is the maximal glucose concentration after four hours, t_f is the final time of the release experiments (in our case 4 h), and t_0 is the initial time (0 h).

The calculated glucose release index (GRI) was the percentage of the ratio of the AUC of each sample (AUC) by the corresponding AUC of the reference sample (control dough) (AUC_C).

$$\mathrm{GRI\ (\%)} = 100 * (\mathrm{AUC/AUC_C}) \quad (3)$$

2.11. Statistical analysis

Unless stated otherwise, all measurements were performed in triplicate. Results are expressed as mean ± standard deviation. Statistical evaluation was conducted using SigmaPlot 12.0 for Windows (Systat Software GmbH, Erkrath, Germany). A one-way analysis of variances (ANOVA) followed by Tukey's test was used to detect significant differences between groups on a significance level of $\alpha = 0.05$. The values were tested for homogeneity of variance (Levene's test) and normal distribution (Shapiro-Wilk test) beforehand. Nonlinear regressions were calculated using OriginPro 2018 for Windows (Origin Lab Corporation, USA).

3. Results and discussion

3.1. Dietary fiber fractions

Images of the eight investigated samples enriched with pectin (P) and the control (C) of doughs (Dough), baked doughs at 180 °C (Muffin), and the extruded doughs at 150 °C and at 180 °C barrel temperatures (Extr150, Extr180) are shown in Table 1 along with the composition of dietary fibers – soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF = IDF + SDF).

Processing increased the amount of IDF, almost doubling from the pectin-enriched dough (DoughP) to the baked and extruded pectin samples (MuffinP, Extr150P, Extr180P) and from the control dough (DoughC) to the extruded control samples (Extr150C, Extr180C), respectively. This increase was also observed in the increasing ratio of IDF/TDF within the control and pectin samples. Simultaneously, SDF slightly increased by processing the control and pectin-enriched doughs. Enriching the samples with pectin led to an increase in TDF and a significant increase ($p \leq 0.05$) in SDF compared to the control. In contrast, the amount of IDF was unaffected. Because of the rise in SDF, the ratio of IDF/TDF decreased in the pectin-enriched samples compared to the respective control. This result was expected, since the pectin used has a high solubility (Bai et al., 2021; Miehle et al., 2022).

The slight increase in SDF due to processing could be due to the transformation of some IDF to SDF. Several studies have shown solubilization of the IDF fraction by the rupture of large molecules due to high-temperature processing as extrusion cooking, causing an increase in SDF and a slight decrease in TDF content, depending on the treatment intensity (Garcia-Amezquita et al., 2019; Huang & Ma, 2016; Larrea et al., 2005; Méndez-García et al., 2011). Solubilization of insoluble pectin compounds was observed by breaking the lateral linkages, whereas the rhamnogalacturonan "backbone" was unaffected (Larrea et al., 2005). Further solubilization was observed by de-esterification of pectin compounds by thermal treatment and beta-elimination degradation reactions (Benítez et al., 2011; Femenia et al., 1999). As the pectin used in the current study is highly soluble (Bai et al., 2021; Nawaz et al., 2021), a transition from insoluble to soluble pectin is rather unlikely, and the increase in SDF could more likely originate from further IDF contained in the dough, e.g., from wheat flour. A slight increase in TDF with a shift from IDF to SDF in extruded white wheat flour was also reported by Björck et al. (1984). The observed increase in IDF due to processing

Table 1
Images and composition of dietary fiber (insoluble dietary fiber (IDF), soluble dietary fiber (SDF), total dietary fiber (TDF = IDF + SDF)) of doughs (Dough), baked doughs at 180 °C (Muffin) extruded doughs at 150 °C and 180 °C barrel temperatures (Extr150, Extr180) enriched with pectin (P) and the control (C); expressed as g/100 g on dry matter of the sample.

	Samples	IDF [g/100 g]	SDF [g/100 g]	TDF [g/100 g]	IDF/TDF [%]
DoughC		1 ± 0.1 ^{a,b}	1.4 ± 0.6 ^{a}	2.4 ± 0.2 ^{a}	41.8 ± 8.7^{b,c}
DoughP		0.7 ± 0.2 ^{a}	3.6 ± 0.1^{b}	4.3 ± 0.1^{c}	16.8 ± 4.3 ^{a}
MuffinC		1.4 ± 0 ^{a,b,c}	1.8 ± 0.4 ^{a}	3.2 ± 0.4^{b,a}	42.8 ± 2.8^{b,c}
MuffinP		1.4 ± 0.1 ^{a,b,c}	4.4 ± 0.5^{b}	5.8 ± 0.5 ^{d}	23.8 ± 3.2 ^{a,b}
Extr150C		1.9 ± 0.4^{c}	1.8 ± 0.2 ^{a}	3.7 ± 0.4^{b,c}	52.2 ± 13.1^{c}
Extr150P		1.5 ± 0.4^{b,c}	4.4 ± 0.4^{b}	5.9 ± 0.4 ^{d}	25.4 ± 8.6 ^{a,b}
Extr180C		1.7 ± 0.1^{b,c}	1.8 ± 0.2 ^{a}	3.5 ± 0.2^{b,c}	49.0 ± 3.8^{c}
Extr180P		1.5 ± 0.4^{b,c}	4.3 ± 0.2^{b}	5.8 ± 0.4 ^{d}	26.2 ± 7.2 ^{a,b}

Different letters indicate significant differences within each column on a $p \leq 0.05$ level basis.

could originate from forming type 3 resistant starch, which is observed by thermal treatment and extrusion of starch-containing products (Vasanthan et al., 2002). The formation of resistant starch and the digestibility of starch will be discussed in more detail in the following section (section 3.2). The differentiation of DF into soluble and insoluble in terms of their functionality is also controversially discussed. Gidley and Yakubov (2019) suggest that the molecule size and the local density/concentration of DF are the key characteristics, which are expected to be related to nutritional functionality such as altered glucose release.

The pectin-enriched products (MuffinP, Extr150P, and Extr180P) contained at least 4.5 g/100 g TDF in the fresh (undried) samples. They could thus be claimed as a "source of dietary fiber", whereby the minimum amount of fiber in the product needs to be 3 g/100 g according to EU Regulation (EC) No. 1924/2006 on nutrition and health claims for foods (Parliament, 2006).

3.2. *In vitro* starch digestibility and SEM images

3.2.1. *In vitro* starch digestibility

In vitro starch digestibility, amount of resistant starch and free glucose of the eight samples are shown in Fig. 1a, and b.

3.2.1.1. Impact of processing conditions. The amount of total free glucose increased with the intensity of processing, with the highest content of 72.6 g/100 g for the control extrudate at 180 °C (Extr180C) and the lowest content in both doughs (Fig. 1b). Simultaneous with the rise of free glucose, the total content of digestible starches – rapidly digestible starch (RDS), slowly digestible starch (SDS), and total digestible starch (TDS) – decreased compared to the contents in both doughs, except for MuffinP (Fig. 1a). Furthermore, processing increased the content of resistant starch (RS) (Fig. 1a), with the highest rise from 0.3 g/100 g in DoughC to 1.5 g/100 g in Extr180C, which resulted in increased insoluble dietary fiber and total dietary fiber contents (Table 1).

The higher content of free glucose determined at the beginning of the intestinal phase might be attributed to the two potential modes of action: Firstly, higher free glucose contents could arise from starch hydrolysis during processing or secondly by increased susceptibility of starch for α-amylase hydrolysis during the first phase of the *in vitro* digestion. A higher degree of starch digestibility in the processed samples might be due to the gelatinization of the starch during heating in the presence of excess water, which made it more readily available for enzymatic degradation (Singh et al., 2010; Wang & Copeland, 2013). The gelatinization of starch also raised the viscosity of the samples (Wang & Copeland, 2013), which is corroborated by the findings of our study (see Section 3.4). Extrusion cooking is known to increase starch digestibility to a greater extent than other processing methods (Singh et al., 2010). The increased shearing action and kneading in the extruder barrel is ascribed to a loss of structural integrity of the starch granules and the formation of a porous, honeycomb-like structure, which increases the susceptibility to enzymatic degradation (Ye et al., 2018). The increase of resistant starch due to processing can originate from several procedures. In previous investigations, the higher gelatinization due to

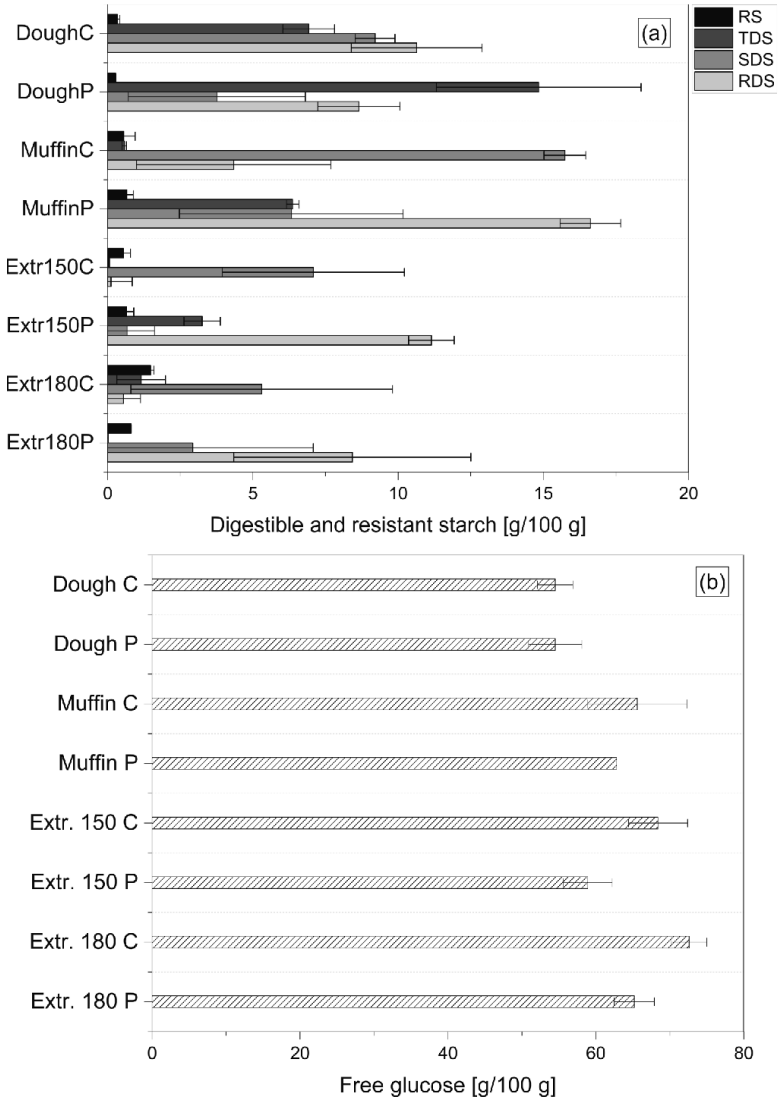

Fig. 1. Contents of digestible starch, resistant starch, and free glucose. Digestible and resistant starch (a) and free glucose (b) content of the untreated doughs (Dough), baked doughs at 180 °C (Muffin), extruded doughs at 150 °C, and 180 °C barrel temperatures (Extr150, Extr180) enriched with pectin (P) and the control (C); rapidly digestible starch (RDS) is hydrolyzed starch after 20 min, slowly digestible starch (SDS) after 120 min, total digestible starch (TDS) after 4 h and resistant starch (RS) remaining starch after 4 h of hydrolysis; free glucose is the glucose content before starch hydrolysis in the small intestinal phase; expressed as g/100 g on dry matter of the sample.

processing led to a higher amount of starch degradation and to increased RS formation (Arcila & Rose, 2015), which was also observed in our investigations (Fig. 1a, Table 1). Besides RS formation due to retrogradation, also amylose–lipid complexes might be formed. These are not digestible and might contribute to RS as they are assigned as RS type 5 (Lockyer & Nugent, 2017; Ye et al., 2018). Extrusion cooking can moreover form starch with lower accessibility due to starch-protein interactions and therefore a rise in resistant starch content. Thereby, strong interactions between protein and starch might occur upon protein denaturation and exposure of reactive side chains (Guha et al., 1997). Those interactions of starch with lipids and/or proteins might also be responsible for the overall lower total starch contents determined in the extruded samples.

3.2.1.2. Impact of pectin enrichment. Adding pectin to the dough and processing the enriched dough led to a lower digestibility of the starch compared to the respective control and a lower amount of RS in Extr180P compared to Extr180C. Thereby, the content of TDS was

higher in all pectin-containing samples than in the respective control – except for Extr180C – and the free glucose content was less than in the control samples. Processing pectin-containing doughs led to a reduction in TDS and SDS contents and increased amounts of both RDS and free glucose. The processed control doughs exhibited higher contents of only free glucose and reduced contents of TDS and SDS.

The addition of pectin can have several effects on starch digestibility. First, pectin as a hydrocolloid can interact with excess water, thus making it less available to starch granulates for gelatinization, as also observed in previous research (Chaisawang & Suphantharika, 2005; Kaur et al., 2008; Song et al., 2006). Gelatinization increases the rate of enzymatic degradation (Wang & Copeland, 2013). The moisture content of the pectin-enriched samples was on average 1.5 % higher than the control samples (DM content Section 2.2). Thus, the minor increase in moisture content of the samples compared to the respective references is judged to be negligible, but the water was less accessible for starch gelatinization (refer to Section 3.3). Therefore, the lower gelatinization due to pectin enrichment led to lower starch digestibility compared to the control samples. Furthermore, the high interaction level of the side chains of pectin with water led to an increase in viscosity (Table 3), which corroborates the results of Morris et al. (1981). Increasing viscosity of the pectin samples could also be related to the slowing down of starch hydrolysis by either reducing the diffusion of enzymes and substrates or by decreasing the diffusion of the hydrolysis products, which then can lead to a higher concentration of amylase-inhibiting substances (Fabek & Goff, 2015; Koh et al., 2009). Similar to our observations, Fabek and Goff (2015) found that *in vitro* digested starch granules from higher viscosity digesta were less degraded than starch granules from low viscosity digesta.

Second, electrostatic interactions between the cationic starch and anionic gums as pectin (Chaisawang & Suphantharika, 2005), the formation of hydrogen bonding between pectin and amylopectin (Sasaki et al., 2015), or the formation of a continuous network by pectin around starch granules (Koh et al., 2009) could also lower starch gelatinization and reduce the digestibility of starches. Wu et al. (2016) observed that the digestion of starch by salivary amylase was delayed in the presence of pectin, which is also supported by our observation of the formation of less free glucose at the beginning of the intestinal phase in the pectin-containing samples. The lower RS quantity in the Extr180P sample than in the control (Extr180C) could originate from the lower overall quantity of gelatinized starch available for retrogradation, as well as the retarded retrogradation of amylose due to pectin-amylose interaction (Rojas et al., 1999). Furthermore, a high water content available for starch leads to a high rate and extend of retrogradation (Wang & Copeland, 2013). The amount of water available for starch was lower in the pectin samples (refer to Section 3.3), despite the fact that pectin samples had a higher moisture content than the control samples (DM content Section 2.2). Less available water for starch is known to lead to a lower amount of starch retrogradation (Wang & Copeland, 2013). The lower amount of retrograded starch subsequently leads to reduced formation of RS. In summary, it can be concluded that pectin enrichment might lead to a lower amount of gelatinized starch after processing, especially at high temperatures during extrusion cooking resulting in lower starch digestibility and, a lower amount of RS.

Altogether, we observed an impact of food processing and pectin enrichment on the digestibility of the starch in our model food recipes. Thereby, food processing showed a more substantial impact on starch digestibility than the enrichment with pectin. Similar results were also previously observed by Fujiwara et al. (2017) and Garsetti et al. (2005).

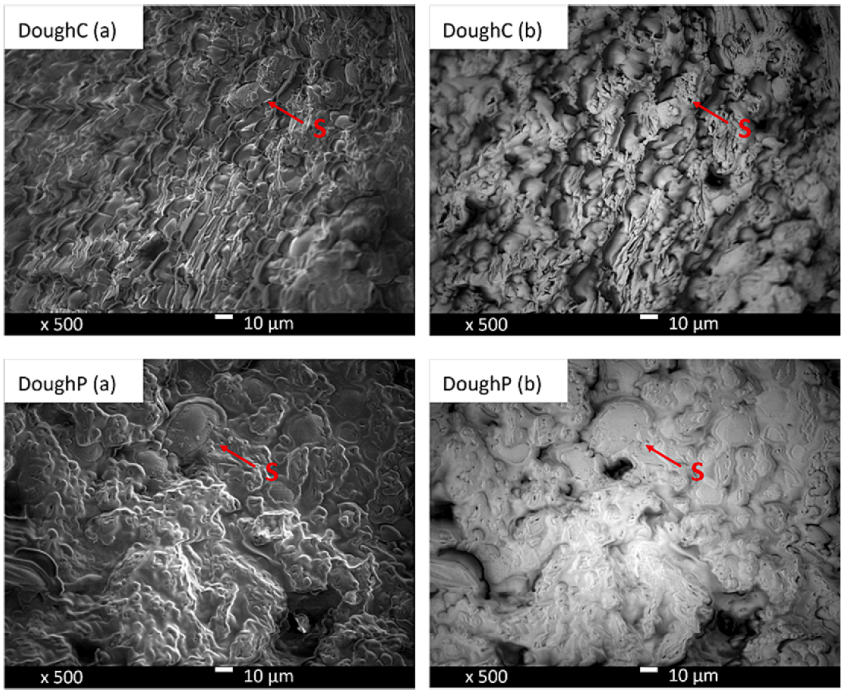

Fig. 2. Scanning electron microscopy images. Scanning electron microscopy images (x500) obtained using two different detectors at the same spot on the sample: the secondary electron detector (SED) (a) and the backscattered electron detector (BED) (b) for doughs (Dough), baked doughs at 180 °C (Muffin) extruded doughs at 150 °C and 180 °C barrel temperatures (Extr150, Extr180) enriched with pectin (P) and the control (C); intact starch granules (S) are marked by arrows.

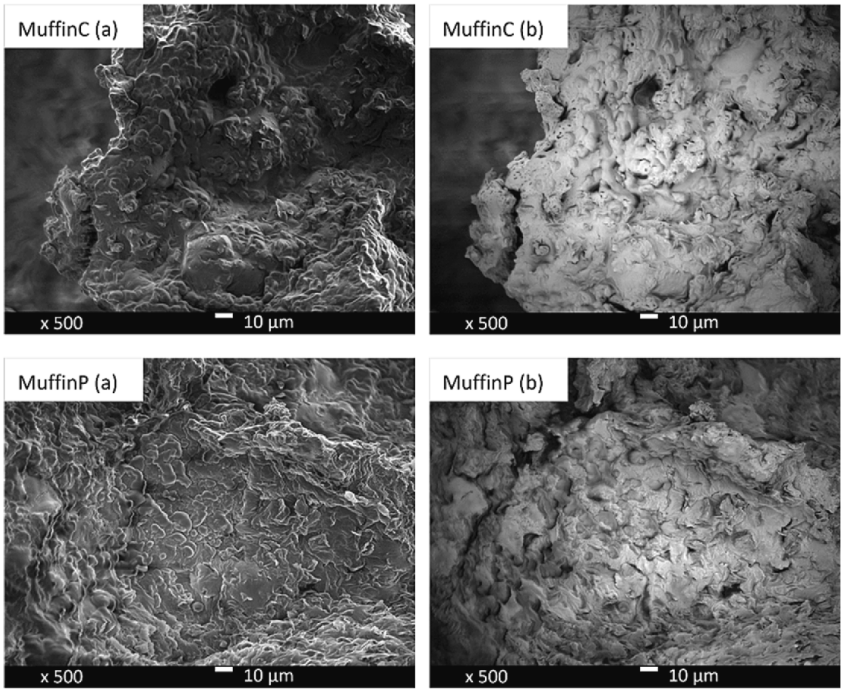

Fig. 2. (*continued*).

3.2.2. SEM images

The breakdown of the starch granules and the formation of different networks due to processing and fiber enrichment was also observed in the images obtained by SEM (scanning electron microscopy), shown in Fig. 2. In both dough samples (DoughC and DoughP), intact starch granules were embedded in a smooth network. The starch granules were oval, with a smooth surface, and of greater size in the pectin-enriched dough. Baking at 180 °C caused both samples (MuffinC and MuffinP) to exhibit a rougher matrix and a decrease in starch granule size, with the pectin samples again exhibiting larger starch granules as well as a rougher, less smooth matrix than the control. For the control sample, extrusion (Extr150C and Extr180C) led to an intense breakdown and melting of the granules, with a very smooth and homogenous network and no visible starch granules in the sample at the higher barrel temperature of 180 °C (Extr180C), which has also been observed by Cesbron-Lavau et al. (2021). Extruded pectin-enriched samples (Extr150P and Extr180P) were less homogenous in the matrix, and small starch granules were still visible compared to the extruded control samples.

The larger sizes of starch granules observed in the pectin-enriched dough (DoughP) could be attributed to an aggregation of starch granules in the presence of pectin, as was also observed by several authors using other soluble DF (Chaisawang & Suphantharika, 2005; Fabek & Goff, 2015). The more significant starch granule sizes in DoughP might have, in addition to the viscosity, contributed to the greater quantity of TDS and a smaller quantity of RDS compared to the control (Fig. 1a). Therefore, the SEM images highlighted the observation that thermal processing increased the digestibility of starch due to increased starch gelatinization, and, in contrast, fiber addition lowered the digestibility of starch due to a lower gelatinization. The gelatinization of starch was further investigated by measuring water mobility in the samples in the next section (Section 3.3).

In order to visualize precise molecular relationships, the obtained SEM images would have to be supplemented by other methods such as confocal laser scanning microscopy (CLSM).

3.3. Mobility and amount of water

To further investigate the amount of gelatinized starch, which is strongly related to the digestibility of starch as discussed in the previous sections, we examined the amount and mobility of water interacting with starch, proteins, sugar, and fiber using ^{1}H NMR measurement.

3.3.1. Impact of processing conditions

Table 2 illustrates the impact of baking and extrusion of the pectin-enriched doughs and the control doughs on the mobility (relaxation times) and amount (intensities) in the four detected proton populations (A, B, C, and D). Both doughs exhibited similar mobility and amount in each population. Baking and extrusion diminished the amount in each population and partially reduced mobility. Thereby, MuffinC showed the lowest amount of all four proton populations ($A_{2,1}$: 5.07 %, $A_{2,2}$: 6.65 %, $A_{2,3}$: 9.08 %, $A_{2,4}$: 2.62 %) and the lowest mobility in populations A, B, and C ($T_{2,1}$: 0.05 ms, $T_{2,2}$: 0.17 ms $T_{2,3}$: 5.24 ms).

The results show that the increased temperature by processing led to a reduced contact of starch and proteins with water (population B) and evaporation of inter- and extragranular water (population C) (Serial et al., 2016), also seen by the lower moisture content of the samples due to processing (see section 2.2). Heating also causes starch gelatinization and egg gel formation. During gelatinization and gelation, water is bound and incorporated into the gel network, as seen in the strongly reduced mobility of proton population C (Assifaoui et al., 2006; Luyts et al., 2013). Starch gelatinization led consequently to a decreased amount of ungelatinized starch, which is associated with the reduced

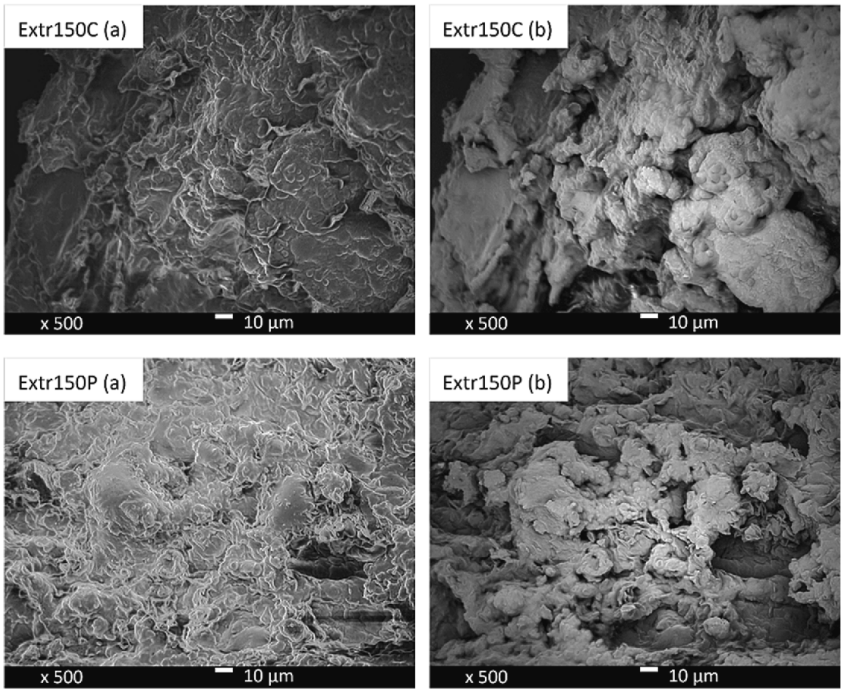

Fig. 2. (*continued*).

population A (rigid CH-protons of starch) in the processed samples compared to the respective doughs (Luyts et al., 2013). While baking cake dough, Luyts et al. (2013) and Serial et al. (2016) also observed strongly reduced mobilities and amounts of protons due to starch gelatinization and egg gel formation.

3.3.2. *Impact of pectin enrichment*

Pectin addition in muffins and both extrudates led to higher amounts in all populations and higher mobilities in populations A, B, and C than the comparable control (Table 2).

Adding pectin affected the amount and mobility of water independent of processing. Pectins – as hydrocolloids – enclose large quantities of water, observed in the higher intensities and higher mobilities in proton populations B and C, compared to the respective control. This observation was also consistent with the pectin samples' higher moisture contents (see Section 2.2) than the control. Water, which is enclosed by the pectins, is less available to the starch granules for gelatinization. This led to higher amounts of ungelatinized starch in the pectin samples than in the respective control, as seen by the higher amounts of population A (rigid CH protons of starch). In contrast, Serial et al. (2016) did not observe a noticeable effect on the mobility and amount in the proton populations due to inulin or oat fiber incorporation in biscuit dough upon heating, indicating that the fibers did not retain a substantial excess of water during the baking process.

Overall, the proton population D was almost unchanged within the different products, as it is associated with oil and egg yolk lipids (Assifaoui et al., 2006; Luyts et al., 2013), which were not affected in our trials.

In conclusion, the lower digestibility of pectin-enriched samples and the higher digestibility of processed samples could most likely be attributed to the degree of starch gelatinization, which is lower in the case of pectin-enriched samples and higher in the case of the processed samples, clearly seen by the amount and mobility in the proton populations of the samples.

3.4. *Viscosity of in vitro digested samples*

A high viscosity of the digesta in the gut is widely associated with a reduction in postprandial blood glucose levels (Goff et al., 2018). Therefore, the viscosity of the eight *in vitro* digested samples was investigated at the beginning (η_B) and end (η_E) of the *in vitro* intestinal digestion lasting for four hours (Table 3). The viscosity was evaluated at a shear rate of 1.7 s^{-1}, corresponding to the mixing rate at 100 rpm during *in vitro* digestion and the glucose release experiments.

3.4.1. *Impact of processing conditions*

Processing significantly ($p \leq 0.05$) increased the viscosity of the *in vitro* digested samples ranging from a twofold (MuffinP, Extr180P) to a ninefold increase (Extr180C) compared to the respective doughs. The effect of processing on viscosity was higher for the doughs without pectin addition than for those comprising pectin. *In vitro* intestinal digestion decreased the viscosity of the samples. The decrease of η_B to η_E was high in all processed samples, with the highest decrease being fourfold for the Extr180C (η_B: 86.5 mPa s to η_E: 22.8 mPa s), whereas the decrease in both untreated doughs (DoughC, DoughP) was minimal, or even neglectable (Table 3).

The heat exposure by baking and the additional pressure and shear exposure by extrusion led to a structural build-up through starch gelatinization and denaturation of the proteins, which formed a network of starch, gluten, and egg (Assifaoui et al., 2006; Luyts et al., 2013), leading again to increased viscosities of the samples. In contrast, the hydrolyzation of starch and protein by pancreatic enzymes during *in vitro*

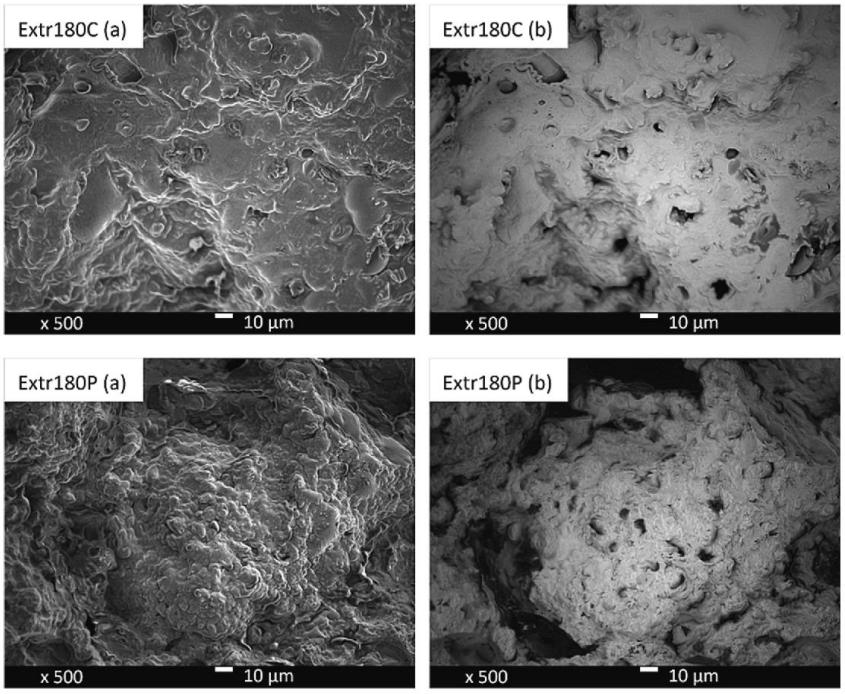

Fig. 2. (*continued*).

Table 2
The four proton populations detected with ^{1}H NMR with their FID relaxation times ($T_{2,1}$) and intensities ($A_{2,1}$), and CPMG relaxation times ($T_{2,2}$, $T_{2,3}$, $T_{2,4}$) and intensities ($A_{2,2}$, $A_{2,3}$, $A_{2,4}$) of the untreated doughs (Dough), baked doughs at 180 °C (Muffin) extruded doughs at 150 °C and 180 °C barrel temperatures (Extr150, Extr180) containing pectin (P) and the control (C).

	Population A		**Population B**		**Population C**		**Population D**	
	$T_{2,1}$ [ms]	$A_{2,1}$ [%]	$T_{2,2}$ [ms]	$A_{2,2}$ [%]	$T_{2,3}$ [ms]	$A_{2,3}$ [%]	$T_{2,4}$ [ms]	$A_{2,4}$ [%]
DoughC	0.05 ± 0.00 ^{a}	8.31 ± 0.12^{f}	0.30 ± 0.00 ^{g}	14.80 ± 0.02 ^{h}	28.45 ± 0.04 ^{h}	29.07 ± 0.02 ^{g}	155.80 ± 0.29^{f}	8.88 ± 0.02 ^{g}
DoughP	0.05 ± 0.00 ^{a}	8.25 ± 0.1^{f}	0.27 ± 0.00^{f}	14.33 ± 0.02 ^{g}	28.39 ± 0.03 ^{g}	33.08 ± 0.02 ^{h}	141.12 ± 0.29 ^{a}	7.26 ± 0.02^{f}
MuffinC	0.05 ± 0.00 ^{a}	5.07 ± 0.04 ^{a}	0.17 ± 0.00 ^{a}	6.65 ± 0.02 ^{a}	5.24 ± 0.02 ^{a}	9.08 ± 0.02 ^{a}	144.28 ± 0.31^{b}	2.62 ± 0.00 ^{a}
MuffinP	0.07 ± 0.00^{b}	7.60 ± 0.05 ^{e}	0.19 ± 0.00^{b}	10.41 ± 0.02 ^{e}	7.55 ± 0.01 ^{d}	17.97 ± 0.02 ^{d}	147.86 ± 0.22^{c}	3.99 ± 0.00 ^{e}
Extr150C	0.07 ± 0.00^{b}	6.15 ± 0.05^{b}	0.21 ± 0.00^{c}	8.92 ± 0.02^{b}	6.41 ± 0.01^{b}	16.18 ± 0.02^{b}	152.91 ± 0.23 ^{e}	3.47 ± 0.00^{b}
Extr150P	0.08 ± 0.00^{c}	6.83 ± 0.05 ^{d}	0.22 ± 0.00 ^{d}	9.96 ± 0.02 ^{d}	9.09 ± 0.01 ^{e}	20.63 ± 0.02 ^{e}	144.27 ± 0.23^{b}	4.01 ± 0.01 ^{e}
Extr180C	0.07 ± 0.00^{b}	6.45 ± 0.05^{c}	0.22 ± 0.00 ^{d}	9.63 ± 0.02^{c}	6.78 ± 0.01^{c}	17.65 ± 0.02^{c}	152.93 ± 0.23 ^{e}	3.68 ± 0.00^{c}
Extr180P	0.08 ± 0.00^{c}	6.89 ± 0.05 ^{d}	0.23 ± 0.00 ^{e}	10.48 ± 0.02^{f}	9.32 ± 0.01^{f}	21.53 ± 0.02^{f}	149.59 ± 0.25 ^{d}	3.90 ± 0.01 ^{d}

Different letters indicate significant differences within each column on a $p \leq 0.05$ level basis.

digestion resulted in structural degradation, as evidenced by decreased viscosity.

3.4.2. *Impact of pectin enrichment*

All samples enriched with pectin (DoughP, MuffinP, Extr150P, Extr180P) exhibited a viscosity that was higher up to a factor of four than the control samples (DoughC, MuffinC, Extr150C, Extr180C), except the η_B of Extr180P.

Pectin belongs to the soluble dietary fibers (Table 1), immobilizes water (Section 3.3), and can form high viscosity as a random coil polysaccharide (Morris et al., 1981).

Pectin is not enzymatically degraded in the phase of the small intestine, which also resulted in a higher viscosity of the enriched samples after *in vitro* digestion compared to the control samples. The reduced viscosity of Extr180P compared to Extr150P might originate from the degradation of pectins due to beta-elimination at higher temperatures during extrusion cooking, especially of highly methylated pectin (Sila et al., 2009). Applying high temperature, shear force and pressure as during extrusion cooking is known to convert dietary fibers into smaller fractions with increased solubility (Bader Ul Ain et al., 2019). Therefore, the potential breakdown of the molecular structure of pectin might lead to lower viscosity (Morris et al., 1981). Nevertheless, the sample Extr180P also had a lower viscosity than the control sample Extr180C, which might be attributed to the lower degree of starch gelatinization in the pectin-enriched sample (see Section 3.3), besides the pectins breakdown.

3.5. *In vitro glucose release*

The time-dependent release of glucose during the *in vitro* intestinal

Table 3
Viscosity ($\dot{\gamma}$= 1,7 s^{-1}) at the beginning (η_B) and end (η_E) of the *in vitro* intestinal digestion (4h) and descriptive parameters of the glucose release over time with the maximal concentration of glucose after the 4 h (c_f), the area under the glucose release curve within the 4 h (AUC), the calculated glucose release index (GRI) (see Equation (3) and amount of released glucose within every hour (Δglucose) of the control (C) and pectin enriched samples (P) of doughs (DoughC and DoughP), baked doughs at 180 °C (MuffinC and MuffinP), extruded doughs at 150 °C (Extr150C and Extr150P) and 180 °C (Extr180C and Extr180P).

			DoughC	DoughP	MuffinC	MuffinP	Extr150C	Extr150P	Extr180C	Extr180P
Viscosity		η_B [mPas]	9.8 ± 4.2 ^{a}	23.9 ± 7.3 ^{a,b}	32.5 ± 4.7^{b,c}	48.9 ± 6.5^{c,d}	56.8 ± 2.7 ^{d}	87.6 ± 0.3 ^{e}	86.5 ± 23.4 ^{e}	55.3 ± 1.8 ^{d,f}
		η_E [mPas]	6.0 ± 0.6 ^{a}	23.5 ± 8.5^{b}	10.2 ± 0.7 ^{a,c}	16.1 ± 0.6^{b,c}	21.2 ± 2.7^{b}	46.6 ± 1.1 ^{d}	22.8 ± 3.5^{b}	33.9 ± 0.5 ^{e}
Glucose release		c_f [mmol/L]	93.9 ± 3.6 ^{a,b}	90.8 ± 2.1 ^{a,b}	94.4 ± 0.8 ^{a}	88.8 ± 4.2 ^{a,b}	93.1 ± 8.6 ^{a,b}	94.5 ± 2.8 ^{a}	69.8 ± 3.6^{c}	83.3 ± 6.0^{b}
		AUC (mmol*h/L)	211.8 ± 6.6 ^{a}	194.1 ± 6.4^{b,c}	198.8 ± 1.1 ^{a,b,c}	193.1 ± 8.4^{c}	206.9 ± 7.9 ^{a,b}	198.8 ± 5.7 ^{a,b,c}	155.3 ± 5.4 ^{d}	187.4 ± 10.1^{b,c}
		GRI (%)	100.0 ± 3.1 ^{a}	91.6 ± 3.0^{b,c}	93.9 ± 0.5 ^{a,b,c}	91.1 ± 4.0^{c}	97.7 ± 3.7 ^{a,b}	93.8 ± 2.7 ^{a,b,c}	73.3 ± 2.5 ^{d}	88.5 ± 4.8^{b,c}
	Δglucose (mmol/L)	1h	31.1 ± 0.5 ^{a}	26 ± 1.7^{b}	26.6 ± 1.1^{b,c}	27.7 ± 0.3^{b,c}	28.8 ± 1^{c}	26.4 ± 1^{b}	22.1 ± 0.5 ^{d}	26.7 ± 1^{b,c}
		2h	23.3 ± 3.2 ^{a,b}	23.4 ± 1.3 ^{a,b}	25.1 ± 1.1 ^{a}	22.8 ± 1.8 ^{a,b}	26.5 ± 1.5 ^{a}	24.9 ± 2.6 ^{a}	19.2 ± 1.6^{b}	26.1 ± 3.2 ^{a}
		3h	23.2 ± 5.8 ^{a}	24.1 ± 6.1 ^{a}	20.7 ± 1.7 ^{a}	18.3 ± 9 ^{a}	20 ± 5.3 ^{a}	21.8 ± 2.3 ^{a}	14.7 ± 3.5 ^{a}	15.6 ± 3.2 ^{a}
		4h	16.2 ± 4 ^{a}	16.9 ± 3 ^{a}	22.4 ± 2.2 ^{a}	20.8 ± 4.5 ^{a}	13.5 ± 10.2 ^{a}	21.6 ± 4.3 ^{a}	13.9 ± 4.9 ^{a}	19.9 ± 7.1 ^{a}

Different letters indicate significant differences within each line on a $p \leq 0.05$ level basis.

phase (4 h) of the eight different samples is shown in Fig. 3. The descriptive parameters of the glucose release curve, with the maximal concentration of glucose after 4 h (c_f), the area under the glucose release curve within the 4 h (AUC), the calculated glucose release index (GRI) (see Eq. (3)) and amount of released glucose within every hour (Δglucose) are given in Table 3. The glucose concentration for all samples after *in vitro* digestion was equal to 344 mmol/L.

3.5.1. Impact of processing conditions

The processing of the doughs influenced the glucose release. Baking resulted in a lower glucose release over time (Fig. 3a) and a lower GRI for MuffinC compared to the untreated dough (GRI of 93.9 % vs. 100.0 %) (Table 3). However, baking did not affect *in vitro* glucose release and GRI of the pectin-enriched dough (GRI of 91.1 % for MuffinP vs. 91.6 % for DoughP). Extrusion at 150 °C of both doughs had no significant ($p \leq 0.05$) lowering effect on the glucose release compared to the untreated doughs, with a GRI of 97.7 % and 93.8 % for Extr150C and Extr150P, respectively. Both samples exhibited a similar glucose release curve over time (Fig. 3b). In comparison, extrusion at 180 °C significantly ($p \leq 0.05$) decreased the glucose release over time (Fig. 3b), the GRI (73.3 %), and the c_f (83.3 mmol/L) of the control sample (Extr180C) compared to all other samples. In contrast, no significantly ($p \leq 0.05$) lowering impact was observed for the pectin-enriched sample (Extr180P) (Table 3).

The lowered glucose release by baking might be the result of enclosed glucose molecules in the formed network (Fig. 2), the higher viscosity of the digesta (Table 3), and the formation of resistant starch (Fig. 1a). The network of the muffin samples might therefore lead to a lower initial Δglucose, but appeared to become less stable after 3 h, as the Δglucose increased again in the fourth hour, compared to the doughs, where the initial Δglucose per h is higher and decreased linearly over time (Table 3). This led to decreased glucose release due to baking in the control sample. The pectin-enriched sample behaved differently. Even though baking increased viscosity, the release remained the same between DoughP and MuffinP. A reason could be a less stable network due to a lower network formation, where the SEM images in Fig. 2, and the lower starch and protein gelation in Section 3.3 give an indication. Extrusion at 150 °C led to a faster or similar glucose release compared to the respective muffin samples, despite a significant ($p \leq 0.05$) increase in viscosity (Table 3). One possible reason might be the higher degree of starch gelatinization and subsequently elevated starch digestibility for the extruded samples, which increased the amount of free glucose at the beginning of the release experiment (Fig. 1b) and, therefore, could diminish the effect of increased viscosity. Raising the barrel temperature to 180 °C during extrusion reduced glucose release compared to all other treatments, particularly for the control sample, despite a higher amount of free glucose before intestinal digestion (Fig. 1b). Thereby, a strong network of proteins and starch was established in the control sample Extr180C, which is visible in the SEM images (Fig. 2). The network of Extr180C bound more water (Table 2) and formed a significantly ($p \leq 0.05$) higher viscosity (Table 3) than the other samples. Therefore, Extr180C constantly released a lower quantity of glucose at all times (Table 3). While food structuring, the barrier properties in the food increase (Gidley & Yakubov, 2019), and Zou et al. (2016) observed that the compact structure of pasta retarded *in vitro* starch digestibility. Also, Shafaeizadeh et al. (2018) showed in a human intervention study with fourteen healthy women that liquid starchy food that turned into solid starchy food due to processing tended to have a more sustained glucose release, whereas the overall glycemic area under the curve remained the same. The observed low glucose release of the extruded samples accompanied by significant changes in native starch is contrary to previous investigations, which have shown that processed food exhibiting minimal changes in the native starch structure and high in SDS fractions led to lower glucose release and thus low glycemic indices (Cesbron-Lavau et al., 2021; Lehmann & Robin, 2007). However, through the dramatic modification of starch structure due to the extrusion process, amylo-lipid complexes resulting in high RS content are formed (Cheetham & Tao, 1998; Godet et al., 1993). These complexes are linked to lower digestion rates (Wang & Copeland, 2013), which could result in lower glycemic indices. Therefore, forming these complexes and RS may play a more important role in attenuating glycemic responses than previously assumed and could be used to produce foods with a low GI.

3.5.2. Impact of pectin enrichment

Pectin enrichment also influenced the release of glucose. The release from DoughP, MuffinP and Extr150P was lower than the respective control samples (DoughC, MuffinC, Extr150C) (Fig. 3a and b). The lower release is also displayed in a slightly lower, but only in the case of the dough significantly ($p \leq 0.05$) different GRI than the respective control samples. In contrast, the result was reversed at 180 °C, in which the control showed a significantly ($p \leq 0.05$) lower GRI than the pectin-enriched sample (Extr180P vs. Extr180C) (Table 3).

The decreased glucose release with lower GRI in the pectin-enriched samples (DoughP, MuffinP, and Extr150P) than the respective control

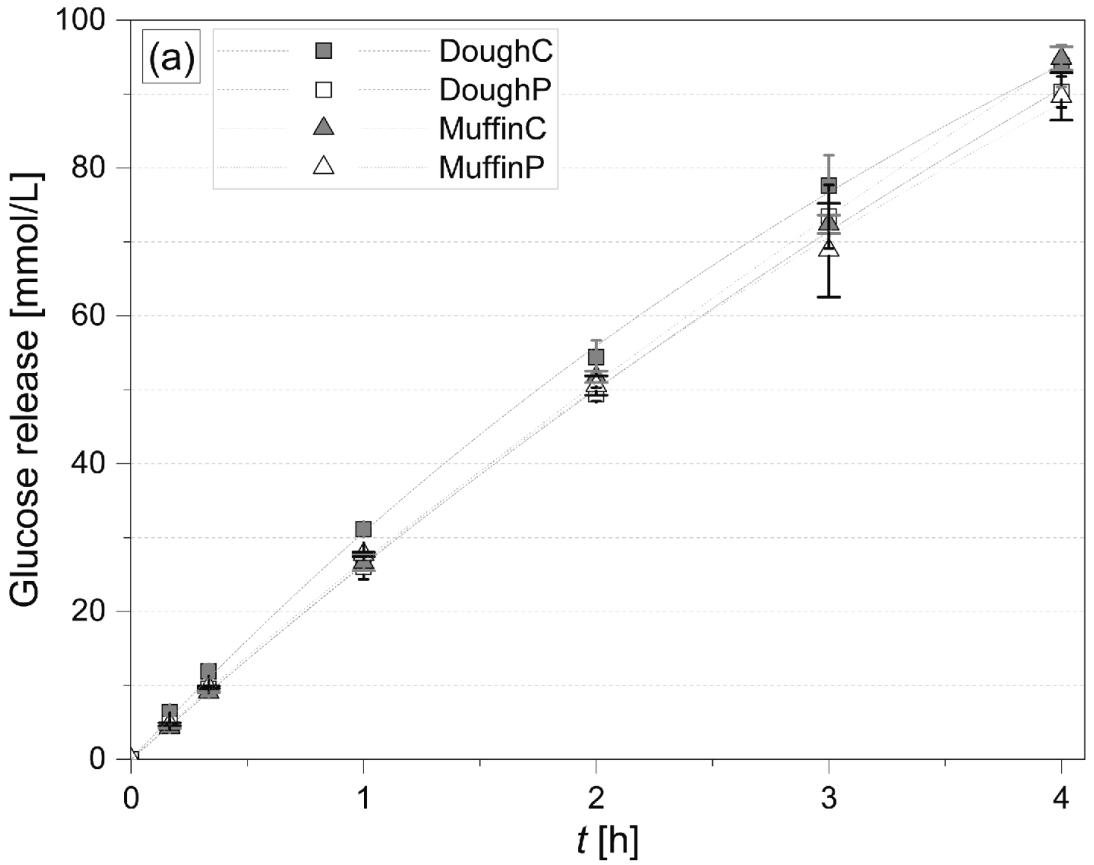

Fig. 3. Glucose release over time. Glucose release over time during *in vitro* intestinal phase of the doughs (Dough) and baked doughs at 180 °C (Muffin) (a), as well as extruded doughs at 150 °C and 180 °C barrel temperatures (Extr150, Extr180) (b) enriched with pectin (P) and the control (C). The symbols in the figures correspond to the experimental data and the lines to fitted data according to Eq. (1) ($R2 \geq 0.96$).

samples probably originated from the higher viscosities after *in vitro* digestion (Table 3). However, the reduction in GRI did not occur to the same extent as the increase in viscosity and was, therefore, lower than expected. This observation was consistent with a preliminary study by Dhital et al. (2014), who showed that the addition of dietary fiber (up to 2 %) resulted in a substantial increase of digesta apparent viscosity by a factor of 100 compared to the control, but only to a modest delay of amylolysis. Another reason for a lower glucose release in the pectin-enriched samples DoughP, MuffinP, and Extr150P could be the lower digestibility of starch compared to the respective control samples (Fig. 1a). Furthermore, sugar molecules with bound water can be entrapped between hydrated pectin molecules, leading to a lower glucose release (Karim et al., 2023). In contrast, pectin sample Extr180P exhibited a faster glucose release at a higher GRI and lower viscosity than the respective control. The faster release might be attributed to different modes of action. Firstly, the heat, shear and pressure exposure by extrusion most likely broke down the pectin molecules, also observed by Wan et al. (2019), as seen by the viscosity drop of the *in vitro* digested samples from Extr150P to Extr180P (Table 3). Secondly, resistant starch formation at the extrusion temperature of 180 °C was minimized due to pectin addition, as compared to the control with 0.8 g/100 g and 1.5 g/100 g, respectively (Fig. 1a). Furthermore, Extr180P formed a less homogenous and more porous network than Extr180C (Fig. 2), probably due to the lack of free water for the gelation process (Section 3.3) or due to phase separation (Kulicke et al., 1996). A porous structure tends to lead to a higher glucose release (Priyadarshini et al., 2022), whereby glucose molecules could penetrate the porous network more easily, leading to increased glucose release. Similar findings were reported by an increased *in vitro* GRI in the presence of hydrocolloids in starch dispersions (Gularte & Rosell, 2011) and *in vivo* by a higher postprandial blood glucose level obtained after the consumption of high fiber spaghetti compared to the control spaghetti (Papakonstantinou et al., 2022). Cesbron-Lavau et al. (2021) observed no difference in processing conditions or *in vitro* starch digestibility on food products with and without bran. Contrary to these findings, there is also some evidence suggesting that incorporating DF into cereal products can lower glycemic responses *in vivo*, including extruded products (Jenkins et al., 2002; Scazzina et al., 2013).

The results of the presented *in vitro* glucose release study suggest a complex interplay between viscosity, the food matrix, and changes in starch digestibility, which are influenced on the one hand by processing and starch gelatinization and, on the other hand, by pectin addition

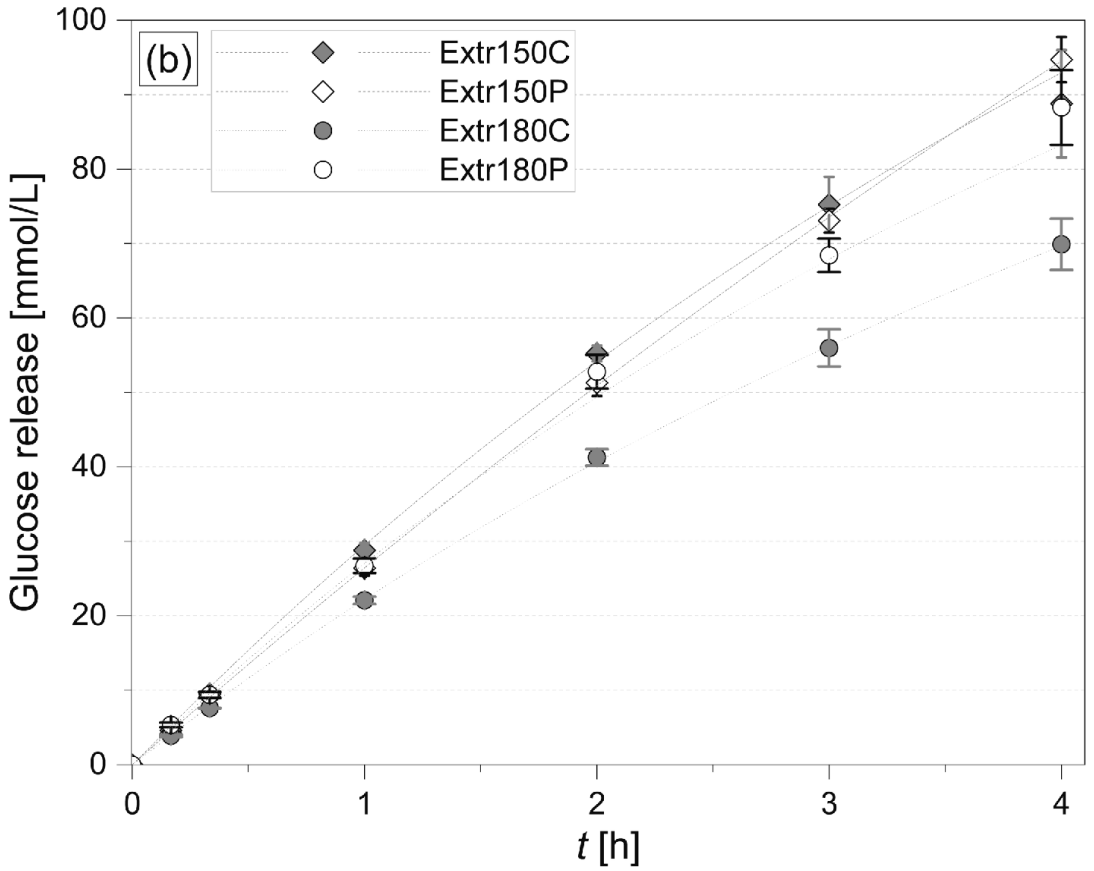

Fig. 3. (*continued*).

itself. Thereby, water availability also played a significant role (Karim et al., 2023). *In vivo* analysis has shown that the plasma glucose response is positively related to the degree of starch gelatinization (Holm et al., 1988; Parada & Aguilera, 2011). In contrast to those findings, we found a strong correlation between the amount of resistant starch and the GRI in our samples with a regression coefficient of $R^2 = 0.86$. The amount of resistant starch showed a more substantial influence on glucose release than the viscosity ($R^2 = 0.35$). Therefore, future research should focus on forming resistant starch, possibly in combination with further soluble dietary fibers, and examining the effects of impact of different types of resistant starch on glucose release.

4. Conclusions

This study aimed to investigate the impact of different food processing methods, namely baking and extrusion cooking, on the *in vitro* glucose release of pectin-enriched cake doughs with a high glycemic load. The results showed that processing had a greater impact on *in vitro* glucose release than enrichment with pectin as a soluble dietary fiber. Specifically, the GRI was reduced by almost 30 % and resistant starch increased fivefold due to processing. An increase in viscosity could mainly be achieved by adding pectin. However, the impact of resistant starch on glucose release ($R^2 = 0.86$) was greater than that of viscosity ($R^2 = 0.35$). The observed variations in *in vitro* glucose release suggest a complex interplay between changes due to processing and the enrichment with pectin. Both factors altered the digestibility of starch, the viscosity of the *in vitro* digesta and the formation of resistant starch as well as the food matrix to varying degrees. Thereby, the availability of water and the degree of starch gelatinization played a meaningful role. In summary, the *in vitro* glucose release was the result of a combination of factors, and could not be attributed to a single cause.

The extent to which the obtained reduction in *in vitro* glucose release can be monitored in human intervention studies will be the subject to future investigations. When developing low glycemic index foods for the industry, it is important to investigate the influence of processing on glucose release and insulin secretion when incorporating soluble or insoluble dietary fibers to develop new functional foods aimed to reducing glycemia.

CRediT authorship contribution statement

Elisabeth Miehle: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Peter Eisner:** Writing – review & editing, Supervision, Conceptualization. **Stephanie Bader-Mittermaier:** Writing – review & editing, Validation, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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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.2023.138331.

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Supplementary material:

Dough formulation of the control (Dough C) and the pectin-enriched dough (Dough P). The recipes are displayed as fresh dough and on a dry matter (DM) basis. The added concentration of pectin corresponds to a pectin concentration two times higher than the critical concentration.

	Dough C	Dough C on DM	Dough P	Dough P on DM
	[%]	[%]	[%]	[%]
Pectin CU	-	-	1.9	2.4
All-purpose wheat flour	27.1	30.4	25.3	29.7
of which starch	20.9	23.4	19.5	22.9
Glucose (monohydrate)	42.3	54.5	39.5	53.2
Baking powder	0.9	1.1	0.8	1.1
Salt	0.1	0.2	0.1	0.2
Sunflower oil	7.1	9.1	6.7	9.0
Egg	15.7	4.6	14.6	4.5
Water	6.7	-	11.0	-

DM for Dough C 76.8±0.5 %; DM for Dough P 75.3±0.7 %

Chapter 4: Impact of Food Processing on the *In Vitro* and *In Vivo* Glycemic Response to Citrus Fiber-enriched Dough Products

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The frequent consumption of processed foods that are high in glycemic index and low in dietary fiber has been linked to an elevated risk of hyperglycemia, hyperinsulinemia, and, ultimately, type 2 diabetes. This study examines the effects of enriching high-glucose doughs with citrus fiber and various processing methods, such as baking at 180 °C and extrusion cooking at 150 °C and 180 °C, on glycemia using a novel combinatory strategy of *in vitro* and *in vivo* methodology as well as their relation to product-specific characteristics.

For this, starch digestibility, dietary fiber composition, structure of the products, and *in vitro* glucose release were analyzed. In addition, *in vivo* glycemia and insulinemia were evaluated in 11 adults at metabolic risk in a randomized, double-blind crossover study using the same products as for the *in vitro* glucose release. All fiber-enriched products significantly lowered *in vitro* glucose release by up to 15 % compared to the control. Intense extrusion at 180 °C raised soluble and total dietary fiber contents by 10 % and the resistant starch content by 60 %, which resulted in impaired *in vitro* glucose release. However, neither fiber enrichment nor different food processing methods of the doughs significantly influenced postprandial glucose and insulin concentrations of the study participants due to inter-individual differences emphasizing the need for combined developmental approaches to evaluate the impact of dietary fiber addition on glycemic response.

Author Contributions:

Miehle, E and Pietrynik, K.: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. Bader-Mittermaier, S.: Conceptualization, Project administration, Validation, Funding acquisition, Writing – review & editing. Skurk, T.: Conceptualization, Project administration, Funding acquisition, Writing – review & editing. Eisner, P.: Conceptualization, Supervision, Writing – review & editing. Hauner, H.: Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing

Miehle, E., Pietrynik, K., Bader-Mittermaier, S., Skurk, T., Eisner, P. & Hauner, H. (2024) Impact of food processing on the *in vitro* and *in vivo* glycemic response to citrus fiber-enriched dough products. Journal of Functional Foods, 106230. doi:https://doi.org/10.1016/j.jff.2024.106230

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Impact of food processing on the *in vitro* and *in vivo* glycemic response to citrus fiber-enriched dough products

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ABSTRACT

Frequent consumption of processed, high-glycemic, low-fiber foods is associated with an increased risk of hyperglycemia, hyperinsulinemia, and ultimately of type-2 diabetes. This work investigated the impact of enriching high-glucose doughs with citrus fiber and various processing methods (baking and extrusion cooking) on glycemia using a novel combination of *in vitro* and *in vivo* methodology and relating to product-specific characteristics. Starch digestibility, dietary fiber composition, product structure and *in vitro* glucose release were determined. *In vivo* glycemia and insulinemia were evaluated in 11 adults at metabolic risk in a randomized, double-blind crossover study. The fiber-enriched products significantly reduced *in vitro* glucose release by up to 15 %. Extrusion at 180 °C increased soluble and total dietary fiber contents by 10 % and resistant starch content by 60 %, impairing *in vitro* glucose release. Neither fiber-enrichment nor processing methods significantly influenced postprandial glucose and insulin concentrations in study participants emphasizing the need for combined developmental approaches.

1. Introduction

Type 2 diabetes is a significant public health concern, with increasing global prevalence (Tönnies et al., 2021). High consumption of refined carbohydrate-rich foods increases glycemia, insulinemia, and the risk of developing type 2 diabetes (Al-Jawaldeh & Abbass, 2022; Stull, 2016). Refined foods with large amounts of rapidly available carbohydrates result in a larger area under the plasma glucose curve in the postprandial phase than an equivalent amount of slowly available carbohydrates (Gropper & Smith, 2017; Ludwig, 2002). Increased dietary fiber (DF) intake can attenuate glycemia and reduce fasting insulin levels, as revealed by several *in vivo* studies (Meyer et al., 2000; Schulze et al., 2004; Stevens et al., 2002). The effect of dietary fiber on glucose tolerance and improved insulin sensitivity is mainly attributed to viscosity formation in the chyme, which reduces rapid elevations in postprandial glucose (Brennan, 2005; Jenkins et al., 2002; Weickert & Pfeiffer, 2008). However, incorporating DF into conventional and widely used products like bread, cookies, and pasta involves several challenges including the alteration of the food's characteristics and processing (Cassidy et al., 2018).

Besides the formulation, food processing can also influence glycemia by affecting the food's structure (Priyadarshini et al., 2022). Different processing methods, such as baking and extrusion cooking, are applied to produce products such as bread (Stamataki et al., 2017), pastry (Cesbron-Lavau et al., 2021; Ferrer-Mairal et al., 2012) or pasta (Di Pede et al., 2021). It was reported that processing can enhance (e.g. Alonso et al. (2000); Fredriksson et al. (1998)) or decrease (e.g. Delcour and Hoseney (2010); Guha et al. (1997); Papakonstantinou et al. (2022))

Abbreviations: MC, control muffin; MCF, citrus fiber-enriched muffin; E150C, control extrudate at 150 °C barrel temperature; E150CF, citrus fiber-enriched extrudate at 150 °C barrel temperature; E180CF, citrus fiber-enriched extrudate at 180 °C barrel temperature.

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glycemic response in humans, depending on processing conditions. During processing, heat, shear, pressure, and moisture can intensely modify the digestibility of starch (Wang & Copeland, 2013). The rate of digestion is mainly measured *in vitro* based on the method by Englyst et al. (1992), which classifies starch into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). Starch processing with heat, moisture, and shear disrupts the highly ordered, crystalline structure of native starch, known as gelatinization, and increases the susceptibility of starch to enzymatic breakdown (Wang & Copeland, 2013). In this regard, the degree of starch gelatinization is strongly positively correlated with glycemia (Holm et al., 1988; Parada & Aguilera, 2009) and insulinemia (Holm et al., 1988). In addition, few researchers reported an increase in soluble DF contents after e.g. extrusion cooking, which also might impact glucose release (Bader Ul Ain et al., 2019; Camire et al., 1997; Naumann et al., 2021).

To date, only a limited number of studies have explored the combined influence of food processing and DF addition on starch digestibility and glycemia by taking into account the composition and structure of the food (Cesbron-Lavau et al., 2021; Ferrer-Mairal et al., 2012; Fujiwara et al., 2017; Papakonstantinou et al., 2022). This study aimed to determine the relationship between food processing and citrus fiber enrichment on glycemia in a translational way. Firstly, starch digestibility, composition, and structure of extruded or baked products with a similar formulation were analyzed for the impact on *in vitro* glucose release. Secondly, based on this data the physiological glycemic and insulinemic response to the high glycemic load products provided as muffins and extrudates was assessed in a randomized, double-blind, crossover trial in adults (45 – 66 years old) at metabolic risk defined by elevated waist circumference. Thereby, the results from both *in vitro* digestion models and *in vivo* studies were compared to increase knowledge on potential transferability of *in vitro* results to *in vivo* studies.

2. Materials and methods

2.1. Materials

Citrus fiber (CF) (Herbacel AQ Plus Citrus – N, derived from citrus fruit pomace) was purchased from Herbafood Ingredients GmbH (Werder, Germany) in food grade. The CF preparation was composed of 71.6 ± 2.9 g/100 g on dry matter (DM) insoluble dietary fiber (IDF), 16.3 ± 3.2 g/100 g on DM soluble dietary fiber (SDF), 5.8 ± 0.1 g/100 g on DM protein, and 1.3 ± 0.2 g/100 g on DM ash. Glucose monohydrate (Roquette GmbH, Frankfurt, Germany), all-purpose wheat flour (Aurora Mühlen GmbH, Hamburg, Germany, starch content 73.7 ± 3.9 g/100 g on DM), baking powder (Dr. August Oetker Nahrungsmittel KG, Bielefeld, Germany), sunflower oil (Nestlé Deutschland AG, Frankfurt, Germany), salt (Südwestdeutsche Salzwerke AG, Bad-Reichenhall, Germany), and pasteurized egg (EIPRO-Vermarktung GmbH & Co. KG, Lohne, Germany) were purchased locally. Alpha-amylase from human saliva (Type IX-A, 1,000–3,000 units/mg protein; A0521), pancreatin from porcine pancreas (8 x USP specifications; P7545), pepsin from porcine gastric mucosa (3,200–4,500 units/mg protein; P6887), bile extract from porcine (B8631), and polyethylene glycol (PEG) (molecular weight of 20 kDa) were purchased from Merck KGaA (Darmstadt, Germany). Carrez reagent I and Carrez reagent II were prepared by dissolving 1.06 g of $K_4[Fe(CN)_6]\cdot 3H_2O$ or 2.88 g of $ZnSO_4\cdot 7H_2O$ in 10 mL of distilled H_2O, respectively. Sodium azide (NaN_3) and all other reagents and chemicals used were purchased from Chemsolute (Th. Geyer GmbH & Co. KG, Renningen, Germany) and were of analytical grade.

2.2. Study product preparation

The doughs for the muffins and extrudates were based on a commercial muffin recipe. They were formulated with all-purpose wheat flour, glucose (instead of sucrose), baking powder, salt, sunflower oil, egg, and water. Samples enriched with citrus fiber (CF) contained 0.9 g CF/100 g fresh product (1.0 g/100 g on DM) and adjusted water content. The concentration was based on a previous study by Miehle et al. (2022), which showed a high rise in the viscosity of CF dispersions at concentrations of 0.4 g/100 g dispersion determined as the critical concentration c^* and a significant retardation of glucose release at concentrations of 2.2 × 0.4 g/100 g. All study foods contained equally 100 g of available carbohydrates per portion, of which 66.6 g were glucose and 33.3 g were rapidly available carbohydrates from wheat flour. The study foods were enriched with 100 µg ^{13}C octanoic acid as described by Ghoos et al. (1993), metabolized to $^{13}CO_2$ to assess $^{13}CO_2$ excretion after consumption of the test meal to evaluate *in vivo* gastric emptying (see section 2.13). The chemical composition of both doughs (control and CF-enriched) was similar, with a protein content of 6 %, a fat content of 12 %, and an ash content of 1 %. The precise ingredient concentrations of the doughs are provided in Table S1 (supplementary material).

The study products were prepared as recently described (Miehle et al., 2024) with minor modifications. The ingredients were whipped in a universal kitchen mixer (Hobart N 50, Illinois Tool Works, Illinois, USA) until a smooth dough was formed. To receive the control muffin (MC) and the CF-enriched muffin (MCF), 86.6 g and 87.8 g were baked in a universal kitchen oven at 180 °C for 30–40 min, respectively. The differences in weight were attributed to the higher water content of the CF-enriched dough (DoughCF), but were adjusted to receive an equal proportion of glucose in both samples. Extrusion was performed by a laboratory co-rotating, intermeshing twin screw extruder (Haake Rheocord, Thermo Fisher Scientific Inc., Waltham, MA USA) equipped with a screw diameter of 16 mm, a smooth barrel, and a length-diameter ratio of 25:1. The dough was placed directly into the center of the feed port during operation and was cooled down to 80 °C in a long die with dimensions of 19 x 2 x 210 mm (*W* x *H* x *L*), attached to the end of the extruder. The control dough was extruded at a barrel temperature of 150 °C (sample E150C), whereas the CF-enriched dough was extruded at two-barrel temperatures of 150 °C and 180 °C (samples E150CF and E180CF), respectively. The screw speed was 250 rpm. For this, the barrel was divided into five temperature-controlled zones. The first four zones increased stepwise from 30 °C to 50 °C, 70 °C, and then 100 °C. The last zone (fifth) was set to the desired cooking temperature of 150 °C or 180 °C, respectively. Once the extruder reached steady-state conditions, samples were collected. Before the cooling die, the extruder responses to pressure (bar) were recorded in-line using Polylab software (Thermo Fisher Scientific Inc., Waltham, MA USA). After extrusion and baking, the samples were cooled to room temperature, and the fresh samples' dry matter content (DM) was analyzed immediately. The DM content of the muffins ranged from 81.5 ± 0.1 % for MC to 79.7 ± 0.1 % for MCF, whereas the extruded samples ranged from 81.9 ± 0.4 % for E150CF to 79.3 ± 0.3 % for E150C and 80.2 ± 0.1 % for E180CF.

Samples for the *in vitro* experiments were lyophilized and ground. Lyophilization of the −50 °C frozen samples was performed at 5–45 °C for 72 h at a pressure of 103 Pa (freeze dryer beta 1–8, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Afterwards, the dry samples were ground to pass through a 500 µm screen using the ZM-200 mill (Retsch GmbH, Haan, Germany), except for observation by SEM. The *in vivo* study products (muffins MC, MCF; and extrudates E150C, E150CF, E180CF) were cooled to room temperature after baking and extrusion and subsequently stored in airtight plastic bags at −20 °C for a maximum of six months until the last subject completed the human study. The study products were thawed overnight in a refrigerator at 4 °C and put at room temperature one hour before the test meal. A possible freeze–thaw effect was neglected (Lan-Pidhainy et al., 2007) as the impact on dietary fiber performance was judged to be small, because the dietary fiber used is mainly insoluble, and only one freeze–thaw cycle was applied.

2.3. Dietary fiber composition

The dietary fiber contents of soluble (SDF) and insoluble (IDF) fibers were determined using an enzymatic–gravimetric analysis (K-TDFR 04/17, Megazyme, Bray, Ireland) according to AOAC 991 (Chemists, 2016). The total dietary fiber content (TDF = IDF + SDF) and the insoluble proportion (IDF/TDF) were calculated.

Nitrogen (N) (AOAC Official Method 968.06) was analyzed according to the Dumas combustion method using a Nitrogen Analyzer TruMac N (LECO Instrumente GmbH, Mönchengladbach, Germany) and the protein content was calculated using the standardized nitrogen conversion factor of N x 6.25. Dry matter and ash contents were determined on a thermogravimetric basis at 105 °C and 550 °C using a TGA 601 analyzer (Leco Instrumente GmbH, Mönchengladbach, Germany) following the official methods, L 18.00–12 and L 15.00–7, respectively (Lebensmittelsicherheit, 2005).

2.4. Scanning electron microscopy (SEM)

A torn fracture of a lyophilized sample was placed onto the surface of double-sided copper adhesive tape and mounted on a sample carrier and SEM-images were taken as recently described (Miehle et al., 2024) with small modifications. The sample was sputter coated with gold from two directions for 1 min (U = 5 kV and I ~ 10–15 mA) in an argon atmosphere (17.3 Pa). Digital images were acquired at an accelerating voltage of 7.5 kV and a magnification of 500 times using a scanning electron microscope (JSM F7200, JEOL Ltd., Tokyo, Japan) with a backscattered electron detector (BED).

2.5. Steady shear viscosity

The steady shear viscosity of the *in vitro* digested samples was measured at the beginning and end of *in vitro* small intestinal digestion (section 2.6) as recently described in Miehle et al. (2024). Viscosity was determined at a constant temperature of 37.0 ± 0.1 °C using a rotational rheometer (Physica MCR 301, Anton Paar GmbH, Graz, Austria) equipped with a parallel plate geometry (diameter: 25 mm, shear gap: 1 mm, PP25/TG-SN23582). Samples were pre-sheared at 2 s^{-1} for 10 s and then allowed to rest for 60 s. Viscosity was monitored using a steady shear mode in a logarithmic scale ranging from 0.1 to 500 s^{-1} (comprising 19 measurement points, measurement point duration from 20 to 10 s). All measurements were conducted in at least duplicate for each separately prepared sample.

2.6. In vitro digestion

The static simulation of oral, gastric, and small intestinal digestion was performed with slight modifications using the harmonized INFOGEST protocol (Minekus et al., 2014) as previously described by Miehle et al. (2024).

The standard samples at the end of the *in vitro* digestion obtained a final dry matter concentration of 12.5 %, and that of the CF-enriched samples of 12.6 % (factor of 1.01 higher), to achieve the same concentration of dough ingredients, primarily glucose and starch (refer to Table S1, supplementary material). Therefore, the concentration of added glucose was equal for all samples at the beginning of the *in vitro* glucose release experiment. For the oral phase, the lyophilized, ground samples were mixed with simulated salivary fluid (37 °C) containing α-amylase (75 U mL^{-1} in the final digestion mixture) (ratio 50:50 *w/w*). The sample was kept for 2 min in a water shaking bath (37 ± 0.1 °C, 100 rpm) before the pH level of the sample was lowered to 3.0 ± 0.1 for the gastric phase. Subsequently, simulated gastric fluid (37 °C) (ratio 50:50 w/w) containing pepsin (2,000 U mL^{-1} in the final digestion mixture) was added and incubated (37 ± 0.1 °C, 100 rpm) for two hours. In the intestinal phase, the pH was increased to 7.0 ± 0.1, and the sample was mixed with simulated intestinal fluid (37 °C) (ratio 50:50 *w/w*) containing pancreatin and bile extract containing bile acid salts. The sample reached an enzyme activity of 100 U mL^{-1} for trypsin, 250 U mL^{-1} for alpha-amylase (15.38 mg pancreatin/mL final digestion mixture), and 10 mmol/L of bile acid salts in the final digestion mixture, respectively. NaN_3 was added as an antimicrobial agent to reach a concentration of 0.1 g/100 g in the final digestion mixture. The digesta was immediately transferred into the side-by-side system's donor cell to perform the *in vitro* glucose release experiment (section 2.8). The rest of the sample was used to determine starch digestibility (section 2.7) and to measure shear viscosity (section 2.5) before the intestinal *in vitro* digestion. After 4 h of simulated small intestinal *in vitro* digestion, shear viscosity was again determined.

2.7. Digestible and resistant starch

The measurement of the digestible and resistant starch contents was based on a method described by Englyst et al. (1992) and conducted using an enzymatic test kit (K-DSTRS 11/19, Megazyme, Bray, Ireland), as described recently (Miehle et al., 2024). Samples of the *in vitro* digested sample solution (section 2.6) were collected to measure the content of digestible starch, including rapidly digestible starch (RDS), slowly digestible starch (SDS), and total starch (TS), as well as free glucose over a 4 h-period. This time frame aligns with the average residence time of food in the human small intestine, which is 4 ± 1 h (Deiteren et al., 2010). After 0 (free glucose), 20 (RDS), 120 (SDS), and 240 min (TS), an aliquot of 100 μL sample solution was mixed with 900 μL of 0.05 M acetic acid to stop further digestion. The samples were then centrifuged at 13,000 rpm for 5 min. Before analysis, a modified Carrez precipitation (Carrez, 1908) was performed, where the supernatant (100 μL) was transferred into fresh reaction tubes (2 mL) containing distilled water (1 mL). Then, Carrez reagent I and Carrez reagent II (100 μL, respectively), NaOH (200 μL, 100 mM), and distilled water (500 μL) were added, vortexed after every addition, and incubated (20 °C, 20 min) (precipitation step), followed by a centrifugation step (13,000 rpm, 5 min) to remove the precipitate. The glucose content of the supernatant (50 μL) was determined following the GOPOD (glucose oxidase/peroxidase) assay. For resistant starch content, a sample (1 mL) was taken after 240 min, washed with an equivalent volume of ethanol (95 % v/v), and further analyzed according to the kit instructions. The absorption of the color change was determined at 510 nm using a microplate reader (BioTek Instruments GmbH, Bad Friedrichshall, Germany). All measurements were conducted in at least duplicate for each sample.

2.8. In vitro glucose release

The *in vitro* glucose release measurements were performed with a side-by-side cell system (SES GmbH, Bechenheim, Germany) using a donor and a receptor cell separated by a dialysis membrane as previously described by Miehle et al. (2021), with slight modifications. After filling phosphate buffer (4 mL, 74.60 mmol/L, with 0.1 g/100 g NaN_3, pH 7.0) into the receptor cell (stirred at 300 rpm), 4 mL of the dispersion at the beginning of the small intestinal *in vitro* phase (section 2.6) was transferred to the donor cell (stirred at 100 rpm). An aliquot of 100 μL sample of the receptor fluid was taken after 10 min, 20 min, 60 min, 120 min, 180 min, and 240 min, respectively, and immediately replaced by 100 μL of phosphate buffer solution. The glucose concentration of the aliquoted samples was determined with an enzymatic test kit for D-glucose (D-Glucose-Food & Feed Analysis, R-Biopharm AG, Darmstadt, Germany) and conducted as described by Miehle et al. (2021).

2.9. In vitro glucose release kinetics

A first-order equation described the glucose release kinetics over time according to Macheras et al. (1986) and Goñi et al. (1997) with slight modifications:

$$c_t = c_\infty[\,1 - \exp(-kt)] \quad (2)$$

where c_t is the glucose concentration at the corresponding time, c_∞ is the maximal glucose concentration at equilibrium, t is the time in hours, and k is the apparent permeability rate constant.

The area under the release curve (AUC) was calculated according to Equation (2) as described by Ferrer-Mairal et al. (2012); and Goñi et al. (1997):

$$AUC = c_f(t_f - t_0) - (c_f/k)\,[\,1 - \exp(-k(t_f - t_0))] \quad (3)$$

where c_f is the maximal glucose concentration after four hours, t_f is the final time (4 h), and t_0 is the initial time (0 h).

2.10. Human study design

The study used a randomized, double-blinded, crossover design. Each participant served as their control and attended five study visits separated with washout periods of at least three days. Study visits were held at the Core Facility Human Studies of ZIEL Institute for Food & Health, Technical University of Munich. Participants were instructed to refrain from physical exercise and alcohol consumption in the evening before every study visit. All participants fasted overnight for at least 10 h (from 10:00 p.m. until consumption of the test product in the morning).

2.11. Human study inclusion criteria

Participants were enrolled in the study if they were 35 to 67 years old, had an elevated waist circumference (> 102 cm in males and > 88 cm in females), and had provided a written informed consent. Exclusion criteria included not providing written informed consent, parallel participation in another interventional study, gastrointestinal diseases, diabetes mellitus, untreated hypertension (> 160/95 mmHg), untreated endocrine diseases, chronic liver diseases, autoimmune diseases, chronic viral infections, cardiovascular diseases, oncologic diseases, acute infectious diseases, allergy or sensitivity to the study products, current dietary fiber supplementation, pregnancy and breastfeeding, and having donated blood in the previous 3 months.

2.12. Human study procedures

Venous blood samples were collected at baseline and at 15, 30, 60, 90, 120, and 180 min after consumption of the test meal to examine plasma glucose and insulin levels. Glucose was assessed with a plasma-calibrated rapid tester (HemoCue Glucose 201+ System, HemoCue AB, Sweden). Insulin was assayed using a commercially available ELISA kit (DRG Instruments GmbH, Marburg, Germany). Complete blood count, lipid profile, liver enzymes, and HbA_{1c} were measured from baseline venous blood sample by a certified laboratory (SynLab, Munich, Germany). Gastric emptying was evaluated with a ^{13}C breath test (Ghoos et al., 1993). To assess $^{13}CO_2$ excretion after consumption of the test meal, breath samples were collected at baseline and at defined time intervals over 240 min following the test meal (Table 1) and analyzed with a nondispersive infrared spectrometer FANci2 (Fischer Analysen Instrumente GmbH, Leipzig, Germany).

Prior to the test meals, physical examination of participants (height, weight, waist and hip circumference, blood pressure, and heart rate) was conducted, and body composition was assessed with a Body Composition Analyzer SECA mBCA515 (Seca GmbH & Co KG, Hamburg, Germany). Participants were asked to evaluate their subjective feelings: hunger, fullness, thirst, appetite, and general well-being at baseline and at determined time intervals (Table 1) after consuming the test products using visual analogue scales (VAS). In addition, participants were asked to assess their digestive symptoms (bloating, increased bowel gas discharge, gastrointestinal sounds, gastrointestinal pain, heartburn, and nausea) along with VAS every 60 min. Immediately after consuming the test meal, participants used VAS to rate the test product (appearance, mouthfeel, taste, and sweetness).

The time when each participant started to consume the meal was defined as 0 min. Participants consumed each test product with 300 mL of water at a comfortable pace within 15 min. Test products were served on a plate that was weighted before and after the test meal to determine the weight of possible meal leftovers.

2.13. Randomization

Before starting the study, all possible orders of 5 study treatments were generated (120 possibilities in total) and stored digitally. If a potential participant met all inclusion criteria during the screening visit, one of the combinations was randomly assigned using the Microsoft Excel® RANDBETWEEN function. Once a combination was assigned to a participant, it was not reused for further randomization.

2.14. Human study outcomes

The primary study outcome was postprandial blood glucose response including blood glucose measurements at 15, 30, 60, 90, 120, and 180 min, baseline-adjusted maximum postprandial glucose concentration (ΔC_{max}), and postprandial incremental glucose area under the curve ($_{i}AUC$). ΔC_{max} was calculated by subtracting the baseline glucose concentration of a participant from the respective peak concentration. $_{i}AUC$ was calculated geometrically as the sum of the areas of the triangles and trapezoids, ignoring the area under baseline (Brouns et al., 2005). Secondary outcomes included the postprandial insulin response (concentrations at 15, 30, 60, 90, 120, and 180 min, postprandial insulin $_{i}AUC$ and insulin ΔC_{max}), pulmonary $^{13}CO_2$ excretion rate and cumulative $^{13}CO_2$ excretion reflecting the rate of gastric emptying (Ghoos et al., 1993).

2.15. Statistical analysis

If not otherwise stated, the results are expressed as mean ± standard deviation. All figures were created with GraphPad Prism version 10.1.2 for Windows (GraphPad Software, Boston, MA, USA). *In vitro* data were analyzed using OriginPro 2018 for Windows (Origin Lab Corporation, USA). One-way analysis of variances (ANOVA) was applied. Tukey's honestly significant difference post hoc test was used to determine the significance of differences between samples on a significance level of $\alpha = 0.05$. Data from the human study were collected and managed using REDCap electronic data capture tools hosted at the Chair of Nutritional Medicine, Technical University of Munich (Harris et al., 2009, 2019),

Table 1
Study procedures during a single visit.

Time [min]	−25	−5	0	15	30	45	60	75	90	105	120	150	180	210	240
Procedure	PE	BS		BS	BS		BS		BS		BS		BS		
		BT		BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT	BT
	BIA	VAS	TM	VAS	VAS	VAS	VAS	VAS	VAS	VAS	VAS	VAS	VAS	VAS	VAS
		DIG					DIG				DIG		DIG		DIG

PE: Physical examination and anthropometry; BIA: Bioelectrical Impedance Analysis; BS: Blood sample; BT: ^{13}C Breath test; VAS: Visual Analogue Scale; DIG: Digestive Symptoms; TM: Test Meal.

and analyzed with IBM SPSS Statistics for Windows, Version 28.0. (IBM Corp., Armonk, NY, USA). All data from the human study were non-normally distributed, and related-samples Friedman's two-way analysis of variance by ranks was applied.

3. Results and discussion

3.1. Product characterization – dietary fiber composition and moisture content

The solubility of the DF can influence the physiological functionality of DF and are, therefore, essential to investigate (Elleuch et al., 2011; Zou et al., 2022). In particular, higher solubility of DF is associated with a reduction in the glycemic response due to an increase in viscosity (Olson et al., 1987; Roehrig, 1988). Images of the control (MC, E150C) and citrus fiber-enriched (MCF, E150CF, E180CF) study products (muffins and extrudates) and their dietary fiber composition and moisture content are shown in Table 2. The appearance of the products was similar among the muffins and extrudates in terms of lightness and color (data not shown). The purchased CF contained around 82 g/100 g insoluble and 18 g/100 g soluble parts on DM of dietary fiber. As expected, the enrichment of the food products with CF resulted in a significantly increased content of insoluble dietary fiber (IDF) and a slight increase in soluble dietary fiber (SDF), which in total led to an increase in total dietary fiber (TDF = SDF + IDF) (Table 2). The amount of TDF slightly increased from the milder baking processing (MC, MCF) to the more intense extrusion processing (E150C, E150CF, E180CF) within the control and the CF-enriched products most likely due to the formation of resistant starch (compare to section 3.2, Table 3) (Guha et al., 1997; Vasanthan et al., 2002; Ye et al., 2018). Furthermore, within the CF-enriched products, the more intense processing of E180CF resulted in an increase in SDF with a simultaneous decrease in IDF compared to the milder processed MCF, in line with the increasing ratio of SDF/TDF from 42.6 % to 53.1 %.

The redistribution of IDF to SDF due to thermal and mechanical effects during extrusion might result from the breakage of glycosidic bonds, leading to smaller and more soluble fractions of polysaccharides, also described in previous studies (Benítez et al., 2011; Larrea et al., 2005; Naumann et al., 2021).

The CF-enriched products (MCF, E150CF, and E180CF) contained at least 4.0 g/100 g TDF in the fresh (undried) sample. Thus, the products could be claimed as a "source of dietary fiber," as the minimum amount of fiber in the product was ≥ 3 g/100 g according to EU Regulation (EC) No. 1924/2006 on nutrition and health claims for foods (Parliament, 2006). The control products almost or just achieved the minimum amount of 3 g /100 g in the fresh (undried) sample (MC 2.6 g/100 g, E150C 3 g/100 g).

3.2. In vitro starch digestibility and in vitro viscosity

3.2.1. Starch digestibility

The digestibility of starch and the free available glucose of the study products are shown in Table 3. The free glucose and rapidly digestible starch (RDS) contents are assigned to the fraction in the digesta that contributes to a rapid increase in postprandial plasma glucose levels (Fraction I in Table 3). On the contrary, the slowly and total digestible starch (SDS + TDS) can be assigned to the fraction of a gradual increase and the resistant starch (RS) of a slowed increase in postprandial glucose levels (Fraction II in Table 3) (Jenkins et al., 2000; Jenkins et al., 1978). Both, inclusion of citrus fibers (CF) as well as processing affected the starch digestibility fractions. The control samples without CF exhibited a higher content of Fraction I than the CF-enriched samples, except for E180CF. Additionally, the content of RS in fraction II was lower for the control samples than for the CF-enriched samples. Furthermore, more intense processing increased the content of free glucose and RS (fraction1) particularly for the E180CF samples, while SDS and TDS decreased. For example, within the CF samples, E180CF showed the highest content in free glucose with 66.1 g/100 g on DM and RS of 1.1 g/100 g on DM and the lowest content in SDS and TDS of 2.8 g/100 g on DM compared to MCF and E150CF. (Table 3).

Food processing – as application of shear forces and thermal treatment – increases the gelatinization of starch (Singh et al., 2010). A higher gelatinized starch is more accessible to digestion (Singh et al., 2010; Wang & Copeland, 2013; Ye et al., 2018) and, thus, can result in a higher content of RDS and free glucose as in our samples. The SEM images also showed a higher degree of gelatinization due to extrusion than due to baking. The extruded samples showed a smoother surface and a more homogeneous matrix than the baked samples, having a rougher surface and a more granulated matrix containing partly intact starch granules (Figure S2, supplementary material). Cooling down the

Table 2
Images, moisture content (MC) and composition of dietary fiber in the test products; composition of dietary fiber is expressed on the dry matter of the sample.

	food products	MC [g/100 g]	IDF [g/100 g]	SDF [g/100 g]	TDF [g/100 g]	SDF/TDF [%]
MC		18.5 ± 0[b]	1.4 ± 0.0[a] *	1.8 ± 0.4[a] *	3.2 ± 0.4[a] *	57.2 ± 15.9[a]
MCF		20.3 ± 0[d]	2.9 ± 0.3[d,c]	2.1 ± 0.4[a]	5.0 ± 0.5[b]	42.6 ± 11.1[a]
E150C		20.7 ± 0.1[e]	1.9 ± 0.4[b] *	1.8 ± 0.2[a] *	3.7 ± 0.4[a] *	47.8 ± 7.7[a]
E150CF		18.1 ± 0.1[a]	3.0 ± 0.0[d]	2.4 ± 0.2[a,b]	5.4 ± 0.6[b]	43.8 ± 4.0[a]
E180CF		19.8 ± 0[c]	2.5 ± 0.1[c]	2.8 ± 0.2[b]	5.3 ± 0.1[b]	53.1 ± 5.4[a]

The different letters a, b, c, d and e indicate significant differences within a column at the $p \leq 0.05$ level basis. IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF = IDF + SDF: total dietary fiber MC, MCF: muffins; E150C, E150CF: extrudates at 150 °C barrel temperature ; E180CF: extrudate at 180 °C barrel temperature; CF: citrus fiber-enriched products; C: control products; data are presented as mean ± SD of 3 experiments each being measured in duplicate to reveal a total of n = 6.

* Republished from Miehle et al. (2024).

Table 3
Free glucose, digestible starches and resistant starch in the test product and the viscosity ($\dot{\gamma}$= 1.7 s^{-1}) of *in vitro* digesta; g/100 g are expressed on the dry matter of the sample.

			MC	MCF	E150C	E150CF	E180CF
Digestibility of starch	Fraction I	Free glucose [g/100 g]	65.6 ± 6.7^{b,c} *	52.0 ± 2.6 ^{a}	68.4 ± 4.0^{b} *	55.6 ± 1.7^{a,c}	66.1 ± 3.1^{b}
		of which added glucose ** [g/100 g]	52.2	51.6	52.2	51.6	51.6
		RDS [g/100 g]	4.3 ± 3.3^{b} *	8.8 ± 2.1^{c}	0.1 ± 0.7^{a} *	1.0 ± 0.1^{a,b}	1.4 ± 1.0^{a,b}
	Fraction II	SDS + TDS [g/100 g]	16.3 ± 0.8^{b} *	12.0 ± 8.5^{a,b}	7.1 ± 3.1^{a,b} *	6.5 ± 3.9^{a,b}	2.8 ± 3.4^{a}
		RS [g/100 g]	0.6 ± 0.4^{a} *	0.7 ± 0.2^{a,b}	0.5 ± 0.2^{a} *	0.8 ± 0.0^{a,b}	1.1 ± 0.0^{b}
Viscosity of *in vitro* digesta		η_B [mPas]	32.5 ± 4.7^{a} *	30.6 ± 0.1^{a}	56.8 ± 2.7^{c} *	48.8 ± 0.5^{b}	49.4 ± 5.3^{b,c}
		η_E [mPas]	10.2 ± 0.7^{a} *	9.3 ± 0.9^{a}	21.2 ± 2.7^{b,c} *	25.5 ± 0.5^{c}	19.5 ± 1.1^{b}

The different letters a, b and c indicate significant differences within a row at the $p \leq 0.05$ level basis. Free glucose: content of glucose before starch hydrolysis in the small intestinal phase; RDS: rapidly digestible starch; SDS: slowly digestible starch; TDS: total digestible starch; RS: resistant starch; Fraction I: Free glucose and RDS; Fraction II: SDS + TDS and RS; η_B, viscosity at the beginning of the *in vitro* intestinal digestion (4 h); η_E, viscosity at the end of the *in vitro* intestinal digestion (4 h); MC, MCF: muffins; E150C, E150CF: extrudates at 150 °C barrel temperature; E180CF: extrudate at 180 °C barrel temperature; CF: citrus fiber-enriched products; C: control products; data are presented as mean ± S.D of 3 experiments each being measured in duplicate to reveal a total of n = 6.

* Republished from Miehle et al. (2024).

** Refer to Table S1 (supplementary material).

gelatinized starch leads to the retrogradation of starch and formation of resistant starch (Goñi et al., 1997), which explains the slight increase in RS with more intense processing conditions.

Fiber enrichment by CF seemed to lower the digestibility of starch. One reason could be a lower gelatinization of starch, as fiber immobilizes water, which is less available for starch granules (Chaisawang & Suphantharika, 2005; Kaur et al., 2008; Miehle et al., 2024; Song et al., 2006; Wu et al., 2016). Another reason could be an aggregation of starch granules in the presence of CF (Chaisawang & Suphantharika, 2005; Fabek & Goff, 2015; Miehle et al., 2024), also indicated by the SEM-images of the enriched muffins, which showed larger starch granules for MCF than MC (Figure S2, supplementary material). However, the pictures do not provide a complete representation of the samples and are merely selective images. Furthermore, the enzymatic hydrolysis could be retarded by coating the surface of the starch granules with CF, building a physical barrier to either enzymatic hydrolysis or release of hydrolysis products (Dartois et al., 2010; Hyun-Jung et al., 2007).

3.2.2. Viscosity

In addition to a low digestibility of starch, a high viscosity of the digesta is also often associated with a reduction in postprandial blood glucose levels and, additionally, with delayed gastric emptying and retarded transit through the digestive tract (Goff et al., 2018; Mälkki, 2001). To estimate the effect of the food products on small intestinal viscosity, a static *in vitro* digestion protocol was used. The viscosity of the *in vitro* digesta before and after the intestinal phase was monitored over the shear rate (Table 3). Flow curves of the *in vitro* digesta showed a shear thinning behavior for all products (Figure S1, supplementary material).

The enrichment with CF showed unexpectedly lower viscosities (η) of the digesta at the beginning (η_B) and end (η_E) of the *in vitro* intestinal digestion compared to the control (Table 3). Only the CF sample E150CF showed a higher η_E than the respective control E150C. More intense processing significantly increased the viscosity. For example, E180CF showed an almost twice as high viscosity than MCF, both at the beginning (49.4 mPas vs. 30.6 mPas, respectively) and the end (19.5 mPas vs. 9.3 mPas, respectively) of digestion. Overall, the four hours of digestion of the samples led to a significant decrease in viscosity for all samples (Table 3).

More intense processing enhances starch gelatinization and protein gelling, increasing viscosity (Wang & Copeland, 2013). In addition to the gelation effect, the increase in the soluble part of dietary fiber due to processing (Table 3) could also increase viscosity. The lower viscosity resulting from CF-addition could originate from a lower gelatinization than the respective control, which led to a lower starch digestibility (Table 3). The hydrolysis of the gel network of starch and proteins due to digestive enzymes led to lower viscosities of the samples after the four hours of the intestinal phase of the *in vitro* digestion. The significantly lower η_E of E180CF than E150CF might indicate the degradation of pectin-like fibers of the CF due to ß-elimination at higher temperatures (Sila et al., 2009).

3.3. In vitro glucose release

A slow glucose release from the digesta to the small intestinal microvilli is associated with reduced postprandial blood glucose levels (Goff et al., 2018). To mimic the intestinal release of amylolytic products, we measured the *in vitro* release of glucose from the *in vitro* digesta for four hours (Fig. 1).Glucose release from both control (C) samples (filled symbols Fig. 1A1&A2) was faster than from the citrus fiber (CF)-enriched samples (empty symbols Fig. 1A1&A2). Thereby, the maximum concentration of glucose after 4 h (c_f) and the area under the release curve (AUC) was significantly higher for the control samples than for the CF-enriched samples ($p \leq 0.05$) (Table 4), with the highest value of 1,699 mg/dL for MC and 3,724 mg*h/dL for E150C, respectively. Therefore, enrichment of the samples with CF led to a decreased *in vitro* release of glucose compared to the respective control samples. Possible reasons could be the lower digestibility of CF-enriched samples due to the retardation of starch gelatinization and starch hydrolysis and the occurrence of larger starch granules (SEM images, Figure S2, supplementary material) This could lead to a lower amount of free glucose and a higher amount of SDS + TDS than in the control samples (section 3.2).

The different processing methods with baking and extrusion at a temperature of 150 °C did not appear to affect glucose release within the control and CF samples, also seen by similar c_f and AUC values (Table 4). However, processing with extrusion at the higher barrel temperature of 180 °C showed a significant effect, resulting in a lower glucose release curve for E180CF (Fig. 1A2). The c_f of 1,170 mg/dL and the AUC of 2,614 mg*h/dL for E180CF showed the significantly lowest values compared with all other samples.

During processing, a network of gelatinized starch and denatured proteins is formed by heat exposure. The network of the extruded samples compared to baked samples in the CF-enriched and control

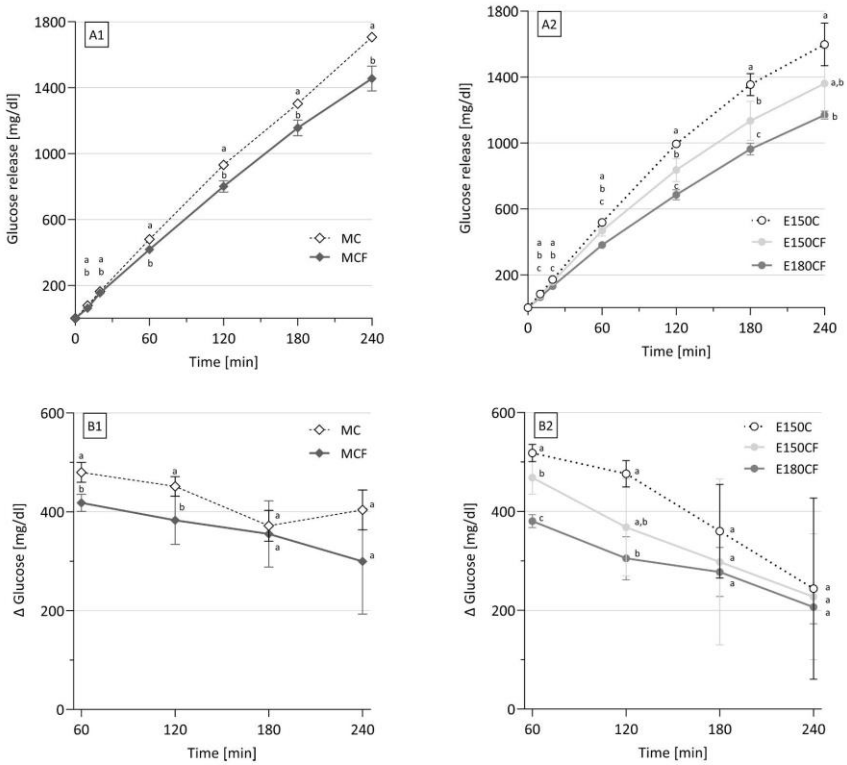

Fig. 1. Glucose release over time (A) and the amount of released glucose within every hour (B) during the *in vitro* intestinal phase of muffins (MC, MCF) (A1 & B1) and extrudates (A2 & B2) at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). The symbols in Figure (A) correspond to the experimental data and the lines to fitted data according to Equation (2) ($R2 \geq 0.96$). Data from the control samples (MC and E150C) are republished from Miehle et al. (2024).

Table 4
Descriptive parameters of glucose release over time: maximum concentration of glucose after 4 h (c_f) and the area under the glucose release curve within 4 h (AUC) (see equation (3)) of the study products.

	MC	MCF	E150C	E150CF	E180CF
C_f [mg/dL]	1699 ± 20^{b} *	1462 ± 135^{a}	1676 ± 155^{b} *	1379 ± 133^{a,b}	1170 ± 90^{a}
AUC [mg*h/dL]	3578 ± 20^{b} *	3112 ± 135^{a}	3724 ± 142^{c} *	3209 ± 133^{b}	2614 ± 90^{a}

The different superscript letters a, b and c indicate significant differences at the $p \leq 0.05$ level basis within the muffins and the extrudates of a row. MC, MCF: muffins; E150C, E150CF: extrudates at 150 °C barrel temperature; E180CF: extrudate at 180 °C barrel temperature; CF: citrus fiber-enriched; C: control; data are presented as mean ± S.D of 3 experiments in duplicate revealing a total of n = 6.

* Republished from Miehle et al. (2024).

samples seems to be stronger, as the viscosity of the *in vitro* digesta before digestion (η_B) was higher (see Table 3). Differences in the networks of the different processed samples were also indicated by the quantity of glucose released over time (Δglucose). The baked control sample MC showed an increase in glucose in the fourth hour (Fig. 1B1), which can be attributed to a less stable network after 3 h of *in vitro* digestion. Rising the barrel temperature during extrusion to 180 °C led to the lowest glucose release of E180CF within all samples (Fig. 1B1&B2). The sample showed the highest amount of SDF and RS. We observed an overall strong negative linear association of RS and AUC within all samples (R^2 of 0.93).

3.4. Human study participants

Thirteen volunteers (10 female, and 3 male) were included in the *in vivo* study. Data from 11 participants who completed the study were used for the analysis. One female participant withdrew her consent from the study due to intolerance of study products, and one male participant was excluded from the study due to a significant weight loss between screening and the first intervention visit. Four participants were overweight (BMI $\geq$ 25 kg/m^2) and 7 were obese (BMI $\geq$ 30 kg/m^2). The demographic characteristics of the participants are shown in Table 5.

3.5. Postprandial glycemic and insulinemic response, gastric emptying

Postprandial blood glucose concentrations did not significantly differ between the CF-enriched and the control products at any time point (p for all > 0.05; Fig. 2 A1 and A2). A modest reduction in mean blood glucose values was observed 90 min after consumption of E150CF and E180CF. However, these differences did not reach statistical significance. At 90 min, the mean glucose concentration for E150C was 146.8 ± 38.4 mg/dL, whereas it was 137.5 ± 36.1 mg/dL for E150CF and 133.4 ± 40.3 mg/dL for E180CF (Fig. 2A2). Similarly, at the 120-minute time point, the mean glucose concentration for E150C was 137.0 ± 42.1

Table 5
Participant demographic characteristics (n = 11).

	Mean	Minimum	Maximum
Age, years	57.1 ± 7.5	45.5	66.0
Weight, kg	89.7 ± 15.3	65.7	113.8
Body mass index, kg/m^2	32.0 ± 5.3	25.3	42.8
Body muscle mass, kg	24.7 ± 5.3	18.2	34.8
Body fat mass, %	37.2 ± 8.4	23.1	55.5
Visceral fat, kg	3.29 ± 2.10	1.50	8.12
Waist circumference, cm	103.5 ± 12.0	89.0	126.0
Waist-to-hip ratio, cm	0.91 ± 0.08	0.78	1.05
Waist-to-height ratio, cm	0.62 ± 0.07	0.52	0.74
Fasting glucose, mg/dL	98.0 ± 6.6	85.0	108.0
HbA1c, %	5.38 ± 0.33	5.00	5.80
Fasting insulin, µU/mL	29.4 ± 12.1	19.5	61.7
Total cholesterol, mg/dL	206.6 ± 24.1	174.0	244.0
HDL-Cholesterol, mg/dL	55.8 ± 9.7	38.0	68.0
LDL-Cholesterol, mg/dL	141.5 ± 22.8	113.0	177.0
Triglycerides, mg/dL	97.7 ± 36.4	45.0	144.0
Systolic blood pressure, mmHg	126.2 ± 21.4	103.0	174.5
Diastolic blood pressure, mmHg	83.4 ± 8.1	74.5	96.5
Heart rate, beats/minute	65.2 ± 14.0	46.5	92.0

mg/dL, whereas it was 125.6 ± 28.1 mg/dL for E150CF and 127.6 ± 31.1 mg/dL for E180CF (Fig. 2A2). At 180 min, the mean glucose concentration for E150C was 114.1 ± 34.6 mg/dL, compared to 107.4 ± 29.6 mg/dL for E150CF and 105.8 ± 28.3 mg/dL for E180CF (Fig. 2A2).

No significant differences were observed in mean glucose $_{i}$AUC (Fig. 2B, $p > 0.05$) and mean glucose ΔC_{max} (Fig. 2 C, $p > 0.05$). Mean glucose $_{i}$AUC and ΔC_{max} were slightly higher for MCF than MC (108.5 ± 54.0 mg*h/dL vs. 95.3 ± 52.4 mg*h/dL and 68.5 ± 21.1 mg/dL vs. 61.7 ± 25.2 mg/dL, respectively) (Fig. 2B and 2C, $p > 0.05$). Mean glucose $_{i}$AUC was slightly lower for E150CF and E180CF than for E150C (99.8 ± 57.0 mg*h/dL and 96.6 ± 54.3 mg*h/dL vs. 107.5 ± 71.6 mg*h/dL, respectively) (Fig. 2 B, $p > 0.05$).

Individual glucose $_{i}$AUC (Fig. 3A) and glucose ΔC_{max} (Fig. 3B) values for particular study products were inconsistent. Individual glucose $_{i}$AUC was highest after consumption of control products in 6 participants (E150C in 4 participants and MC in 2 participants) and in 5 participants after consumption of CF-enriched products (E180CF in 3 participants and MCF in 2 participants). Individual glucose $_{i}$AUC was lowest in 5 participants after consumption of control products (E150C in 1 participant and MC in 4 participants) and in 6 participants after consumption of CF-enriched products (E150CF in 2 participants and E180CF in 4 participants) (Fig. 3B). Regarding individual glucose ΔC_{max}, the highest values were seen in 3 participants after consuming control products

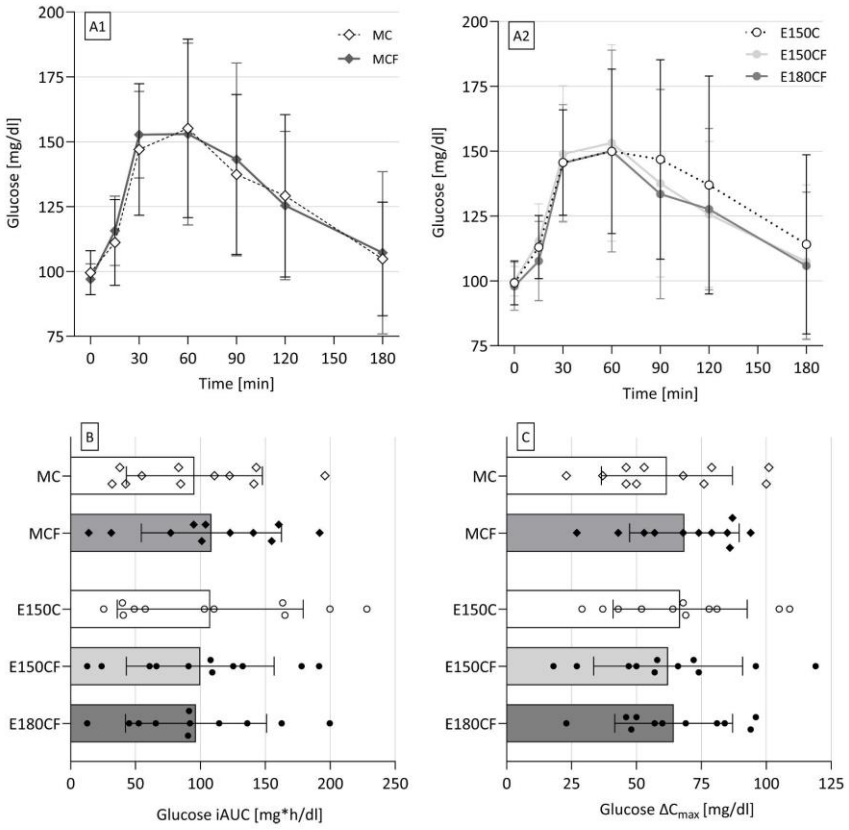

Fig. 2. (A1) Postprandial glycemic response curves for muffins (MC, MCF). (A2) Postprandial glycemic response curves for extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). (B) Postprandial glucose incremental area under the curve ($_{i}$AUC) for muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). (C) Baseline-adjusted maximum postprandial glucose concentration (ΔC_{max}) for muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). The points in Figures (B) and (C) represent values of individual participants.

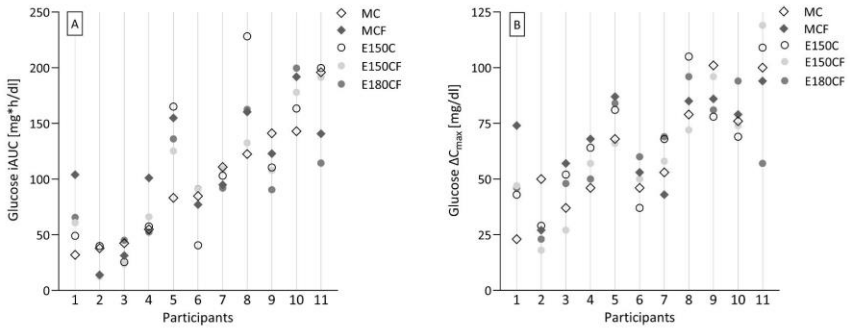

Fig. 3. Individual glucose responses to the study products. (A) Postprandial incremental area under the curve ($_{i}$AUC) for muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). (B) Baseline-adjusted maximum postprandial glucose concentration (ΔC_{max}) for muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). The data have been ordered by $_{i}$AUC values for MC.

(E150C in one participant and MC in 2 participants), while 8 participants exhibited the highest values after consuming CF-enriched products (E150CF in one participant, E180CF in 3 participants, and MCF in 4 participants), as shown in Fig. 3C. The lowest individual glucose ΔC_{max} values were recorded for control products in 5 participants (E150C in 3 participants and MC in 2 participants) and in 6 participants after the consumption of CF-enriched products (E150CF in 4 participants, E180CF in one participant, and MCF in one participant), as depicted in Fig. 3C.

No significant differences between CF-enriched and control products were observed in the postprandial insulinemic response. However, mean insulin concentration was slightly, but not significantly elevated at 90, 120 and 180 min after the consumption of E150C in comparison with E150CF and E180CF (Figure S3, supplementary material). Likewise, no significant differences were found for postprandial insulin incremental AUC and insulin ΔC_{max} levels. Again, a rather large variation of these parameters of insulin secretion was observed (Figure S3, supplementary material).

No significant differences between CF-enriched and control products were observed in pulmonary $^{13}CO_2$ excretion rate and $^{13}CO_2$ cumulative pulmonary excretion rate showing similar gastric emptying. However, the CF-enriched products exhibited slightly lower values, with this difference being particularly visible in muffins (Figure S4, supplementary material).

4. Correlation of *in vitro* and *in vivo* data

The *in vitro* glucose release was significantly lowered by CF addition and intense processing by extrusion at 180 °C. Thereby, the lowered *in vitro* glucose release is attributed to an interplay of the amount of TDF, the, the digestibility of starch, the formation of resistant starch, and the viscosity of the *in vitro*-digested chyme. The high pressure, shear, and heat exposure during the extrusion of E180CF increased the TDF content, with a redistribution from IDF to SDF, by an increased amount of water held in the matrix. Thereby, the viscosity of the *in vitro* digesta is raised. Food products with high SDF, and viscosity are often associated with reduced postprandial blood glucose levels (Elleuch et al., 2011; Goff et al., 2018). Extrusion also increased the digestibility of starch due to higher gelatinization by intense breakdown and melting of the starch granules, simultaneously increasing the amount of free glucose. In contrast to processing, enrichment of the products with CF led to a lowered digestibility of starch and a decreased viscosity of the *in vitro* digesta.

A food product with a high amount of rapidly digestible starch and free glucose is likely to cause blood sugar levels to rise quickly (Jenkins et al., 2000). However, sample E180CF resulted in the lowest *in vitro* glucose release curve with the significantly lowest values of c_f and AUC compared with all other samples (Fig. 1A2). Extrusion at 180 °C formed a strong and homogenous matrix of proteins and starch, permeated with CF (SEM image Figure S2, supplementary material). The compact structure of E180CF could decrease the glucose diffusion in the food matrix (Gidley & Yakubov, 2019), particularly under *in vitro* conditions. Furthermore, the increase in RS by extrusion (Table 3) could also lower glucose release (Wang & Copeland, 2013). The high amount of free glucose in E180CF seemed to play a more subordinate role in *in vitro* glucose release than the strong matrix and high amount of RS, as the release was low despite the elevated amount of free glucose.

As recently shown, the same observation was made for the extrudate at 180 °C barrel temperature of the control sample (Miehle et al., 2024). Thereby, the glucose release curve was lowered to a similar extent as for the E180CF. That leads to the assumption that the processing effect seemed higher than that of CF-addition. The same observations were previously made by Papakonstantinou et al. (2022), where the type of food (spaghetti vs. bread) had a higher impact than the addition of dietary fibers. Previous research supported our findings that a strong network (Jenkins et al., 1987; Petitot et al., 2009; Yang et al., 2019), a higher amount of resistant starch (Bravo et al., 1998; Miehle et al., 2024), and the incorporation of partly soluble fibers (Adiotomre et al., 1990; Goff et al., 2018) may reduce the glycemic response. However, the results of our human study were not in line with these observations. Although both CF enrichment and food processing significantly reduced *in vitro* glucose release, our *in vivo* findings yielded inconclusive results. The mean postprandial blood glucose values did not significantly differ between the CF-enriched and control products at any given timepoint, nor were they significantly affected by the food processing method (baking vs. extrusion). Additionally, we observed a high level of individual variation in the glycemic response to specific study products, making it difficult to identify a consistent response pattern among the participants. These discrepancies may be due to an oversimplification of the physiological conditions in the gastrointestinal tract in the *in vitro* model, as it is impossible to simulate all parameters relevant for digestion, such as particle size (which may be influenced by mastication intensity and time), mixing with digestive enzymes, gastric and intestinal pH values, gastric emptying times as well as insulin response and clearance (Bellmann et al., 2018), which may have varied across individuals participating in the human study.

The association of high DF consumption with improved glycemic control and decreased risk of developing type 2 diabetes has been previously described in observational and long-term interventional studies (McRae, 2018; Yao et al., 2014). These effects may be attributed to the quality of carbohydrates in the diet and to increased general intake of wholegrain foods (Della Pepa et al., 2018; Reynolds et al., 2019; Tieri

et al., 2020; Weickert & Pfeiffer, 2018). To date, positive impact of isolated DF (including intake of DF supplements) on blood sugar regulation has been observed in long-term interventional studies (Jovanovski et al., 2019; Xie et al., 2021), while there is little evidence on favorable effects of DF-enriched foods on acute glycemic response after single exposure to a DF-enriched product. The purpose of our study was to compare the immediate glycemic response to the study products. Participants were exposed to each product only once, and postprandial glycemia was measured for 180 min, which is a considerable limitation that may have contributed to the inconclusive results. However, our findings are in agreement with a systematic literature review on the impact of dietary fiber in starchy food products on acute postprandial glycemic response, where a clear relationship between DF consumption and blunting of postprandial glycemia could not be observed (Tsitsou et al., 2023). In the same review, both soluble DF and resistant starch showed mixed results for postprandial insulinemia with most studies lacking an effect. There is limited evidence that resistant starch may improve insulinemic responses in a dose-dependent manner. In our study, the proportion of resistant starch was rather low (<1 g/100 g) in all products and therefore unlikely to exert a significant effect on insulinemia.

Participants in the present study consumed a substantial portion of 100 g of available carbohydrates. Previous studies on DF-enrichment of starchy foods which have reported favorable effects of DF-enrichment on postprandial glycemia used foods containing 50 g of available carbohydrates (Chillo et al., 2011; Jenkins et al., 2008; Matsuoka et al., 2020; Robert et al., 2016; Thondre & Henry, 2009), while in other studies that utilized more than 50 g of available carbohydrates, no effect of DF-enrichment on postprandial glucose could be observed (Panahi et al., 2014; Papakonstantinou et al., 2022; Thondre & Henry, 2009). In a study where muffins containing 74 – 93 g of available carbohydrates were used, postprandial glycemia and glucose area under the curve were higher following the consumption of DF-enriched products compared to control products (Willis et al., 2011). CF-enriched products in the present study contained 2.5 g – 2.75 g fiber per 50 g available carbohydrates, which is a relatively low amount compared to the previous studies which employed up to 16 g fiber per 50 g available carbohydrates. Our findings suggest that solely increasing the DF content in high-glycemic products may not be sufficient to effectively reduce postprandial glycemia. When developing novel products, it is important to consider not only the elevated DF content but also the macronutrient composition of DF-enriched foods, with particular attention to the ratio of DF and available carbohydrates.

It is important to mention that previous studies utilized different types of DF, mostly β-glucan (Chillo et al., 2011; Matsuoka et al., 2020; Panahi et al., 2014; Thondre & Henry, 2009; Thondre & Henry, 2011; Yokoyama et al., 1997) and different kinds of water-soluble fiber (Frost et al., 2003; Papakonstantinou et al., 2022; Robert et al., 2016; Willis et al., 2011), whereas in our study a partly insoluble DF ingredient was used. In addition, different food matrices were used in the previous studies: freshly made pasta (Yokoyama et al., 1997), spaghetti (Chillo et al., 2011; Frost et al., 2003; Papakonstantinou et al., 2022), flatbread (Robert et al., 2016; Thondre & Henry, 2009; Thondre & Henry, 2011), bread (Matsuoka et al., 2020) biscuits (Jenkins et al., 2008), muffin (Willis et al., 2011) and bar (Panahi et al., 2014) making it challenging to compare findings of those studies with our results and provide an explanation for our inconclusive outcomes.

The key finding of our study is the high individual variation in glycemic responses to identical study products. Notably, some participants exhibited a more favorable glycemic profile after consuming the control products rather than the DF-enriched products. (Fig. 3). Inconclusive results on acute postprandial glycemia and glycemic index of particular foods have been previously described in interventional studies (Matthan et al., 2016; Vega-López et al., 2007; Williams et al., 2008; Zeevi et al., 2015). However, to our knowledge, no previous study on the influence of DF on blood glucose has examined individual glycemic responses and only mean glucose values of all participants have been reported. The glycemic response can be influenced by the habitual diet (Reik et al., 2022) and the meal preceding the test meal (Wolever et al., 1988). To reduce this potential confounding factor, participants in the present study were instructed to consume and record a defined dinner meal prior to each test day. However, participants consumed their dinner at home without supervision, which could have resulted in inconsistencies in reporting and impact the glycemic response on the following day. Several other factors can influence the individual glucose response, including age, physical activity level, gut microbiota, body composition (lean body mass and body fat percentage), metabolic parameters such as fasting glucose and insulin levels, triglycerides, and blood cholesterol (Matthan et al., 2016; Zeevi et al., 2015). Our study participants were defined by age, waist circumference, absence of chronic diseases, and untreated metabolic conditions, while their metabolic parameters exhibited high variability (see Table 5). As the number of participants was low (n = 11), it was impossible to perform a sub-group analysis and seek correlations between the individual metabolic situation above and glucose metabolism.

No significant differences between CF-enriched and control products were observed in postprandial insulinemic response, and a high variability was observed among participants, as no consistent pattern in insulinemic response to CF-enriched products was observed on individual level. Mean insulin concentrations for E150C remained elevated starting from 90 min after test meal in comparison with CF-enriched extrudates, which mirrored the postprandial glycemia. The mean pulmonary $^{13}CO_2$ excretion rate was slightly, but not significantly lower after consumption of CF-enriched products. The values were consistent for all time points, which indicates that gastric emptying rate could have been impacted by CF and is in agreement with previous research (Müller et al., 2018). However, a higher number of study participants would be required to potentially obtain statistically significant results.

5. Conclusions

The promising results obtained in *in vitro* experiments could not be confirmed *in vivo* in a randomized, double-blind, crossover intervention trial with single exposure to defined carbohydrate-rich food products. While both CF addition and food processing methods significantly influenced glucose release in the *in vitro* model, acute postprandial glycemic and insulinemic responses did not significantly differ *in vivo* after consuming the study products. Furthermore, the human study outcomes showed high variability among the participants, suggesting that this individual variability may play a greater role for the glycemic and insulinemic response than the composition and processing of defined carbohydrate-rich foods. However, it is noteworthy that *in vitro* models simulating all phases of digestion or including *in silico* simulation could become a valuable tool to investigate the principal effect of added fibers and processing technologies on glucose availability in the development of carbohydrate-containing foods. The novel learning from this study was that results from this approach require confirmation in human studies. As the present study only investigated the acute response to test products, the effects of CF addition and food processing should be additionally examined in long-term intervention studies better reflecting the "real world" situation.

Ethical Statement

The human study was registered in the German Clinical Trials Register (DRKS00020983), and the study protocol was approved by the Ethics Committee of the Faculty of Medicine of the Technical University of Munich (approval no. 515/19). Before participation, all individuals provided written informed consent. The study procedures followed the principles outlined in the Declaration of Helsinki.

CRediT authorship contribution statement

Elisabeth Miehle: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Katarzyna Pietrynik:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Stephanie Bader-Mittermaier:** Writing – review & editing, Validation, Project administration, Funding acquisition, Conceptualization. **Thomas Skurk:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Peter Eisner:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hans Hauner:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2024.106230.

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Supplementary material

Table S1 Dough formulation of the control (Dough C) and the citrus fiber enriched dough (Dough CF) of the fresh dough and on dry matter (DM). The dough of one serving of the test foods contained 100 g of available carbohydrates, of which 66.6 g were glucose and 33.3 g were rapidly available carbohydrates. Therefore, all test products equally contained the same amount of available carbohydrates.

	Dough C	Dough C on DM	Dough C per serving (muffin/ extrudate) (173.2 g)	Dough CF	Dough CF on DM	Dough CF per serving (muffin/ extrudate) (175.6 g)
	[%]	[%]	[g]	[%]	[%]	[g]
Citrus fiber (CF)	-	-		0.9	1.1	1.5
All-purpose wheat flour	27.1	32.0	46.9	26.7	31.6	46.9
of which starch	20.9	24.6	34.8	20.6	24.4	35.6
Glucose	38.5	52.2	66.6	38.0	51.6	66.6
Baking powder	0.9	1.2	1.5	0.9	1.1	1.5
Salt	0.2	0.2	0.3	0.2	0.2	0.3
Sunflower oil	7.1	9.6	12.2	7.0	9.5	12.2
of which ^{13}C octanoic acid	0.06	0.08	0.10	0.06	0.08	0.10
Egg	15.7	4.8	27.2	15.5	4.8	27.2
Water	10.5	-	18.4	10.9	-	19.2

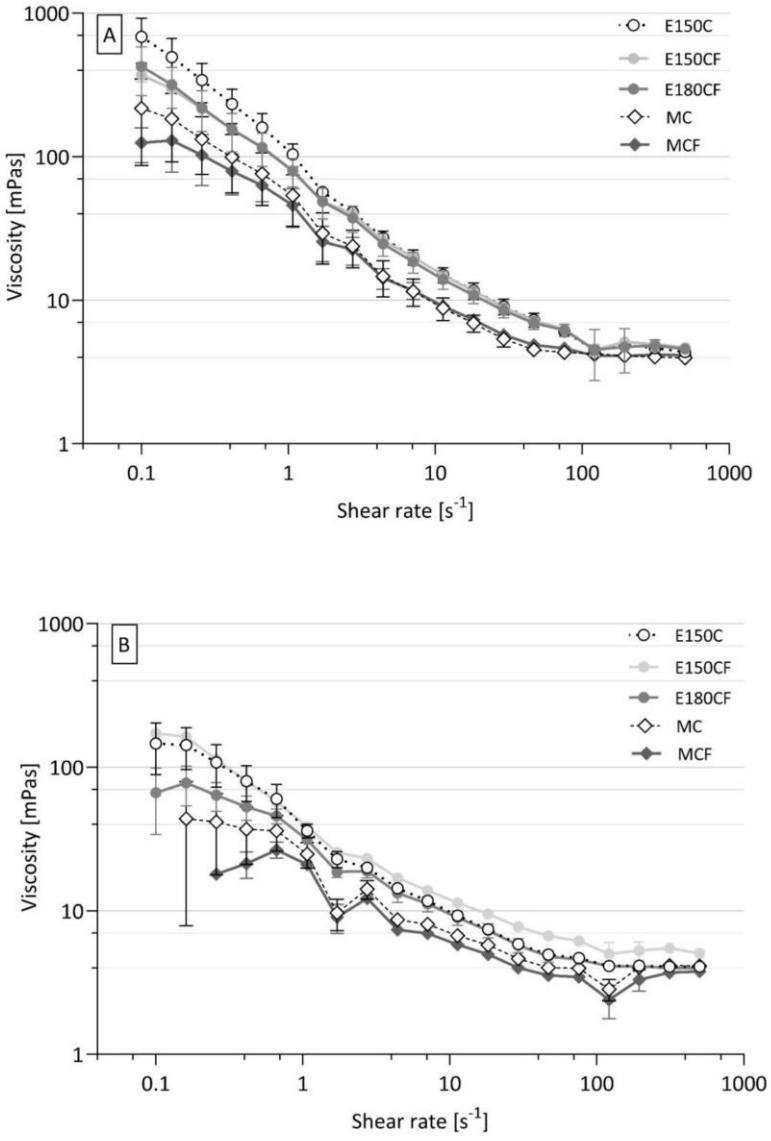

Figure S1 Flow curves at the beginning (A) and end (B) of the *in vitro* intestinal digestion (4h) of the muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF).

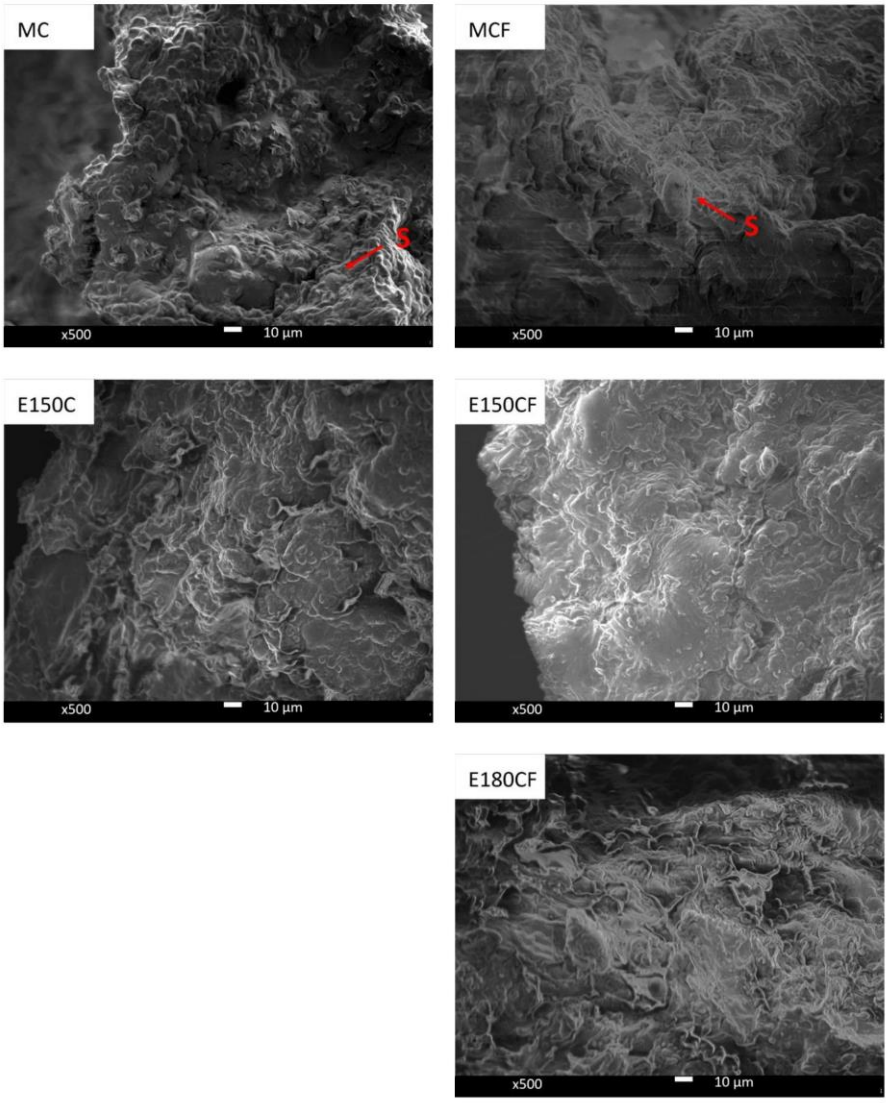

Figure S2 Scanning electron microscopy images (x500) obtained using a backscattered electron detector (BED) for muffins (MC, MCF) and extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF); intact starch granules (S) are marked by arrows. Images of MC and E150C are republished from Miehle et al. (2024).

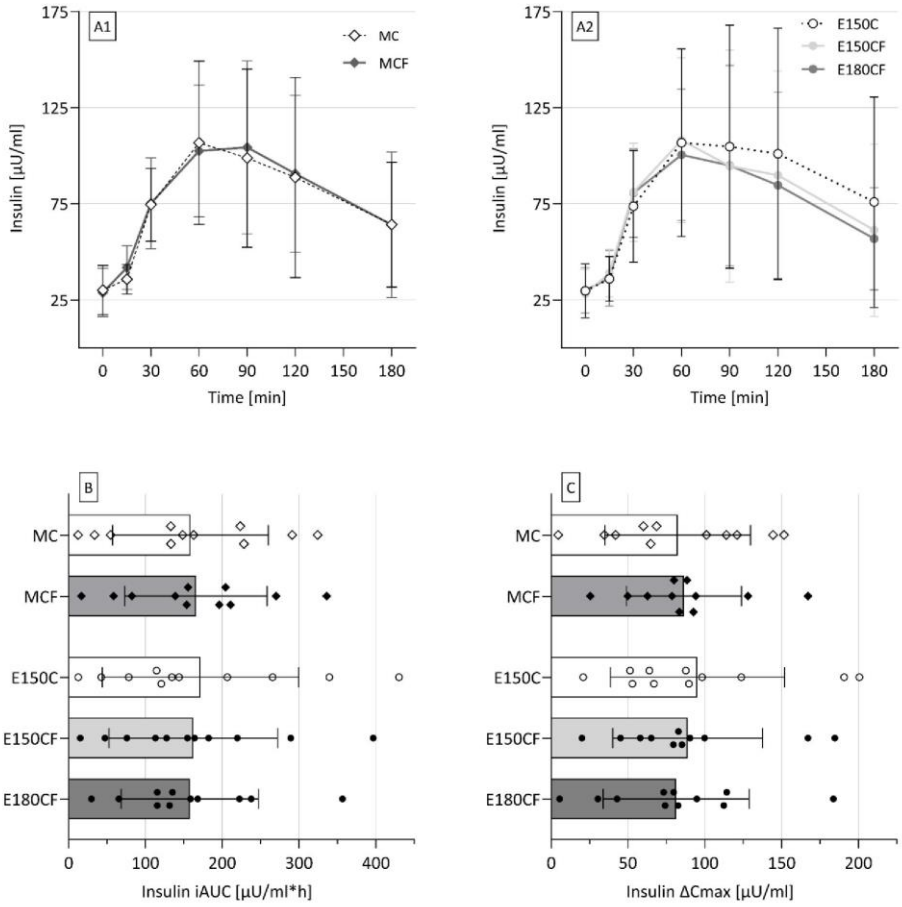

Figure S3 (A1) Postprandial insulinemic response for muffins (MC, MCF). (A2) Postprandial insulinemic response for extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). (B) Postprandial insulin incremental area under the curve (iAUC) for muffins (MC, MCF) and extrudates (E150C, E150CF, E180CF).(C) Baseline-adjusted maximum postprandial insulin concentration (ΔCmax) for muffins (MC, MCF) and extrudates (E150C, E150CF, E180CF).

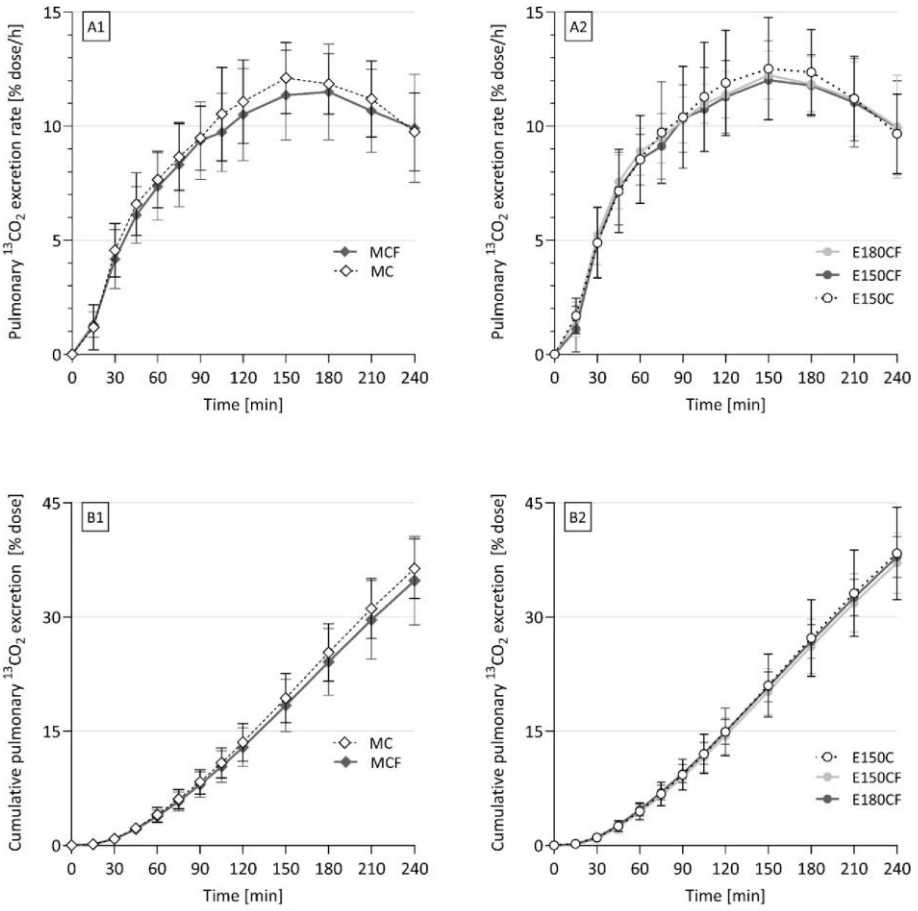

Figure S4 (A1) Time-course for pulmonary $^{13}CO_2$ excretion rate for muffins (MC, MCF). (A2) Time-course for pulmonary $^{13}CO_2$ excretion rate for extrudates at 150 °C and 180 °C barrel temperatures (E150C, E150CF, E180CF). (B1) Cumulative $^{13}CO_2$ excretion rate for muffins (MC, MCF) and extrudates (E150C, E150CF, E180CF (B2).

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Miehle, E., Eisner, P., & Bader-Mittermaier, S. (2024). Effects of food processing on in vitro glucose release of high methylester pectin-enriched doughs. *Food Chemistry*, *442*, 138331. https://doi.org/https://doi.org/10.1016/j.foodchem.2023.138331

Concluding Remarks and Outlook

The growing number of people with type 2 diabetes places a significant burden on healthcare systems and society. The positive influence of dietary fibers (DF) on human health was enlightened in both intervention trials and epidemiological studies in the past decades (Goff, Repin, Fabek, El Khoury & Gidley, 2018). The reduced risk of type 2 diabetes and the improved glycemic control associated with high DF consumption are considered a given. To achieve positive effects, a diet should include at least 25 to 50 g of DF, as suggested by Anderson, Randles, Kendall and Jenkins (2004). For instance, increasing DF intake by 10 g per day can reduce the relative risk of type 2 diabetes up to 75% (Consortium, 2015). However, in many highly civilized countries such as Germany the average total intake of dietary fiber is low, with an average of only 24 g (Nationale Verzehrsstudie, 2015). This is due to a preference for refined foods high in white flour, sugar, and fat, which are favored for their taste, while foods high in DF are not as widely accepted (Meuser, 2008). The positive effects on blood glucose levels related to the addition of DF after consuming high-glycemic foods can be explained by several mechanisms of action. These mechanisms include the delay of amylolysis and sugar absorption by viscous DF. However, their contribution levels and the interactions with potential other mechanisms are still not fully understood. It is also well known that not all types of DF behave similarly in relation to their impact on maintaining blood glucose levels, as the source of fiber with different molecular structures exhibits different physicochemical properties and, thus, also physiological characteristics. However, to date, this link between the molecular structure of DF and its physiological role in the small intestine still remains unclear (Goff et al., 2018). Furthermore, the impact of other food ingredients, as well as changes in the food matrix and the interactions between all ingredients and DF during food processing and storage, are still unknown. This is evident in the diverging results of various studies that compare investigations using isolated DF to those investigating food products (Cassidy, McSorley & Allsopp, 2018). Additionally, emphasizing the importance of *in vivo* studies is valuable, as they serve to validate and enhance the credibility of results obtained through *in vitro* methods. Therefore, the food industry faces the challenge of defining 'new DF' that do not negatively affect color, texture, or palatability, while also providing significant health benefits, such as maintaining blood glucose levels. Furthermore, these DF should remain stable during food processing to retain their positive effects.

Thus, the present thesis enlightened the role of selected soluble DF and citrus fiber, which are neutral in color and taste, in solutions as well as in model food systems using an *in vitro* digestion model to measure their effect on glucose release in the small intestine and the delay of amylolysis. For this purpose, a deeper understanding of the mechanistic principles and the molecular

structure-function relationship was gained through the use of a mimicked chyme in the intestine containing DF, starch, sugars, and digestive enzymes. Since the food components and enzymes are transported by diffusion and convection in the chyme during digestion, depending on the intestinal motility, the first two studies examined the effect of adding DF to aqueous solutions with varying concentrations and milieu conditions on the transport of sugar (glucose) to the mucosa by two different mechanisms: diffusion and convection. The third and fourth study further explored the impact of various food processing methods on the amylolysis and glycemic impact of DF-enriched foods.

The outcomes of the *in vitro* investigations were further validated by *in vivo* studies conducted by the research partner ‘core facility human studies’ of the ZIEL Institute for Food & Health at the Technical University of Munich.

1. *In Vitro* Analysis of Glucose Release

An *in vitro* approach was applied to investigate the effect of the tested DF on glucose release. Various static *in vitro* methods are available to study glycemic response with DF supplementation (Priyadarshini, Moses & Anandharamakrishnan, 2022). Most commonly are restricted methods, where released glucose out of a dialysis bag in a buffer solution is measured (Dhital, Gidley & Warren, 2015; Ou, Kwok, Li & Fu, 2001; Qi et al., 2016; Srichamroen & Chavasit, 2011). In this regard, some research lacks the transferability of the *in vitro* results to the physiological *in vivo* state, for example, when DF are tested in distilled water or in buffer solutions (Dhital et al., 2015; Espinal-Ruiz, Parada-Alfonso, Restrepo-Sánchez & Narváez-Cuenca, 2014; Liu et al., 2021; Ou et al., 2001; Qi et al., 2016; Srichamroen & Chavasit, 2011). The physiological conditions, such as digestive electrolytes, influence the physicochemical properties of DF, e.g., the viscosity and, therefore, their physiological outcome.

This thesis aimed to find an *in vitro* approach to measure glucose release over time under unstirred (static) and stirred (non-static) conditions for soluble and insoluble DF samples. The standardized INFOGEST *in vitro* digestion protocol was applied, including pH changes, dilution during digestion, and digestive electrolytes and enzymes with typical physiological concentrations (Minekus et al., 2014). The use of dialysis bags came with some challenges. The soluble DF (various pectins) absorbed the buffer solution surrounding the dialysis bag to its inside during dialysis due to the high hydration capacity of the soluble DF. The volume inside the dialysis bag changed linearly, whereas the viscosity dropped exponentially, which was the highest in the first three hours (refer to Figure A1 in the appendix). Therefore, the area of mass transfer also changed

over time. With the change of the viscosity and the mass transfer area, the diffusion coefficient also changed over time (refer to Equation 2 in General Introduction and Equation 5 in CHAPTER 1). Dialysis bags also impede the experimental setup with stirring conditions inside the bag, making non-static experiments challenging to model. Therefore, an *in vitro* release system with two temperature-controlled cells arranged side-by-side, connected by a dialysis membrane with a constant area, was used to mimic the glucose release in the small intestinal content (refer to Figure 1 in CHAPTER 1). The two-cell system also had the advantage that each cell could be stirred individually. This system allowed trials under static and non-static conditions, temperature control, a constant area of mass transfer, and a small area of mass transfer to volume ratio of each cell. This small ratio resulted in a negligible dilution and constant viscosity of the sample over time. By calculating the diffusion coefficient (*D*) with Higuchi's kinetic model (refer to Equation 5 in CHAPTER 1), five restricting conditions must be given in the system (Crank, 1979; Higuchi, 1962): (1) Unstirred, semi-infinite donor solution, (2) well-stirred receptor solution with infinite absorption capacity, (3) substance release of the donor solution <30 %, (4) established equilibrium at the membrane surface, (5) negligible membrane resistance. The setup of the cells provided the first two conditions, as the amount of diffused glucose (max. 0.05 mmol/mL) is negligible to the volume of the receptor and donor solution (4 mL each) over the diffusion time. We also determined the *D* under a released amount of glucose <30 % out of the donor solution (condition 3) and a uniform linear release over time after a first lag phase (10 – 20 min) (condition 4). The resistance of the membrane was assumed negligible, as the density and regularity of the pores were high while the cross section (thickness) of the membrane remained low (15.54 ± 1.23 µm), which was determined by SEM imaging (refer to Figure A2 in the appendix). Thus, a suitable *in vitro* approach was found to mimic the different conditions of release experiments in this thesis, such as static and non-static conditions and applied osmotic pressure.

2. Effects of Physicochemical Properties of Dietary Fiber on Glucose Release

A profound characterization of the DF is essential for understanding their physiological role in the small intestine and their function within each food matrix (Cassidy et al., 2018; Meuser, 2008). To date, numerous studies have confirmed the positive impact of DF addition, particularly soluble fiber, on controlling glycemia by rising the viscosity of the chyme. Both the molecular weight (M_W) and concentration (*c*) impact viscosity, but the individual extent of each parameter on glucose release, independently from viscosity, has not been elucidated to date (Cassidy et al., 2018; Wood, 2004). Therefore, DF were often added to food at very high concentrations to obtain an *in*

vivo effect, but despite high *c,* the study outcomes were contrary (Cassidy et al., 2018; Rieder, Knutsen & Ballance, 2017). Moreover, even DF solutions with high viscosity did not achieve the expected *in vivo* effects (Dhital, Dolan, Stokes & Gidley, 2014; Shelat et al., 2010). This suggests that additional factors related to DF also affect controlled glycemia.

The first study investigated the influence of M_W, *c,* and the resulting viscosity of soluble DF on *in vitro* glucose release by diffusion and convection. Combining *in vitro* release and diffusion calculation, the study showed a clear correlation between the critical concentration *c**, M_W, and glucose diffusion. The number of DF molecules in the solution had a higher impact on the diffusion than the viscosity. Under convection conditions, viscosity had a greater impact on glucose release by significantly reducing the flow behavior compared to diffusion conditions. In convection conditions, high molecular weight chains were also found to be more effective than low molecular weight chains in reducing glucose release. As the results of the study were achieved by one type of soluble DF (carboxymethyl cellulose (CMC)), studying further soluble DF would be revealing for a broader understanding. The simulation of the flow behavior in the intestine by an *in situ* approach could further enlighten the effect of *c*, M_W, and viscosity of DF on the transport processes in the small intestine. Nevertheless, the results of the study suggested that a high number of low M_W chains would be more effective in reducing the diffusion of glucose in the unstirred mucosal layer and, therefore, delaying glucose absorption. On the other hand, a highly viscous solution of high M_W chains would be more effective in inhibiting turbulences and providing laminar flow in the center of the chyme, potentially delaying sugar and enzymatic transport (Lentle & Janssen, 2008). A further simulation could provide data to better understand the contribution of reduced glucose diffusion and glucose convection to the overall reduction in glucose absorption in the small intestine.

For several decades, in addition to M_W, *c*, and viscosity, researchers have focused on the hydration capacity of DF, mainly in terms of the impact of DF on processes in the large intestine, such as stool weight and bulking. The impact of hydration properties on glucose diffusion in the small intestine has been part of the research more recently and to a minimal extent (Liu et al., 2021). Therefore, the second study took a closer look at DF's hydration capacities and their influence on *in vitro* glucose diffusion in an osmotically driven system. In the frame of this thesis, a new approach for *in vitro* glucose release experiments was applied by mimicking the osmotic pressure on the DF due to water resorption and dissolved nutrients in the chyme (Rehner & Daniel, 2010) using an osmotic gradient in the receptor cell. The results implicated that high hydration properties of several (five) soluble DF are essential to lower glucose diffusion by forming osmotic pressure and high water holding capacities. The findings were compared with the partly insoluble

citrus fiber (CF), which exhibited different hydration properties, but affected glucose diffusion also on a high level. The hydration capacity of CF is mainly defined by its swelling capacity and gelling character (Elleuch et al., 2011). Research on the hydration capacities with further insoluble and partly insoluble fibers and their impact on glucose diffusion could enlighten the detailed mechanism for such types of DF.

High hydration properties of DF increase the total volume of the intestinal content as DF bind water and the aqueous phase increases. Therefore, the transport and mixing processes of the chyme can be influenced. Further trials in a convective system or simulation could expand the findings of the study of the impact of hydration properties on glucose absorption in the small intestine. Gastric emptying (GE) is regulated in the duodenum by chemoreceptors that measure the osmolarity of the stomach contents, whereby low osmolarity leads to a high GE rate (Mackie et al., 2017; Rehner & Daniel, 2010). The high hydration capacity of DF may have an ambivalent influence. On one hand, binding a high amount of water dilutes the concentration of nutrients, resulting in a lower osmolarity and faster GE. On the other hand, soluble DF with high hydration capacity typically increases the viscosity of gastric content, which slows down the GE rate (Mackie et al., 2017). For a better prediction of the GE rate and the resulting blood glucose response, it may be beneficial to utilize *in vivo* data or simulations of stomach response (Somaratne et al., 2020). For instance, the 3D-printed ARK® (Artificial-stomach Response Kit) dynamic digestion model developed by Gopirajah and Anandharamakrishnan (2014) could provide valuable insights. In the *in vivo* study conducted in this thesis, no delayed GE was observed after consuming food products or drinks supplemented with soluble DF and CF (refer to CHAPTER 4 and Pietrynik, Skurk and Hauner (2024b)).

3. Effects of Food Processing on Glucose Release

The impact of food processing on DF and glucose release was examined in this thesis using the *in vitro* method. *In vitro* and *in vivo* studies have often investigated food processing in the context of affecting glycemic response (GR). In most studies, starch's digestibility was focused on estimating the GR (Elleuch et al., 2011; Singh, Dartois & Kaur, 2010). Different food processes, such as boiling, frying, baking, microwave cooking, extrusion, and roasting, were applied to different food categories, e.g., potatoes (Nayak, Berrios & Tang, 2014), cereal-based food products (Cesbron-Lavau, Goux, Atkinson, Meynier & Vinoy, 2021), or west-Indian carbohydrate-rich foods (Bahado-Singh, Wheatley, Ahmad, Morrison & Asemota, 2006). Furthermore, extensive research has been conducted on the impact of different DF applied to one type of processed food

on GR. The DF used in those studies were primarily soluble, such as beta-glucan, guar gum, arabic gum, inulin, xanthan gum, locust bean gum, gellan gum, sodium alginate and guar-galactomannan, while only a few were (partly) insoluble, such as oat bran, psyllium, and pea fiber. The food types tested were diverse, including pasta (C. S. Brennan & Tudorica, 2008; Foschia, Peressini, Sensidoni, Brennan & Brennan, 2015; Papakonstantinou et al., 2022; Tudorica, Kuri & Brennan, 2002), bread (C. Brennan, Blake, Ellis & Schofield, 1996; Peressini & Sensidoni, 2009; Scazzina, Siebenhandl-Ehn & Pellegrini, 2013), cooked rice (Hyun-Jung, Liu & Lim, 2007), muffins (Tosh, Brummer, Wolever & Wood, 2008), and extruded snacks (Reis & Abu-Ghannam, 2014). However, many studies which use dialysis bags fail to distinguish between a lowered glycemic impact caused by reduced starch degradation or by a delayed diffusion of the amylolytic product (such as glucose) (Chau, Huang & Lee, 2003; Ou et al., 2001; Qi et al., 2016; Srichamroen & Chavasit, 2011). This is due to the fact that both observations are measured by dialysis of glucose out of dialysis bags.

This thesis explored various food processes and applied two different types of DF to investigate their impact on *in vitro* glucose release and starch digestibility in foods. Therefore, the GR of several processed foods with a high glycemic load and different sources of DF was examined. To date, very few studies have addressed this issue (C. S. Brennan, 2005; Ferrer-Mairal et al., 2012; Fujiwara, Hall & Jenkins, 2017). Those studies mainly lack a detailed characterization of the physicochemical properties of DF or the food matrix.

Therefore, in the third and fourth study (CHAPTER 3 and CHAPTER 4), both the characteristics of the DF, the glucose release and starch digestibility of baked and extruded foods enriched with pectin or citrus fiber, as well as the food matrix created during the processing were examined. For this purpose, the studies combined a restricted and an unrestricted *in vitro* method, which means that the glucose diffusion and the amylolysis of starch were separately investigated to distinguish between those two modes of action. As the second study (CHAPTER 2) showed a different behavior in the hydration properties and viscosities of pectin (HMP) and citrus fiber (CF), the aim of the third and fourth study was to gain more information about the interactions of those types of DF in a food matrix. The results of the studies showed multiple interactions between processing, food ingredients, matrix, and GR, revealing that the inclusion of DF into the food matrix impacted GR and significantly differed from GR from aqueous solutions. In aqueous solutions, a clear correlation between the properties of the DF and the *in vitro* GR was observed (refer to CHAPTER 1 and 2), which was also similar to the investigated doughs (refer to CHAPTER 3 and 4), where DF addition reduced the GR (refer to Figure 3 in CHAPTER 3 and Figure A3 in the appendix). The effects of DF addition in food matrices were more complex, making it impossible to establish a

clear correlation between fiber characteristics and GR based on the achieved data. As a result, the gelatinization of starch was influenced by the DF, leading to in a modified food matrix. However, in the performed studies, the partially insoluble CF was more suitable to decrease the GR in a food matrix than the soluble pectin, even though the viscosity of the *in vitro*-digested chyme was higher for pectin. A different study outcome was observed by C. S. Brennan (2005), where the soluble DF (guar and locust bean gum) appeared to decrease the GR of bread and pasta to a greater extent than the insoluble DF (pea fiber). In addition, the combination of both soluble and insoluble DF, as with CF, even resulted in antagonistic effects on GR in pasta (Foschia et al., 2015).

The results of this thesis show that it is essential for food development to select the DF used in the food products carefully and to consider the processing methods employed. Further studies are needed to better understand the effects of well-characterized DF on differently processed foods. In addition to examining the impact of individual food components on the glycemic response (GR) in simple model food systems, there is still a need for research on the interaction of all food components in complex food systems. Future research should focus on studying the interactions between food ingredients and the formation of food structure, as it was found to be essential within the presented studies of this thesis. Additionally, novel processing methods should be explored to decrease the availability of carbohydrates for digestion by forming slowly digestible starch, resistant starch, and dietary fiber in the food.

4. *In Vivo* and *In Vitro* Approaches: Challenges of the Transferability

In vitro approaches are more cost and time-efficient than *in vivo* and *ex vivo* approaches. As a result, *in vitro* methods have been improved and standardized over time. The protocol developed by Englyst, Kingman and Cummings (1992) highlighted the importance of the digestibility of starch, whereby *in vitro* starch digestibility is highly correlated with *in vivo* GI values (R = 0.76). Combining the digestibility of starch with a dialyzed glucose-release method can provide an even more accurate explanation of the physiological glycemic response (R = 0.91) (Goñi, Garcia-Alonso & Saura-Calixto, 1997). Additionally, in order to achieve a reliable physiological prediction of the GR, it is essential to characterize the *in vitro* digesta by its rheological properties (Woolnough, Monro, Brennan & Bird, 2008). Thereby, a wide range of shear rates ($\dot{\gamma}$ = 0.005–120 s^{-1}) was used by different researchers to compare the viscosity of the *in vitro* digesta (Dikeman, Murphy & Fahey Jr, 2006; Fabek, Messerschmidt, Brulport & Goff, 2014; Hardacre, Yap, Lentle & Monro, 2015; Tharakan, 2009). However, it is important to note that *in vivo* viscosity can differ significantly from

its *in vitro* measurement. Predicting the shear rates *in vivo* based on *in vitro* measurements is challenging due to the variability caused by segmentation contractions and peristalsis in the small intestine. In addition to viscosity, other physicochemical properties, such as the hydration capacities of DF, may differ from their predicted *in vitro* values due to the influence of digestion conditions. Therefore, any changes to the physicochemical properties of DF during *in vivo* digestion may affect the expected *in vivo* outcomes predicted beforehand by *in vitro* studies.

One aim of the present thesis was to determine if the results obtained *in vitro* could be verified by *in vivo* data. Therefore, in addition to the *in vitro* experiments, short-term human intervention trials were conducted to investigate the *in vivo* effect of selected DF on postprandial glycemic and insulinemic response. The foods tested in the studies were high glycemic. They also contained low concentrations of DF compared to studies providing DF contents as high as 10 g per portion/serving. However, the concentration of DF was well above the critical concentration c^* to obtain realistic concentrations for formulation and consumption of foods that should still be physiologically effective, according to the *in vitro* results of this thesis and previous studies (Rieder et al., 2017). Based on the *in vitro* results of the first and second study (CHAPTER 1 and CHAPTER 2) the products for the first short-term human intervention study were developed. These products comprised drinks containing glucose enriched with CF, HMP, and CMC and a control drink without DF. The drinks were consumed by adults (n = 12) with a higher risk for type 2 diabetes (Pietrynik et al., 2024b). The concentrations of the DF in the drinks were based on the *in vitro*-determined critical concentration c^*. The second human study was conducted with matrix-based model food products containing CF and control products, as described in CHAPTER 4. and with the same study group as in the first human study.

The *in vitro* results of the solutions and dispersions were corroborated by the findings of the *in vivo* study, suggesting the importance of c^* for attenuating blood glucose levels. The drinks with all three types of DF (CF, HMP, and CMC) lowered postprandial blood glucose levels and plasma insulin c_{max} values compared to the control glucose drink by tendency (Pietrynik et al., 2024b). However, most of the results were not statistically significant due to the high interindividual variability of the test subjects. Although the drinks with CMC and CF at a concentration of 2 x c^* appeared to have a lower viscosity than HMP at 1 x c^* (refer to Figure A4 in the appendix), the c_{max} values for CMC and CF were lower than for HMP. This highlights the significance of the critical concentration c^*. Further, the blood glucose curves varied in shape depending on the type of DF used, which may be attributed to differences in their physicochemical properties (Pietrynik et al., 2024b). The viscosities of the tested solutions seem to play a subordinate role *in vivo*, indicating the significance of diffusion in glucose metabolism. In contrast, the critical concentration c^*, which

is influenced by the concentration and molecular weight, appears to have an effect *in vivo*. The results of Kwong, Wolever, Brummer and Tosh (2013) confirmed, that a variation in viscosity did not show an *in vivo* effect, unlike differences in the M_W, which did show an effect.

Contrarily, the *in vivo* study outcomes conducted with the matrix-based products could not verify the *in vitro* results and did not show a clear difference between the various study products (refer to CHAPTER 4). The differences in the *in vitro* and *in vivo* results could be based on the limitations in mimicking the applied *in vitro* approach. The digestion and glucose release of the matrix products *in vivo* appeared to differ more significantly from the *in vitro* model than the tested drinks. Furthermore, in addition to static *in vitro* digestion, various other factors *in vivo* could impact the glycemic response of the study products in the gastrointestinal tract. Among others, the length of chewing (bakery goods vs. drink), the retention time in the stomach, gastric and intestinal pH, peristalsis and various shear forces, mixing with digestive enzymes, and dilution could impact the properties of the intestinal chyme, including its viscosity, as well as particle size or swelling of the added DF. Furthermore, the mucus layer at the intestinal mucosa, the absorption of sugars through the intestinal mucosa, digestive hormones, insulin response and clearance, distribution and interactions between glucose and insulin in several organs (e.g., liver, muscles, brain), and feedback mechanisms can affect the *in vivo* results. The food composition of the bakery products, could also clearly affect various *in vivo* factors related to glycemia, such as gastric emptying and nutrient absorption. The bakery products contained 12 % fat and 6 % protein, which were in contrast not present in the drinks. A delay of gastric emptying caused by DF is probably not responsible for the attenuation of postprandial glycemic and insulinemic responses observed in the present studies, as food products and drinks supplemented with CF significantly did not reduce the percentage dose recovery of C^{13} value compared to control food products and drinks (refer to CHAPTER 4 and Pietrynik et al. (2024b)). Also, the results of Hlebowicz, Darwiche, Bjorgell and Almér (2008) showed a significant reduction in postprandial glycemic response by an enrichment of a meal with beta-glucan, but no observed effect of the GE rate, also indicating that the GE rate does not regulate the blood glucose level. The individual physiological impact on the postprandial glycemia and insulinemia of the matrix-based study products was also shown through the variability among participants consuming matrix-based products. These findings may also have implications for the differences observed *in vitro* (refer to CHAPTER 4).

Besides the different effects of physiological digestion on the liquid and solid study products, the total amount of fibers consumed can further influence physiology. The solid products enriched with CF contained a lower total amount of CF (1.5 g) compared to the drinks (3.3 g), but the concentration of CF in the product was equal (0.9 g/100 g) (refer to Table S1 in CHAPTER 4).

This difference in CF content could potentially result in a lower physiological effect of the solid products. Human studies conducted with the partly soluble psyllium made similar observations. Psyllium enriched in drinks showed a glycemic *in vivo* effect (Dow, Pritchett, Hawk, Herrington & Gee, 2012; Rigaud, Paycha, Meulemans, Merrouche & Mignon, 1998; Sierra et al., 2001) but enriched in a solid food matrix did not (Frost, Brynes, Dhillo, Bloom & McBurney, 2003).

Based on the findings of the *in vitro* and *in vivo* human trials of the thesis, it can be concluded that the primary mechanisms involved in the attenuation of postprandial glycemic and insulinemic responses after consuming DF could be the alteration of sugar transport from the small intestinal luminal bulk to the intestinal brush border and a delayed amylolysis. Further, the consumption mode (liquid/solid) may influence the glycemic response. The present human studies did not find a significant delay in GE caused by DF. The low power of the first and second human studies due to a small study group (n = 12) combined with the high individual variability may explain the results. Therefore, further studies will be necessary to gain more insight into the postprandial glycemic behavior of DF. To gather additional information on the long-term impact of DF on glucose regulation and to better reflect the ‘real world’ situation, a two-week human study was subsequently conducted using smoothies enriched with 5 g of CF (Pietrynik, Skurk & Hauner, 2024a). Although, the evaluation of the study results is still ongoing, previous studies have already shown positive results regarding the postprandial glycemic response of DF-enriched drinks before a high-carbohydrate test meal (Dow et al., 2012; Rigaud et al., 1998).

5. Closure

Epidemiological studies have demonstrated that DF have a clear protective effect on glucose tolerance and insulin resistance. Therefore, increased fiber consumption can lead to improved health among the population. However, due to uncertain evidence regarding the cause of this positive effect, health claims related to DF have not been widely approved. Health agencies currently recommend a daily consumption of 30 g of DF to maintain good health. The present thesis highlights the importance of the physicochemical properties of DF in attenuating glucose release. It was found, that the DF concentration thresholds to achieve a positive effect differed significantly between the investigated DF types.

The results suggest that in order for health claims to be approved by EFSA, a specific recommendation for the amount of DF consumed to reduce postprandial glycemic response needs to be provided. This recommendation might also consider the physicochemical properties of DF in aqueous environments. In this regard, the critical concentration could be used as a standardized parameter to evaluate the amount of DF used in a meal to reduce glycemia. When incorporating DF into foods, it is essential to evaluate the altered properties of both the fiber and the food to ensure a positive effect on blood glucose. Approval of additional positive health claims of DF for lowering postprandial glycemic response could address the current lack of DF-enriched foods on the market which are well-accepted by consumers.

While many short-term studies show attenuation of postprandial glycemic response after a carbohydrate load, longer clinical trials are needed to prove that prolonged attenuation of glycemic response with DF leads to a reduction in the incidence of type 2 diabetes. In addition, future *in vitro, in silico*, and *in vivo* studies should further clarify the causes of the beneficial properties of DF on human physiology by studying the mechanistic principles and structure-function relationship. Finally, it should be noted that fiber consumption alone is insufficient to reduce the prevalence of diet-related diseases such as type 2 diabetes. A sustainable reduction can be supported by combining physical activity, a balanced diet, low GI foods with slowly digestible carbohydrates, and a high fiber consumption.

Appendix

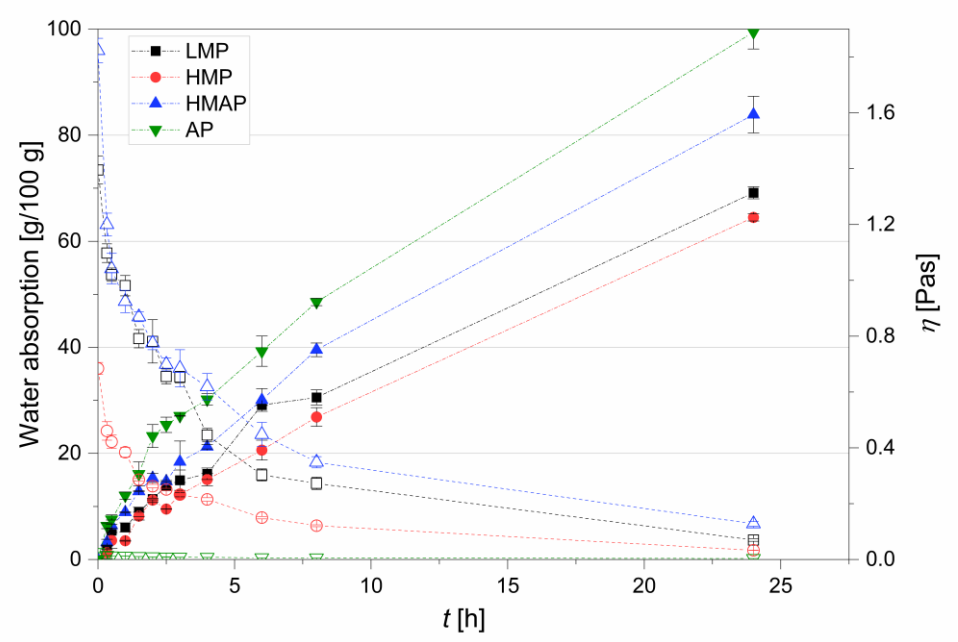

Figure A1 Water absorption (filled symbols) and viscosity ($\dot{\gamma}$ = 50 s^{-1}) (empty symbols) of four *in vitro* digested pectin solutions (3.13 g/100 g) in a dialysis bag over glucose release time: Three high molecular weight low methylester citrus pectin (LMP), high methylester citrus pectin (HMP), and high methylester apple pectin (HMAP), and the low molecular weight apple pectin (AP). Mean ± SD

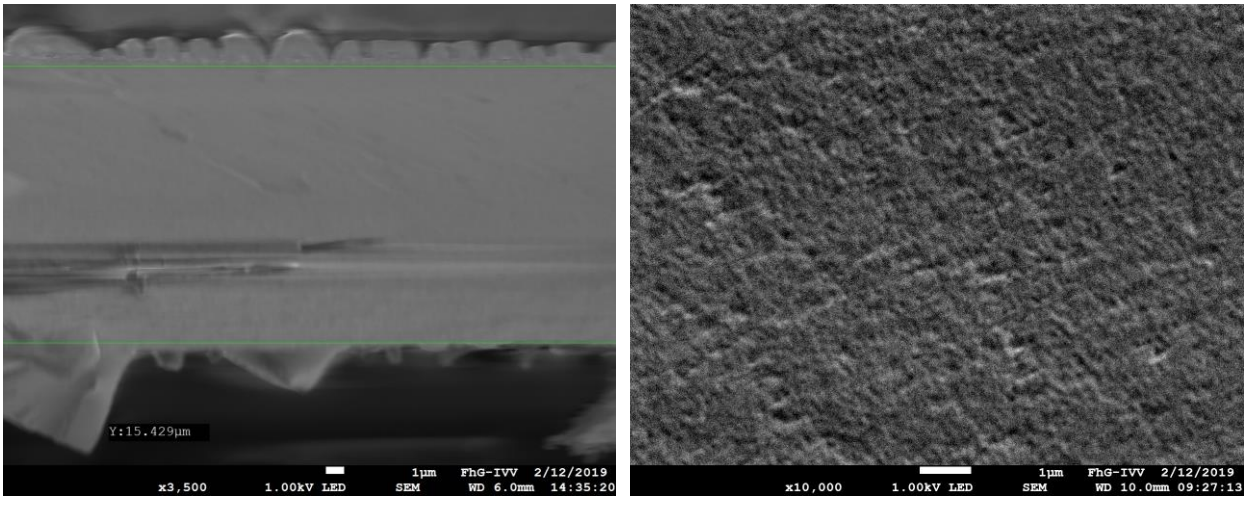

Figure A2 Cross-section (left) and surface (right) of the cellulose membrane (CO 14 kDa), used for the *in vitro* release experiments, imaged by scanning electron microscopy. The sample was washed in distilled water and dried before imaging. For image A, the sample was freeze-fractured in super-cooled slushed nitrogen, and for image B, the sample was sputter-coated with Au for 30 seconds.

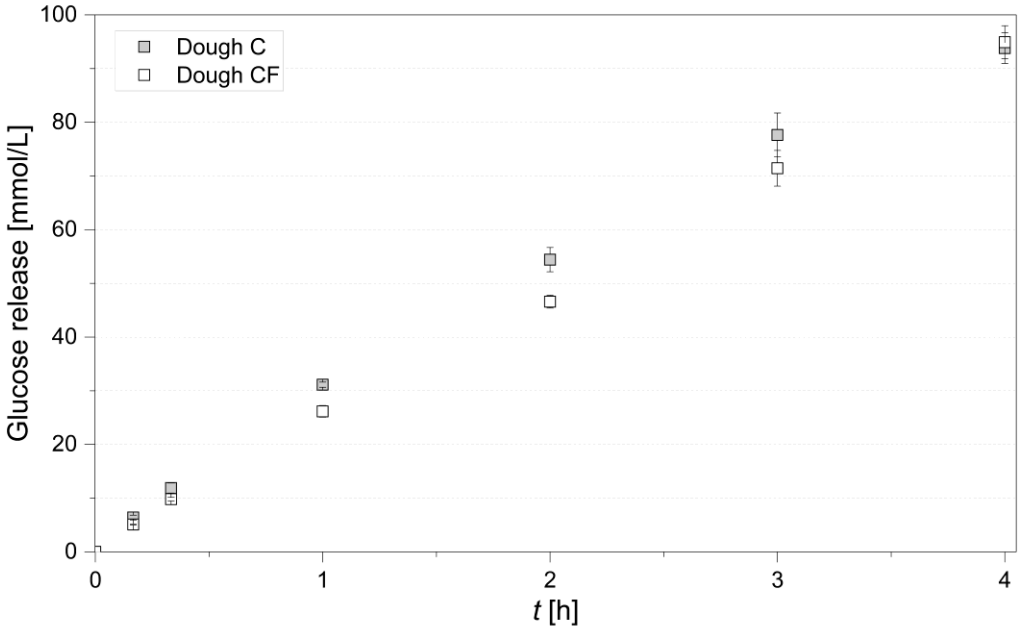

Figure A3 Glucose release over time during the *in vitro* intestinal phase of the citrus fiber-enriched (CF) and control (C) dough prepared for study 4 (CHAPTER 4). Mean ± SD

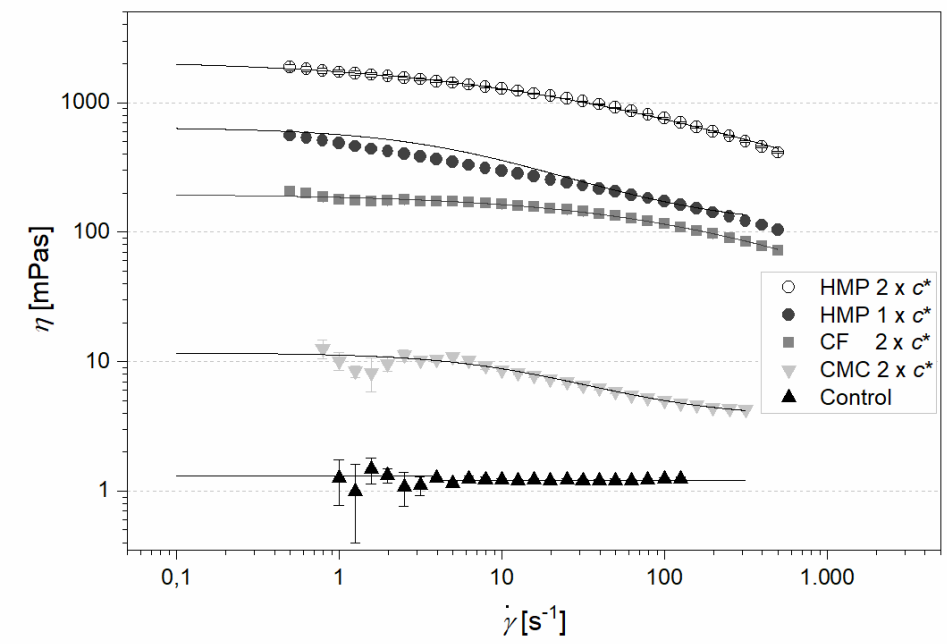

Figure A4 Flow behavior of the drinks with DF in concentrations based on the critical concentration (*c**) given at the short-term human study: Carboxymethyl cellulose (CMC) in 2 x *c** (0.9 g/100 g), citrus fiber (CF) in 2 x *c** (0.8 g/100 g), high methylester pectin (HMP) in 1 x *c** (0.9 g/100 g) and 2 x *c** (1.8 g/100 g), and control (0.0 g/100 g). Points correspond to experimental data and lines to fitted data according to the Cross equation (Equation (7), CHAPTER 1) for the DF solutions and Newtonian equation (Equation (8), CHAPTER 1) for control (R2 ≥0.98). Mean ± SD

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