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Microfluidics-based observations to monitor dynamic processes occurring in food emulsions and foams

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Sebastien Marze³, Meinou Corstens¹ and Emma Hinderink⁴

Food design is often done based on a trial-and-error basis, using structure properties as an indicator of product quality. Although this has led to many good products in the market, this 'cook and look' approach could benefit from insights into dynamic processes as they occur during food formation, storage, and digestion. Currently microfluidic devices are being developed to allow these types of observations, and here we show the latest examples in the field of emulsions and foams, including effects that occur during digestion. We expect that these techniques will supply a stepping stone to thorough understanding at various length and timescales that are all instrumental in designing high-quality food products, and ultimately creating foods with health benefits.

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Introduction

In literature, many 'beautiful' pictures can be found of various structures present in foods, such as droplets, bubbles, and soft solid matrices, as very nicely reviewed by Heertje [1]. These images represent the situation *at a*

given moment in time, while in reality, such systems are generally highly dynamic during their formation, keep changing gradually over storage time, and even more extremely during digestion. The interpretation of these images needs to be done with great care, as they are easily influenced by, for example, the choice of location, or any treatment needed to get the image (e.g. use of fluorescent markers for confocal laser scanning microscopy, sputter coating of a thin layer, or extreme drying of samples as is customary for scanning electron microscopy) [2].

In our view, the next level of understanding of food structure formation and its development in time can be obtained using microfluidic techniques [3,4]. These devices are known for their precise control over process conditions, leading to identical events that can be used to derive representative parameters in a statistically relevant way. Microfluidic systems usually involve high bubble or droplet formation rates, which implies that a tremendous number of observations can be made — providing the systems are connected to ad hoc visualization and recording equipment. For example, for droplet coalescence in highly concentrated emulsions, up to 10 000 droplets could be generated and analyzed within a very short time, which is not possible through any other technique. This also implies that if coalescence does not occur very often, it can still be monitored. In microfluidic devices, the flow conditions as well as the length and timescales are similar or close to the ones that occur in large-scale processing (see also *Outlook* section) [5], and obtained insights therefore closer to practical applications compared with classic approaches (e.g. drop tensiometry to measure interfacial tension, thin-film balance to understand film drainage during coalescence [6]). Therefore, microfluidic insights are expected to bring closer to understanding many practical applications.

In the current review, we highlight the use of microfluidics to monitor two-phase food systems, such as foams and emulsions under (highly) dynamic conditions. It is good to mention that the bubble formation rate in foams is even much higher than the droplet formation rate in emulsions, due to the low viscosity of the gas phase. Although both processes have a common basis, they differ in expansion rate and initial interfacial energy [7]. We give suggestions on how to study bubble and droplet formation in the respective sections. Besides

formation, we also report on processes that occur between distinct bubbles or droplets, and how they influence the stability. We pay special attention to interfacial processes that ideally supply stability to the product during production and storage. To cover processes as they occur over the full lifespan of a food product, we also describe how microfluidic techniques can be used to investigate digestive processes. This is expected to lead to insights in targeted delivery from a fundamental point of view, which ultimately may facilitate providing health benefits to consumers. Based on all these insights, it is expected that food structures can be investigated using microfluidics with the ultimate goal that future food design could be targeted at creating specific health benefits.

Microfluidic techniques used for dynamic evaluation of processes as they occur during the lifespan of foods

In literature, many reviews can be found on the formation of droplets and bubbles in microfluidic devices, and they detail on the various geometries that are used [8–10]. These reviews generally distinguish active devices that use shear forces to make bubbles and droplets such as T- or Y-junctions, coflow devices, and passive devices that use spontaneous formation, such as terrace-based, EDGE (Edge-based Droplet GENERation), and step systems that are based on interfacial tension gradients. Very often, these papers compare process performance based on the droplet/bubble size, but do not give details on the dynamics of droplet/bubble formation, and that is what we highlight in the current paper.

Microfluidic devices have been widely exploited for making bubbles (e.g. [11,12]) and droplets (e.g. [13,14]), and various device geometries have been even upscaled to some extent [15]. Typically, the formation mechanisms of individual droplets and bubbles are determined by the device geometry in combination with local process conditions. The findings are not always translatable, for instance, the scaling relations for the initial size of droplets and bubbles differ widely [16]. Compared with droplets, the scaling relations for bubbles need to incorporate the compressibility of the gas phase [23]. Besides, bubble formation is much faster than droplet formation due to the marked low viscosity of the gas phase. Depending on the process conditions, this makes the bubble formation process inherently more difficult to quantify since it includes dynamic surface tension effects at even shorter timescales than those that would occur for droplets.

We first address dynamic effects reported for bubbles, followed by those occurring in droplets, highlighting the dynamic processes underlying interface stabilization. In

a third section, we report on processes that occur during digestion of droplets.

Foams

Bubble formation and stability

Only very recently, insights were obtained in how the combination of bubble formation and immediate re-coalescence leads to the final bubble size [12] in so-called partitioned-EDGE devices (Figure 1a,b). To produce monodisperse foams that are more stable than their polydisperse counterparts, it is crucial to tune bubble formation and stabilization at relevant length and timescales including those for emulsifier adsorption (see also *Outlook* section). For partitioned-EDGE devices, one bubble formation cycle can be split up into two consecutive subprocesses [19], both of which having a typical timescale, with the pore-filling time mainly setting the bubble formation frequency and the subsequent bubble growing time mainly determining the initial bubble size. The characterization of these subprocesses provides insights into how process conditions influence the initial properties of the bubbles, and the final properties of the foam (e.g. through bubble coalescence).

Surface and interfacial tension measurement

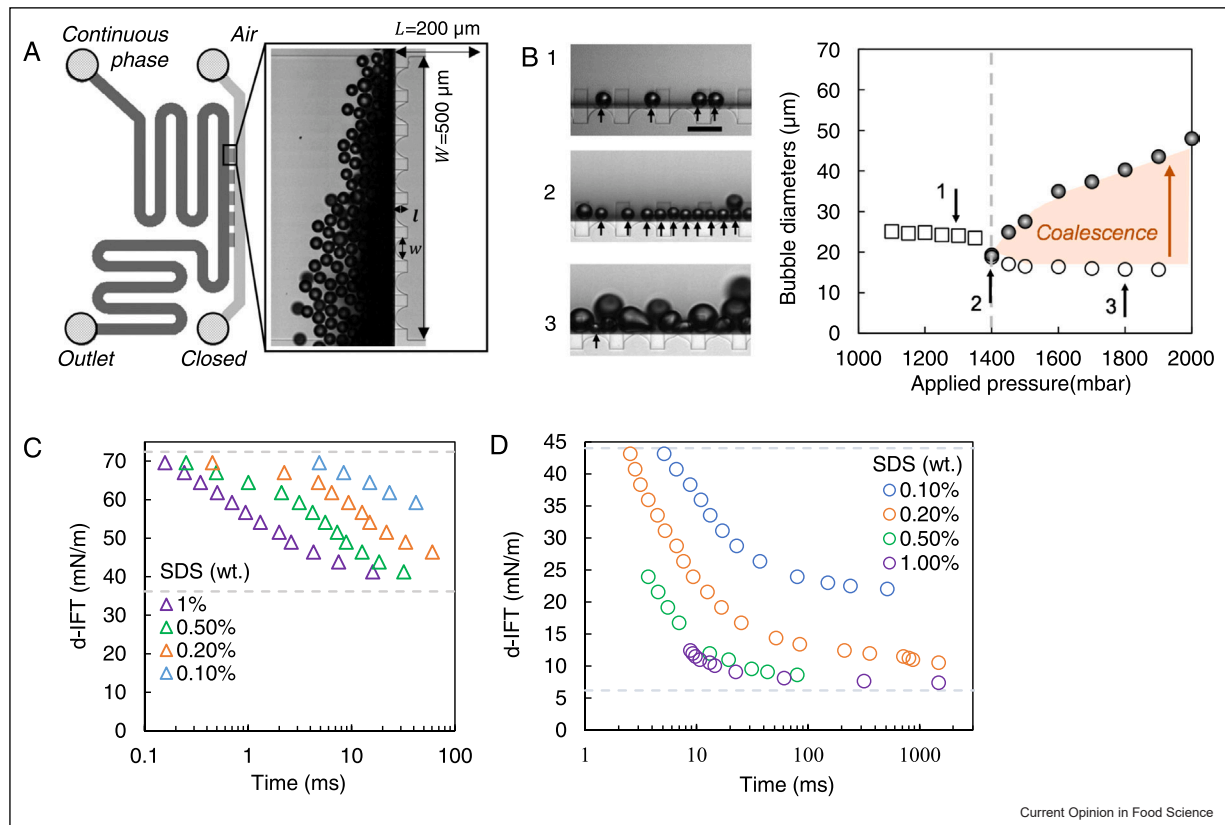
Microfluidics have been used to probe emulsifier adsorption kinetics [20,21] and the resulting dynamic interfacial tension at timescales down to (sub)milliseconds [22–24], although it is good to mention that most work has been done on droplets, and rarely on bubbles [25–27]. Very recently, we showed that the bubble formation frequency follows from the dynamic surface tension. The bubble formation frequency (i.e. the reciprocal value of the bubble formation time) can be obtained easily by counting the number of bubbles formed at the pore within a certain period of time and increases with the dynamic surface tension, as demonstrated for both bubbles (Figure 1c) and droplets (Figure 1d) in the presence of low-molecular-weight surfactant SDS [28]. This allows a direct connection of the dynamic surface tension to the short-term coalescence stability of the bubbles since *both* observations can be done in the partitioned-EDGE simultaneously. The partitioned-EDGE devices are expected to further aid food formulation (e.g. the type and concentration of emulsifiers) through high-throughput experimentation under process conditions relevant to industrial applications, please also see the *Outlook* section for our view on how close devices currently are to facilitating food formulation.

Emulsions

Interfacial-layer properties

Various emulsifiers are commonly used, of which proteins are paramount for food emulsions. Since proteins adsorb much slower than conventional low-molecular-

Figure 1



Ma) Schematic overview of the partitioned-EDGE microfluidic device. The air phase is forced to flow through the shallow plateau and pores under an effective pressure drop, leading to bubbles formed from the pores' exit (see inset). **(b)** Snapshots of bubble formation behavior at different applied pressures. At low applied pressure, individual bubbles are formed that are stable against coalescence; at 1400 mbar, the first coalescence events start taking place, while at 1800 mbar, many coalescence events take place at the pore. The rich bubble behavior can be observed and analyzed with partitioned-EDGE devices. **(c)** and **(d)**. Dynamic surface and interfacial tension results as a function of the bubble and droplet formation time, respectively, obtained with the 'EDGE tensiometer' operating at low applied pressures (< 1400 mbar) for the indicated sodium dodecyl sulfate (SDS) concentrations.

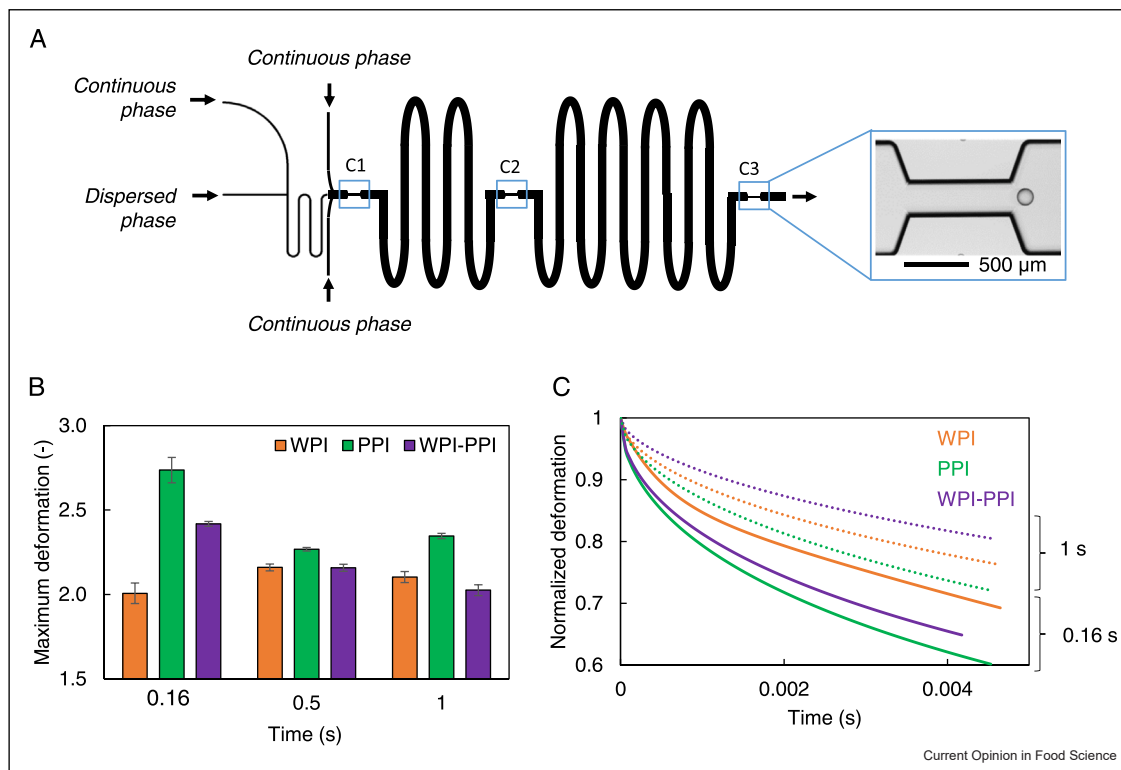
Figures **(a)** and **(b)** are redrawn based on images from [12], and Figures **(c)** and **(d)** are adapted from [28].

weight emulsifiers, it is particularly important to characterize the initial stages of interface stabilization over a relevant timespan [29]. When given sufficient time, proteins form an interfacial layer that protects droplets against coalescence due to the formation of a stiff interfacial layer. The onset thereof is expected to start at timescales similar to those of droplet formation in conventional emulsification devices (i.e. in milliseconds [30]). The formation of the interfacial network may be instrumental in subsequent droplet stabilization after droplet formation. A microfluidic rheology chip can measure these effects through droplet deformation that can be used to determine interfacial rheological properties within (milli)second timescales [31], which cannot be probed using classical methods such as drop tensiometry. Information can be retained regarding the interfacial stiffness (from the maximum deformation) as well as the interfacial viscous and elastic contributions (from the relaxation patterns) (Figure 2). Ideally, these

contributions would be converted to elastic and loss moduli in the near future.

The microfluidic rheology chips can also be applied more generally to compare various protein sources. This is especially relevant for emerging plant protein ingredients of which a large fraction remains insoluble and can only anchor at the interface when their kinetic energy exceeds the adsorption energy barrier. Their interfacial properties can thus not be measured using conventional model interfaces that rely on adsorption of the emulsifiers by diffusion. The interfacial rheological properties of such particle-stabilized droplets could thus be assessed with the microfluidic rheology chips that operate under convective mass transfer conditions [31]. This would open new perspectives to study interfacial properties of plant protein particles or other bio-based particles. Insights can be linked to the coalescence stability of droplets at the same timescale [32,33]. For the

Figure 2



(a) Microfluidic droplet rheology device of Hinderink et al. [31] to study interfacial rheological properties within the (milli)second timescale. (b) Example of maximum deformations measured for droplets stabilized with 0.5 g/L whey protein isolate (WPI), pea protein isolate (PPI), and their 1:1 mass ratio blend (WPI-PPI) at constrictions C1, C2, and C3 that correspond to 0.16, 0.5, and 1 s, respectively, under the used flow conditions. (c) Examples of normalized deformation curves for droplet stabilized by the same proteins after constriction C1 (solid lines) and C3 (dashed lines). All images are redrawn based on information available in [31].

latter, coalescence cells can be used where droplets can collide and if not sufficiently stabilized coalesce. These cells have been proven to provide insights into the coalescence stability of surfactant- [32], protein- [34,35], and even particle-stabilized droplets [36].

Phase inversion

Microfluidics can be used to gain fundamental insights into other aspects of emulsification, for example, shear-induced phase inversion (e.g. going from water-in-oil to oil-in-water emulsions, or vice versa). *In situ* analysis at the droplet level, as done in microfluidics, is fundamental to understanding the physical factors that drive the process [37,38]. Coalescence is expected to be the basis of phase inversion. Using microfluidics, an interesting phenomenon referred to as ‘cascade coalescence’ or ‘coalescence avalanche’ was observed in emulsions with a high dispersed phase fraction flowing through a constriction [38,39]. During a coalescence cascade, one coalesce event may propagate to neighboring droplets therewith destabilizing a large part of the droplet assembly, which may ultimately lead to complete phase inversion. The conditions under which these cascades

occur should be investigated as a function of shear, pressure difference, liquid and surfactant-type wall effects, and droplet size to generalize the insights and translate them toward industrial applications.

Digestion kinetics studied in droplet microfluidic platforms

Besides phenomena related to droplet formation and stability, droplet microfluidics are very well suited to study physiological processes in which interfacial phenomena are involved. Initially, oral drug delivery and release was considered, focussing mainly on the *production* of drug-loaded particles (biopolymers and/or lipids) with controlled structural and physicochemical features [42]. Only a few studies focused on monitoring gastrointestinal release directly on the microfluidic chip, for example, for colloidal particles. Most microfluidic approaches in this field were recently reviewed [3,43]. Here, we focus on microfluidic devices for the kinetic study of droplet digestion and evaluate its potential.

The first investigation of lipid droplet digestion on a microfluidic chip was reported by Marze et al. [44], for

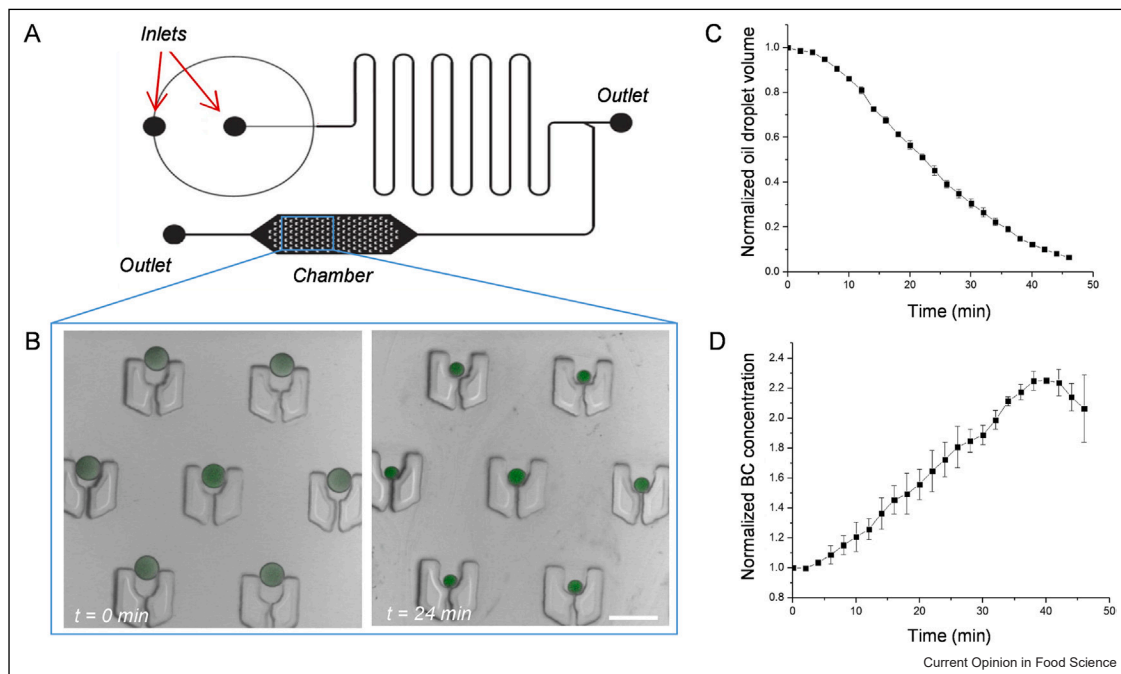
protein-stabilized triglyceride droplets (135 μm) captured in a digestion chamber through individual traps. Digestive fluids containing enzymes (and bile salts in the intestinal fluid) were flown continuously and sequentially through the chamber to mimic gastrointestinal digestion. Oil digestion was monitored continuously through droplet-size reduction in time and this takes these types of investigations away from microscopic images of aliquots of simulated digestive fluids at defined time points or measuring an average rate of digestion product formation with standard static digestion techniques. The microfluidic technique ensures similar events occurring through the use of monodisperse droplets, as well as systematic variations of, for example, pH, electrolytes, bile salts, enzymes, and so on, and monitoring their influence on digestion.

The effects of various parameters as investigated with microfluidics were consistent with usual emulsion digestion results, but quantitatively the surface-normalized kinetic rates were about 10-fold faster for these isolated droplets compared with droplets in emulsion. This was attributed to droplet monodispersity and the absence of droplet interactions, such as flocculation or coalescence. This was confirmed by Scheuble et al. [45] for smaller medium-chain triglyceride droplets (25–65 μm). Coalescence events during the gastric step

decrease the total specific surface area, and for single oil droplets of different sizes, it was found that the extent of digestion increased as the droplet size decreased. Emulsion flocculation and coalescence under digestive conditions could be further studied using a coalescence chip (mentioned in the *Emulsion* section, e.g. [31]).

When microfluidics were coupled to a confocal fluorescence microscopy method to image both oil droplets and their lipophilic bioactive content (vitamin A, retinol, or β -carotene (BC)) in the microfluidic chip ([46], Figure 3), it could be shown that BC degradation could be differentiated from its release by imaging both droplet size and BC autofluorescence. BC degradation kinetics and extent were in agreement with those obtained for conventional emulsions, and controlled by triglyceride digestion. The kinetic rates were higher for isolated droplets compared with droplets in a conventional emulsion, but when corrected for the specific surface area, the systems turned out to be identical. This highlights that both droplets and digestive fluids should be accounted for [47]. A recent study where droplet digestion was monitored in a quartz microfluidic device by small-angle X-ray scattering [48] showed that dynamic phase transitions of the molecular assembly within phytantriol–triglyceride emulsion droplets were very similar and more resolved in space and time compared

Figure 3



(a) Droplet microfluidic device of Nguyen et al. [46] to study triglyceride oil droplet digestion and lipophilic vitamin release kinetics simultaneously. (b) Confocal fluorescence images of trapped tricaprylin droplets containing BC before and after 24 min of intestinal digestion. Using image analysis, the (c) volume of the triglyceride oil droplets and (d) the concentration of BC was measured. Scale bar is 200 μm . Adapted from Schroën et al. [3] under the terms and conditions of the Creative Commons Attribution CC BY 4.0 International Public License.

with bulk pH-stat method. Such microfluidics-analytical instrument coupling is very promising for short-time kinetics investigation of digestion-induced structural phase transitions, mainly occurring for lipids.

To gain understanding of the interfacial behavior during digestion, the microfluidic droplet rheology device of Hinderink et al. [31] (described in the *Emulsion* section) could be used under digestive conditions. In this context, interfacial tensiometry with phase exchange has previously been used [49,50], but in this case, adsorption is mainly based on diffusion, which does not capture convective mass transfer [22]. In principle, the digestion behavior of other types of food colloidal particles could be studied in microfluidic platforms as well, provided they are of a characteristic size that can be observed with a light microscope (2–200 μm). This is the case for oil bodies (oleosomes), starch granules, and some protein–protein and protein–polysaccharide particles, which may potentially open new fields of research.

Outlook

As put forward in the paper, microfluidic techniques can be applied under conditions that are relevant for the entire lifespan of food products, from the hectic formation phase, to conditions as they occur during further processing, and even digestion. In all these stages, the most relevant dynamic processes can now be assessed (although some challenges remain as detailed later), which is a huge step forward, and of essence to design food in a rational way, not only to obtain products with enhanced shelf life, but most probably to design food that creates health benefits in the future.

It is important to note that microfluidic approaches have taken a next step from understanding fundamental effects of the dynamics of droplets and bubbles, toward effects that are relevant for the stability of colloidal food structures. This also goes beyond the dynamic effects of interfacial and surface tension addressed in the last decade; currently, the link with, for example, coalescence stability is being made, including interfacial film formation. When looking further into the future, it is expected that the underlying mechanisms will become more and more known, and that is not only important for the relatively simple systems that we discuss here, but even more so for complex systems such as double emulsions, gelled systems [51], water-in-water emulsions, and so on. The basis established for simple systems can be used as design rules for more complex systems, and thus speed up their design, although it also should be kept in mind that various synergistic and antagonistic effects may occur, and those can also be identified using the techniques mentioned earlier.

Translation of microfluidic emulsion insights to industrial processes

Droplets in current microfluidic devices typically have sizes ranging from 2 to 200 μm , and are deformable if the interfacial tension is low and the viscosity ratio between dispersed and continuous phase is high [19]; bubbles typically range from tens to hundreds of micrometers. Droplet and bubble formation times measured in microfluidic devices are similar as those that would occur in industrial processes (0.1–1 ms [30]), and that would also hold for interfacial expansion rates and adsorption times (depending on the formulation used, see table in [16]). It is however not possible to recreate turbulent conditions (especially relevant for recoalescence) in a microfluidic device given its small dimension, and that would also hold for direct observation of small droplets as would be desired for some foods such as, for example, milk (sub-micron-size), and that are more resistant to deformation upon collision. Preliminary results in our lab show that it is possible to produce submicron-sized emulsion droplets with EDGE devices, but obviously, productivity would be rather low, and many upscaling challenges remain [13,40,41], including development of new observation methods since this would lead out of the realm where convenient light microscopy in combination with high-speed imaging can be used. In contrast, the bubble size is in good accordance with that obtained in industrial settings.

Next steps in device design and production

We point out that the microfluidic devices described in this article are made of glass or silicon, requiring clean room technology, which allows ultimate control over the geometrical and chemical features, but comes with a cost. There are a lot of developments that allow for 3D printing of devices, production in paper or in thin film, and these techniques have also been reviewed recently [52,53], and even commercialized, which brings microfluidics within reach of many. Irrespective of the production method used, it is always important for multiphase systems to monitor wettability changes [54,55] that may occur because of the adsorption of components at the device surface, which influence its operation greatly.

Toward application of microfluidic devices

To wrap up, the use of microfluidic techniques comes with many opportunities, as well as challenges. Many have suggested their use for large-scale (food) product formation, and although this is possible, we feel that, as would be the case with any new technology, this would require courage from industry. Most probably, products with a more attractive margin of profit would qualify sooner for microfluidic application for production purposes. It is still good to point out that the production of

relatively small amounts of highly defined droplets is relevant, for example, in the investigation of lipid oxidation as is currently done in the Wageningen labs.

Where we see a high added value for microfluidic techniques is in their use as analytical tools. The examples reviewed in the paper are a clear indication of the uniqueness of measurements that can be done, and are (far) beyond the reach of other techniques. We are convinced that when microfluidics can be used in a high-throughput setting, and produced at reasonable costs, they can bring food formulation to a next level, also in terms of ingredient functionality screening/comparison.

Data Availability

No data were used for the research described in the article.

Conflict of interest statement

The authors declare no conflict of interest. The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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