Plant Protein Structuring: Recent Developments, Opportunities, and Challenges

Clyde Don[1,2] and Atze Jan van der Goot[3]

ABSTRACT

Inverting the balance of animal versus plant protein in our consumption pattern is needed to keep producing foods sustainably. Since the turn of the century, novel technologies have been developed that allow development and production of next generation meat analogues. Protein structuring using the shear cell has provided a route to mimic whole cut meat, while innovative industrial research has resulted in the development of a plant-based heme ingredient that provides a taste and cooking experience that is similar to comminuted beef products. In addition, crop processing technologies to produce protein concentrates and enriched fractions are paving the way to a more sustainable protein supply. Clever blending of plant proteins improves meat analogue structure, and it potentially improves the protein nutritional quality score. In combination, these technologies give us the opportunity to meet supply and consumer demand for attractive plant-based products. At the same time, a couple of challenges remain; however, these are expected to be solved in the near future.

A recent article published in *Cereal Foods World* (*CFW*) (1) outlines the exciting scope of plant-based protein foods and snacks from 2015 and their projected consumption toward 2050. The early days of vegetarian product development (1980s and 1990s) were mostly focused on consumers who already were vegetarians or vegans, meaning they were already used to eating alternative products for meat. These groups became familiar with the preparation, taste, and texture of the first generation of meat alternatives, such as tofu and seitan. Over the last 5–10 years, the group of consumers interested in plant-based proteins has grown steadily (1,2). Vegetarians for one day a week, such as a meatless Monday, and flexitarians entered the consumer market during the first decades of the 21st century. These consumers have the strongest memory of the texture and taste of meat products, and highly appreciate the unique sensory perception offered by meats. Current plant protein structuring research, therefore, is very focused on mimicking meats in all their sensory attributes, more closely than did the meat alternatives of the late 20th century. The paradigm is to try to keep the large group of current "early adopting" consumers attached to plant-based meat alternatives—even if consumption of plant-based alternatives is not an immediate full-time lifestyle. Mainstream consumers accustomed to a Westernized diet will probably indulge in eating meat at specific times (e.g., festive days) while balancing their menu with greater amounts of plant-based alternatives, which will help to reduce the global demand for animal-based products. The underlying goal is to invert the balance between animal versus plant protein consumption toward 2030 (Fig. 1) in order to meet the long-term target that globally the average portion of plant proteins consumed will rise to a level at which the food protein production system is sustainable by 2050. Clearly, over the next 10 years the high level of animal-protein consumption in developed countries must decrease, especially considering the fact that meat consumption is likely to increase on other parts of the world, leading to an overall increase in meat consumption. In the poorest areas of the world a slight increase in consumption of animal-derived products could alleviate protein malnutrition, which is the reason that (higher) meat consumption is considered a step forward in these areas of the globe. Hence, a truly global approach for sustainable food proteins for the longer term needs to consider not only changing consumption patterns developed over the last 100 years, in order to adequately feed part of the global population, it also has to consider the larger part of the global population that must deal with a limited supply of high nutritional quality food proteins.

Replacing meat in developed countries is seen as a major step forward in closing the protein gap. Plant protein structuring is a pivotal technology that can be used to entice meat-eating consumers to increase their plant protein consumption. In this technical review, we will focus on developments in structuring of plant-based whole meat cuts using the shear cell and the formulation of comminuted meat analogues since the turn of the century. The opportunities these developments have provided to further improve plant-based products are discussed, as well as a couple of the technical challenges that lie ahead.

Meat—A Short Recap

Mimicking meat is the big protein structuring question of today. So, we will first recap a few things about the original product: meat. At first glance, meat seems a simple food material, but when taking a closer look with a microscope, the multiphasic and fibrous nature of meat becomes clear. Similar to what is familiar to cereal scientists with regard to separating wheat proteins, the proteins in meat can be differentiated by a fairly simple, crude fractionation scheme:

- Sarcoplasmic proteins soluble in low salt concentration solution (0.3–0.7% NaCl)
- Muscle proteins solubilized in high salt concentration solution (2–5% NaCl)
- Collagens—insoluble in water

The unravelling of a piece of chopped beef with this fractionation scheme results in three protein phases, as shown in the upper panel of Figure 2. The multiple protein phases of meat all have different water-holding capacities and respond differently to heat-processing. Myofibrillar proteins upon cooking form a firm and elastic gel that holds water, while collagen first shrinks and expels fluid and sarcoplasmic proteins form small aggre-

[1] FoodPhysica Lab, Malden, The Netherlands.
[2] Corresponding author. E-mail: clyde.don@foodphysica.com
[3] Laboratory for Protein Structuring, Wageningen University & Research Center, Wageningen, The Netherlands.

https://doi.org/10.1094/CFW-65-4-0037

gates with low water-holding capacity. The lower panel in Figure 2 illustrates how these protein phases behave (3). In short, meat is a highly complex multiphasic material consisting of various proteins that all respond differently to cooking. These properties give experienced chefs ample opportunities to control the sensory perception, or "*cuisson*," of a piece of meat. Therefore, mimicking such a product is really challenging and not always successful. Some may still remember the first generation of meat replacers that were based on a single plant protein source (e.g., soy), often originating from Asian cuisine, which appeared on the market about 20 years ago. The texture, taste, and juiciness of such monophasic protein-based alternatives were perceived to be too dissimilar from meat.

Recent Developments in Mimicking Meat: From High-Moisture Extrusion toward Shear-Cell Technology

High-moisture food extrusion is a technology that is closely associated with traditional meat analogue manufacture. Of course, the technology of food extrusion is not new, as described in articles previously published in *CFW*, spanning the 1970s through the 2010s, and the extensive book *Extrusion Cooking* published by AACC International (4). Really understanding what happens during extrusion processing—the dynamics of destructuring and restructuring the protein phase into a fibrous material—is still very difficult. Competence and craftmanship in operating an extruder are needed to produce a consistent product with the desired textural features. Even today, after decades of experience, the meat analogues produced by high-moisture extrusion have drawbacks in perceived texture and juiciness. According to Noguchi (5), shearing near and at the extruder die, die shape, and temperature at the die are important aspects in structuring a proteinaceous mass. Taking empirical extrusion knowledge into account, and to gain a better understanding of the effect of the uniaxial shearing process on a doughy mass, the shear cell was developed (6,7). A shear cell applies unidirectional shear in a controlled manner, allowing a more precise study of cause-and-effect when processing a high-viscosity dispersion or viscoelastic mass. The first demonstration of protein fiber formation using a shear cell was with a concentrated (30%, wt/wt) protein dispersion of calcium caseinate (8). When the cross-linking enzyme transglutaminase was added, the observed fibrous structure improved further (Fig. 3A and B). According to the authors, the formation of shear- and enzyme-induced anisotropic structures was explained by the aligning of protein aggregates due to shear and concurrent solidification by enzymatic cross-linking. More recently, the importance of air bubbles was shown (9,10). This layering of fibrous insoluble protein phases or strands, in a high-moisture environment, opened an alternate route to create meat mimics or meat-cut analogues. At the time, it was also realized that there was a need for better meat mimics to entice more meat-eating consumers to diversify their menus with plant-based proteins, thereby inverting the animal versus plant balance (11). With the proof of concept in hand, the creation of palatable meat analogues that are 100% plant-based seemed to be within

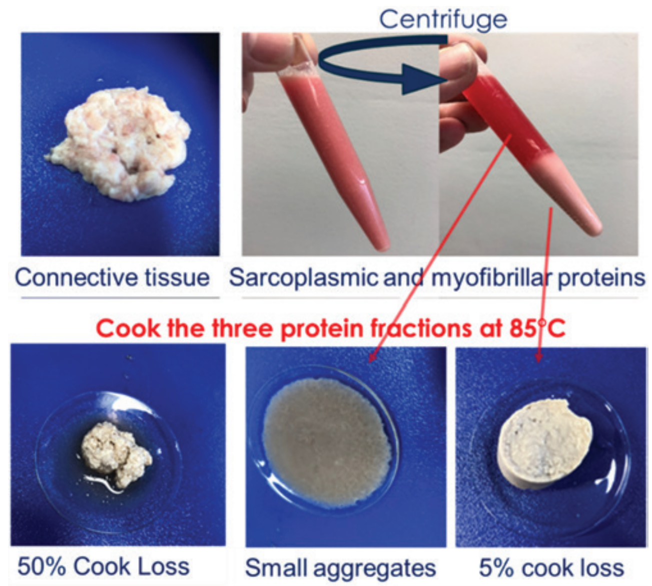

Fig. 2. Upper panel: multiple protein fractions of meat; lower panel: response to cooking of the three main protein fractions in meat.

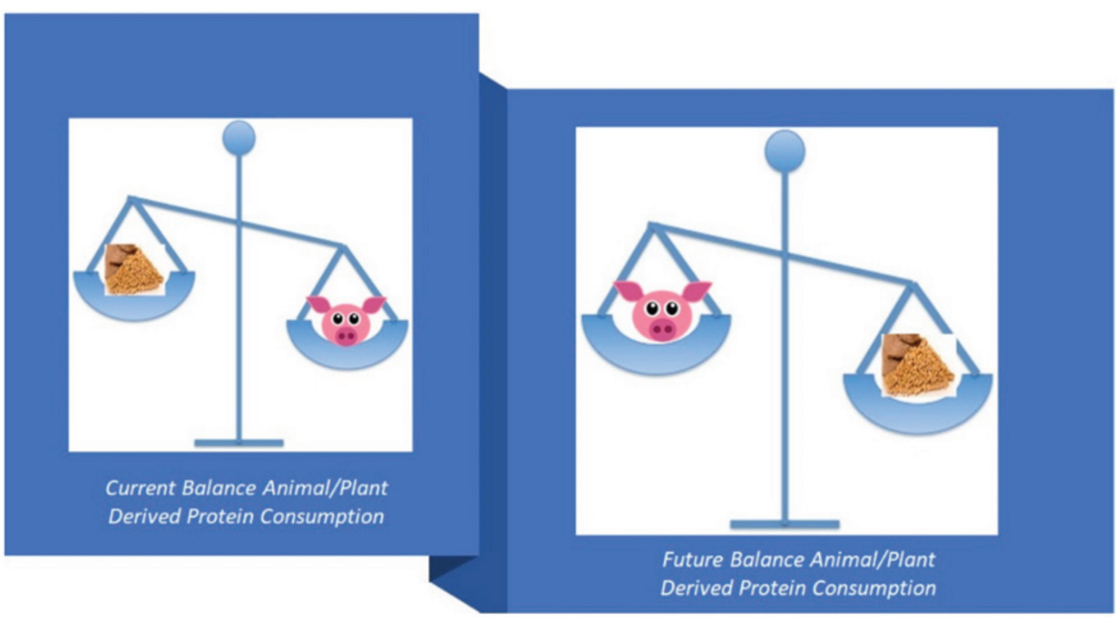

Fig. 1. Inverting the balance of animal versus plant protein consumption.

reach. The next steps were to induce anisotropy, fibrous structure in plant-based proteins using shear-cell technology. Initially single proteins (mainly soy) were used, and soon more proteins were added into the mix to create more complex meat-like structures in the shear cell. An example of a soy–gluten blend is shown in Figure 3C and D. The list of proteins and protein blends that have been explored and will be explored is becoming larger: soy protein, pea protein, gluten protein, soy–gluten blend, pea–gluten blend, and even ternary blends of protein–protein–hydrocolloid (12). Further experimentation is likely to continue using rice, mung bean, and other new protein sources to better mimic meat structures. The blending strategy actually better reflects the multiple protein phases described earlier for meat. These pioneering steps with the shear cell and further exploration of this structuring technology led to the ultimate mimic of a fresh meat cut—a plant-based steak (Fig. 4)—that has the closest approximation of beef structure produced to date (13). After a period of around 10 years since the first proof of concept, shear-cell technology has been taken out of the lab and into industry. From 2018 to 2020, the technology has matured into a proven technology; in collaboration with a food machinery company, the first prototypes of the ultimate in plant-based meat cuts are rolling from the production line (Rival-Foods start-up company, The Netherlands [https://rival-foods.com]).

Opportunities—Enriched Fractions, Protein Quality, Minerals, and Fibers

With improvements in technology, more has been learned about the requirements of protein purity. For a long time, the generally accepted notion was that purified plant protein sources were a prerequisite to be able to extrude the best meat analogues. Indeed, still today the preference for applications such as extrusion processing is to work with protein ingredients that have the highest level of purity. This is probably due to the fact that protein ingredients with a high level of protein purity and solubility are intuitively assumed to have superior functional properties. Concentrating plant proteins to a high content and purity to obtain the functional protein does have its drawbacks, including water and energy use and protein loss (14). Contrary to assumptions about purity and functionality, while experimenting with the shear cell it was revealed that less pure plant proteins, such as protein concentrates, blends of proteins, and fibers, resulted in better meat-like structures. This saves the burden of extensively purifying plant-protein sources, which requires less water and energy. Another opportunity shear-cell technology (and extrusion) has brought to light is that even non- to mildly processed plant crops can be introduced into formulations and result in acceptable final products. A good example of non- to mild processing is winnowing, a process used to sift dry matter from peas, lentils, and cereal grains in protein-enriched and fiber enriched fractions.

Energy requirements for each transformation and purification step for protein foods have been identified as a critical issue (15). Milder processing clearly will help reduce energy use and CO_2 emissions involved in the protein food production chain. Next to sustainability attributes, the concentrates, enriched fractions, and raw materials also contain minerals, vitamins, and fibers that could contribute to healthier meat analogues. The use of fuel for transportation is another aspect of sustainability with which the shear cell can help. Shear cells can be made in custom sizes that allow semi-finished to finished products to be processed closer to a crop harvest location. As a result there is no need to first purify the crop, isolate and dry proteins from it, and then at the meat analogue production site rehydrate plant proteins to allow for high-moisture extrusion processing to create the meat analogue.

As recently stated by Ingram (16), there is a difference between food security and nutrition security. In the world of new plant-based meat analogues, this difference may be overcome. Similar to the structuring strategy followed by blending proteins that respond differently to processing, clever blending of plant

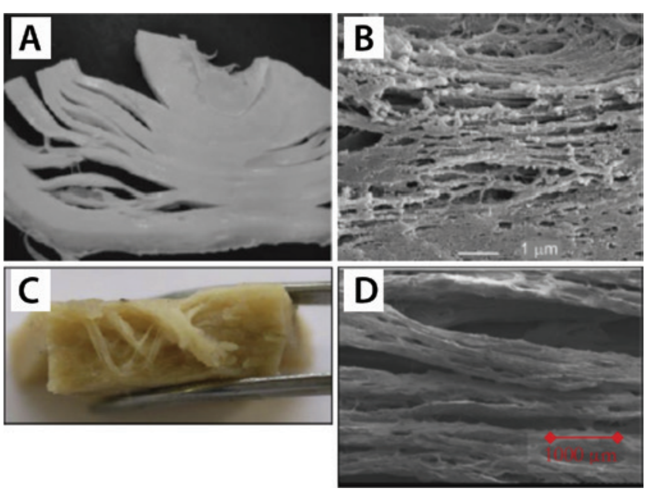

Fig. 3. A, Example of the first fibrous structures made by shear-cell technology with calcium caseinate; **B,** microscope image of the fibrous structure of calcium caseinate; **C,** example of plant-based fibrous structures made by shear-cell technology blending soy protein and wheat gluten; **D,** microscope image of the soy protein and wheat gluten blend. (Courtesy A. J. van der Goot)

Fig. 4. Plant-based steak (held by Atze Jan van der Goot).

proteins can provide a better quality mixture in, for example, the protein digestibility–corrected amino acid score (PDCAAS). This is a FAO/WHO (Food and Agriculture Organization of the United Nations and World Health Organization) adopted method for the measurement of protein value in human nutrition. The method is based on comparison of the concentration of the first limiting essential amino acid in the test protein with the concentration of that amino acid in a reference scoring pattern (17). An important detail in protein quality scoring is the effect of the lack of just one essential amino acid pushing the protein score far below 1 (1.0 = highest score and 0.0 = lowest score in truncated PDCAAS). The effect of a limiting amino acid seems to be factored in quite rigorously, which may discourage the pursuit of achieving better scores by blending low-PDCAAS proteins. When combining low-quality proteins that compensate each other for the lack of a limiting amino acid, a reasonable to good quality score can be achieved (>0.7). An example is given in the review by Friedman (18), in which reference is made to a blend of proteins (rice, mungo, sesame, and carrots) that approaches the protein quality score of casein. Hence, by

Table I. Texture profile analysis peak compression forces and expelled juice measured for commercial cooked plant-based versus meat-based sausages and burgers

Sample	F1 (Peak 1) (g·force)	(F2/F1) × 100 (%)	Expelled Juice (mg Juice/ g Product)
Vegetarian sausage	1,200	93	4
Pork-based sausage	1,150	59	18
Vegetarian burger	910	84	5
Beef-based burger	830	42	19

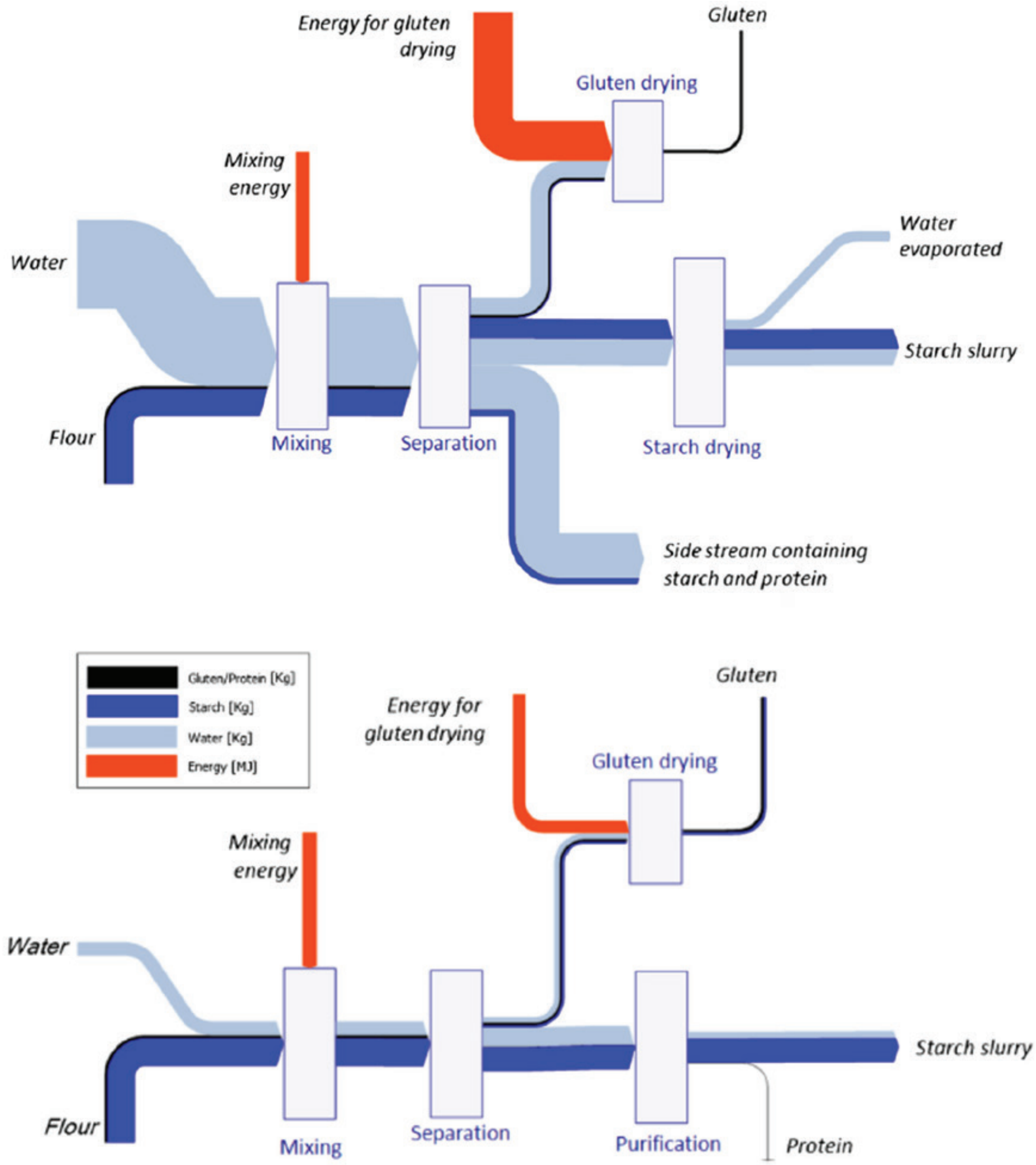

Fig 5. Sankey diagram of more sustainable gluten–starch separation. Upper part: conventional process; lower part: concentration process on the same scale. The widths of the arrows indicate the relative size of the stream (internal recycle streams not included). (Reproduced, with permission, from A. J. van der Goot)

blending proteins strategically and processing in a shear cell, a plant-based meat can be tuned toward alternatives with a protein quality score that is similar to animal-derived protein casein. Many more opportunities with nonanimal protein blends are being researched to raise the protein quality of, for example, corn, soy, and peanut protein blends (21). Applying a similar strategy to meat analogues can bring it to the same level as traditional meat. There are plant-based examples being developed that could potentially have an even better protein nutritional profile than meats. In addition, there are meat alternatives that contain dietary fibers, which are often present in insufficient quantities in Western diets.

Remaining Challenges

Product Acceptance. Juiciness is one of the sensory properties of meats that has been used in product evaluation for decades. Measuring expelled juice in a compression test is considered an objective method for predicting juiciness (20). In a recent short survey of extruded plant-based sausages and hamburgers (Table I), texture analyses combined with catching expelled juice on filter paper confirmed that the meat-based products were juicier (22). The average resistance on first stroke (F1) was within ±10% variation for plant-based and meat-based products, suggesting that the mimics showed a similarity in initial bite. However, the plant-based products appeared to be tougher, showing a higher peak force at the second stroke (F2) of the texture profile analyzer than the meat-based products. With regard to short-term juiciness, the comminuted meat analogues need to be closer to that of meats. Independently, based on sensory research, Hoek et al. also suggest that the sensory quality and resemblance to meat of meat analogues needs to be improved further (23). Expelling "meat-like" juices containing water, oil. and salt helps deliver flavors to the palate, and, thereby, deliver better taste as well. Nonetheless, commercially available plant-based comminuted meat analogues are getting closer and closer to meats in texture and taste; a good example is the addition of plant-based heme protein (24). Myoglobin and hemoglobin belong to the low-salt soluble sarcoplasmic proteins described earlier as one of the multiple protein phases of meat. Therefore, the idea of creating a plant-based heme to improve the taste and juiciness of meat analogues is quite logical from a multiphasic protein standpoint. As our insights deepen and biotechnology progresses, it is only a matter of time before the juiciness test results of meat analogues will be similar to those of meat.

Global Protein Supply. With all the good news about meat analogues, the challenge to make the transition to plant proteins a global one still remains. In developed regions of the globe, such as Europe and North America, the transition to plant proteins is gaining traction. However, we face an additional challenge on how to nourish the rest of the globe with sustainable plant-based food proteins. This is one of the current questions in the agri-food system that urgently needs to be resolved. With the recent growth in plant-based foods, the demand for plant-based ingredients has risen to a level that is difficult to reach with conventional sources, such as cereal proteins (gluten), soy proteins, and pea proteins. New processes will be needed; an example of how gluten and starch can be separated more sustainably is illustrated in Figure 5. Several interesting initiatives are experimenting with harvesting protein from aquatic biomass, but there are challenges ahead with other crops as well, including lentils, seaweed, hemp, and others (19). There are certainly major challenges ahead. Less focus on protein purification and concentrating with excessive use of water and more on extracting enriched fractions sustainably are keys to success (25).

Conclusions

Giant steps forward have been made in using shear-cell technology in combination with new protein-enriched fractions. However, in the short term, there remain exciting opportunities for applied science to help further improve the texture and juiciness of all kinds of meat analogues. In the longer term, innovations in crop processing technologies will help to create a sustainable global plant-protein supply.

References

1. Hood-Niefer, S. Insights into extrusion of protein. Cereal Foods World 62:148. DOI: https://doi.org/10.1094/CFW-62-4-0148. 2017.
2. Mehmet, S. US plant-based market growing five times faster than total food sales. New Food Magazine. Published online at www.newfoodmagazine.com/news/106862/us-plant-based-market-growing-five-times-faster-than-total-food-sales. March 5, 2020.
3. Don, C. Syneresis, water-holding and pressed juice: Indicators of juiciness. Conference Presentation at Meat Analogues 2018, October 31–November 1, Wageningen, The Netherlands, 2018.
4. Mercier, C., Linko, P., and Harper, J. M. *Extrusion Cooking*. AACC International, St. Paul, MN, 1989.
5. Noguchi, A. Extrusion cooking of high moisture protein foods. In: *Extrusion Cooking* C. Mercier, P. Linko, and J. M. Harper, eds. AACC International, St. Paul, MN, 1989.
6. Peighambardoust, S. H., van der Goot, A. J., Hamer, R. J., and Boom, R. M. A new method to study simple shear processing of wheat gluten–starch mixtures. Cereal Chemistry 81:714, 2004.
7. Pietsch, V. L., Karbstein, H. P., and Emin, M. A. Kinetics of wheat gluten polymerization at extrusion-like conditions relevant for the production of meat analog products. Food Hydrocoll. 85:102, 2018.
8. Van Der Goot, A. J., Peighambardoust, S. H., Akkermans, C., and Van Oosten-Manski, J. M. Creating novel structures in food materials: The role of well-defined shear flow. Food Biophys. 3:120, 2008.
9. Wang, Z., Tian, B., Boom, R. M., and van der Goot, A. J. The effect of air and protein phase on mechanical anisotropy of calcium caseinate fibers. Food Res. Int. 121:862, 2019.
10. Tian, B., Wang, Z., van der Goot, A. J., and Bouwman, W. G. Air bubbles in fibrous caseinate gels investigated by neutron refraction and X-ray tomography. Food Hydrocoll. 83:287, 2018.
11. Hoek, A. Will novel protein foods beat meat? Consumer acceptance of meat substitutes—A multidisciplinary research approach. Ph.D. thesis. Wageningen University, The Netherlands, 2010.
12. Dekkers, B. L., Boom, R. M., and van der Goot, A. J. Structuring processes for meat analogues. Trends Food Sci. Technol. 81(May):25, 2018.
13. Krintiras, G. A., Gadea Diaz, J., van der Goot, A. J., Stankiewicz, A. I., and Stefanidis, G. D. On the use of the Couette Cell technology for large scale production of textured soy-based meat replacers. Van Der Goot, A. J., Pelgrom, P. J. M., Berghout, J. A. M., Geerts, M. E. J., Jankowiak, L., Hardt, N. A., and Boom, R. M. Concepts for further sustainable production of foods. J. Food Eng. 168:42, 2016.
14. van der Weele, C., Feindt, P., van der Goot, J. A., van Mierlo, B., and van Boekel, M. Meat alternatives: An integrative comparison. Trends Food Sci. Technol. 88:505, 2019.
15. Ingram, J. Nutrition security is more than food security. Nat. Food 1:2, 2020.
16. Schaafsma, G. The protein digestibility-corrected amino acid score. J. Nutr. 130:1865S, 2000.
17. Friedman, M. Nutritional value of proteins from different food sources: A review. J. Agric. Food Chem. 44:6, 1996.
18. Tamayo Tenorio, A., Kyriakopoulou, K. E., Suarez-Garcia, E., van den Berg, C., and van der Goot, A. J. Understanding differences in

protein fractionation from conventional crops, and herbaceous and aquatic biomass—Consequences for industrial use. Trends Food Sci. Technol. 71:235, 2018.
19. Tannor, B., and Chriswold Clark, N. Mechanical determination of the juiciness of meat. J. Agric. Res. 66:403, 1943.
20. Manary, M. Protein quality and growth in malnourished children. Food Nutr. Bull. 37:S29, 2016.
21. Don, C. Factors affecting the quality of hybrids and plant-based meat analogs. Presentation at The Meattech Association's Annual Protein Seminar 2019, Doorwerth, The Netherlands, 2019.
22. Hoek, A. C., Luning, P. A., Weijzen, P., Engels, W., Kok, F. J., and de Graaf, C. Replacement of meat by meat substitutes. A survey on person- and product-related factors in consumer acceptance. Appetite 56:662, 2011.
23. Fraser, R., O'Reilly Brown, P., Karr, J., Holz-Schietinger, C., and Cohn, E. Methods and compositions for affecting the flavor and aroma profile of consumables. U.S. Patent No. US20170295833A1. 2017.
24. Vanham, D., and Leip, A. Sustainable food system policies need to address environmental pressures and impacts: The example of water use and water stress. Sci. Total Environ. 730:139151, 2020.