Towards more biomimetic and sustainable infant formula: challenges and future opportunities

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ABSTRACT

Background: Infant formula (IF) requires further optimization as there are still differences in health consequences between human milk (HM) and IF.

Scope and approach: The present review addresses the challenges and future opportunities inherent in the production of more biomimetic and sustainable IF. After presenting the targets, limitations and challenges for IF optimization, process innovations that could contribute to designing the next-generation of IF are discussed. The final section describes how such improvements should be addressed by means of a more systemic approach.

Key findings and conclusions: Gaps in our knowledge of the compounds and structures in HM and their effects on digestion and health still exist, rendering the biomimicry of HM more difficult. Overall, optimizing IF is complex and requires trade-offs between synergistic and conflicting objectives, which include HM biomimicry, safety, functionality, ingredient sourcing as well as environmental, economic and social sustainability issues. Process innovations and optimized technological routes, including minimal processing, offer opportunities to implement new ingredients and improve the preservation of IF compounds, while ensuring microbial safety and addressing several pillars of sustainability through energy costs or reductions in gas emissions. Given the complexity of producing biomimetic and sustainable IF, a multi-objective optimization strategy is proposed, reliant on a multidisciplinary approach, where nutrition and process engineering would play pivotal roles with assistance from other disciplinaries such as biochemistry, microbiology, pediatric medicine, data and consumer sciences and public health. This rethinking of IF production should be driven by a multidisciplinary, non-profit consortium involving the entire value chain.

1. Introduction

Although the World Health Organization recommends exclusive breastfeeding for the first 6 months of life of an infant, only 44% of them worldwide are breastfed, indicating that 56% receive a human milk (**HM**) substitute (UNICEF, 2020). Although HM remains the best option for an infant, when breastfeeding is not possible or desired, the most adequate substitute is infant formula (**IF**). The latter is generally based on bovine milk (**BM**) proteins and designed to cover the infant's nutritional needs during the first 6 months of life (European Union, 2016). Since the first commercial IF was launched in 1867 by Julius Von Liebig, tremendous improvements have been made to IF formulation and processing. To mimic the nutritional content of HM, numerous ingredients are added to IF and a succession of unit operations is included in the process to comply with both dairy industry constraints and customer expectations for a stable and safe product. IF must primarily be nutritionally adequate, microbiologically safe and beneficial to health, and secondly it needs to be environmentally-friendly, culturally acceptable, economically viable and accessible to all; in other words, IF must be a sustainable food (FAO & WHO, 2019).

The first 1000 days of life, including infant nutrition, are recognized as crucial to the short- and long-term development of health and disease. The health consequences of consuming HM and IF continue to differ (Lemaire et al., 2018); breastfeeding reduces the occurrence of

Abbreviations: ARA, Arachidonic acid; BM, Bovine milk; DHA, Docosahexaenoic acid; HM, Human milk; HMO, Human milk oligosaccharides; IF, Infant formula; WP, Whey protein.

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respiratory and gastrointestinal infections during the first year, but also the risk of developing inflammatory and metabolic disorders such as obesity, diabetes or inflammatory bowel disease later in life (Lemaire et al., 2018; Victora et al., 2016). It is therefore necessary to continue improving IF. While the nutrient contents of IF and HM are relatively close, their fine composition and structure (*e.g.* casein micelle size, fat droplets, *etc.*) differ. A closer mimicking of the composition and structure of HM could help to mimic its nutritional and health impacts. At the same time, the IF production chain needs to address not only the challenge of biomimicry (which is specific to this food) but also other issues such as environmental, economic and social sustainability, which must be taken into account by the food industry as a whole in order to contribute to global transition, particularly in the context of climate change. The simultaneous challenges of biomimicry and sustainability can lead to contradictory objectives but also to synergies. More biomimetic IF could become more complex, requiring additional processing steps that are less sustainable (higher energy costs with impacts on the environment and economic viability, higher prices affecting social equity). However, optimizing the production process could respond to the objectives of reducing energy costs and improving process sustainability while limiting the impact of technological treatment and ensuring high nutrient quality((

This review aims to address the challenges and future opportunities inherent in the production of more biomimetic and sustainable IF. After defining the targets, limitations and challenges relative to their production, we will examine how rethinking the actual process might help

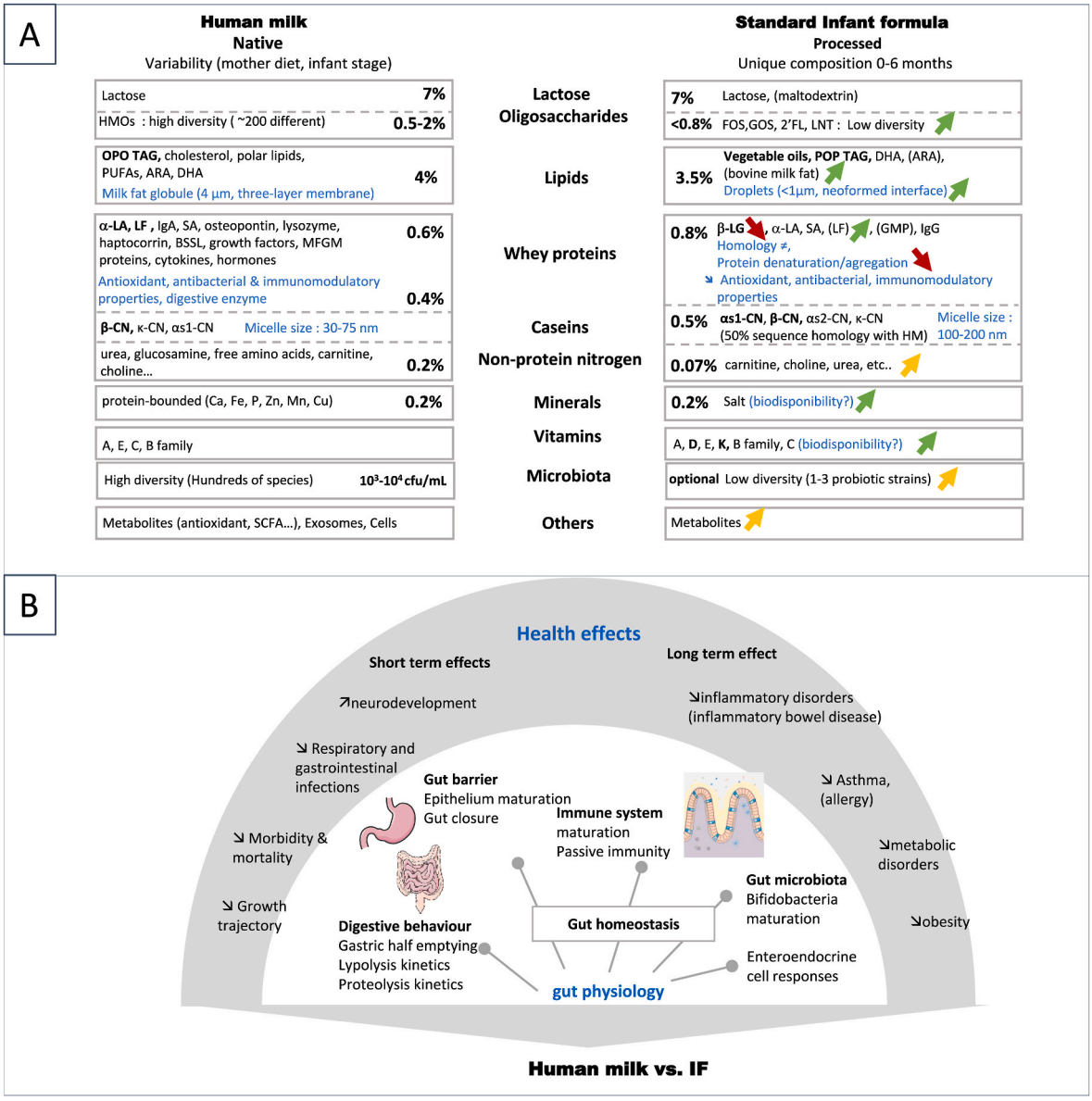

Fig. 1. (A) Principal differences between human milk (HM) and standard infant formula (IF) in terms of composition [in black, with average nutrient proportions (%, wt/wt)] and structure/function (in blue). Major compounds are in bold. The main avenues for IF improvement are indicated with arrows: green (to be increased), red (to be reduced), yellow (to be increased after further evaluation on the infant impact). (B) Health benefits of HM compared to IF. 2′FL: 2′-*O*-fucosyllactose, a-LA: a-lactalbumin, ARA: Arachidonic acid, BSSL: bile salt-stimulated lipase, CN: casein, DHA: docosahexaenoic acid, FOS: fructo-oligosaccharides, GMP: glycomacropeptide, GOS: galacto-oligosaccharides, HMO: human milk oligosaccharides, Ig: Immunoglobulin, LF: lactoferrin, LNT: lacto-*N*-tetraose, MFGM: milk fat globule membrane, O: oleic acid, P: palmitic acid, PUFA: polyunsaturated fatty acids, SA: Serum Albumin, SCFA: short chain fatty acids, TAG: triacylglycerol. Parts of the figure were drawn using images from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to design more biomimetic formulas while complying with the current food challenge of sustainability. Finally, we will discuss how such improvements should be considered as a multi-objective optimization strategy that is addressed throughout the global value chain, from milk production to the consumer.

2. Biomimetic infant formulas: targets and limitations

Improving IF necessitates better knowledge of the target, *i.e.* HM. The latter has been characterized in detail in terms of its composition, structure and effect on neonatal physiology and health; however, this knowledge is constantly evolving, so the target remains indistinct. Far from being exhaustive, this first part aims to provide an overview of the principal gaps between IF and HM (Fig. 1), highlighting the challenges and strategies that are starting to be explored regarding the development of biomimetic IFs in response to customer demand. An overview of the major innovations in IFs since they were first made commercially available is given in Fig. 2. It should be noted that IF stands for a formula for specific use during the first 6 months of life (European Union, 2016) and usually targets a mature and stable HM, *i.e.* from the third to fourth weeks postpartum, as presented in Fig. 1.A.

2.1. Targeted composition and structure

2.1.1. Carbohydrates

The most important carbohydrate in HM (in mass terms) is lactose, an important energy source for the infant (Fig. 1.A); this is easily supplied in IF from BM, although sometimes added with maltodextrins, despite their absence from HM. The other source of carbohydrates, although non-digestible, are HM oligosaccharides (**HMO**), which are 10 times less concentrated than lactose (Fig. 1.A). These are crucial to the infant, acting as prebiotics and orientating the gut microbiota towards a dominance of *Bifidobacterium*, displaying antimicrobial properties and altering epithelial and immune cell responses (Bode, 2015; Cheng & Yeung, 2021). HMO are present in much larger quantities and greater diversity than in BM, with about 200 different structures (Cheng & Yeung, 2021). Because the production of synthetic human oligosaccharide-like structures is challenging and expensive, IFs are usually supplemented with non-human oligosaccharides such as galacto-oligosaccharides and fructo-oligosaccharides. The addition of 2′-*O*-fucosyllactose (one of the most abundant HMO) combined with lacto-*N*-neotetraose was recently authorized in Europe; this enables the microbiota to be more similar to that of a breastfed infant (Alliet et al., 2016). The implementation of HMO in IF is definitely a goal to be achieved, with promising outcomes for infant gut microbiota. Challenges also remain regarding the complexity and variability of these HMO and full assessment of their roles.

2.1.2. Lipids

Lipids are the second most abundant nutrient in mass terms but the leading energy provider in HM (Fig. 1.A). In HM (and in raw BM), lipids are dispersed as micron fat globules (modal diameter of 4–6 μm), but not in IF (submicron fat droplets, < 1 μm). HM (and BM) fat globules contain a core of triacylglycerols (98–99%) surrounded by a typical trilayer milk fat globule membrane , containing polar lipids (0.2–1% wt/wt; glycerophospholipids and sphingolipids) conveying valuable bioactive functions for the infant (Gallier et al., 2015) in association with cholesterol (0.4%) and other minor sterols and also with minor proteins (Bourlieu et al., 2015). As for the fatty acid profile, oleic (18:1n-9) and palmitic (16:0) acids are the most abundant in HM (1/3 and 1/4 of total fatty acids) with a specific regiodistribution on the glycerol backbone (*sn*-1,3 and *sn*-2 positions, respectively; Delplanque et al., 2015). Short and medium chain fatty acids (including C14) are also present in substantial quantities in HM (10–20% of total fatty acids). To a lesser extent but of nutritional importance, HM contains functional polyunsaturated fatty acids such as arachidonic acid (**ARA**, 0.47% of total fatty acids) and

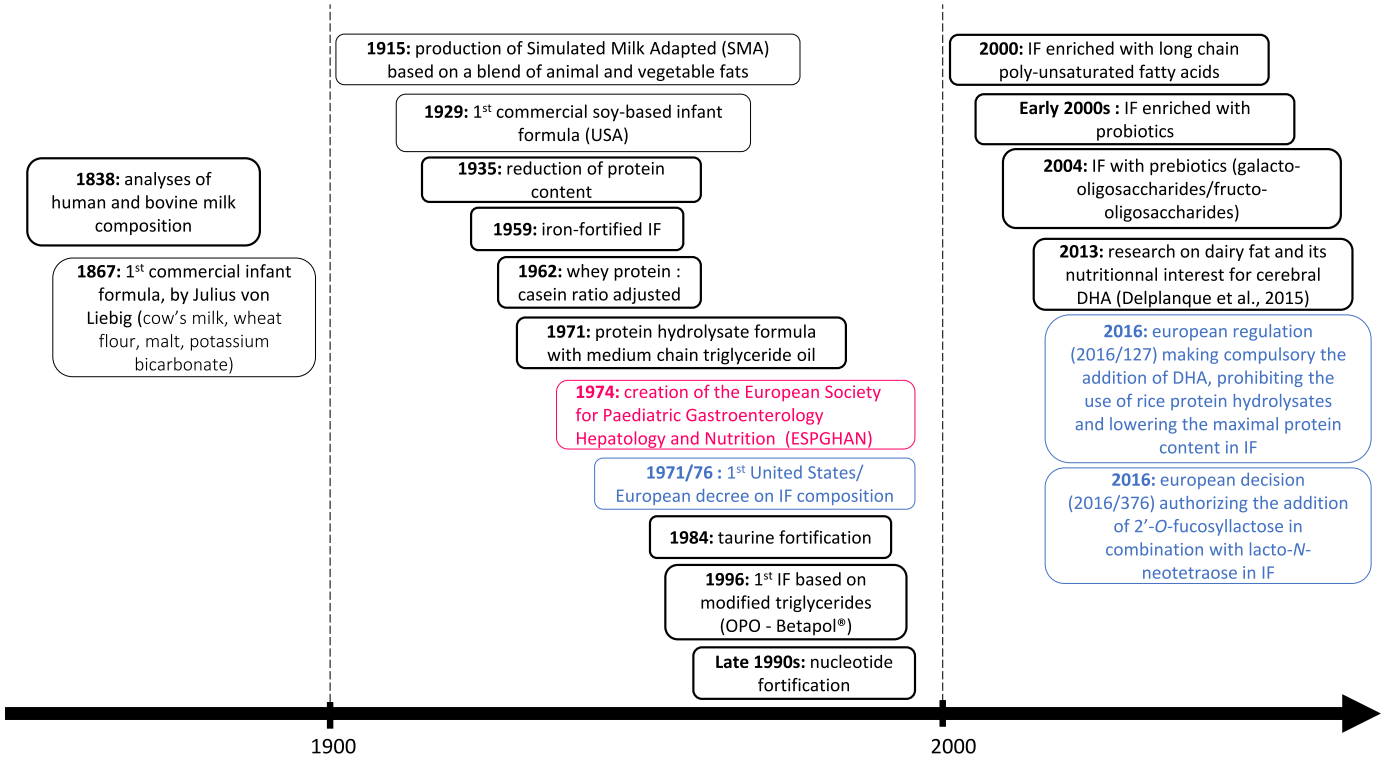

Fig. 2. A short history of infant formula (IF) optimization. Major achievements (in black) and regulations (in blue) are shown as well as the creation of the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN, in pink), a multi-professional organisation whose aim is to promote the health of children through, among other tasks, dissemination of science-based information for pediatric clinicians and professionals. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

docosahexaenoic acid (**DHA**, 0.32%) (Brenna et al., 2007), which are now somewhat better mimicked in IF through compulsory supplementation in DHA (European Union, 2016), although this is still optional for ARA. HM contains other bioactive fatty acids such as *trans*, odd-chain or branched-chain fatty acids, probably from dairy food intake, and some lipid mediators such as endocannabinoids, oxylipins (Wu et al., 2016) and etherlipids. For economic reasons, most IFs are solely based on a mix of vegetable oils (palm, coconut, rapeseed, sunflower) that can mimic the fatty acid profile but with a different regiodistribution of the fatty acids. Modified lipids such as "β-palmitate", offering a regional distribution of palmitic and oleic acids similar to that seen in HM (OPO structure, Fig. 2), may be an alternative, but they are demanding in terms of biotechnology and process. By contrast, BM fat (40–50% of total fat, combined with rapeseed/sunflower oils) offers a minimally processed solution to better mimic HM fat complexity, including fatty acid regiodistribution (OPO structure) and specific lipids such as cholesterol and milk fat globule membrane bioactive compounds, absent from vegetable oils. Mimicry of the milk fat globule microstructure, which is of importance to health effects (Baars et al., 2016), remains challenging due to the homogenization step that forms submicron droplets in addition to the neo-formed interface (dairy proteins and potentially soy lecithin). This nevertheless seems achievable as patented in 2017 (EP 2825062B1), but then raises the question of accessibility for all infants as it is currently restricted to the depositary company

2.1.3. Nitrogenous compounds

The protein content in HM is one of the lowest among mammal milks (0.8–1.2 g.100 mL^{-1}), in line with the slow human infant growth rate (Jenness, 1986), but not with its importance to achieving optimal development. Most IFs are BM protein-based, despite differences between HM and BM proteins, starting with a 2 times lower proportion of caseins in HM proteins (Chatterton et al., 2013). This can be easily mimicked in IF by adding whey proteins (**WPs**) to skimmed BM, but differences remain in terms of protein profile and structure, as stated in Fig. 1.A. Additionally, glycoproteins (*e.g.* lactoferrin) may display different glycosylation patterns between species, which could partly affect their functionality. The origin of protein ingredients may also modulate protein quality, with cheese whey (a major source of WPs in IF) containing the glycomacropeptide released from κ-casein, which unbalances the essential amino acid profile of WP but supplies some O-glycan chains with potential bifidogenic properties, although these may be limited because of their low content (Fukudome et al., 2021). Overall, a higher protein content is needed in IF to cover amino acid requirements (European Union, 2016), while this should be lowered to reduce the risk of obesity (Totzauer et al., 2018). Both HM and BM convey hundreds of minor proteins, although with different concentrations and structures (Zhang et al., 2017). They exert major bioactive functions, such as immune-related (cytokines, immunoglobulins, osteopontin), antioxidant (glutathione peroxidase), growth-promoting (growth factors), antibacterial (lactoferrin) or even digestive (bile salt-stimulated lipase solely in HM) functions (Gila-Diaz et al., 2019; Vizzari et al., 2021). However, most of them are heat-sensitive and may be inactivated during IF production. Finally, unlike BM, HM contains a high proportion of non-protein nitrogen, mainly composed of urea, having some bifidogenic properties (Atkinson & Lönnerdal, 1995), but this is not considered when formulating IF. Free amino acids account for a small proportion of this non-protein nitrogen, mostly as glutamate and taurine. While taurine is frequently added in IF, this is not the case for glutamate, despite its potential role in later taste acceptance (Schwartz et al., 2013) and its immunomodulating properties (van Sadelhoff et al., 2020). Other HM nitrogenous compounds, *e.g.* carnitine and choline, are mandatory in IF (European Union, 2016), while glucosamine nitrogen from oligosaccharides and glycoproteins, small peptides, ammonia, amino alcohols, nucleic acids, nucleotides and polyamines (Atkinson & Lönnerdal, 1995) are not necessarily found in IF. It is necessary to improve mimicry of the IF protein profile in the future, taking account of protein composition and structure and the subsequent impacts of heat treatments, which, among other consequences, may inactivate bioactive proteins. Greater consideration should also be given to the role of the non-protein fraction in terms of overall IF health consequences.

2.1.4. Other compounds

Besides macronutrients, there are several other differences between HM and IF relative to micronutrients and metabolites, the microbiota and cells and exosomes. HM contains vitamins and minerals in quantities sufficient to cover the infant's needs, but the levels differ from those found in BM. Because of the use of skimmed and heat-treated BM and demineralized WPs, vitamins and mineral salts need to be added to the mix. However, and particularly for minerals such as calcium, bioavailability remains uncertain, while the addition of iron may have adverse consequences by favoring lipid oxidation in IF, notably due to the presence of small droplets that are no longer protected by the trilayer membrane. HM also contains a specific profile of metabolites, including short chain fatty acids, amino acids such as tryptophan and its derivatives (*e.g.* kynurenines), polyamines (*e.g.* putrescine) and antioxidant compounds (Gila-Diaz et al., 2019; Gomez-Gallego et al., 2018). These metabolites originate from the mother's metabolism and diet, and from metabolism of the HM microbiota (Gomez-Gallego et al., 2018; Ojo-Okunola et al., 2020). They play a potential role on maturation of the gut, and immune and nervous systems (Ojo-Okunola et al., 2020), with later consequences on health outcomes such as allergies (Paparo et al., 2021). The HM metabolomic profile differs from that of BM, thus rendering it difficult to mimic in IF.

It is now acknowledged that HM also contains a microbiota, characterized by a low microbial load but high diversity (Fig. 1.A), with several hundreds of bacterial and fungal species (Oikonomou et al., 2020). Interestingly, several taxa are shared between HM and BM, including *Staphylococcus, Streptococcus, Bifidobacterium* and several gut obligate anaerobic species (Oikonomou et al., 2020). At present, BM microorganisms are inactivated by heat treatment during IF manufacture; the microbial fraction in IF is limited to the addition of a few probiotic strains, generally belonging to the *Bifidobacterium, Lactobacillus* and/or *Streptococcus* species. However, despite the promising beneficial health impacts, the considerable variability of trial results has led to the lack of systematic recommendation by the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN; Braegger et al., 2011).

Milk exosomes, derived from mammary gland epithelial cells, contain multiple proteins, lipids and ribonucleic acids (Melnik et al., 2021). Some milk exosomes can impact the gut microbiota and intestinal maturation. Other exosomes can reach the systemic circulation, where their micro-RNAs may affect the epigenetic programming of various organs (Melnik et al., 2021). Recent research has also revealed the presence of stem cells in breastmilk. Animal studies have demonstrated that after milk ingestion these stem cells can enter the systemic bloodstream and integrate various target organs (*e.g.* central nervous system, thymus, pancreas, spleen and kidney), where they differentiate into functional cells, further contributing to the development and maintenance of the newborn's bodily functions (Vizzari et al., 2021).

Overall, HM contains a complex metabolic, immune and endocrine signaling system for postnatal growth and programming (Melnik et al., 2021). The integration of some of the aforementioned substances is impossible due to the absence of safe substitutes (cells, exosomes). One could question the role of these substances provided by BM to IF. The bioavailability of vitamins and minerals should be further investigated to enable their optimization. For other substances, such as more complex microbial communities or metabolites (except HMOs and vitamins), their implementation, albeit premature, is worthy of exploration and more data are necessary to clarify their contribution (or not) to the health effects of HM.

2.2. Nutritional and health impacts

2.2.1. Digestive behavior

HM and IF behave differently during digestion. Firstly, gastric half-emptying time is delayed for IF, with 78 *vs.* 47 min for IF *vs.* HM in full-term infants (Bourlieu et al., 2014); this results from numerous parameters such as emulsion stability and structure, stomach coagulum hardness, a difference in hormonal feedback, *etc.* Gastric emptying is a key parameter in the subsequent kinetics of digestion and absorption, and in turn for the metabolic fate of nutrients. Because of protein denaturation/aggregation, heat treatments affect the microstructure of the gastric digesta (de Oliveira et al., 2016; Halabi et al., 2022), potentially modulating the gastric emptying rate. The lipid structure is also of importance; the large native fat globules (4–6 μm) found in HM are more resistant to digestion than the submicron droplets in IFs, the latter inducing a drastic and early increase in lipolysis due to their different interface and elevated specific surface available for lipases to anchor at the oil-water interface (Bourlieu et al., 2015; de Oliveira et al., 2017). This faster lipolysis may elicit a hormonal feedback (cholecystokinin) that slows down the gastric emptying rate, in the same way as with homogenized HM (de Oliveira et al., 2017). At the molecular level, the regiodistribution of fatty acids impacts their release, due to the *sn*-3 and *sn*-1,3 stereospecificity of the gastric and pancreatic lipases, respectively. Unlike in HM, palmitic acid, which is mainly located in a *sn*-1,3 position in palm/coconut-based IFs, and thus released as free fatty acid, can form an insoluble soap with calcium in the intestine, thus decreasing its absorption and inducing harder stools (Delplanque et al., 2015). As for proteins, the true ileal digestibility of total nitrogen has been reported to be lower with HM than with IF in a piglet model, with values of 91 *vs.* 98% (Charton et al., 2023), due to the higher proportion of undigestible non-protein nitrogen in HM, potentially acting as a prebiotic. By contrast, true ileal amino acid nitrogen digestibility was found to be high and similar (97–98%) in both HM and IF. Regarding proteolysis kinetics, conflicting results have been reported from *in vitro* studies, with a slower (Maathuis et al., 2017) or faster (Abrahamse et al., 2022) intestinal rate for IF than for HM. Among IFs, the protein denaturation level has been shown to impact proteolysis kinetics, as observed *in vitro* (Halabi et al., 2020, 2022). In addition, the protein location in the emulsion, either in solution such as in HM, or at the oil-water interface such as in homogenized IF, has been shown to impact their digestion kinetics (Macierzanka et al., 2009; Zhai et al., 2012). *In vivo*, the more rapid and greater appearance of plasma amino acids has been reported for IF than for HM in preterm infants (Moro et al., 1999). Among IFs, the protein denaturation level was shown to slightly modulate the postprandial plasma amino acid concentration when WP solutions were administered to piglets (Welch-Jernigan et al., 2018). Whether this remains true with complete IF is still unknown. Further, heat treatment during IF production favors the Maillard reaction which impairs lactose and amino acid (mostly lysine) bioavailability, but may also modulate the physiological impacts (allergenicity, microbiota, epithelial maturation).

Further investigation is necessary to better understand what is expected for HM proteolysis kinetics and how IF manufacturing processes impact its digestive behavior.

2.2.2. Intestinal homeostasis

Intestinal homeostasis relies on the equilibrium between its external environment (microbiota) and internal environment (immune system), mediated by a tight and functional epithelial barrier. This equilibrium matures after birth under the influence of diet (Fig. 1.B) (Figueroa-Lozano & de Vos, 2019), with different gut closure dynamics in HM- or IF-fed infants (Le Huerou-Luron et al., 2010). The infant immune system probably develops more rapidly in breastfed than in IF-fed infants (Andersson et al., 2009), as well as passive immunity being transferred by HM immunoglobulins, particularly with colostrum. These differences likely result in part from the impact of diet on the gut microbiota. HM promotes a higher proportion of *Bifidobacteria* in the gut microbiota, while IF causes an accelerated evolution towards an adult-like profile (Davis et al., 2017). Differences have also been reported in the fecal metabolome, as a result of both diet and gut microbiota metabolism (Sillner et al., 2021). Finally, infant's diet may also modulate the secretory response of its intestinal entero-endocrine cells (Le Huerou-Luron et al., 2010).

Many HM bioactive compounds can influence development of the intestinal barrier and gut microbiota, including growth factors, immune-related proteins (cytokines, immunoglobulins, lysozyme, lactoferrin), HMOs, HM microbiota, metabolites and micro-RNAs (Boudry et al., 2021; Figueroa-Lozano & de Vos, 2019). The recent development of *in vitro* models that combine digestion and the gut epithelium, such as multicellular models, pediatric intestinal organoids or organ-on-chip, may help to decipher the effects of HM counterparts on gut homeostasis (Noel et al., 2021).

2.2.3. Short- and long-term health impacts

Epidemiological studies have reported the short- and long-term nutritional and health-promoting effects of breastfeeding compared to IF-feeding (Fig. 1.B). Breastfed infants have a slower growth trajectory than IF-fed infants during the first months of life, which may be associated with a lower risk of obesity in infancy and later in adulthood (Lemaire et al., 2018). Breastfeeding has been reported to decrease the risk of morbidity and mortality during the first year of life and to protect against respiratory and gastrointestinal infections, as well as reducing the risk of developing inflammatory and metabolic disorders such as obesity and diabetes, inflammatory bowel disease, asthma or allergy, although the findings remain controversial with respect to allergy (Lemaire et al., 2018; Victora et al., 2016). A positive impact of breastfeeding on reducing cardiovascular risk, on neurodevelopment and more generally on cognitive development has also been suggested in premature infants (Lechner & Vohr, 2017). Overall, these observations support breastmilk as the "gold standard" for optimal infant development and health, although the components in HM, their synergistic effects and the underlying mechanisms are not fully understood, thus remaining a challenge in terms of producing a substitute. Some of the long-term health-promoting effects of breastfeeding are likely related to the impact of diet on the establishment of intestinal homeostasis during the so-called "window of opportunity" of the first months of life (Amenyogbe et al., 2017).

2.3. Limitations to biomimetic formulas

While the first 2 sections above clearly highlight the gaps between IF and HM in terms of composition and, more importantly, their nutritional and health effects, several further limitations preclude a fully biomimetic IF and are discussed below.

2.3.1. Variability and complexity of human milk

While IF targets an average mature HM and remains similar between 0 and 6 months, HM is a dynamic biologic fluid with a varying composition that depends on the gestational and postnatal ages, maternal diet and other minor influencing factors such as the mother's age, pregnancy weight gain or parity (Andreas et al., 2015). Colostrum is rich in immunologic components and developmental factors, whereas the total protein content and the proportion of WP decreases in transitional milk (Lönnerdal et al., 2017). By contrast, the lipid content increases over lactation, together with milk fat globule size (Michalski et al., 2005). HM content also varies during a single meal, with a gradual increase in fat content concomitant with a decrease in lactose content (Andreas et al., 2015). Strong inter-individual variabilities have been reported for lipids (*e.g.* the ratio between linoleic and α-linolenic acids and the DHA content), mainly in relation to diet (Delplanque et al., 2015), and for HMOs in relation to the mother's genetics (Andreas et al., 2015). The HM microbiota and metabolites, including vitamins (such as

A, D, and B excluding folates) may also vary according to lactation stage, parity, delivery mode, mother's health status, diet, lifestyle or geographical location (Allen, 2012; Gomez-Gallego et al., 2018; Oikonomou et al., 2020). Finally, the maternal diet may also affect the unique HM flavor profile to which neonates respond through physiological and behavioral means (Loos et al., 2019), with potential consequences regarding the establishment of food preferences in later life, although it is still difficult to demonstrate the differential impact of HM- *vs.* IF-feeding (Sina et al., 2019).

Mimicking variations in HM composition over lactation, and notably during the first weeks of life, could be addressed by producing age-tailored IF during the first 6 months of life, as has been proposed in some patent applications (*e.g.* WO2015085551A1). It remains difficult to take account of the inter-individual variability of HM composition for as long as the relationships between HM composition and infant health impacts have not been fully elucidated.

2.3.2. Ensuring the safety and stability of infant formula

The development of new IF or the implementation of new technological processes must always ensure microbial safety, *i.e.* involving no pathogenic microorganisms such as *Salmonella* spp., *Staphylococcus aureus*, or *Cronobacter* spp. Any bacterial contamination can have dramatic effects on infant health, so therefore needs careful monitoring. The cardinal growth parameters of the most common pathogens in IF are relatively well known, so that heat treatment parameters can be set to ensure microbial risk control; however, they are not optimized regarding the concomitant preservation of heat-sensitive components. If such optimization is not possible, alternative treatments that assure both microbial safety and the preservation of native components, such as membrane microfiltration, could be of value.

In terms of functionality, customers demand IF powders that are slightly off-white in color and homogeneous in appearance (meaning no flecks or colored dots), and have free-flowing and fast rehydration properties during storage and ageing. Liquid and reconstituted IF powders should be homogeneous, show no signs of creaming, no "white flecks", no sediment and no defects regarding viscosity, flavor or color that would reduce customer acceptance and possibly the nutritional properties of the IF.

A creaming instability of reconstituted IF is observed when oil droplets flocculate or their diameter increases above a critical size, such as in raw BM and HM. Flocculation occurs in IF because the oil droplet surface is mainly covered by amphiphilic heat-sensitive WPs that can aggregate during heat treatment, leading to oil droplet bridging (McCarthy et al., 2012). These adverse evolutions could be reduced by the controlled pre-denaturation of WPs (Schmidmeier et al., 2019), although this is not compatible with objectives concerning protein preservation, or by modifying the WP profile (Buggy et al., 2017). Nevertheless, if well controlled during production, IF creaming within a bottle, due to the presence of large droplets, could constitute a strategy to mimic HM variability throughout a meal, for as long as the IF flow through the teat is not impaired. In addition, protein aggregation during heat treatment (mainly induced by soluble calcium salts), and insoluble mineral fortification, can trigger the formation of sediments in certain long-life, ready-to-feed IF products (Crowley et al., 2014; Barone et al., 2021).

White flecks are insoluble spongy powder particles of several hundreds of μm in diameter that are sometimes observed in reconstituted IF. They are prone to cling to the feeding bottle surface and/or clog the bottle teat, which may result in nutrient loss for the infant. Insoluble salts are often associated with sensory defects in reconstituted IF, such as chalkiness, grittiness and bitterness, and limit mineral bioavailability. The use of micronized insoluble salts with a smaller mean particle diameter, can reduce these sensory defects and the sedimentation rate in liquid IF (Barone et al., 2021). Maillard reaction and lipid oxidation products are responsible for off-flavors and color changes during the processing and shelf life of IF, particularly if hydrolyzed proteins are used (Yang et al., 2020). Increasing the amounts of free fat (McCarthy et al., 2013; Saxena et al., 2021) and minerals in the formulation, and notably soluble forms of metal ions (Gharibzahedi & Jafari, 2018) also promote oxidative flavors (Wang et al., 2020).

Compromises concerning all the aforementioned defects and their effects on both functionality and nutritional properties need to be borne in mind when developing a new IF.

2.4. The challenges of mimicking human milk

Given the considerable complexity of the composition of HM and constantly evolving knowledge in this area, closing the compositional gap between HM and IF may seem unachievable. One alternative might be to mimic nutritional, physiological and long-term health outcomes rather than composition. One major challenge is then to fill the knowledge gaps concerning the effects of all HM components, including minor ones, on infant development and health. Beyond their composition in HM, their structures as well as their bioavailability and bioactivity need to be considered. Deciphering the mechanisms of action of HM components would assist in proposing relevant alternatives that would mimic their physiological, nutritional and immune effects and confer health benefits similar to HM. If data on all these elements are not available, then biomimicry of the nutritional and health effects of HM could be achieved by mimicking these different components. A second challenge would then be to design new strategies to implement substitutes for all these HM components of interest. Finally, the challenge of HM mimicry - in terms of nutritional and health effects - should be addressed in a more global context, in light of the other challenges faced by the IF market, including environmental, economic and social sustainability.

Several improvements to IF with potential positive health outcomes have already been proposed (Fig. 2) (Ahern et al., 2019; Davis et al., 2017; Lemaire et al., 2018). Further improvements still need to be made, strategies already partially implemented should be continued and others fully explored (see arrows on Fig. 1A). Regarding proteins, a lower total protein content and lower casein:WP ratio are now widely applied in IF. The addition of specific bioactive proteins (*e.g.* lactoferrin, α-lactalbumin, lysozyme, osteopontin) is more challenging and remains limited because of the high cost and limited availability of these dairy ingredients, but also due to the technological hurdle linked to protein heat-sensitivity, potentially impacting their bioactivity. One alternative might be the dry blending of these heat sensitive proteins into the final product, which needs to be fully controlled to prevent any microbial contamination. Another issue concerns the composition and structure homology between dairy and human proteins, which is far from perfect. This is even more true for plant proteins, including soy proteins, where the health outcomes of such plant-based IFs remain poorly documented (Westmark, 2017). The use of recombinant HM proteins has been proposed but this raises ethical issues. As for lipids, some products on the IF market have already been modified (dairy lipids and/or milk fat globule membrane extract, ARA, DHA). The production of IF containing large droplets has indicated promising health effects (Brenk et al., 2017) but the production and stability of these emulsions remains a technological challenge. Efforts have been made to improve the carbohydrate moiety through the addition of a few bifidogenic oligosaccharides, alone or combined with probiotics, with some beneficial effects on the gut microbiota, immune system and health (Ahern et al., 2019). However, the pre and probiotics added to IF are far from being able to mimic the diversity of HM oligosaccharides and bacteria. In the latter case, as well as the challenge of mimicking the bacterial profile, certain technological hurdles (*e.g.* growth conditions, stability) and regulatory requirements (*e.g.* Qualified Presumption of Safety status) need to be tackled. It will be very difficult to mimic several other components in IFs, such as immunoglobulins and immune cells, specific exosomes with human miRNA, growth factors and metabolites.

Further investigation is necessary to elucidate the roles of bioactive

components in HM, to explore their implementation in IF through the better use of existing ingredients (*e.g.* BM fraction) or alternatives (*e.g. de novo* synthesis of HM proteins) conferring benefits similar to HM. It is even more necessary to evaluate the risk/benefit and cost/benefit ratios and address all concerns regarding the safety, efficiency and regulatory aspects of this implementation, which may differ depending on the target population (preterm or full-term neonates, neonates with a high risk of developing metabolic or immune diseases later in life). The introduction of new ingredients or processes to obtain this highly regulated food product often requires extensive and costly clinical studies; companies are reluctant to initiate this type of development which hinders innovation. Further, IF manufacturers need to address numerous, and sometimes contradictory, customer demands. The more biomimetic IF of the next generation will also have to consider other growing concerns regarding sustainability and/or less processed food products.

Processing plays a central role in the IF production chain. The next section will highlight how processing innovations can contribute to the production of more biomimetic IFs while preserving or enhancing their safety, stability and sustainability. The principal challenges regarding processing concern the addition of new bioactive components while minimizing the number of steps, improving the preservation of bioactive components in IF and mimicking the HM macrostructure while simultaneously reducing energy costs and greenhouse gas emissions.

3. How processes can enable more biomimetic and sustainable infant formulas

3.1. Impacts of processes on ingredient and infant formula functionality

IF manufacture involves a succession of unit operations that usually use a wet-mixing approach (Fig. 3) or more rarely a dry blending method. Wet-mixing consists in rehydrating powdered ingredients before other unit operations, ending with spray-drying to produce IF powder. In order to better understand how IF processes could be reconsidered, it is important to understand the influence of each unit operation on the physicochemical properties of the wet-mix and of the final product, as discussed below.

3.1.1. Preparation of the wet-mix

The water-soluble ingredients (lactose and WPs) are rehydrated in fresh skimmed milk or in rehydrated skimmed milk powder to reach the desired composition. The choice of ingredients is key, as it can lead to different protein profiles and/or protein denaturation/aggregation levels as well as to different levels of advancement of the Maillard reaction. High shear mixers at temperatures up to 50 °C (Bylund, 1995)

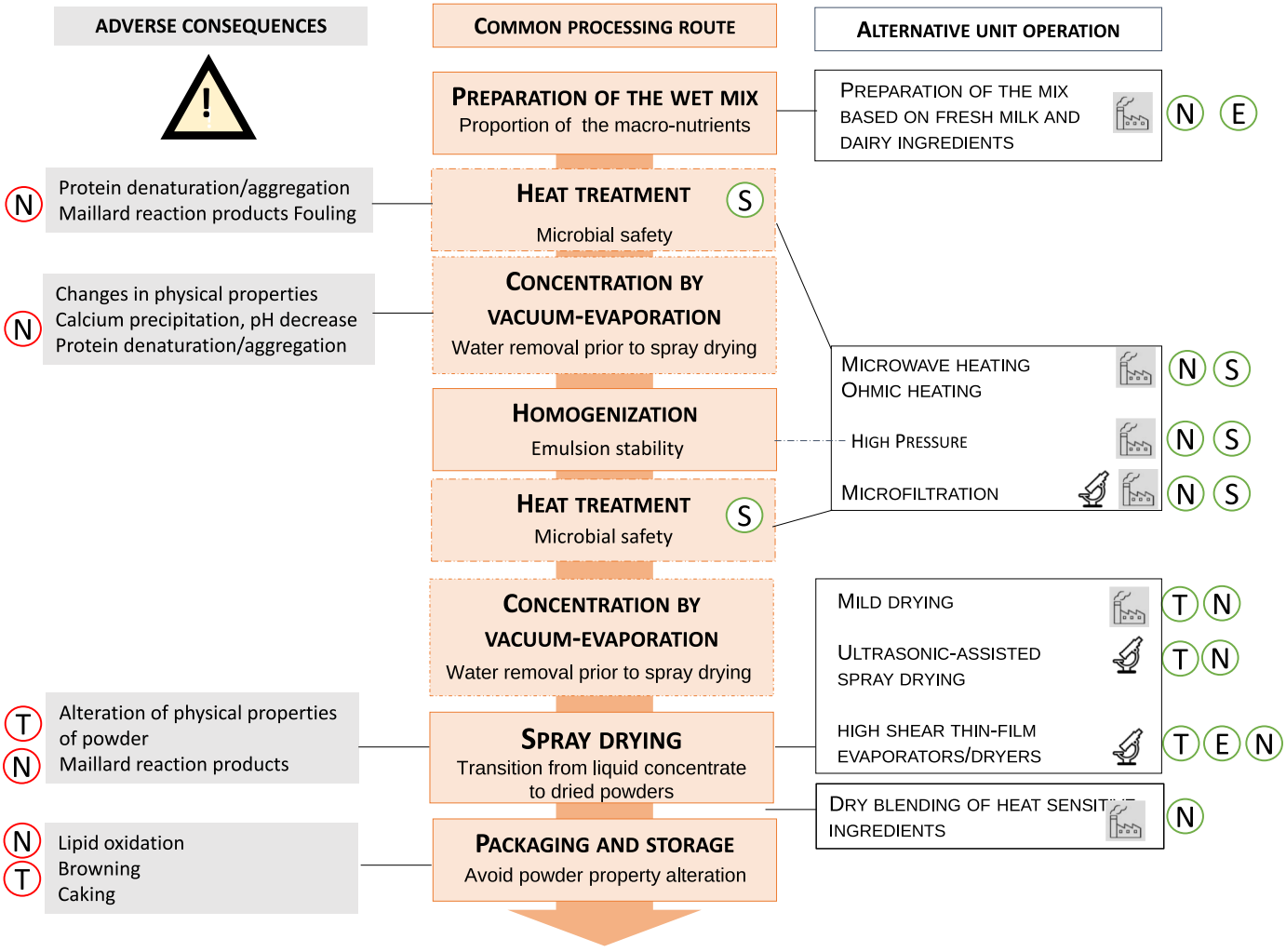

Fig. 3. Successive unit operations in the industrial production of powdered infant formula (IF) with adverse consequences and their alternatives. Positive (green) and negative (red) potential impacts are indicated. N: nutritional impact, S: sanitary impact, T: techno-functional impact and E: energy saving. Dotted orange lines indicate unit operations that may occur at different time points during processing, depending on the manufacturer. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

are used to disperse raw materials in order to reduce rehydration time (McCarthy et al., 2013). A temperature higher than 50 °C enables the dissolution of crystalline lactose as a function of its solubility curve (Schuck et al., 2004).

3.1.2. Heat treatment

Heat treatment is a critical control point in IF processing for microbial quality, and may be performed either directly (*e.g.* steam infusion or injection) or indirectly (*e.g.* use of tubular or plate heat exchangers). Pasteurization is applied when producing powdered IF, the aim being to inactivate the vegetative forms of bacteria, viruses, protozoa, molds and yeasts. The temperature/time combinations for pasteurization in the dairy industry vary depending on the processing step (Jeantet, 2016). Sterilization, which enables the additional destruction of bacterial spores, is applied to liquid IFs which can then be stored at ambient temperature for up to 12 months.

The positioning of heat treatment within the process is flexible (Fig. 3). If applied before homogenization it may be more effective in producing a more stable emulsion (Varnam & Sutherland, 1994). When heating is scheduled after homogenization, oil droplets coated with native WPs may flocculate and coalesce, potentially leading to the formation of white flecks (Schmidmeier et al., 2019). The effect of heat treatment on the physical stability of IF also depends on the fat/protein ratio (Buggy et al., 2017). However, this could offer a way to increase the droplet size and thus slow down the lipolysis kinetics.

Heat-induced protein structural changes in IF (Buggy et al., 2017; Crowley et al., 2016) depend on time, temperature and protein content (*i.e.* before or after concentration). This can lead to the formation of soluble WP aggregates and/or casein micelle-bound WP aggregates, the latter being favored by a higher heating temperature and lactoferrin content (Halabi et al., 2020). This may modulate the digestive behavior of proteins, and, in the presence of lactose, favor the Maillard reaction, leading to adverse consequences as discussed in section 2.2.1.

It should however be noted that whichever process is used, microbial destruction does not preclude any microbial risk, as deleterious bacterial compounds (*e.g.* toxins, biogenic amines) may remain active and post-contamination may occur.

3.1.3. Concentration by vacuum evaporation

Concentration by evaporation under vacuum (~50–70 °C) is carried out to eliminate most of the water from heat sensitive wet-mixes, thus reducing the energy required for subsequent spray-drying. The level of concentration is limited by the increase in the viscosity of the concentrate, which should not exceed 60–100 mPa.s to enable subsequent spraying and drying (Westergaard, 1994). Evaporation can affect the physical state of wet-mix constituents. The concentration of skimmed milk increases the quantity of calcium associated with casein micelles. Moreover, the insolubilization of calcium phosphate, combined with an increase in ionic strength, may lower the milk pH. These evolutions reduce the hydration and zeta potential of casein micelles and may affect protein aggregation during evaporation (Liu et al., 2012). However, the latter is limited when vacuum evaporation is conducted at a mild temperature (Yu et al., 2021).

3.1.4. Homogenization

The oil-soluble phase (oil mixture, lipophilic vitamins) is usually mixed with the water-soluble system to form a pre-emulsion which is then homogenized, generally using high-pressure valve-type homogenizers (Bylund, 1995). These generate high shear forces that break down the fat droplets into those of submicron size. The positioning of homogenization within the process is flexible (Fig. 3), either before concentration when fat is present in the wet mix, or after when fat is added after concentration. In many cases, homogenization is performed in 2 stages, the first (~8–15 MPa) reducing fat droplet size in the emulsion and the second (~2–3 MPa) preventing and disrupting fat globule clusters (McCarthy et al., 2012). Homogenization increases the total surface area of lipid droplets, so that milk proteins and other emulsifiers must be adsorbed on the newly formed surface. The affinity of proteins to adsorb at the oil-water interface is dependent on many factors, such as their nature (caseins, WPs), structure (native, denatured) (Dickinson, 2013) and the type of lipids (Zhai et al., 2012). If there is a high proportion of native WP in the formulation, there is a greater risk of oil droplet flocculation during post-homogenization treatments (heat treatment, evaporation, spray drying) when the homogenization pressure rises because more heat-sensitive WPs are adsorbed at the surface of the oil droplets. This homogenization step also has nutritional consequences, as discussed in section 2.2.1.

3.1.5. Spray drying

Spray drying converts the liquid concentrate into dried powder. Typically, concentrated solutions are fed to a nozzle atomizer that breaks the liquid stream into a spray of droplets of almost homogeneous size (100–400 μm) (Westergaard, 1994). These droplets are characterized by a high surface to volume ratio and high temperature/low vapor partial pressure conditions that favor rapid solvent evaporation and the progressive shrinkage of droplets to reach the final particle state. Water removal leads to skin/crust formation and finally to complete solidification within a few seconds. The spray drying of IF is generally carried out using multistage spray dryers, composed of a large drying chamber where the bulk water is removed, followed by supplementary drying stages assured using an internal and/or external fluidized bed. Specific nutritional, physical and technological properties such as limiting lysine loss, rehydration properties (Selomulya & Fang, 2013) or moisture content as it affects product stability and shelf life and nutrient encapsulation ability (Schuck et al., 2016), can be achieved by modulating the main operating parameters for spray drying. However, despite recent technological advances, optimizing the evaporation capacity and thermal efficiency of the dryer still represents a complex challenge because of the multiple factors involved, related to environmental conditions (*e.g.* temperature, relative humidity and flow rate of the hot air stream), feed concentrate properties (*e.g.* viscosity, sticking tendency) and dryer configuration.

3.1.6. Packaging and storage

Because the quality of IF powders can be compromised by lipid oxidation, browning and moisture-induced caking, the use of appropriate packaging is of the utmost importance. Thus, in order to minimize IF oxidation, materials with a high gas barrier (*e.g.* coated metal cans) and nitrogen gas are mostly employed. IF powders should generally be stored under cool (20–25 °C) and dry conditions (< 25% relative humidity). Higher storage temperatures result in more physicochemical changes to IF powder (Tham et al., 2017), particularly due to amorphous lactose crystallization and the concomitant release of moisture. Furthermore, lactose crystal growth may potentially disrupt the oil droplet membrane, thus favoring free fat formation and affecting IF solubility (Toikkanen et al., 2018). This can be mitigated by the addition of maltodextrin (Masum et al., 2019), but this will compromise IF biomimicry.

3.2. Rethinking processes: consequences and opportunities for more biomimetic and sustainable IF

The aforementioned unit operations, which involve considerable energy consumption, impact the IF components with consequences on their physico-chemical, nutritional, and thus potentially health properties. Different strategies have been explored to improve IF biomimicry and process sustainability: i) optimizing key unit operations in terms of process efficiency and energy costs, ii) developing innovative processes (Fig. 2), or adapting existing processes to IF production. One of the main challenges lies in scaling-up from innovative scientific advances in the laboratory to industrial production (Shirkole et al., 2021). Rethinking processes by reinvestigating IF production stages from the macroscopic

to molecular scales should provide new opportunities to develop more biomimetic IF and address the growing demand for more sustainable production.

3.2.1. Alternatives to heat treatments: ensuring microbial safety while preserving native nutrients and reducing energy costs

As previously mentioned, although heat treatments ensure the microbial safety and shelf life stability of IF, they induce numerous physicochemical modifications such as protein and mineral alterations, leading to processing constraints (*e.g.*, increase in fluid viscosity that affects spray drying efficiency) and change to functional and nutritional properties. Innovative, alternative or complementary (both thermal and non-thermal) processes have been proposed (Fig. 3) and are described below, although to date their implementation at the industrial production scale remains challenging in some cases. By improving the preservation of IF components, some of these alternatives offer new opportunities for the implementation of labile components such as immunoglobulins, growth factors, cytokines or hormones, provided their benefice for infant health can be established.

3.2.1.1. Microwave heating. Since the 1990s, microwave heating (300 MHz–400 GHz) has often been employed – alone or coupled to traditional methods – at both the laboratory and industrial scales. Although this process has been proved effective in terms of the microbial control of dairy liquids, the effects on milk components require further investigation. As recently highlighted in the case of HM, microwave heating can affect the secondary structure of proteins such as α-lactalbumin, which may cause changes to certain nutritional/bioactive properties, and also some minor modifications to the properties of IF powder (Martysiak-Żurowska et al., 2022). However, the use of microwave heating at an industrial scale has diminished during recent decades because of the fire risk.

3.2.1.2. Ohmic heating. Promising outcomes have been achieved at the laboratory scale using ohmic heating (current passage tube). This method ensures a uniform thermal distribution and constant difference in temperature between the hot wall and the liquid in the equipment, enabling the better control of microbial inactivation and protein denaturation than using standard High-Temperature Short-Time pasteurization. Furthermore, ohmic heating markedly mitigates the fouling phenomena observed in heat exchangers, whose consequences constitute the main contribution to production costs. Ohmic heating enables more energy saving (< 82%) compared to conventional heating, while microwave heating only achieves an energy saving of up to 65% (Sakr & Liu, 2014).

3.2.1.3. High pressure processing. Applying high pressures to dairy fluids (100–600 MPa) favors the tight control of microbial activity (Moschopoulou, 2021). These specific operating conditions contribute to the fragmentation of casein micelles through the dissolution of colloidal calcium phosphate and the disruption of hydrophobic interactions (Anema et al., 2005), resulting in a smaller casein micelle size. As well as enhancing the rehydration properties of the final powders, this smaller micelle size may be of interest when trying to better mimic those in HM. Further, a single high pressure pasteurization process could replace both heat treatment and homogenization (Sousa et al., 2020). The principal limitations to its industrial implementation are its high cost (including that of equipment and a lower throughput compared to thermal treatments) and the fact that it is a batch process. For this reason, high-pressure pasteurization is often combined with other pasteurization methods and with homogenization.

3.2.1.4. Microfiltration. Microfiltration is a standard unit operation in the dairy industry. In the manufacture of IF, it can be used to produce a native WP concentrate using a 0.1 μm pore size membrane. Although a higher cut-off size (0.8 μm) has not yet been implemented in IF production for bacteria removal, it might offer a promising alternative to heat treatment. Yu et al. (2021) recently validated the possibility of replacing the multiple thermal processes generally involved in IF processes with this type of microfiltration (0.8 μm pore size membranes) on fresh milk, coupled with the production of a liquid WP concentrate from raw skimmed milk microfiltration (0.1 μm pore size membrane). The outcome of this research, conducted at a semi-industrial scale, showed that coupling the membrane filtration of fresh milk was a very promising strategy to ensure the bacteriological safety of IF while preserving a high native compound content in the final product. These pioneering results open the way to further experimental investigations before scaling up to the industrial level. Preliminary findings have demonstrated the beneficial nutritional and physiological impacts of minimally-processed IF in rats, as well as modulation of the organoleptic properties (unpublished results). It is worth mentioning that microfiltration (0.1 μm) at cold temperatures has also been proved promising. Cooling the temperature enables the permeation of both WPs and β-casein that can then dissociate from the casein micelles, particularly at 4 °C (France et al., 2021). Cold microfiltration therefore enables the generation of a novel ingredient with improved functionality, microbial quality and sustainability; less cleaning water use are possible because of reduced fouling, despite more electricity being required to cool the system and more water required for the diafiltration (Crowley et al., 2015; McCarthy et al., 2017). Further research is still necessary before such promising processes can be used industrially.

3.2.2. Spray drying optimization and alternatives: improving functional properties, process efficiency and energy costs

3.2.2.1. Spray drying optimization. Numerous studies have focused on the spray drying step. Most drying equipment uses pressure nozzles to atomize concentrated dairy solutions into micron drops (~100 μm) in order to maximize evaporation kinetics. After spraying and until the water has evaporated, the droplet temperature remains at wet bulb temperature, below the protein denaturation temperature; conversely, droplet/particle temperature rises at the end of drying towards the outlet air temperature, as water evaporation no longer balances the energy transfer from the air to the droplet. It has been shown at a semi-industrial scale that applying mild temperatures (inlet and outlet temperatures of 160 °C and 70 °C, respectively) enabled a low level of protein denaturation and sticking behavior (Yu et al., 2021), although lower temperatures come with lower dryer productivity and a higher specific energy cost of drying. Beetz et al. (2020) drastically changed the design of the drying chamber to allow a reduction in the inlet temperature to below 100 °C. The injection of secondary air streams at numerous locations ensured localized turbulent zones in the tower. The simultaneous effect of enhanced hydrodynamics and a shorter residence time would contribute to reducing energy consumption and hence capital costs. This method might be potentially useful at an industrial scale once cleaning requirements have been optimized.

To prevent thermal setbacks, the most promising method is probably ultrasonic-assisted spray drying (Khaire & Gogate, 2021), which consists in feeding a concentrated dairy solution into a narrow capillary that ends in a piezo-ceramic plate, allowing the formation of a homogenous film before breaking down into small regular droplets and producing small dry particles (down to few nanometers). Such a slow atomization process reduces material losses on the walls (*i.e.* sticking) and the size necessary for drying chambers. This setup holds promise in terms of preserving native structures and hence the nutritional properties of the powder. However, some important limitations need to be pointed out: i) the restricted use of Newtonian fluids with low viscosity to enhance cavitation phenomena and droplet formation, ii) the large quantities of hot air necessary for evaporation, iii) the fine particle size distribution that might impair rehydration properties, and particularly iv) the low

throughput capacity due to smaller equipment size. Experiments have mostly been conducted on WP solutions (Chegini & Taheri, 2013) and further investigations using complex dairy solutions are now required.

Improvements to the drying process are still subject to significant limitations because of a lack of knowledge regarding its impact on the physicochemical properties of the powder. It is crucial to investigate and predict the evaporation mechanisms inside drying chambers. The machine learning-based predictive approach (Khan et al., 2022) and artificial neural networks used for the biological predictive modeling of complex, dynamic and highly nonlinear scientific and engineering problems in food drying (Mattar et al., 2004) could contribute to predicting the physicochemical properties of the powder as a function of the feeding solution and experimental conditions.

3.2.2.2. Alternatives to spray drying. Very few innovative technologies have explored alternatives to spray drying. Technologies based on implementing high shear thin-film evaporators/dryers (*e.g.* Tixotherm, towerless drying or agitated thin-film drying), which have been tested on WP isolate solutions or high lactose dairy streams, represent possible breakthroughs that deserve further investigation (Patil, Tanguy, Floch-Fouéré et al., 2021; Qiu et al., 2019). Elimination of the traditional falling film vacuum evaporator by using more energy efficient in-line high shear mixing technologies before spray drying have been investigated (Murphy et al., 2013). This approach makes it possible to maintain high-solid dairy concentrates (80–86% wt/wt dry matter) in a fluid form that exploits their shear thinning behavior. At the end of the superconcentration towerless drying process, the thick paste obtained is granulated into discrete particles by the addition/recirculation of dry powders (97% wt/wt dry matter), resulting in non-sticky granules (> 88% wt/wt dry matter) that can be further dried up to 97% wt/wt dry matter using either a fluidized bed or the same thin-film rotary evaporators. Similar outcomes have been achieved using the Tixotherm and agitated thin-film drying processes, but only at the laboratory scale. The bottlenecks that affect these innovative processes are related to the rheological behavior of the processed fluids at increasing concentrations, which determines their ability to be superconcentrated. Patil, Tanguy, Le Floch-Fouéré, et al. (2021) recently showed that onset of the cohesive state that limits superconcentration occurs at a lower dry matter with a higher protein-to-lactose ratio, making the towerless drying process more applicable to high lactose dairy streams. This opens new opportunities for IF production using an appropriate dairy powder base with respect to caseins, WPs and lactose to granulate a superconcentrated demineralized whey paste. IF production according to this approach would have several benefits, starting from significant energy savings (10–30%) and costs (up to 40%) and including easier maintenance and cleaning procedures because of the compact setup configuration.

These disruptive technologies that bypass standard spray drying still need to be tested extensively to demonstrate their reliability for industrial production and to prove that the properties of the final products will be well preserved, including from a nutritional standpoint.

3.2.3. Minimal processing to improve biomimicry and sustainability?

The aforementioned advances in IF processing have not only focused on reducing energy and maintenance costs, but also on the mitigation of the dairy component alteration by the different treatments. In this context, the use of less processed ingredients, starting with raw or minimally heat-treated milk, may be preferred over the widely used spray-dried skimmed milk that has already undergone several heat treatments even before the start of IF production; in this way, the preservation of nutrients and bioactive components in a native state would be assured. Likewise, the strategies mostly used for IF production, consisting in milk fractionation before the ingredients are recombined must be challenged by simpler approaches starting from fresh milk and limiting its transformation and/or fractionation. For instance, demineralized whey powder is often over-demineralized in terms of its IF mineral content; consequently, a fraction of the minerals removed needs to be reincorporated in the wet mix. As for proteins, adjustment of the casein:WP ratio is generally achieved by incorporating WP powder into rehydrated skimmed milk powder. If using raw milk, adjustment of the protein ratio could be achieved by implementing the microfiltration steps applied to raw skimmed milk (as described above), while adjustments to the mineral content adjustment could be achieved through partial demineralization. Regarding the final drying step, disruptive technologies such as the towerless drying process are also promising at the laboratory scale and require investigation to achieve industrial implementation. However, the need to produce IF as a powder might also be questioned, as liquid IF could be advantageous in obtaining more biomimetic IF, but more controversial regarding its storage, transport and distribution.

Overall these disruptive technologies for IF production deserve further attention in terms of evaluating their impacts regarding functionality and nutritional and health properties, while taking account of environmental, economic and social sustainability. In particular, their impact on energy costs and greenhouse gas emissions needs to be fully evaluated.

4. Towards an integrated approach to the production of biomimetic and sustainable infant formulas

Rethinking processing and technological routes, as presented in section 3, will undoubtedly open avenues for the improvement of IF. Yet these new routes also highlight the complexities of optimizing IF. This complexity arises from multiple objectives that include biomimicry of the health effects of HM, economic, environmental and social sustainability and the contradictory demands of customers (Fig. 4). A multidisciplinary approach, based not only on nutrition and process engineering as pivotal disciplines, but also including microbiology, biochemistry, pediatric medicine, consumer and data science and public health is necessary to integrate all dimensions of the issue. This cannot be addressed solely through improvements to processes. In this section, we suggest that a broader and more systemic approach that takes account of the entire value chain from ingredient sourcing to IF distribution to the customer could help to design the next generation IF by fully rethinking the IF production system. Simultaneous consideration of the different objectives can be achieved by modeling and multi-objective optimization of the system. This systemic approach should make it possible to achieve the acceptance and development of innovative and disruptive processes at the industrial scale while considering different parameters inherent to the food industry, *i.e.* technical, economic, environmental and organizational challenges. Issues related to public health must also be addressed, based on the importance of early nutrition to the future health of the infant.

4.1. Towards multi-objective process optimization

In the coming years, it will be essential to shift the paradigm when designing the new generation IFs and related processes. Process design is often a compromise between minimizing process-related inputs (water, energy and chemicals), environmental impacts and related costs, and maximizing productivity and product qualities. At present, these objectives are generally considered as single constraints added iteratively, thus limiting the degrees of freedom and leading to highly restricted process control and design. The production of biomimetic and sustainable IFs will require the development of multi-objective optimization strategies to integrate the notion of compromise between conflicting or synergistic objectives (Fig. 4). This optimization will have to consider technological innovations as well as sourcing new ingredients, and discern the optimal solutions to be implemented. In this area, design based on multi-objective optimization within the chemical industry should be viewed as fruitful and worthy of study and transposition.

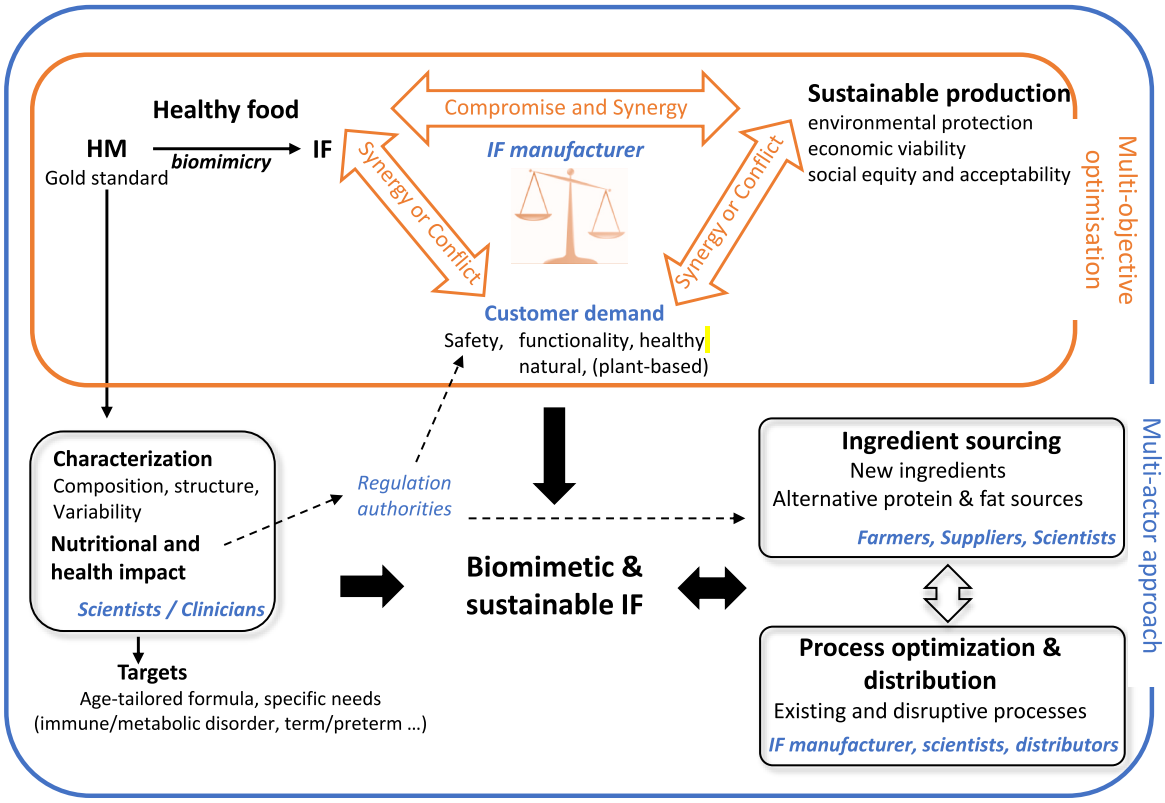

Fig. 4. Interplay among multiple objectives and actors for the production of a biomimetic and sustainable infant formula (IF). HM: human milk.

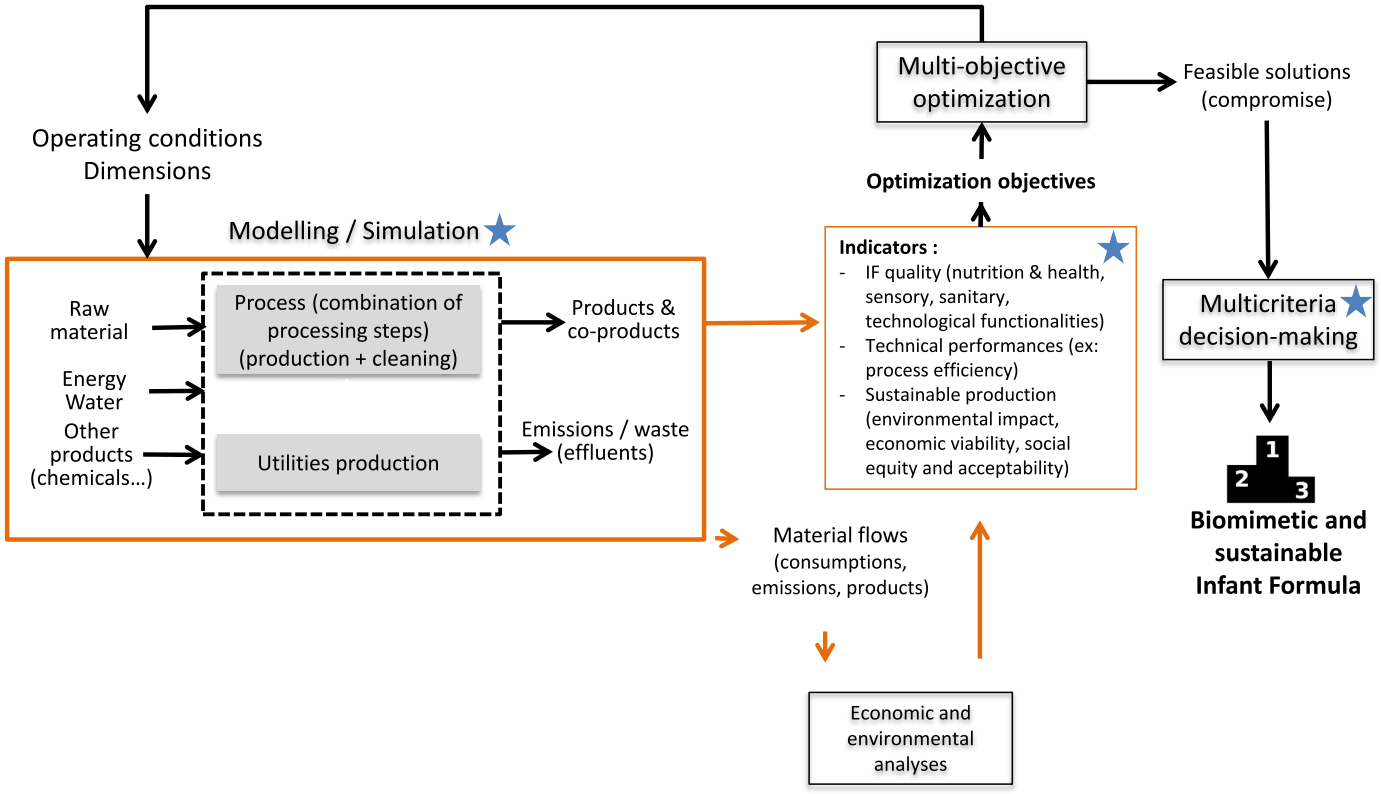

Fig. 5. Multi-objective optimization strategy to produce more biomimetic and sustainable infant formula (IF). The involvement of stakeholders is indicated by a blue star. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In terms of research, attention should be paid to formulating the multi-objective problem and mathematical modeling of the objectives, before the optimization step is actually implemented (Fig. 5). Problem formulation requires identification of the optimization objectives, decision variables and their influential relationships (Belna et al., 2022), which is complex in the context of IF production due to: i) the need to simultaneously consider the different aspects of IF quality (nutrition, health, sensory, sanitary), functionality, the design and operating conditions of the production process, and cost and environmental inputs, ii) competing contradictory objectives, iii) the large number of heterogeneous variables involved (ordinal, cardinal, discrete, continuous), iv) the lack of models (Trystram, 2012) and the complexity (non-linearity) of certain relationships between variables and objectives, and v) the diversity of technological alternatives. The coupling of knowledge integration and optimization might offer an interesting strategy to solve multi-objective problems in a field where knowledge is incomplete. The idea is to use expert knowledge to define the influential relationships between variables and model processes (Belna et al., 2022). A co-creation process involving different experts in the field should therefore be encouraged in order to collect and structure dispersed knowledge. Multidisciplinary efforts will be necessary to determine the causal relationships between different steps in IF processing and final product qualities and translate them into models. Disciplines such as nutrition and process engineering would undoubtedly play a pivotal role in the multi-objective optimization of IF, but contributions from the other disciplines listed above are necessary to enable a proper definition of the objectives, their transcription into mathematical models and finally optimization of the multi-objective model.

4.2. Rethinking the infant formula value chain

To ensure the successful development of next-generation IF and foster solutions that maximize co-benefits for all stakeholders, including infants and the public health policy makers, a systemic approach must be developed, implying a clearer understanding of the interactions between the different components of the current system. A multi-actor approach, from the farmer down to the infant and its parents, is needed to bring about such changes to the system. The current IF value chain includes numerous steps between milk production and IF consumption, with several logistical constraints but also a limited number of IF companies, which thus exert considerable influence. Thanks to a systemic and more sustainable approach to the system, innovative IF production processes could become viable business models, thus enabling their broad adoption by stakeholder communities and practitioners. This systemic assessment will enable the proposal of innovative alternatives and rethinking of the entire IF production process while considering the specificities of the location of the production unit, its surroundings and the expectations of external stakeholders. Scientists are expected to play a key role in this multi-objective and multi-actor approach by continuously improving our knowledge on the nutritional and health effects of HM components and the substitutes used in IF, exploring new ingredient sources and/or processes, and also objectifying the indicators associated with each objective, thus enabling system modeling and optimization.

4.2.1. Ingredient sourcing

Ingredient sourcing could be challenged by evaluating the nutritional advantages of BM from other cow races or other mammalian milks, such as donkey milk, which has seen a regain in interest but where current production cannot meet demand, or camel milk, which presents a protein profile much closer to that of HM, but is only produced in arid regions (Africa or Middle East). Dairy fat should perhaps be partly reintroduced in IF, with the positive infant health consequences being considered in the optimization objectives at the same level as other objectives. Bovine colostrum, which is still underexploited, might also be a promising source of several bioactive molecules such as immunoglobulins or growth factors, but the added value and possible adverse effects for the human infant need to be investigated. Alternative ingredient sourcing based on synthetic biology techniques enabling the *de novo* synthesis of HM compounds (*e.g.* proteins, HMO) by engineered cells, microorganisms or mammals should also be considered (Feng et al., 2015). While they may simultaneously address biomimicry and sustainability issues, questions remain as to their safety, acceptability and industrial implementation. Plant proteins (other than the soy already authorized for IF or hydrolyzed rice protein which is no longer authorized) (European Union, 2016) might offer an alternative source of WPs, as recently proposed (Le Roux et al., 2020). The use of plant-based rather than animal-based ingredients in IF may improve the overall sustainability of the system, but the potential allergenicity and impact on infant development of plant proteins still need to be assessed. Such hybrid products (plant and animal proteins) could nevertheless be of interest for infants who are already receiving a diversified plant-based diet, *i.e.* $>$ 6 months of life.

4.2.2. Processing

Several incremental or disruptive innovations, as presented in section 3, are likely to contribute to the multi-objective optimization of IF production. Regardless of sourcing, the objective of saving energy necessitates a reduction in processing steps. New routes based on minimal processing are promising. They would not only save energy but also better preserve compounds in their native state, which would offer new perspectives for the implementation of sensitive bioactive molecules (*e.g.* immunoglobulins, cytokines, growth factors, hormones, *etc.*). Similarly, the optimal fractionation of dairy ingredients, as proposed in section 3.2.3 for WPs and minerals, would mean that their target concentration in IF in their native state could be achieved directly.

4.2.3. Transport and distribution

Innovations could also focus on reconsidering the logistics involved throughout IF production in order to limit to a minimum the transport of dairy ingredients and the number of unit operations required. The transformation from fresh BM to IF could be performed more systematically in the same plant, thus avoiding spray drying in between. Such a scheme might require the development of IF production plants throughout milk production regions, and consideration of the implantation of small or medium-sized plants adapted to the local production volumes, thus avoiding long-distance transport of the main ingredients. Nevertheless, the balance between economies of scale and those of transportation needs to be evaluated.

Finally, shelf-ready products could also be changed. The current market is largely dominated by powdered IF because of its ease of transportation and storage and extended shelf life. Other formats and uses could be extended, such as tableted powders, already launched in 2021 in the UK. Interest in liquid IF concentrates, mainly because less energy is required for production, needs to be confirmed, and a solution found regarding the issue of their thermal stabilization without extensive protein degradation.

4.2.4. Regulatory authorities and policy makers

Because of the nutritional and health issues surrounding this unique alternative to breastfeeding, IF are among the most regulated foods, with numerous nutritional and safety requirements (Fig. 5). The regulatory authorities and also public health policy makers should be involved when innovation processes are proposed, in order to improve and facilitate evaluation of these next generation IFs. However, these IFs should not be promoted against breastfeeding, and customers should be reminded that replacing HM with IF should preferably be done when breastfeeding is not possible or not recommended. Furthermore, while IF still need to be improved, an unintended effect of such development may be higher in prices and therefore less socially equal. Given the importance of the early nutrition on the future infant health, which, if improved, could reduce the population disease burden and thus

generate savings for the public health care, one could consider some financial support of the next generation IFs by the health care system such as done for medicine.

4.2.5. Customers

Finally, customers should be involved in this rethinking process. Their contradictory demands for a healthy, natural, easy to use, sustainable or sometimes plant-based diet will necessarily require compromises. Optimizing the multi-objective demands of customers also raises the question of willingness to pay, and social equity regarding this essential food. As mentioned above, due to the importance of nutrition during the first 1000 days of life to the development of health and diseases in both the short and long terms, the purchase of IF could partly be supported by public policies. This would contribute to a greater sustainability of IF through better access to these products and to improving population health.

Living labs involving all stakeholders could offer a forum to discuss tradeoffs between the different objectives, define the gold standard IF based on scientific evidence and drive innovations with the involvement of customers, moving them from the final target to the informed consumer-actor. One limitation to such a multi-actor approach is the power relationship between stakeholders. A limited number of companies produces IF (and its ingredients), so it is difficult for other stakeholders to have a strong impact on what happens throughout the chain. Such a global approach should be driven by a non-profit interdisciplinary consortium with the participation of all stakeholders. The objectives of IF manufacturers and the constraints affecting them could then be taken into account in multi-objective optimization while preventing the dominance of their interests alone.

5. Conclusions and future perspectives

Since the first IFs were developed at the end of the 19th century, their composition has evolved considerably to become as close as possible to that of HM; IFs offer newborns an alternative way to meet most of their nutritional needs while guaranteeing their safety. Despite these improvements, IFs do not strictly mimic the composition and structure of HM, or its health benefits. IF optimization is however not straightforward for various reasons, the first one being the extreme complexity of HM. There remain some gaps in our knowledge of HM compounds and structures and their effects on digestion and health, rendering the biomimicry HM more difficult. Because IF production does not solely target this objective, any optimization within industry is not straightforward and may require incremental evolutions or a more complete rethinking of the production processes. Conversely, technological innovations may open opportunities for the implementation of new ingredients in IF, or improved consideration of the structure of some nutrients. Complexity also arises from the different and sometimes contradictory demands of customers for a HM substitute that is healthy (safe and providing health benefits) and at the same time natural, sustainable, easy to use, and affordable; on the other hand, IF producers aim for process efficiency and economic viability. The challenges of next-generation IF will require compromises between synergistic and conflicting objectives. This can be achieved with the help of multi-objective optimization, provided the objectives are translated mathematically into variables. This needs collaborative efforts by a variety of scientific areas such as process engineering and nutrition, as well as biochemistry, microbiology, pediatric medicine, data and consumer sciences and public health. For all these reasons, the optimization of IF cannot and should not be addressed by IF producers alone but should be managed by a multidisciplinary, non-profit consortium involving the entire value chain from producers down to customers.

Author contribution

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Declaration of competing interest

None for all the authors.

Data availability

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

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