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Combining extrusion, electron microscopy and rheology to study the product characteristics of meat analog products

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Key words

Plant-based, meat analog, soy protein, wheat gluten, food extrusion, electron microscopy, rheology

Introduction

High moisture extrusion offers a great potential to produce a wide range of meat analog products from various plant protein sources. Products that can be achieved from this process are supposed to resemble the product texture of muscle meat. Typically, high moisture extrusion yields intermediate products, which are further processed to produce meat analog, ready-to-eat products using conventional meat processing operations such as mincing, marinating, and mixing.

It has been proposed that the desired muscle meat-like texture can be achieved by imparting an anisotropic, fibrous structure into plant proteins through a shear-induced struc-turing of a multi-phase system [1]. Such mechanism can take place if a dispersed phase is present together with a continuous phase, as shown in Figure 1. While the exact composition of the dispersed phase depends on the plant protein used as source, it typically consists of free water, water-insoluble proteins, or residual polysaccharides. The continuous phase is usually comprised of water-plasticized plant proteins. During a shear-induced structuring process, such as extrusion processing, these phases are mixed with each other, leading to the formation of a water-in-water type emulsion. Based on the flow characteristics, the dispersed phase is deformed in the direction of the flow. Upon cooling, the matrix solidifies and the deformed structure is retained.

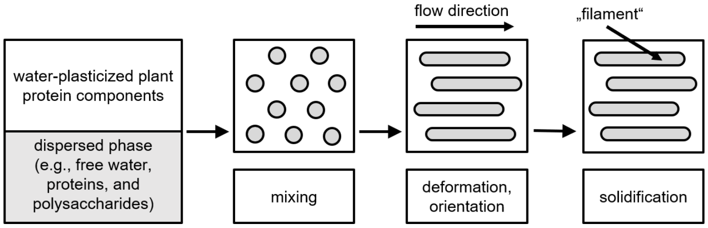


Figure 1: Scheme of shear-induced structuring of a multi-phase system described by Tolstoguzov [1], [2].

To achieve the desired anisotropic, fibrous structures, the high moisture extrusion process is generally operated using a co-rotating twin-screw extruder at water contents similar to meat, i.e. between 40 - 80%, and with a cooled slit die attached to the end of the extruder. During the extrusion process, plant proteins are fed to the extruder, mixed with water, and transported along the rotating screws. In the screw section of the extrusion process, the material will be exposed to thermomechanical stresses due to the shearing of the screws and the heating of the barrel. These stresses can affect the material properties of the plant protein-water matrix in terms of protein denaturation (reaction behavior), miscibility, and rheological properties. The flow characteristics prevailing in the cooling die will determine the deformation and orientation of the dispersed phase in flow direction.

From this general understanding of shear-induced structuring, it can be derived that the final product properties of meat analog products depend on the final morphology of the dispersed phase. The morphology of the dispersed phase further depends on the volumetric fraction and the deformation of the dispersed phase. As current state of research, it is considered that these factors can be influenced by the ingredient composition as well as extrusion process conditions [2-4]. To tailor the product properties of meat analog products to match consumers expectations, it is therefore necessary to consider the influence of ingredient composition as well as extrusion process conditions on the final product characteristics.

Typical ingredients used to produce meat analog products comprise plant protein concentrates or isolates from various plant protein sources. The purity of the protein ingredients as well as their technological properties depend on the processing technology applied. Herein, protein concentrates and isolates typically exhibit a protein content of approx.

70% and 90%, respectively. The most widely used protein sources utilized to produce meat analogs currently are soy protein, wheat gluten, pea protein, or mixtures thereof. Furthermore, interest is rapidly growing in producing meat analogs from plant protein sources that are novel to this application, such as canola seed protein, peanut protein, or algae. Although not vegetarian, even insects are currently considered as promising protein source for proteins for human nutrition that can be imparted into meat analog products.

With the wide range of protein sources available, the applicability of existing as well as novel protein sources to produce meat analog products needs to be evaluated on a regular basis. In terms of material development as well as quality control, the Thermo Scientific™ Process 11 Hygienic Extruder offers a solution to produce meat analog products on a lab scale and test new ingredient compositions with reduced testing time, sample size, and waste [5]. In this report, it is demonstrated how the Process 11 Hygienic Extruder can be applied in combination with rheological tools and electron microscopy to evaluate the influence of ingredient composition on the final product characteristics of meat analog products. Microstructural characterization of the fibrous, anisotropic structure was carried out using a Thermo Scientific™ Quattro S Environmental Scanning Electron Microscope (ESEM), an ultra-versatile high resolution SEM with unique environmental capabilities, which provide the flexibility to accommodate any type of sample. Quantitative measurements of rheological properties were conducted using the Thermo Scientific™ Haake™ MARS™ 60 Rheometer, a research grade rheometer for extended material characterization.

Material and methods

Extrusion experiments

Meat analog products were produced with the Process 11 Hygienic Twin-screw Extruder and a cooled slit die. Plant-protein powders were fed into the extruder with a volumetric Twin-screw feeder. A peristaltic pump was used for water feeding. The schematic process setup is shown in Figure 2. The dimensions of cooled slit die were H x W x L: 5 x 20 x 200 mm.

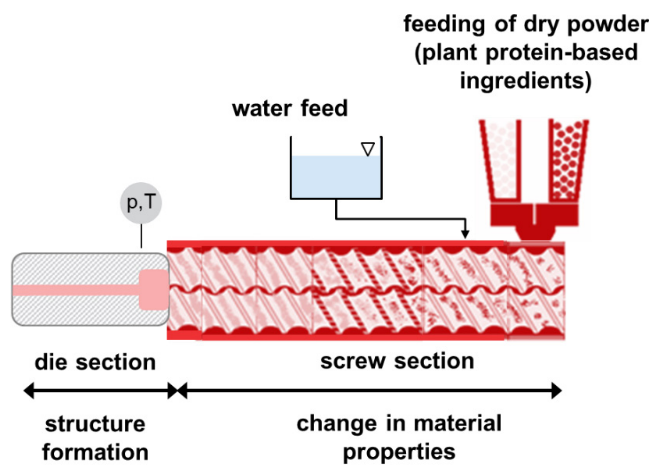


Figure 2: Schematic setup of the Process 11 Hygienic Twin-screw Extruder with length to diameter ratio of 40 and cooled slit die.

Three different formulations were analyzed to investigate the influence of ingredient composition on the product characteristics of meat analogs. As indicated in Table 1, wheat gluten and soy protein were used as well as a mixture thereof. The trial was performed at otherwise constant process parameter settings, where screw speed was kept at 400 rpm, total feed rate at 1.1 kg/h, and a water content at 60%. Material temperature of the melt was measured at extruder exit (before entering the cooling die) and accounted for 135 °C for all three formulations. The temperature of the cooled slit die was kept constant at 80 °C. After

Table 1: Ingredient composition used for extrusion trials.

Soy Protein Concentrate (SPC)	vital Wheat Gluten (WG)
100%	0%
70%	30%
0%	100%

extrusion trials, samples were taken and stored at - 8 °C in airtight plastic bags to prevent drying and spoilage of the samples. For analysis, samples were defrosted overnight and taken out of the plastic bag directly before the measurement.

Visual appearance and SEM analysis

The typical characteristics of a meat analog productin terms of fibrousness, anisotropy, and texture were observed by the visual appearance of a sample. The analysis of the microstructure of the extruded samples was carried out using a Thermo Scientific™ Quattro S ESEM. The samples were prepared for SEM analysis by means of freeze-fracture, a method allowing to expose the cross section of the material without creating any artifacts due to the use of cutting tools. A small piece of the frozen extruded sample was dropped into liquid nitrogen to further cool it and increase its brittleness. Once fully cooled the sample was broken along the flow axis in order to expose a fracture surface along the middle axis of the sample. The fractured sample was then thawed at room temperature and then fixed onto an aluminum SEM stub using carbon tape. Lastly, the sample was sputter coated with Iridium to make the surface electron conducive. The SEM analysis was carried out in High Vacuum mode using Everhart-Thornley Detector (ETD) to collect secondary electron images, which provide topographic information. An acceleration voltage of 5 keV was selected to reveal enhanced surface details.

Rheological characterisation

Compression and oscillatory measurements were conducted to characterize the rheological properties of the extruded meat analog products. All measurements were carried out at room temperature. As shown in Figure 3, compression tests were conducted with the HAAKE MARS 60 Rheometer equipped with a plate-plate geometry with 8 mm diameter at a compression speed of 0.1 mm/s. For

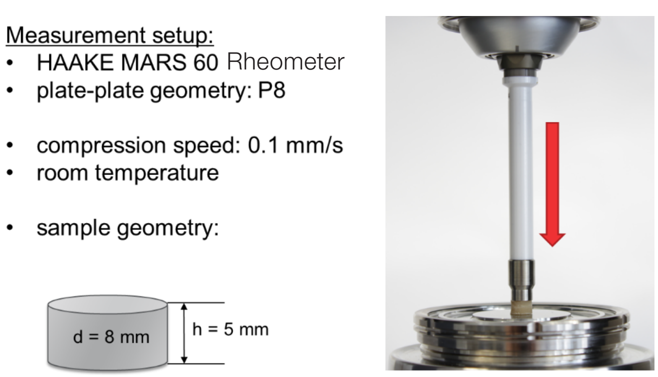


Figure 3: Measurement setup for compression tests using a HAAKE MARS 60 Rheometer and plate-plate geometry P8.

the tests, samples were cut with a circular punching iron into a round sample shape with a diameter of 8 mm. The height of the sample resulted from the original height of the cooling die, which was 5 mm.

Using a plate-plate-geometry with a diameter of 35 mm, oscillatory measurements (amplitude and frequency sweeps) were conducted. For this purpose, two stripes of extruded sample were taken and placed under the plate-plate geometry (see Figure 4). The length of the samples was cut to approx. 35 mm. This way it was ensured that the measurement gap is completely filled. Height and width of the sample resulted from the original height and width of the cooling die, which was 5 and 18 mm, respectively. The samples were loaded into the measuring geometry by applying a constant normal force of 20 N. After reaching a constant normal force, oscillatory measurements were conducted at the resulting gap height. The respective strain and frequency settings used for amplitude and frequency sweeps are listed in Table 2.

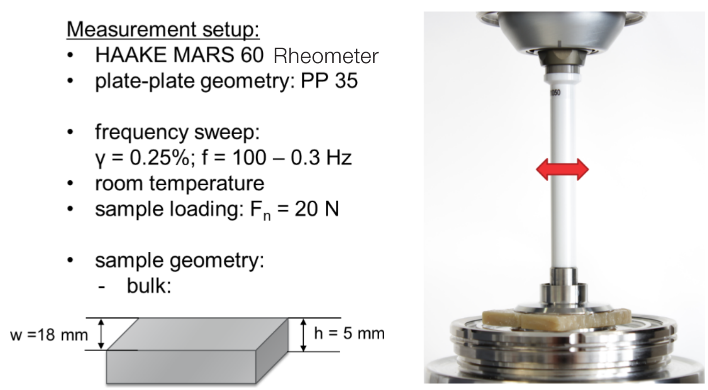


Figure 4: Measurement setup for amplitude and frequency (bulk) using a HAAKE MARS 60 Rheometer and plate-plate geometry P35.

Table 2: Measurement settings for amplitude and frequency measurements (bulk).

	Amplitude sweep	Frequency sweep
Strain γ	10^{-2} - 10^{3}%	1 Hz
Frequency f	0.25%	100 - 0.3 Hz

Results

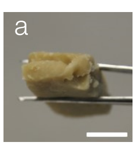
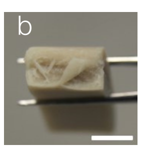
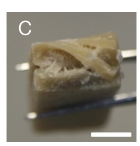
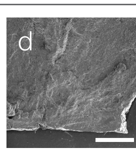
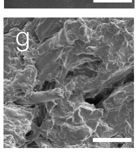
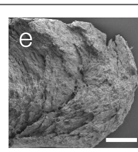
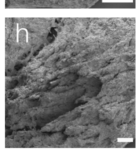
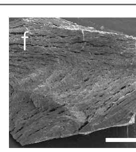
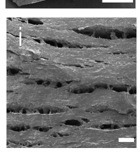
Visual characterization of macro- and micro-structure

The results for the visual characterization of the macro- and micro-structure of the extruded meat analog products are given in Table 3. The view of the macrostructure of all samples shows that all extruded samples exhibit an anisotropic, fibrous structure. With a first visual and haptic analysis it could be observed that the samples exhibit overall different product properties. The samples made from 100% Wheat Gluten (WG), for example, was very elastic and chewy. At the same time, the sample made from 100% Soy Protein Concentrate (SPC) seemed to be very brittle and hard. In contrast to SPC as well as WG, it can be observed that the mixture of SPC and WG with a ratio of 70% to 30% results in an extruded product with a soft texture.

Having a closer look at the microstructure, the visual appearance of all extruded samples derives from the presence of a multi-phase system. For all samples, a dispersed phase could be observed, which is oriented in flow direction. As explained earlier, this type of structure is considered as a prerequisite for the formation of a meat analog product with a muscle meat-like texture. This is in agreement with previous studies [6], where it has been shown that the SPC to WG ratio significantly affects the structure formation and product characteristics. Moreover, it was suggested that meat analog samples containing 20 to 30% WG are close to the texture of boiled chicken meat [6].

Overall, these results demonstrate that not only the extrusion process parameters, but also ingredient composition affect the product characteristics of meat analog products. In the following, rheological analysis was used as standardized operation procedure for the quality assessment of meat analog products.

Table 3: Macro- and micro-structure of meat analog samples produced with Process 11 Extruder using varying ratios of Soy Protein Concentrate (SPC) and Wheat Gluten (WG). The scalebar in the images are 100 mm (a, b, c), 1 mm (d, e, f), 100 µm (g, i) and 200 µm (h), respectively.

Ratio of SPC / WG	100 / 0	70 / 30	0 / 100
Macro-structure			
Micro-structure (SEM)	 	 	 
Visual and haptic description	Anisotropic Chewy, elastic	Anisotropic Soft	Anisotropic Brittle, hard

Rheological analysis as quantitative measuring tool

The aim was to use rheology as comparative tool to identify the extruded product with a targeted product quality, e.g. similarity to chicken meat [6]. First, compression analysis was conducted by subjecting uniaxial strain to the extruded samples. The result in Figure 5 depict the typical force-distance curve that were recorded for the different meat analog samples. The force distance curves first show a linear increase, which corresponds to the elastic deformation of the material. Then, the applied force approaches a maximum yield point, at which the material cannot sustain its original structure anymore. In the non-linear region of the force-distance curve, the material begins to flow and can rupture. From the Linear Viscoelastic Region (LVR) of these measurements, it is possible to compare the stiffness and elasticity of the extruded samples. The Young's modulus E can be calculated from this part of the force-distance curve. An increase in Young's modulus describes the increase in stiffness of a sample, whereas a decrease in Young's modulus indicates the increase in elasticity of a sample [4].

The comparison between the different samples shows that the Young's modulus increases with increasing Soy Protein Concentrate (SPC) content. These results are in good accordance with the haptic and visual description of the sample. Accordingly, the results show that this method can be used quantitatively to compare the texture of meat analog samples with respect to their elasticity and stiffness.

Second, amplitude sweeps were conducted. The aim was to quantify the structural strength and chewiness of the meat analog samples. The results are depicted in Figure 6. All samples show a distinct range, where storage and loss modulus G' and G'' are constant and independent of the applied deformation. This region is typically referred to as the Linear Viscoelastic Region (LVR). The width of the LVR depends on the structural strength of the material. A change in storage and loss modulus indicates that the applied deformation exceeds the LVR, causing a change in the samples structure. In Figure 6, the results show that, after a certain deformation, the non-linear deformation starts with a non-linear increase in loss modulus G'' and following the cross-over of G' and G''. Typically, the cross-over point is defined by the balance of G' = G''. The loss factor tan d can be used as characteristic value to determine the cross-over point. It is given by the ratio of G'' and G'. At the cross-over point, tan d equals 1 or $\delta = 45°$.

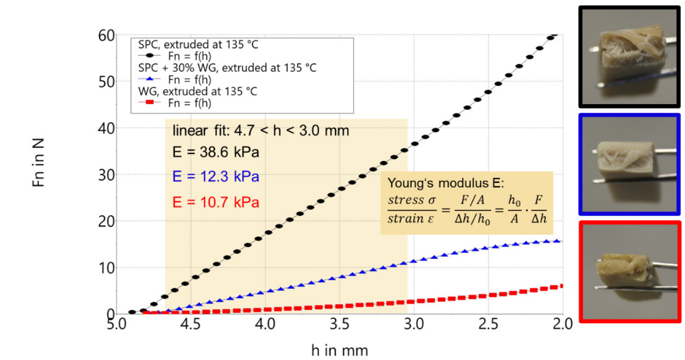


Figure 5: Change in the elasticity and stiffness of meat analog samples made from varying ratios of Soy Protein Concentrate (SPC) and Wheat Gluten (WG) characterized by compression analysis using a HAAKE MARS 60 Rheometer and plate-plate geometry P8.

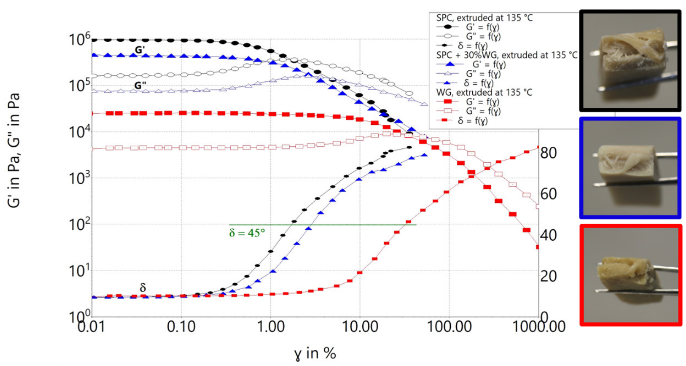


Figure 6: Change in the structural strength of meat analog samples made from varying ratios of Soy Protein Concentrate (SPC) and Wheat Gluten (WG) characterized by amplitude sweeps (bulk) using a HAAKE MARS 60 Rheometer and plate-plate geometry P35.

The observed increase in G'' before approaching the cross-over point can be caused by the inner friction between structural elements, e.g. between emulsion droplets in liquid systems. In solid systems this increase can also results by the occurrence of micro cracks. To gain further insight on what is happening during the deformation on a micro-structural level, additional amplitude sweep measurements were conducted using the RheoScope module. As explained in [7], this module comprises an optical (polarization) microscope, which can be applied to record microscopic images during shearing of samples. The applied measurement procedure and resulting RheoScope images are depicted in Figure 7. To attain an optical image, it was necessary to cut the samples in thin layer (see Figure 7 a). Sample images were recorded during the deformation of Soy Protein Concentrate (SPC) and Wheat Gluten (WG). Similar to the SEM images depicted in Table 3, the micro-graphs of both samples show a dispersed phase, which is embedded into a continuous protein matrix. The results in Figure 7 b and c depict the microscopic images recorded during an amplitude sweep of SPC at a deformation of 30% and 230%. As highlighted in both images, micro cracks propagate with increasing deformation. Similar findings are observed for WG, where a larger crack appears with increasing deformation from 40% to 120%. Thus, it is very likely that the observed increase in G'' (see Figure 6) following the cross-over of G'' over G' is related to the break-down of the distinctive microstructure with increasing deformation.

Regarding the characterization of product properties, the results depicted in Figure 6 provide information on the structural strength and chewiness of the extruded meat analog products. While SPC shows a cross-over point at a deformation of 1.7%, the cross-over point of the SPC-WG mixture and WG are shifted to 2.9% and 33%, respectively. The observed shift in cross-over point indicates that the structural strength of meat analog products produced from WG is higher compared to SPC. The fact that the structural break-down of the WG sample requires higher deformation implies that consumers will perceive the texture of meat analogs made from this protein source as chewier.

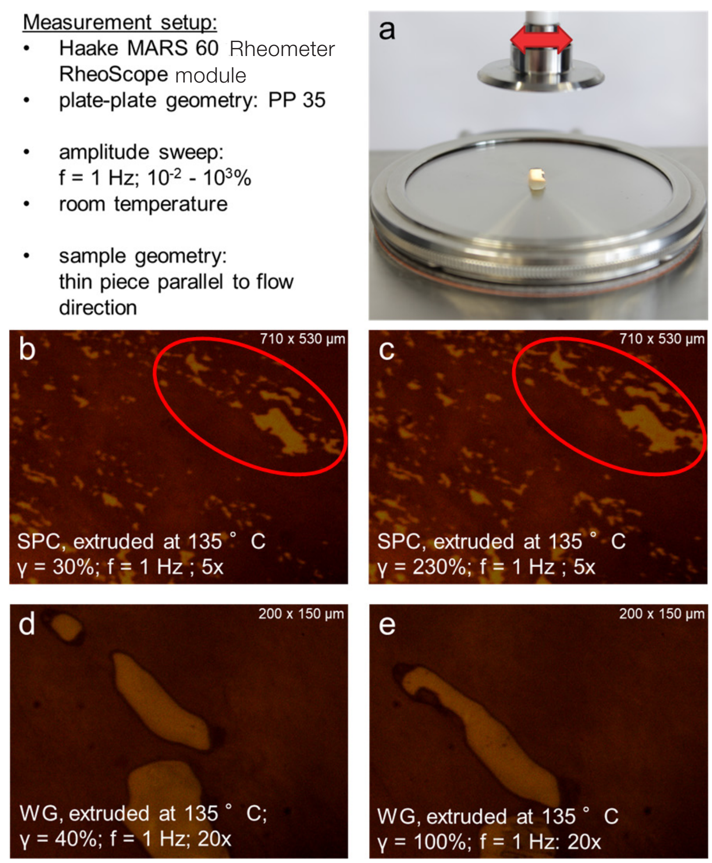


Figure 7: Change in micro-structure elements with increasing deformation, characterized by amplitude sweeps (thin layer) using a HAAKE MARS 60 Rheometer with RheoScope module and plate-plate geometry P35.

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Thus, the results from the rheological measurements are not only in very good alignment with the visual and haptic impressions of these samples (see Table 3), but they also show that the results (e.g., the applied deformation and rheological properties at cross-over point) from amplitude sweep analysis can be used as quantitative value to compare the product properties (chewiness) of meat analog products.

Finally, frequency sweep measurements within the Linear Viscoelastic Region (LVR) can be used to describe the hardness or softness of the samples. The results for the different meat analog samples are displayed in Figure 8. The material shows shear thinning behavior and is predominantly elastic as G' > G''. Moreover, the meat analog sample made from SPC shows the highest viscosity. The complex viscosity further increases from the meat analog SPC-WG mixture to WG. Since these measurements were conducted on samples in bulk, they indicate that the samples with highest complex viscosity are more resistant to shear stresses. Thus, the samples with a higher viscosity are likely to be perceived by the consumer as harder and less easy to bite. These findings are also in very good accordance with the qualitative observations summarized in Table 3. Therefore, these results (e.g., complex viscosity) give a quantitative value to compare the product properties (hardness) of meat analog products.

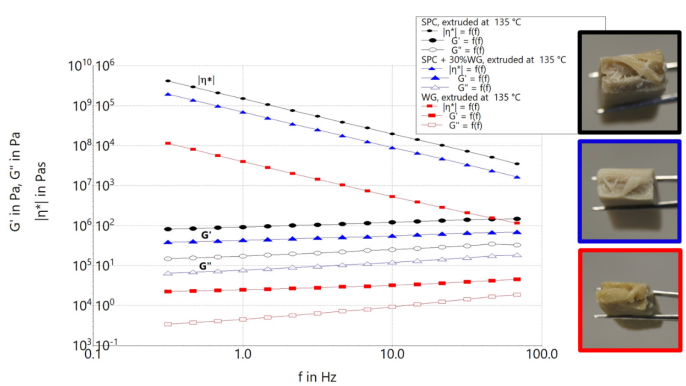

Figure 8: Change in the hardness/softness of meat analog samples made from varying ratios of Soy Protein Concentrate (SPC) and Wheat Gluten (WG) characterized by frequency sweeps (bulk) using a HAAKE MARS 60 Rheometer and plate-plate geometry P35.

Conclusion

The results of this study show how the product characteristics of meat analog products can be analyzed by the combination of microscopy and rheological tools. As summarized in Figure 9, the results from SEM analysis using the Quattro S ESEM show that meat analog products with typical characteristics can be produced using a Process 11 Hygienic Twin-screw Extruder with cooled slit die. Results from rheological measurements using a HAAKE MARS 60 Rheometer allowed to determine quantitative values that describe the elasticity, chewiness, and hardness of the meat analog products. Overall, the product characteristics of the resulting meat analog product depend on the ingredient composition and extrusion process conditions. With the presented analysis tools, the target product quality of the resulting meat analog products can be selected according to the consumers' requirements.

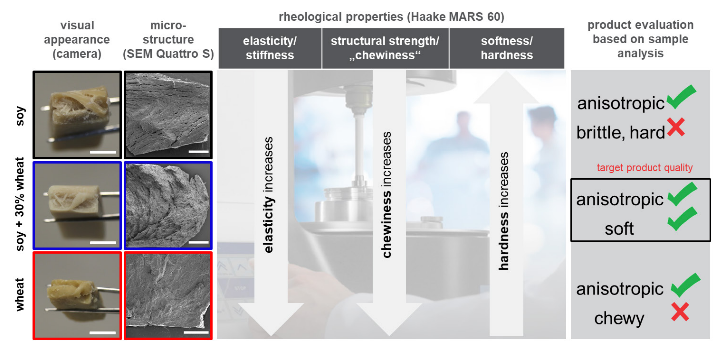

Figure 9: Evaluation of meat analog product quality based on sample analysis via optical and rheological tools.

References

1. Tolstoguzov, V.B., 1993. Thermoplastic extrusion the mechanism of the formation of extrudate structure and properties. Journal of the American Oil Chemists' Society 70 (4), 417–424.
2. Pietsch, V.L., 2019. High moisture extrusion: Investigations on the reaction behavior of plant proteins. PhD Thesis, Karlsruhe Institute of Technology.
3. Osen, R.J.J., 2017. Texturization of pea protein isolates using high moisture extrusion cooking. PhD Thesis, Technical University of Munich.
4. Dekkers, B., 2018. Creation of fibrous plant protein foods. PhD Thesis, Wageningen University.
5. Guy, R., 2000. Extrusion cooking. Technologies and applications. Woodhead Publishing.
6. Chiang, J.H., Loveday, S.M., Hardacre, A.K., Parker, M.E., 2019. Effects of soy protein to wheat gluten ratio on the physicochemical properties of extruded meat analogs. Food Structure, 19, 100102.
7. Oldörp, K., Application Note V-228, What happens when rheological properties change? Looking into Rheological Properties with Simultaneous Collection of Microscopic Images.

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