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Food product development with spirulina (*Arthrospira platensis*) – Sensory profiling, product perception and consumer acceptance

Dissertation

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Paper I:

Grahl, S., Palanisamy, M., Strack, M., Meier-Dinkel, L., Toepfl, S. & Mörlein, D. (2018): Towards more sustainable meat alternatives: How technical parameters affect the sensory properties of extrusion products derived from soy and algae. *Journal of Cleaner Production*, Vol. 198, 962-971. DOI: 10.1016/j.jclepro.2018.07.041

Paper II:

Grahl, S., Strack, M., Weinrich, R. & Mörlein, D. (2018): Consumer-oriented product development: The conceptualization of novel food products based on spirulina (*Arthrospira platensis*) and resulting consumer expectations. *Journal of Food Quality*, Vol. 2018, Article ID 1919482, 11 pages. DOI: 10.1155/2018/1919482

Paper III:

Grahl, S., Strack, M., Mensching, A. & Mörlein, D. (2020): Alternative protein sources in Western diets: Food product development and consumer acceptance of spirulina-filled pasta. *Food Quality & Preference*, Vol. 84, 2020, Article 103933. DOI: 10.1016/j.foodqual.2020.103933

List of abbreviations and chemical formulas

CH_4	Methane
CO_2	Carbon Dioxide
DIN	Deutsches Institut für Normung
DM	Dry Matter
DoE	Design of Experiment
EU	European Union
FAO	Food and Agriculture Organization
FCR	Feed Conversion Ratio
GfK	Gesellschaft für Konsumforschung
GHG	Green House Gas
HMEC	High Moisture Extrusion Cooking
LCA	Life Cycle Assessment
MIB	2-Methylisoborneol
N_2O	Nitrous Oxide
PBR	Photobioreactor
PCA	Principal Component Analysis
TVP	Texturized Vegetable Protein
WHO	World Health Organization
WP	Work Package

1 Introduction

Proteins are the most abundant macromolecules in living cells of animal and plant tissues. For humans, dietary protein is essential for growth, development, reproduction, lactation, and health (Wu, 2010). Across Europe, about 61 % of the dietary protein is supplied by animal derived foods (de Boer et al., 2006), which are nutritious and meet dietary requirements due to adequate amounts and proper ratios of amino acids and micronutrients (FAO/WHO, 1991). The global demand for protein is continuously rising due to population growth, increasing incomes and a consequently growing middle-class (Sabaté & Soret, 2014; Wu et al., 2014). During this development, an increase of demands for more nutrient-dense foods, mainly of animal origin, can be observed. The underlying phenomenon is described by Bennett's law who described the positive correlation between increasing wealth and higher consumption of meat and dairy while consuming less protein from staples (Bennett, 1941).

Global food systems have responded to growing demand through increased productivity based on intensification, fertilization and genetic modification (Fedoroff, 2015). However, growing demands cannot be met by a simple increase of agricultural produce without harming the environment and depleting natural resources. Today's intensive agricultural systems already influence the environment through its increased emissions of greenhouse gases (GHG) like nitrous oxide (N_2O) which is linked to the use of nitrogen fertilizers, methane (CH_4) from enteric fermentation of ruminants as well as carbon dioxide (CO_2) emissions from the combustion of fossil fuels (Guyomard et al., 2012; Yusuf et al., 2012). Securing food production under planetary constraints is consequently one of the urgent tasks to be solved in recent times. A transformation of the food sector, or more aptly a transition to sustainable[1] production, is therefore highly needed to secure the provision of food while limiting the impact on the environment (Vinnari & Vinnari, 2014).

The production of animal-source foods occurs at high environmental cost and generates more GHG emissions than the production of plant-based foods (Di Paola et al., 2017; Sabaté et al., 2015). Even though meat reduced or vegetarian diets are more environmental friendly, plant proteins are not accepted by a broad community (Apostolidis & McLeay, 2016; Hoek et

[1] The statement by the World Commission on Environment and Development serves as a definition of sustainability: "Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Brundtland, 1987). While environment, economy and society are interdependent and equally relevant within sustainability (Langhelle, 2000), this dissertation refers to the environmental aspect of sustainability only.

al., 2017a). Nonetheless, a stepwise transition towards more sustainable ingredients, dishes and diets is possible through increasing availability and a broader range of meat alternatives that attract consumers who are oriented towards health, sustainability and convenience alike (de Boer & Aiking, 2019; Schösler et al., 2012; Wild et al., 2014).

Soy is frequently used to produce meat alternatives for human consumption (Asgar et al., 2010). Soybean cultivation however leads to deleterious effects for the ecosystem in cultivating countries due to land use change (Boerema et al., 2016; Taelman et al., 2015). Additionally, soybeans are the most prominent genetically modified crop (Burssens et al., 2011). However, consumers express concerns related to genetically modified organisms, which are associated with undesirable effects on human health and/or the environment (Hu et al., 2004). Therefore, the search for alternatives – both in terms of protein source and plant protein processing – is all the more urgent to overcome consumers' reluctance to change diets (Slade, 2018). As an alternative to soy, high-protein microalgae such as spirulina (*Arthrospira platensis*[2]) are of particular interest. The advantages of spirulina over soy are numerous: it can be grown on land otherwise not suitable for agriculture (Barka & Blecker, 2016), it contains high amounts of protein on dry matter (DM) basis (Becker, 2007), it is much more efficient than terrestrial plants (Wang et al., 2008), and it offers the chance for Europe to become less dependent on protein imports that also harm the environment (Meier et al., 2014).

This dissertation revolves around the development of foods based on the microalga spirulina. Taking manufacturability and consumer opinion into account, products were investigated that are suitable to promote a meat-reduced diet and thereby exploit spirulina's health and environmental benefits. It starts with the idea of using the microalga in high moisture extrusion to produce meat alternatives. On the basis of the derived spirulina-soy-extrudates, various product ideas were generated in which the intermediate product could be processed. Product ideas screened by experts were also conceptually pursued and presented to consumers for evaluation. Consumers' opinion supported the convergence towards a specific product, i.e. filled pasta. The development of pasta filled with a spirulina-based meat alternative is based on analytical and hedonic sensory evaluations of ingredients as well as prototype pasta variants.

[2] Hereafter, *Arthrospira platensis* is referred to as spirulina.

The analytical evaluation by selected and trained sensory panelists through profiling is inevitable in new product development since it helps to deduce the "sensory space" of novel food ingredients. Also, the impact of different process parameters of the production process of meat alternatives on the sensory quality is revealed so that it is possible to translate sensory perception into actual actions in terms of recipe and process adjustments. In the course of new product development, the early elicitation of consumer opinions guides the development process into a promising direction and increases chances for market success. Finally, a hedonic evaluation of newly developed pasta recipes by naïve consumers sheds light on actual liking and in combination with sensory profiling through trained sensory panelists enables the translation of consumer opinions into recipe modifications for optimized products.

Driven by forecasts of an continuously increasing world population and potential food protein shortage (Henchion et al., 2017), the scope of the dissertation contributes to the repertoire of plant-based protein food in Western European diets in order to promote reduced meat consumption without compromising on sensory quality and diversity of food. Thereby, critical dietary nutrient gaps, such as protein, can be mitigated, and a diet supplemented by these novel foods can potentially contribute to health as well as environmental and resource protection.

2 Research project and objectives of the dissertation

The project was based on the concept of "sustainability transitions", a scientific field that has been increasingly researched during the past 20 years (Markard et al., 2012). It is motivated by the recognition that environmental problems, such as climate change, loss of biodiversity and resource depletion call for profound improvements of established socio-technical systems (Köhler et al., 2019). Sustainability transitions imply the transformation towards more sustainable modes of production and consumption. Originally, research has focused primarily on energy issues but has paid less attention to food (Markard et al., 2012). The work presented in this dissertation was done in the course of the project "*Sustainability transitions in food production: alternative protein sources in socio-technical perspective*" which aimed to establish alternative protein sources, i.e. microalgae and insects, in animal and human nutrition by investigating the entire value chain. Thus, sustainability transitions shall also find their way into the food sector. The interdisciplinary project was financed through the funding initiative "*Niedersächsisches Vorab*" by the Ministry for Science and Culture of Lower Saxony (Germany).

The research network comprised collaborators from the **German Institute of Food Technology (DIL e.V.)** in Quakenbrück, the **University of Greifswald**, the **University of Vechta** and from the **University of Göttingen** working on 8 different work packages (WP). Three WP focused on exploitation and usage of the alternative protein sources spirulina and larvae of black soldier flies (*Hermetia illucens)* for feed and food. Five other WP dealt with the analysis of production networks for food and the social environment of sustainability innovation from a spatial perspective.

The objectives of the project were twofold:

1) Research on algae-based meat alternatives to support a meat-reduced diet and directly exploit the sustainability advantage of lower meat consumption.
2) Research on algae and insects as protein feed for poultry and swine to achieve increasing independence from soy imports.

The research presented in this dissertation focuses on the microalga spirulina and its use in food. Based on sensory and consumer science, the work covers the food product development process starting with raw algae biomass and ending with prototype products including marketing concepts that attract consumers.

The objectives of this dissertation are:

- The identification of the optimal parameters for the high moisture extrusion of spirulina without compromising on sensory quality and texture of meat alternatives.
- The consumer-oriented identification of promising product ideas based on the previously developed meat alternatives together with matching marketing concepts.
- The development of recipes that take algae flavor into account and prototype production of spirulina-based food products.
- The determination of the link between the objective evaluations of sensory attributes of spirulina-based food products by trained sensory panelists and subjective consumer acceptance to derive approaches for product optimization.

In total, **chapter 3** comprises background and theory related to new product development based on alternative protein sources. **Chapter 3.1** explains why industrialized agriculture is such an environmental burden and why it is therefore necessary to turn towards novel proteins and to foster a transit towards more sustainable food consumption. Following, a close-up on the microalga spirulina will be presented that reveals, why the microorganism has potential to pave the way towards more sustainable food production (**chapter 3.2**). **Chapter 3.3** gives an introduction into the process of high moisture extrusion cooking which is the process of choice to process spirulina into meat alternatives. To understand the relevance of consumer orientation and sensory perception in new product development, **chapter 3.4** touches upon relevant fields during the process, i.e. sensory profiling, consumer acceptance, individual consumer differences like familiarity and neophobia as well as appropriate marketing strategies of novel products.

Papers I to III represent practical applications of the previously presented insights. Because high moisture extrusion cooking (HMEC) is a novelty related to the processing of spirulina in food, Paper I (**chapter 4**) explores the microalga in extrusion based on a Design of Experiment (DoE). The DoE considers various parameters: the spirulina and soy content in the extrudate, the screw speed of the extruder, the moisture content in the extrudate and the process temperature. By means of descriptive analysis using a trained sensory panel, the effect of the parameters on the sensory properties of the extrudates is examined; thus, deriving the possibilities for controlling the desired texture and flavor. Paper II (**chapter 5**) investigates consumer perception of three different product categories (pasta, sushi, or jerky) incorporating the spirulina-soy-extrudates. The aim is to detect the most popular product according to consumers and to identify promising marketing opportunities based on three different benefits (sustainability, health, or innovation). Being most familiar and therefore most wanted, pasta is followed up upon in Paper III (**chapter 6**). Here, three different pasta variants filled with different shares of spirulina-soy-extrudate are evaluated for their consumer acceptance and sensory quality. The pasta recipes are developed based on masking or flavor-flavor learning approaches related to spirulina. Finally, the consumer study identifies favored flavor combinations as well as accepted amounts of spirulina in the pasta filling. Individual differences among consumers like familiarity with spirulina or food neophobia lead to a different performance of the pasta variants regarding acceptance and should be considered during further development. All-in-all, the research conducted as a part of this dissertation offers starting points to derive possible changes for the processing of spirulina powder in general and filled pasta in particular.

In **chapter 7** challenges of the research are presented and assessed, as well as topics for future research are discussed. **Chapter 8** addresses some project limitations. Conclusions are summarized in **chapter 9**.

3 Background and theory

In the following, fields related to the food product development based on the alternative protein source spirulina will be described to enable a better understanding how spirulina could contribute to a transformation of the food system we know today. Starting off by the environmental impact of food production in general and protein supply in particular, the role of meat and meat alternatives will be explained. The next section will give an introduction to spirulina's cultivation requirements. It will be explained why research about spirulina and the incorporation in food is worthwhile and promising in order to contribute to environmental protection and the security of food availability. Finally, sensory profiling, consumer perception of product concepts and marketing categories as well as consumer acceptance testing are described in relation to consumer-oriented food product development. Finally, it will be highlighted that consumer orientation in new product development is crucial to make use of the biomass in successful food products.

3.1 Environmental impact of food production

Human action is the main driver for global environmental change (Steffen et al., 2007), which is induced through pollutants and GHG emissions, land use change and depletion of limited resources. Global food systems, through which humans influence the environment, account for approximately one third of all GHG emissions (Vermeulen et al., 2012). While direct emissions account for N_2O, CH_4 and CO_2, indirect emissions are released through land use change, i.e. deforestation, forest degradation and peat land degradation (Vermeulen et al., 2012) in order to make land available for industrialized agriculture. Changing land use practices to produce soy for animal feed put environmental pressure on already impaired ecosystems in the global south, and intensive agriculture in these areas causes soil degradation, reduces fertility and leads to biodiversity loss (Foley et al., 2005; Henchion et al., 2017). More than 80 % of newly created agricultural land was derived from deforestation of rainforests between 1980 and 1990 so that cropland and pastures meanwhile occupy approx. 40 % of the land surface (Foley et al., 2005; Gibbs et al., 2010). Augmenting area used as cropland has the aim to increase harvests to meet the immediate need for food and feed, but long-term ecosystem losses with sometimes hard to foresee consequences are the result (Foley et al., 2005). However, arable land is finite so that an increase through the agricultural cultivation of terrestrial plants is limited. Therefore, the establishment of agricultural cultivation independent from fertile area is deemed necessary to feed a growing world population. In addition to soil and land, modern agriculture uses substantial amounts of other natural resources, i.e. nutrients and water (Holden et al., 2018; Rockström et al., 2009). Furthermore, it is also the most energy-intense part of the food system, mainly relying on non-renewable resources, particularly fossil energy (Holden et al., 2018).

Consequently, food production has nearly reached its planetary boundaries and faces a two-fold challenge: it needs to secure food for a growing world population, while simultaneously ensuring it is done in an environmentally sustainable manner (Foley et al., 2011). This will include closing yield gaps, increasing efficiency, changing diets and reducing waste to potentially double food production (Foley et al., 2011). Of the above mentioned advances to contribute to climate and environment protection, altering diets and consumption patterns has recently attracted increasing amounts of attention (Behrens et al., 2017; Parodi et al., 2018; Willett et al., 2019). While some research states that it would already be enough to follow dietary guidelines (Behrens et al., 2017; Willett et al., 2019), others promote a dietary shift towards plant-based diets (Sabaté & Soret, 2014). More recently, the assessment and consumption of future foods like insects, cultured meat and microalgae entered the debate about environmental friendly food consumption (Parodi et al., 2018). Through the incorporation of the protein-rich microalga spirulina, protein supply can be diversified, propelling a stepwise transformation of diets in the Western European population.

3.1.1 Protein supply

The environmental impact of food is tightly connected with its production and the demand of dietary protein, both from animal and plant origin. The present global protein demand for 7.3 billion people is approximately 202 million tons (Henchion et al., 2017). It is assumed to increase due to population growth and increasing wealth, resulting in a per capita consumption far exceeding the current-day, as projected by the FAO (2009). It is predicted that demand will increase by 78 % if the world population rises to 9.6 billion people by 2050 and current eating habits remain unchanged (Henchion et al., 2017). Research indicates that animal-based protein foods play a relevant role in the discussion about protein supply in a sustainable manner (Dagevos & Voordouw, 2013; Sanchez-Sabate & Sabaté, 2019). The debate is related to conversion efficiencies on the one hand and GHG emissions on the other hand.

The feed to food conversion entails losses and is particularly viewed critically, if feed is used that is principally edible for humans. Estimated feed conversion ratios (FCR) defined as the ratio between feed intake and weight gain vary between animal species and feeding regimes (Fry et al., 2018; Wilkinson, 2011). Commercial feeds and intensive production methods provided, Fry et al. (2018) reported the FCR for terrestrial animals to be 6.0 to 10.0 for beef cattle, 2.7 to 5.0 for pigs and 1.7 for 2.0 for chickens. The FCR usually accounts for the weight of feed inputs and disregards the nutritional content of the feed, the inedible portion of animals, or the nutritional quality of the final product (Fry et al., 2018). Wilkinson (2011) therefore presented values for one kg of protein that is required to be converted into animal protein: while chickens need 2.1 kg of protein from feed, pigs need 2.6 kg and cattle needs 3.0 kg. The author concludes that conversion ratios larger than 1.0 highlight the need to improve efficiency of feed use and to adjust feeding patterns towards less edible crops. After all, increased efficiency and changed livestock feeding patterns would support sustainable food systems (Schader et al., 2015).

Approximately 10 % of European GHG are emitted by livestock production through direct emissions from animals and manure and indirect emissions through feed production including land use (Westhoek et al., 2011). Aiking (2014) reported that protein consumption in the European Union (EU) is about 150 % of the dietary recommendations by the WHO (0.83 g protein per kg of bodyweight). The protein consumed originates mostly from animal origin. Under the assumption that reduced consumption of meat leads to lower production rates, it can be supposed that compliance with nutrition guidelines would already contribute somewhat to reducing GHG emissions in the EU (Behrens et al., 2017). In addition to reducing meat consumption, replacing part of animal protein with plant protein would further help to comply with dietary guidelines.

Being rich in protein and other nutrients, plants like cereals and pulses are a major source of dietary protein in many regions of the world (Henchion et al., 2017). However, consumption of plant derived protein in the EU is rather low and originates mostly from cereals (de Boer et al., 2006). Generally, the environmental impact of the protein supply would benefit from more frequent consumer choices towards plants like pulses, which have been shown to be less resource intensive (Aiking, 2014; Sabaté et al., 2015). From a nutritional point of view, the digestibility of animal-derived protein is higher and the composition of amino acids is more favorable compared to plant protein. But by consuming a variety of cereals and pulses, nutritional needs can be met (Henchion et al., 2017; Smil, 2002). Nevertheless, plant proteins are also not entirely uncritical with regard to environmental impact. This is mainly due to the massive use of protein-delivering plants like soy in animal nutrition and the large-scale and intensive cultivation (Aiking, 2011; Henchion et al., 2017).

Protein-rich microalgae are discussed as a sustainable alternative to previously established protein sources. However, microalgae like spirulina cannot always be considered more sustainable than conventional protein production. Sustainability is dependent upon production conditions and mostly constrained due to still rather high energy consumption (Smetana et al., 2017). Nonetheless, microalgae cultivation systems do not compete with arable land (Rösch et al., 2019) and require less land than livestock: <2.5 m^2 per kg of protein are required compared to 47–64 m^2 for pork, 42–52 m^2 for chicken, and 144–258 m^2 for beef production (Caporgno & Mathys, 2018). In the debate about decreasing the consumption of meat and the ration of animal over plant protein in order to meet dietary requirements in a more environmentally friendly manner, microalgae can be considered.

3.1.2 The role of meat alternatives

Meat alternatives have a long tradition in human nutrition (Joshi & Kumar, 2015). Products like tofu, tempeh or seitan have played a substantial role in Asian food culture for centuries. The Western market for manufactured meat alternatives however, only started in the 1960s (Sadler, 2004). Consumer interest in a partial replacement of meat protein with alternatives has been triggered by higher awareness about environmental, ethical and health issues and leads to continuous market growth (Aiking, 2011; Godfray et al., 2018; Joshi & Kumar, 2015; Reijnders & Soret, 2003; Sadler, 2004; Wild et al., 2014). This development contributes to dietary change towards meat-reduced, vegetarian or vegan diets (Apostolidis & McLeay, 2016; Dagevos & Voordouw, 2013).

More and more consumers look for products that replace the function of meat in a dish and contribute similar protein content to the diet (Van Mierlo et al., 2017). On the one hand, there is the growing awareness that eating large quantities of red meat in particular is unhealthy because it probably increases the risk of mortality from cardiovascular diseases and cancer

(Godfray et al., 2018; Pan et al., 2012). On the other hand, ethical concerns about animal welfare during animal production also play a role when it comes to the amount of meat consumed. The industrial way of meat production including factory farming has raised doubts on whether the meat industry can respond to growing demands in an animal-friendly manner (de Boer et al., 2013; Rothgerber, 2015). These doubts let people make more conscious consumption decisions or even abstain from meat (Rothgerber, 2015). Being based on traditions, emotions and learning, eating behavior is rather conservative and therefore not so easy to change, even if health and ethical concerns related to meat consumption are spreading (Beverland, 2014). Even if there is an intention to change diets, it is difficult for consumers to include new foods in a diet, which was developed and shaped over many years (Guerrero et al., 2009; van't Riet et al., 2011). The advantage of meat alternatives comply with the wish for a sufficient protein supply without compromising on habits, convenience, familiarity and sensory enjoyment (Hoek et al., 2017b) because they can be easily integrated into already established eating habits. A combination of pulses and cereals can be understood as a meat alternative; at least as far as the reduction in protein intake from animal origin is concerned. However, pulses are perceived as old-fashioned and might only be accepted if benefits are emphasized, the image is modernized and if various convenient dishes are created (de Boer & Aiking, 2019).

The offer of and demand for plant-based meat alternatives is increasing steadily and the market volume with it, at least in Germany (GfK, 2016). Therefore, it appears that meat alternatives will be a part of the solution when it comes to reducing meat consumption in favor of plant-oriented diets (Alexander et al., 2017; van der Weele et al., 2019). The emphasis should clearly be on plant origin products because soy-based meat alternatives have been shown to have a lower environmental impact than dairy-based products (Smetana et al., 2015). Looking at these developments, it seems that the cultural dominance of meat may be less robust than initially thought (Aiking & de Boer, 2018; Dagevos & Voordouw, 2013; de Bakker & Dagevos, 2012).

Microalgae in the context of meat alternatives form a relatively new range of products. Due to their nutritional profile, microalgae like spirulina are worthwhile to consider for product development. Consequently, an introduction to spirulina as a food source will be given in the following sections.

3.2 Spirulina (*Arthrospira platensis*) as a food source

Spirulina has been extensively studied in terms of usage in food as well as dietary supplements. A search query on the bibliographic database PubMed on the 5th of August 2019 resulted in 309 hits for the keywords "(*Arthrospira platensis*) AND food". The resulting publications date back to the year 1973. More than half of these publications (167 articles) were published in the last 5 years. Despite the growing interest in spirulina, it is still somewhat new (Belay, 2008), particularly to the European market. It has been used as a food for centuries, but has only been commercially available for the past 50 years (Borowitzka, 1999). The key features of the microorganism will be shown in the following subchapters.

3.2.1 Characteristics

Morphology

Spirulina is a multicellular, photosynthetic and filamentous cyanobacterium (Belay, 2008; Jung et al., 2019) and not a true alga. Nevertheless, it is considered a microalga[3]. It forms unbranched helical trichomes (Figure 3.1) of varying size (diameter of the helix between 35 to 60 µm) and degree of coiling, which explains the origin of the name (Belay, 2008; Ciferri, 1983). Spirulina's blue-green biomass is mostly used in powder form after drying a filtered suspension (Hu, 2004).

Figure 3.1: Microscopic view of spirulina. Photo credit: Divine Organics.

[3] The term "microalgae" comprises both plants and bacteria: some are eukaryotic and commonly identified as algae, e.g. *Chlorella vulgaris*. Unlike eukaryotes, bacteria have no nucleus and belong to prokaryotic organisms. Owing to their photosynthetic activity, cyanobacteria can be considered intermediates between bacteria and plants (Barka & Blecker, 2016). Spirulina is at best plant-like.

Taxonomy

In the class of cyanobacteria, spirulina belongs to the family of *Oscillatoriaceae*. Regarding taxonomy, there has been confusion about the two genera *Spirulina* and *Arthrospira* which were regarded separate until 1932, when Lothar Geitler unified them under the name *Spirulina* due to their similar helical morphology (Habib et al., 2008). However, they are distinguishable through the presence of septa. While the genus *Arthrospira* has them, *Spirulina* has been attributed the aseptate form and it took until 1989 when these microorganisms were taxonomically separated again (Tomaselli et al., 1996). Nowadays, there is consent that the two genera are distinctively different; Nelissen et al. (1992) has proven it by analyzing rRNA sequences. Nonetheless, the confusion led to the situation where spirulina is the commercial market name to refer to the taxonomic *Arthrospira*.

3.2.2 Cultivation

Spirulina can be found in highly selective habitats like alkaline (pH of 9.5 to 10.5), brackish and saline water (Borowitzka, 1999; Ciferri, 1983; Shimamatsu, 2004). Optimal growth can be achieved at temperatures between 35 °C and 37 °C (Habib et al., 2008). Due to extreme growing conditions that are unfavorable for other microorganisms, spirulina cultures are predominantly pure cultures and at low risk for being contaminated with toxin-producing microorganisms (Ciferri, 1983; Habib et al., 2008). Naturally, the microalga can be found in arid areas of the tropics and subtropics with high solar radiation (Ciferri, 1983; Habib et al., 2008). Commercial mass cultivation started in the late 70s in the Lake Texcoco in Mexico (Belay, 2008; Shimamatsu, 2004). Other records of spirulina cultivation from the 1940s point to the exploitation of naturally blooming cultivation in the Republic of Chad (Belay, 2008; Chaumont, 1993; Ciferri, 1983; Soudy et al., 2018).

Meanwhile, spirulina is produced all over the world with a focus on the Asia-Pacific region (Hu, 2004). Cultivation is driven by modern biotechnology and different cultivation systems have been established. These developments have led to the situation where cultivation in non-arable regions is possible, so that crop yields on underperforming land are improved (Foley et al., 2011), agriculture can be transformed and yield gaps can be closed. Cultivation systems are typically classified as open- or closed-culture systems and will be briefly described as well as advantages and disadvantages will be contrasted.

Open-culture systems

Ponds and lakes serve to cultivate spirulina in open systems and are the oldest experimental systems for spirulina cultivation (Chaumont, 1993). Their major advantage is that they are less costly to build and operate compared to closed systems; operation costs are lower because of a free source of energy (Chaumont, 1993; Jiménez et al., 2003; Mata et al., 2010). Furthermore, higher capacities enable larger production volumes; however at the same time this is disadvantageous as more extensive land area is occupied (Mata et al., 2010). Ponds, be it long raceways (Figure 3.2) or circular ponds, can have a surface area of up to 5000 m^2 and a depth between 15 and 30 cm (Borowitzka, 1999; Mata et al., 2010). Cultivation is affected by water depth, rate of mixing and population density. Finding the right compromise is important for maximizing the biomass output (Borowitzka, 1999; Hu, 2004). A disadvantage of this production system is its susceptibility to weather conditions which causes difficulties to control water temperature, evaporation and lighting (Borowitzka, 1999; Mata et al., 2010). The risk for contamination with toxin-producing microalgae is higher in open-culture systems than in closed-culture systems as the former is exposed to the elements, which makes increased quality control in open systems inevitable (Borowitzka, 1999; Jung et al., 2019). Because of the highly selective growing conditions, i.e. unfavorable conditions for other organisms, and the lower costs, spirulina is currently most often cultivated in open-culture systems (Borowitzka, 1999; Delrue et al., 2017; Small, 2011).

Figure 3.2: Open raceway ponds of Earthrise in California, U.S. Photo credit: Earthrise.

Closed-culture systems

Photobioreactors (PBR), often flat plate or vertical tubular systems (Figure 3.3), have been constructed to optimize biological and physiological characteristics of microalgae in general (Borowitzka, 1999; Mata et al., 2010), and spirulina in particular. Because there is no direct contact of the algae cultivation with the surrounding environment, PBRs offer a more protected and a safer environment. Advantages relate to a better control of growth conditions like pH or temperature, limited water evaporation and prevention from contamination as well as higher population density resulting in better productivity than ponds (Mata et al., 2010). Furthermore, PBRs require less land and can be operated over a much wider climatic range than open-culture systems (Borowitzka, 1999). PBRs disadvantages are related to the risk of overheating, bio-fouling, oxygen accumulation and higher costs to build and operate such systems (Drexler & Yeh, 2014; Mata et al., 2010; Tredici & Materassi, 1992). Higher operation costs are mainly due to higher energy expenditure for artificial lightning compared with open-culture systems, even though they are designed in a way that the amount of light available per cell is increased (Borowitzka, 1999). In Western Europe, the cultivation of spirulina is most commonly done in PBRs.

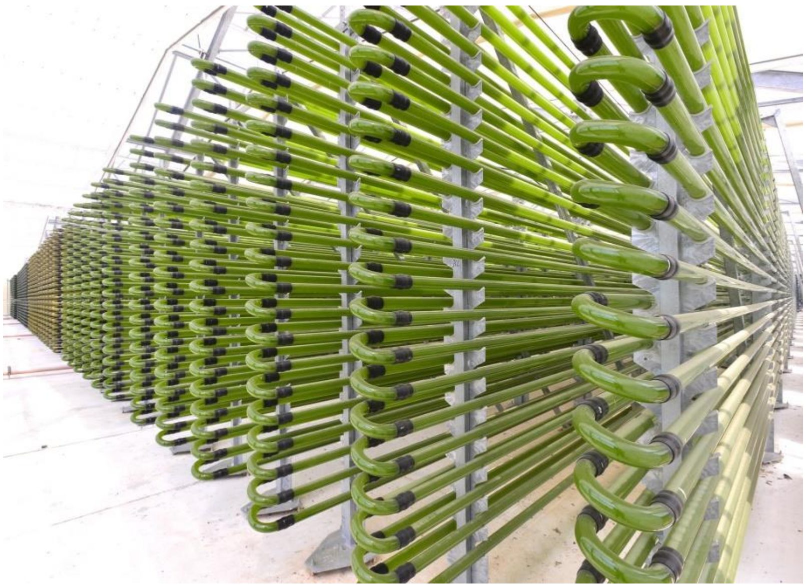

Figure 3.3: Photobioreactor of Roquette Klötze in Sachsen-Anhalt, Germany. Photo credit: Investitions- und Marketinggesellschaft Sachsen-Anhalt.

Post-cultivation processing and market volume of spirulina

After cultivation, spirulina is harvested, dried and packaged (Hu, 2004). For commercial harvesting, filtration devices are used that are sensitive enough to prevent filament breakage which could introduce bacterial contamination (Hu, 2004). A suspension of 8 to 15 % DM is then attributed to spray drying yielding a spirulina powder with 3 to 4 % of moisture (Hu, 2004). For food production, spray drying is done where the powder is exposed to heat for a few seconds to preserve heat sensitive nutrients like pigments or enzymes (Belay, 2008). Immediately after drying, the spirulina powder is sealed under vacuum to limit oxidization (Hu, 2004).

About 15 years ago, around 5,000 tons of microalgal DM were produced annually (Pulz & Gross, 2004). Spolaore et al. (2006) reported the annual production of spirulina DM to be 3,000 tons per year. The highest amounts are produced in Asia and Australia; companies in Europe are estimated to produce only a share of about 5 % (Vigani et al., 2015). In comparison to the production of seaweed, which is reported to be 24 million tons per year (WRAP, 2015), the microalgae sector is still at its infant stage (Vigani et al., 2015). Recent production volumes are not yet competitive with traditional agricultural commodities (Vigani et al., 2015).

Spirulina's productivity is influenced by cultivation system, growth medium and environmental conditions (de Jesus et al., 2018; Delrue et al., 2017). Dismukes et al. (2008) compared the productivity of spirulina grown in an open pond with the productivity of land-based plants and found productivity rates of 27 tons DM/ha and year. Similar values were reported by Jiménez et al. (2003). Delrue et al. (2017) managed to produce spirulina at a rate of 58.4 g/m^2 and day in a PBR which corresponds to 213 tons DM/ha and year; this would deem spirulina 30 times more productive than corn (Dismukes et al., 2008). As mentioned previously, PBR is the more expensive cultivation system so that one can conclude that production has to compromise on cost, productivity and quality.

Prices for dry microalgae biomass in general and spirulina in particular range from 4 to 10 € per kg (Delrue et al., 2017; Draaisma et al., 2013). Higher production costs in a PBR increases the price to approx. 19 € per kg (Delrue et al., 2017). Provided the production matures and algae biorefinery is making progress, the future market price of microalgal biomass will be able to compete with conventional commodity prices (Draaisma et al., 2013).

3.2.3 Nutritional composition

While foods of animal origin contain 40 % and more protein based on DM, plants with exception of pulses contain up to 15 % DM (Wu et al., 2014). Spirulina contains comparably high amounts of protein, i.e. up to 65 % DM, which is why it is mostly cultivated for the use in human food, but also animal and fish feed (Becker, 2007; Buono et al., 2014). Most plant proteins do not contain all essential amino acids required for human nutrition (Wu et al., 2014). However, plant-like spirulina contains a balanced composition (Lupatini et al., 2017; Mišurcová et al., 2014), comparable to animal derived protein (Wu et al., 2014). Sulfur amino acids (methionine and cysteine) are considered as limiting in plant proteins from pulses due to their insufficient amounts regarding human nutrition. However, the content of methionine and cysteine in spirulina covers 73.8 % of the recommended daily intake (RDI) for these amino acids (Henchion et al., 2017; Mišurcová et al., 2014).

Phycobiliproteins, i.e. photosynthetic pigments belonging to the protein fraction, are of interest for the food industry, mainly as a colorant. Phycocyanin (20 % of the total crude protein in spirulina) is mainly extracted from blue-green algae, like spirulina (Buono et al., 2014; Panutai et al., 2018). It has been attributed to antioxidant properties, but it is unstable to heat and light (Stanic-Vucinic et al., 2018). Health-promoting effects are consequently only assured if spirulina is added after thermal food-processing.

Spirulina has a fat content of 5 to 10 % DM (Batista et al., 2013; Gutiérrez-Salmeán et al., 2015). Approximately 20 % of the fat content constitute of γ-linolenic acid and spirulina is therefore considered one of the best sources providing this essential polyunsaturated fatty acid for human nutrition (Gutiérrez-Salmeán et al., 2015; Sajilata et al., 2008).

Carbohydrates, of which spirulina contains about 15 % DM, constitute mainly of branched polysaccharides composed of glucose, rhamnose, mannose, xylose and galactose (Ciferri, 1983; Rösch et al., 2019; Shekharam et al., 1987). Because cell walls of spirulina do not contain cellulose, spirulina is easily digestible, even for patients with impaired intestinal absorption (Beheshtipour et al., 2013; Cuellar-Bermúdez et al., 2017). While spirulina is not mainly consumed because of its carbohydrate content, the carbohydrate content is important for biofuel production, particularly for bioethanol. Salla et al. (2016) were able to increase the carbohydrate level of spirulina up to 58 % while reducing the protein content at the same time.

Spirulina has been reported as being rich in minerals and vitamins (Matos et al., 2017). Abundant minerals in spirulina are sodium and potassium, followed by phosphorous, calcium, magnesium and iron (Carcea et al., 2015; Tokusoglu & Ünal, 2003). Spirulina is a natural source of β-carotene (Vitamin A) which is important for the immune system and also known for its antioxidant capacity (Soudy et al., 2018; Wu et al., 2016). Often, spirulina is associated with considerable amounts of vitamin B_{12} (Beheshtipour et al., 2013; Matos et al., 2017; Santos et al., 2016; Vaz et al., 2016). However, spirulina only contains an inactive vitamin B_{12} analogue that is not bioavailable for the human intestine (Watanabe et al., 1999, 2002). Consequently, consumption of spirulina does not improve the vitamin B_{12} status in deficient consumers, e.g. vegans.

Due to its functional ingredients and bioactive metabolites like phenolic compounds (Matos et al., 2017), spirulina was shown to modulate biochemical pathways related to antioxidant (Hirata et al., 2000), anti-inflammatory (Pulz & Gross, 2004), anticancer and immunomodulatory activities (Wu et al., 2016), mostly in animal studies. Overall, the nutritional composition depends on culture media and cultivation conditions and can vary accordingly (Carcea et al., 2015; Lupatini et al., 2017). This likely explains the variability of reported values across scientific publications.

3.2.4 Flavor

Spirulina has a particular flavor that is not well-established in Western European diets. Small (2011) argued that the flavor does not justify using spirulina without incorporation in dishes or mixing it with other ingredients. In some parts of the world, the microalga is consumed as sun-dried cake, called dihé – either pure or in sauces (Carcea et al., 2015; Kay & Barton, 1991; Tefera et al., 2016). However, the consumption is geographically and ethnically restricted and influenced by the availability of other, presumably more popular, foodstuffs (Ciferri & Orsola, 1985).

Previous research shows that the aroma of cyanobacteria is mainly constituted by geosmin and 2-methylisoborneol (MIB), which are typical cyanobacterial metabolites (Smith et al., 2008) and known for their earthy and musty odor (Liato & Aïder, 2017; Mahmoud & Buettner, 2017). Sensory studies have categorized these aroma compounds as off-flavors (Johnson & Kelly, 1997; Smith et al., 2008). Here, it is important to note that there are no reports of MIB or geosmin production by spirulina (Cuellar-Bermúdez et al., 2017; Milovanović et al., 2015) but the flavor and odor of spirulina have nevertheless been described as fish-like, musty, or earthy (Aguero et al., 2003; Becker, 2007; Stanic-Vucinic et al., 2018). Protein- and lipid degradation are likely involved in fish-like odor development in spirulina (Cuellar-Bermúdez et al., 2017). To diminish the undesirable odor and flavor of spirulina, fermentation has been investigated by Bao et al. (2018). Compared to the unfermented spirulina, mixed fermentation with *Lactobacillus plantarum* and *Bacillus subtilis* led to a decrease of most aromatic compounds to an undetectable level. The authors claim 2,5-dimethylpyrazine and 2-methylpyrazine together with the alcohols 1-hexanol, 3,3,5-trimethylcyclohexanol and the ketones 2,2,6-trimethylcyclohexanone and 3,5,5-trimethyl-2-cyclohexenone to be responsible for the off-odor of spirulina. In the same order they are attributed earthy, green, woody, musty or tobacco-like sensations. The compounds were no longer detectable after fermentation. Accordingly, fermentation has been shown to be a possible method to improve the sensory perception by modifying most of the aromatic profile.

The aroma of spirulina implies that the microalga cannot be used throughout the food industry but only in specific applications. If the entire biomass is used, concepts must be found that are compatible with the sensory perception the alga provokes as well as the composition of macronutrients. The high protein content makes spirulina an interesting ingredient for high moisture extrusion cooking.

3.3 High moisture extrusion cooking

Extrusion cooking was originally established in the 1960s to texturize vegetable protein (TVP) in order to produce dried, puffed products with 10 to 30 % moisture (Cheftel et al., 1992). If used in a meat replacing context, TVP have to be rehydrated before being introduced in final food or dishes (Arêas, 1992). Such products are used in dishes to substitute minced meat due to the comparatively small size of the pieces. Since the 1980s, it has been possible to texturize protein mixes containing 40 to 80 % moisture in the continuous process of extrusion cooking (Cheftel et al., 1992; Noguchi, 1990). Instead of product expansion leading to a dry and porous structure of small pieces, the high moisture extruded products have multilayer and partly fibrous structures that resemble whole-muscle meat (Cheftel et al., 1992; Noguchi, 1990) and mimic bite and mouthfeel.

3.3.1 Extrusion process

High moisture extrusion cooking (HMEC) is a continuous process where proteins need to unfold, cross-link and align themselves to form fibers. During extrusion, proteins transform and interact so that a three-dimensional network is formed (Arêas, 1992). The process can be influenced by varying process parameters such as moisture (viscosity), barrel as well as cooling temperature (thermal energy), screw diameter and speed of the twin screws as well as die geometry (velocity) impacting pressure, shear and friction (mechanical energy) (Noguchi, 1990).

Extruders with co-rotating twin screws and a cooling die are used to process a low-viscous mix of protein and water into a fibrous protein strand (Akdogan, 1999; Cheftel et al., 1992). At first, the protein mass (up to 70 % water) is fed into the long extruder barrel and heated up to a temperature of 140-180 °C. The twin screws mix the ingredients thoroughly and move it continuously towards the cooling die. The mixing and shearing through the twin screws results in unfolded proteins and benefits the formation of both covalent disulfide and non-covalent bonds. The cooling die serves to gradually reduce the thermal energy in order to increase protein–protein interactions and the alignment of the proteins into the flow direction while viscosity rises. The temperature gradient from the die wall to the core of the solidifying mass results in a lower flow of the layers in contact with the die than of the still fluid internal layers. Consequently, the shear force of the mass increases. Finally, cooling avoids product expansion which would otherwise be caused by the evaporation of water (Cheftel et al., 1992; Liu & Hsieh, 2008; Wild et al., 2014). The resulting intermediate meat alternative is a densely layered and fibrous band with an elastic consistency, as well as a smooth and homogeneous surface (Cheftel et al., 1992).

Properties of high moisture extrudates are not only influenced by process parameters but also by the processed ingredients. Various plant materials have been investigated in terms of usability in extrusion. Most often studied and best understood are soy protein concentrate or isolate (Lin et al., 2000; Pietsch, Bühler, et al., 2019). The reason might be that soy protein is equivalent to animal protein (Kumar et al., 2017), particularly if the protein digestibility corrected amino acid score (PDCAAS) is compared (Hoffman & Falvo, 2004; Rizzo & Baroni, 2018). However, its incorporation into food still plays a minor role in Europe where around 2 % of inhabitants report soy food consumption in general and where an intake of 30 g of soy protein can only be reported for vegetarians and vegans (Rizzo & Baroni, 2018). Without a doubt, transformation through extrusion contributes substantially to the circumstance that soy plays an ever bigger role in human nutrition (Arêas, 1992). However, some drawbacks associated with the use of soy are the presence of anti-nutritional factors, its allergenic potential, and the introduction of genetically modified organisms (Burssens et al., 2011). Therefore, other protein sources to use in HMEC are under investigation, either pure or as a mix together with other protein ingredients. These include wheat gluten (Pietsch, Werner, et al., 2019), peas (Osen et al., 2014), lupins (Palanisamy, Franke, et al., 2018), spirulina/lupins (Palanisamy et al., 2019) or insects/soy (Smetana et al., 2018).

Studies about intermediate meat alternatives often focus on the physical and chemical aspects of HMEC (mechanical energy, thermal energy, moisture) and investigate the relationship between texture formation and molecular restructuring of the proteins (Lin et al., 2000; Liu & Hsieh, 2008; Palanisamy, Töpfl, et al., 2018; Sandoval Murillo et al., 2019). Such studies add to a better understanding of the underlying protein interactions and separation of water-rich and protein rich domains when it comes to texture formation. Thereby, the settings leading to desired texture have mostly been identified. Consequently, the production process can be optimized and the end product can be improved (Liu & Hsieh, 2008).

In this dissertation HMEC was researched for the processing of spirulina with a focus on sensory properties. Various extrudates incorporating spirulina and soy were produced based on a DoE to explore the usability of the microalga in the extrusion process. To gain an understanding of the effect of selected process parameters on sensory perception, the human perception of spirulina based meat alternatives was investigated (**Paper I**). Next to physical and chemical investigations, this is a relevant approach when it comes to further food product development.

3.3.2 Extruded food products

Material derived from HMEC is versatile and can be consumed in most dishes as an alternative for meat ingredients. HMEC technology enables the production of a wide range of shapes, sizes and textures, leading to chunks, mince, dices or strips of protein material that can be further processed into burgers, sausages, schnitzels, meatballs, nuggets or toppings and fillings (Joshi & Kumar, 2015; McIlveen et al., 1999). This versatility of extrudates from rather neutral plant proteins like soy, pea or lupines comes in handy, since it carries the opportunity to open up a wide range of products for different consumer demands.

However, previous studies showed that meat alternatives appear more appropriate in small pieces in fillings and sauces instead of large pieces of meat, such as steaks or cutlets (Elzerman et al., 2015, 2013) which implies that "context fit" is important and emphasizes the difficulty of imitating whole muscle meat. While sensory properties haven been shown to not meet expectations of non-vegetarian consumers due to problems in mimicking meat-like properties (Hoek et al., 2013), the development has made a leap when US-based companies like Beyond Meat or Impossible Foods recently launched plant-based burger patties that seek a direct duel with meat. Such companies aim to offer meat alternatives made of soy and bean protein that look and taste like the original and thereby add to the constantly growing product selection (Keefe, 2018). While still new to the market, such animal-like meat alternatives contribute to a differentiation of available products into these that convince meat lovers and those that are targeted at vegetarians. Their motivation to abstain from meat originates not only from concerns about ethics and personal health but also from disgust of meat sensory properties (Ruby, 2012). If disgust for meat is involved, animal-like meat alternatives are avoided.

As previously mentioned, spirulina has a distinct algae flavor (chapter 3.2.4) and is therefore not suitable to be used as steak or chicken nugget analog. The musty-earthy algae flavor is not conducive to match consumer expectations if marketed as such. Nonetheless, it is still possible to incorporate spirulina in food in order to create new taste sensations and to further differentiate meat alternative products (**Paper II**). Neutral and versatile pasta dough, sushi or dark-colored jerky strips were investigated for acceptability by consumers in an online survey. As other research on context of meat alternatives showed, familiar products are more likely to be accepted (Elzerman et al., 2011, 2015). After all, convenience of the final product has also been important for consumers (Gravely & Fraser, 2018); the three products mentioned above differ regarding familiarity and are easy to prepare or ready-to-eat.

Since meat alternatives based on microalgae are still uncommon and a novelty to the market, Weinrich and Elshiewy (2019) investigated preferences and willingness to pay (WTP) for such products in an online survey. They found that if consumers choose microalgae-based meat alternatives, it is generally irrelevant how much microalgae are incorporated. At this point it must be emphasized once again that microalgae are not as mild tasting and familiar (Buono et al., 2014; Chacón-Lee & González-Mariño, 2010) as other ingredients examined in the study (peas, lupine, soy, egg and milk) and that the consumers in the study by Weinrich and Elshiewy (2019) therefore have no real idea how such a product would taste like. In order to be able to elicit actual acceptance, real food products have to be developed and presented to consumers.

3.4 Consumer oriented new product development

In the development process, it is of importance to elicit consumer opinions as early as possible. During the process, sensory profiling enables the translation of consumer wishes into implementable instructions regarding recipe adjustment. Consumer acceptance and sensory profiling are of particular relevance when incorporating novel ingredients like spirulina, where development cannot be based on previous experience or insights. In the following, it will be briefly shown how sensory analysis is a useful tool to translate the mind of the consumer into tangible elements that lead to final products based on spirulina.

3.4.1 Conventional sensory profiling

Conventional sensory profiling is a descriptive technique where human senses of between eight and twelve trained panelists are used to describe sensory variation (DIN, 2015). It should be noted that it is similar to a quantitative descriptive analysis (QDA) but can only be called that, if specific rules are followed, e.g. an unstructured line scale of 15 cm length or anchors that are indented (Stone et al., 2012). Just like in a QDA, product specific vocabulary is defined and the panelists are exposed to many different products as well as suitable reference standards in order to align and finally attain the understanding of the product set during training of the panel for conventional sensory profiling. Training progress and performance of the panel is measured based on consensus among panelists, discriminability between panelists and repeatability within each panelist (Lawless & Heymann, 2010a).

Descriptive analysis techniques are useful, if sensory-instrumental relationships should be revealed or if a detailed specification of sensory attributes is desired, particularly with regard to product development. The aim is to not only describe the product set, but also to measure intensities of the selected attributes. The attained data set containing at least duplicate measurements of a product set based on agreed upon attributes can then be analyzed through a principal component analysis (PCA). PCA is a reduction procedure to capture the relationships among the products within the set based on the attribute ratings describing the products. Provided that the first two components explain a substantial amount of variance, i.e. about 90 % as in **Paper I** and **Paper III**, the products of investigation are similar if they are mapped closely to each other in the variable factor map of the first two components. Products located far apart are rather dissimilar (Lawless & Heymann, 2010a, 2010b).

Conventional sensory profiling was conducted in two of the three studies attached. In **Paper I**, the PCA was used to explore the sensory-instrumental relationships of spirulina-soy-extrudates that were developed based on a DoE. Instead of describing individual products, it was used to explore the general impact of extrusion parameters and to consequently derive recommendations for the future production of extrudates. In **Paper III**, the PCA had a slightly different purpose. Here, it was used to understand the sensory perception of the pasta variants incorporating the spirulina-soy-extrudate at different levels and to specific related attributes. Through that, sensory-based optimization was promoted. Consequently, conventional sensory profiling was used to investigate both food ingredients and the effect of the production process on sensory perception as well as prototype products.

3.4.2 Consumer orientation and acceptance

Important motives that influence whether a product will be accepted or rejected are sensory properties, anticipated harmful consequences and ideational factors, i.e. knowledge about the food origin and the assumption that it is (in)appropriate to eat (Rozin & Fallon, 1987). Understanding both products and consumers is therefore equally important during food product development. Considering consumer opinions and preferences is deemed a success factor to limit the risk of novel product failure on the market (Grunert & van Trijp, 2014; Moskowitz & Hartmann, 2008).

However, there are ambivalent opinions at which stage consumers should be integrated in the product development process. Lilien et al. (2002) describe consumers as a poor source of information based on the limited imagination that results from the contact with the limited number of products that every consumer has previously encountered. In other words: consumers do not know what they want, unless they have faced something similar before. That would imply that consumers' opinion is not reliable before actual products can be presented.

On the contrary, Cox & Delaney (2009) suggest that the involvement of the consumer is of good help to make better products, even if no real products exist yet. The authors assume that consumers have a strong desire to contribute to food product development. Additionally, today's society can be described as multicultural meaning that consumers are getting constantly into contact with many different food cultures and habits, either in their own countries or while traveling (Sijtsema et al., 2002).

Instead of involving consumers at the very beginning of the development process, expert interviews were conducted during the pursuit of this dissertation research. In fact, this approach took into account that different consumer opinions, for instance collected in focus group interviews, could have resulted in a blurred picture of which products should be pursued further. From expert opinions, multiple clear and concrete ideas for promising products were expected. The interviewed experts were initially confronted with photos of a collection of previously compiled product ideas, i.e. sushi, filled pasta, nuggets and crispy bites, jerky, salad, stir fry, polenta or burek-like crunchy sticks (Figure 3.4). Finally, they were asked about their opinions on the possibility and success for such products.

Based on the results from the expert interviews, three of these products were chosen to be exposed to consumer opinions. Consumer opinions were collected based on photos in an online survey. Through the survey, pertaining to sushi, pasta and jerky products it was sought to let the consumer generate the further direction of development (**Paper II**). Instead of only an online survey, consumers have to have the chance to encounter and experience novel products. Experience with food influences preferences as well as requirements and consumers are reported to be less satisfied with mediocre product experiences. An explanation for this phenomenon might be that consumers in Europe are meanwhile used to the circumstance that everything is available in abundance at all times. Consequently, there is no need to compromise on mediocre food experience or just "a belly filling meal" (Cox & Delaney, 2009). That makes acceptance testing in order to produce satisfying products even more crucial. Since filled pasta was the product category of consumer's choice in the online survey (**Paper II**), different formulations were developed on the basis of various theories for the fit of spirulina with different flavoring ingredients (**Paper III**).

Figure 3.4: Ideas, how to process the spirulina-soy-extrudate. Samples were produced and evaluated at internal tastings at DIL e.V. in Quakenbrück (Germany). Photos depict: a) sushi, b) filled pasta, c) nuggets, d) crispy bites, e) jerky, f) salad, g) stir fry, h) polenta or i) burek-like crunchy sticks. Photo credits: proprietary, except for h) Spirulina Academy and i) Oste.

To gain a better understanding of the European market, consumers in Germany, the Netherlands and France were asked for their opinion about the product ideas investigated in **Paper II** and the pasta recipes in **Paper III**. Research pointed out that Europe does not have a homogeneous food culture and that differences exist regarding food preferences, habits, food-related behavior, and attitudes (Askegaard & Madsen, 1998; Guerrero et al., 2009). To take account of such differences in product development, the studies were carried out in various European countries.

The success of spirulina-filled pasta or other spirulina-based foods is obviously influenced by eating habits and food culture. The environment where consumers grow up is decisive when it comes to decide what is palatable or not, particularly when mimicking animal proteins (Hocquette, 2016). No matter if animal or plant origin: the establishment needs time and positive experience to minimize the reluctance to try new food within a broad range of consumers. The triumphal march of sushi into western eating routines is a good example (Cwiertka, 2005). While raw fish and cold rice were culturally unusual in Western Europe only 20 years ago, sushi is meanwhile broadly accepted (House, 2018).

A shift towards plant-based or at least meat reduced diets comes along with either the incorporation of novel foods or ingredients that are not established in diets yet or it revives declining consumption of ingredients like pulses (Guyomard et al., 2012; Schneider, 2002). Product acceptance, however, depends not only on the product properties but also on the ease of adoption of different cuisines and new eating habits, as well as the time needed to change them into familiar ones. In order to optimally accompany this process, food manufacturers are well advised to listen to consumers.

3.4.3 Impact of familiarity and neophobia on acceptance

Individual attitudes among consumers impact the acceptance of novel products. Neophobia, i.e. the reluctance to eat unfamiliar food, is a protective human behavior to avoid eating hazardous foods (Pliner & Hobden, 1992). Familiarity with food and neophobia are closely related and the latter has been shown to have no influence on the extent to which consumers evaluate familiar foods (Raudenbush & Frank, 1999). The authors found that neophobia is particularly noticeable when novel foods are studied. With new and unfamiliar ingredients such as spirulina, it is therefore important to rely on familiar food concepts so that the neophobic behavior does only have a mitigated or even no effect. It is therefore not surprising that consumers chose the most familiar concept, i.e. pasta to be most liked when incorporating spirulina.

A widely spread theme to overcome neophobia with the help of familiarity is the idea that novel protein ingredients should be invisibly incorporated into familiar foods. Research related to processing insects in food for the European palate makes also use of familiarity and invisibility (Hartmann et al., 2015; Verbeke, 2015). The approach to disguise the green-black spirulina-soy-extrudate in familiar pasta was therefore somewhat obvious.

Repeated exposure to food products leads to familiarity and consequently to higher acceptance (Zajonc, 1968). Exposure frequency combined with actually letting consumers taste the product has also been shown to reduce neophobia (Birch et al., 1987). Particularly, repeated exposure has been investigated in relation to plant-based meat alternatives (Hoek et al., 2013) and it has been shown that it bears the chance to increase acceptance by consumers. Nonetheless, initial product experience should be positive and liking should be high in order to increase the chance that actual meat alternatives or spirulina pasta are recurrently consumed.

Further, information about health or taste have been shown to reduce perceived novelty and uncertainty (Martins et al., 1997; Pelchat & Pliner, 1995). Accordingly, product concepts that convey familiarity rather than novelty are seen as beneficial in terms of minimizing the effects of neophobia on the success of novel products.

3.4.4 Marketing strategies for novel products

Product information regarding novel protein foods like spirulina pasta is conveyed best with coherent marketing concepts highlighting the benefits of spirulina. Product-oriented marketing approaches that attract and attain consumers and lead to a positive perception have a key role during the establishment of novel protein sources (de Boer & Aiking, 2019). Spirulina's potential to contribute to a better environment, to a healthy diet as well as its innovative character should be at the forefront when marketing this novel product, as there are not yet comparable products available. As Wild et al. (2014) already argued with respect to LikeMeat products, alternative products are better off if information is provided that helps consumers to understand deviations from conventional products. That means that alternative products are tolerated as being different from the norm in order to meet the different needs and expectations of consumers if consumers have a better understanding what the products are all about. As with sensory experience, contrast effects in perception should be avoided and care should be taken to ensure that expectations provoked through marketing concepts fit with experience. Sensory appeal, health, convenience and price are the most important factors related to food choice (Steptoe et al., 1995). While appearance, taste, convenience and price are rather obvious for consumers to experience as they are delivered by its features, health and environmental friendliness are valued by consumers but cannot be verified - so called credence characteristics. Credence characteristics can be communicated through packaging, labels and claims (Grunert & van Trijp, 2014). Both experience and credence characteristics have to match with consumer beliefs so that products with spirulina will be widely accepted.

When establishing marketing concepts for alternative products human health and environmental sustainability should be intertwined, e.g. through naturalness (Hoek et al., 2017b; Willett et al., 2019). However, health orientation of foods contributing to meat reduced dietary guidelines helps consumers to consider a dietary change regarding aspects other than only environment (Behrens et al., 2017; Weinrich & Elshiewy, 2019). That health is more relevant for consumers than the environmental impact of food has once more been shown in the comparison of the three marketing concepts investigated in **Paper II**. Innovation only attracts consumers combined with truly innovative and supposedly unfamiliar foods like jerky.

Also relevant in terms of marketing of novel products is the fact that the whole algal biomass was used to produce the pasta, with all its aromatic pitfalls. This originates from the idea that processing the natural ingredient without concentrating or isolating any specific compounds would add to the natural and thereby healthy and environmentally friendly image of such products.

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Paper I

5 Paper I: Towards more sustainable meat alternatives: How technical parameters affect the sensory properties of extrusion products derived from soy and algae

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Abstract

High moisture extrusion cooking is a well-established method to develop soy-based meat alternatives. Algae are increasingly researched into as an alternative protein source, particularly the micro-algae spirulina (*Arthrospira platensis*) is known for its high protein content. However, little is known how spirulina behaves during the extrusion process. This study investigates the sensory properties of spirulina (10 %, 30 % or 50 %) and soy-based meat alternatives (16 samples) produced according to a Design of Experiment (DoE) with different levels of moisture (57 %, 67 % or 77 %), screw speed (600 rpm, 900 rpm or 1200 rpm), temperature (140 °C, 160 °C or 180 °C) and two production days (independent replicates). Conventional profiling by a trained sensory panel (n = 12) based on a product-specific vocabulary was carried out and the samples were also subjected to instrumental texture profile analysis (TPA) and razor shear measurement (MORS). The data revealed that high spirulina content caused a black color, an intense flavor with earthy notes and an algae odor that is rather musty. Additionally, the higher the share of spirulina, the lower were the elasticity, fibrousness and firmness of extruded samples. High moisture content resulted in products evoking a juicy and soft mouthfeel plus a moist appearance. Instrumentally measured texture parameters matched with the sensory profile regarding texture: high moisture induces a decrease in shear force, shear energy, hardness, cohesion, springiness and chewiness. Particularly firmness perceived by the panel correlates with both MORS and TPA measurements while sensory elasticity correlates with springiness. When keeping moisture low, but screw speed and temperature high during extrusion, it is possible to partly substitute soy with spirulina in order to produce firm and fibrous products with a decent algae flavor that will contribute to shape a more sustainable future for food.

Introduction

The world food supply needs to increase by 70 % to feed expected 9 billion people until 2050 (Hermansson & Lillford, 2014). To provide enough nutrients for everybody, world food supply needs to undergo considerable change within the next 30 years. To feed a plus of 1.5 billion people, incremental changes, e.g. changing livestock diets (Röös et al., 2016), may not be enough but ground-breaking changes must be implemented into various cultures (van der Linden & Foegeding, 2015). The establishment of applications based on alternative protein sources like microalgae represent such a change which may help to solve one of the most urgent tasks in the near future: producing a sufficient supply of protein in a sustainable manner. Most animal-derived proteins are more easily digested by humans than plant-derived proteins (FAO/WHO, 1991). Meat and other livestock products are preferred foods in most countries, not only due to their high nutritional quality and sensory aspects like taste and tenderness, but also because the entire animal protein production system has been able to respond to the growing demand through an increase in animal numbers and productivity (Boland et al., 2013; FAO, 2006; Grunert et al., 2004).

Consequently, livestock production accounts for ca. 12 % of global greenhouse gas emissions (Westhoek et al., 2011). The cultivation of protein-rich plants, like soy, peas, lupines, etc. for feed and food is not necessarily more sustainable than meat production. Particularly the high land occupation in South America puts environmental pressure on impaired ecosystems of main producing countries like Brazil or Argentina (Meier et al., 2014). To sustainably feed the growing world population we need to grow more on the same amount of land while using less water, energy, and chemicals, which means substituting animal proteins with non-animal proteins (Di Paola et al., 2017). The adoption of genetic modifications has largely resulted in productivity gains of plant protein production (Fedoroff, 2015); however these solutions are not without their own problems. The large impact of protein production on the environment needs to be reduced accordingly to remain sustainable within our planetary constraints (Rockström et al., 2009).

A protein source is considered as more sustainable if it can be grown on acreage that is otherwise not arable, if it has low water expenditure, and if it can maintain high yields (Pimentel & Pimentel, 2003; Tilman et al., 2002). It should not be unnoticed that a protein source alone does not define a sustainable diet. The underlying concept is rather a shift in thinking about foods as to become accessible, affordable, environmentally sustainable and culturally acceptable (Johnston et al., 2014). In terms of novel protein sourcing, algae come into play so as to enlarge the repertoire accessible to human consumption and to enable substitution of plant proteins which have a substantial impact on the environment, e.g. soybeans (Taelman et al., 2015). *Arthrospira platensis*, hereafter referred to as spirulina, contains up to 63 % of protein based on dry matter (Becker, 2007) while soy beans contain on average 35 % to 40 % of protein (Malav et al., 2015). Up to 20 % of the protein fraction consists of phycocyanin (Liao et al., 2011) which is known for its high antioxidant activity (Hirata et al., 2000). Besides, spirulina, which is classified as cyanobacterium, is one of few algae amenable to large scale production, not least because it grows in an alkaline milieu which is unfavorable for traditional crops (Devi & Venkataraman, 1984; Fradique et al., 2010; Habib et al., 2008). The high pH of an alkaline habitat implies that the growth of most other organisms is prevented so that spirulina grows in more or less pure cultures (Small, 2011). Furthermore, the environmental footprint of spirulina protein compared with traditional protein crops is smaller in terms of water use, land occupation and energy consumption (Habib et al., 2008). Therefore, spirulina, as a non-animal-based protein, is well posed to decrease our overall protein-based environmental impact (Di Paola et al., 2017; Smetana et al., 2017) while still maintaining important sensory aspects.

To smoothen the way for spirulina into consumers' diets, a procedure to process the biomass needs to be established. High moisture extrusion cooking is already a well-established production method in the food industry to process plant proteins into food products with a meat-like texture (Asgar et al., 2010; "Sustainable Protein Production and Consumption: Pigs or Peas?," 2006). Extrusion is a continuous process comprising mixing and kneading through rotating twin screws within a tightly fitting barrel and final shaping and structure generation while cooling through a die. The texturization occurs due to structural modifications such as denaturation, aggregation and disruption of the protein structures under high shear, pressure and temperature (Akdogan, 1999; Bouvier & Campanella, 2014; Žilić et al., 2014). After the processing zones, the melted protein material enters the cooling die. Due to the shear actions, the molecules are aligned in laminar flow manner and phase transition from liquid to gel state takes place which finally causes fiber formation (Noguchi, 1990). Resulting products are often referred to as meat analogs (Lin et al., 2000; Liu & Hsieh, 2008) or meat alternatives (Wild et al., 2014). For the extrusion process of meat analogs, a strong research focus was put on the three protein-rich ingredients soy, wheat gluten and pea. These plant protein concentrates or isolates are meanwhile well known for their good properties regarding the formation of fibers that resemble chicken or turkey breast (Yao et al., 2004).

However, little is known how spirulina biomass behaves during the extrusion process. A pretest showed that a 100 % replacement of soy by spirulina will not lead to a desired product by extrusion, most of all due to lacking fibrousness and firmness. Therefore, this study investigated the sensory and textural properties of spirulina and soy-derived meat alternatives that were systematically produced according to a Design of Experiment (DoE) with different levels of a mixture of spirulina and soy, moisture, screw speed, temperature and a batch replicate factor.

The main research questions in this study were

i) To what extent do the extrusion parameters temperature, screw speed and moisture influence the sensory properties of meat alternatives derived from soy and spirulina?
ii) How much soy can be substituted with spirulina during extrusion without compromising sensory properties?
iii) How are sensory texture attributes correlated with instrumental ones?

We hypothesized that higher production temperature and lower moisture both additively promote fiber formation (Cheftel et al., 1992; Liu & Hsieh, 2008) and improve textural properties towards a texture resembling poultry meat in firmness and elasticity – even if spirulina is present. Secondly, higher moisture is expected to cause less springy and cohesive samples (Lin et al., 2002). Thirdly, we expected a higher content of spirulina biomass to cause visually darker samples and a pronounced aroma profile without affecting the textural properties. Finally, it was assumed that the analytical texture attributes of the samples are correlated with the sensory data.

Material and methods

Experimental design and sample production

The experimental design varied spirulina content [%], temperature [°C], screw speed [r/min] and moisture [%]. The extrusion parameters (Table 5.1) and their levels were selected according to literature (Lin et al., 2000, 2002; Yao et al., 2004) and based on expertise and pretests done by the German Institute of Food Technology (DIL e.V.). Spirulina was mixed with a "structuring" protein (soy) that is known for its good textural properties (Akdogan, 1999; Arêas, 1992; Cheftel et al., 1992; Kumar et al., 2017; Lin et al., 2000; Yao et al., 2004).

Table 5.1: Extrusion parameters of meat alternatives with spirulina.

	Factor Levels		
Spirulina [%]	10	30	50
Temperature [°C]	140	160	180
Screw Speed [r/min]	600	900	1200
Moisture [%]	57	67	77

In order to investigate the repeatability of the extrusion process (i.e., the batch effect), the four factors of Table 5.1 were enhanced by a replication factor (two production days). A full combination of all extrusion parameter factor levels of Table 5.1 with a replication would have resulted in 162 products. Through the use of a Design of Experiments (DoE; e.g. Carlsson and Martinsson, 2003; Kuhfeld et al., 1994), only 16 different samples had to be produced (Appendix A). A major advantage is the possibility to estimate the properties of 162 products by evaluating just 16 of them by a linear model. The DoE was created as an orthogonal design with the help of IBM SPSS Statistics 23 (IBM Corp., 2015). The three levels of spirulina content, temperature, screw speed and moisture were construed as respective linear continuous factors (-1,0,1), whereas the two production days were effect coded (-1,1).The design is not balanced, i.e. not every factor level occurs equally often, but main effects have perfect zero correlations and two-way interactions only weakly correlate with main effects. Thus it is possible to estimate the main effects of the different factors with sufficient reliability.

Functional soy protein concentrate ALPHA 8 IP was obtained from Solae, LLC (St. Louis, Missouri., U.S.A.). Spirulina premium II powder was produced in open ponds in Asia and procured as complete dried biomass via the Institute for Food and Environmental Research (ILU e.V.) in Nuthetal, Germany. According to the manufacturer's data, spirulina biomass contained 6 % of moisture, 70.3 % of protein and 6.4 % of fat. Soy protein concentrate contained 4.6 % of moisture, 66.5 % of protein and 2 % of fat. Extrusion trials were conducted using a twin screw extruder (ZSK 26 Mc, Coperion). The diameter of the screws is 25.5 mm and the external / internal diameter ratio is 1.55. The length of the extruder process part is 700 mm. The extruder has 9 processing zones and the temperatures in these zones were set at 40, 60, 90, 160, 160, 160, 150, 130 and 125 °C, respectively. The temperature of the 4^{th}, 5^{th} and 6^{th} processing zone was modified in our study, as these zones are considered to be important zones for texturization. At the end of the processing part, a cooling die is attached where the laminar flow fibrous structure formation takes place. The internal dimensions of the cooling die are 4.8 × 1.2 × 100 cm (W × H × L). The blends of soya protein concentrate and spirulina biomass were mixed using a blender (Stephan Machinery GmbH, Germany) before being transferred into the feeder and then extruded. Raw material feeder and water pump were set according to the desired moisture. That means that water is injected into the mix of soy and spirulina powder prior to the actual extrusion. The water content of the samples after extrusion was calculated based on the weight of the extrudates. For instance, if 16 kg of a sample with 57 % of moisture is aimed to be produced, 6.9 kg of powder were processed and 9.1 kg of water were injected. An additional assessment of the moisture of the samples was done. To do so, the samples were defrosted over night at 4 °C. A weighed portion of 5 g per sample was put on filter paper and dried at 105 °C over night in a compartment dryer (Heraeus Holding GmbH, Germany). The dried samples were weighed again and dry mass as well as moisture content were determined. The measured moisture of the 57, 67 and 77 % moisture samples was 57.6 % (std 4.0 %), 64.6 % (std 2.8 %) and 68.7 % (std 2.0 %). With Pearson's correlation coefficient of $r = .84$ ($p < .0001$), the calculated and measured moisture differ somewhat, which might be due to freezing and defrosting the samples or due to irregular fluctuation in the extrusion process.

Conventional sensory profiling

In the present study, conventional profiling was conducted not only to describe the samples qualitatively but also to quantify the intensities and magnitude of the sensations (Costell & Duran, 2009). With novel products that as such do not yet exist on the market, a conventional profile seemed to be most appropriate to thoroughly describe the product space.

The sensory panel consisted of 12 panelists who were selected according to ISO 8586-1 (ISO, 1993). In order to perform the descriptive analysis, they were trained compliant with DIN 10967-1 (DIN, 1999). The panel leader served as a facilitator to structure and guide the sessions and did not participate in the profiling. A pre-selection of descriptive vocabulary through the panel leader for the samples was on the one hand based on the samples and their inherent differences and on the other hand on the literature available on texture properties of meat alternatives (Elzerman et al., 2013; "Sustainable Protein Production and Consumption: Pigs or Peas?," 2006) as well as processed meat products (Beilken et al., 1991). The pre-selection of attributes saved time during training and guided the panel towards objective texture evaluation. The panelists generated the final list of descriptors through consensus to ensure agreement upon the attributes. During training sessions, samples from the sample set and other foods were served (Table 5.2) to exemplify stimuli for the consensus language development and to define the exact sensory concept behind each attribute term (Costell & Duran, 2009; Stone et al., 2012). The list of descriptors comprised odor (O_), appearance (A_), flavor (F_), texture (TX_), mouthfeel (M_) and aftertaste (AT_) attributes (Table 5.2). Panelists decided on the references, the definitions and the order of assessment of the descriptors.

During training, references were fixed on the scale through consensus, i.e. the panel agreed on the intensities of the reference samples and thereby learned how to set the samples under investigation into relation.

As a following step, the eating regime had to be established; i.e. to standardize how often is chewed on the samples until a certain term is evaluated and how, for instance, the fork is handled to divide the sample into pieces (Beilken et al., 1991; Civille & Liska, 1975; Civille & Szczesniak, 1973; Muñoz, 1986).

To evaluate each descriptor, the panelists used unstructured line scales with varying verbal anchors (Table 5.2). During the training, focus was put on discriminability between samples, consensus amongst panelists regarding the samples and the ability to repeat the evaluation of the products. The training was held during ten sessions comprising a total of 18 h, followed by five sessions for data collection where the sample set was assessed in duplicate by each panelist in a randomized order. During measurement sessions, each panelist sat isolated in individual booths in the sensory lab, constructed in concordance with ISO 8589 (ISO, 2007). Both training and measurement took place in the sensory lab of the Georg-August-University in Goettingen where the panel met twice a week for 90 to 120min.

Table 5.2: Overview of attributes, scales, reference products and the assessment of the attributes.

Attribute	Scale (Anchors)	Reference [4]	Assessment
O_overall	weak – strong	sample 1132[5] (rather weak); sample 3321 (strong)	smell the bottom part, holding it 2cm under the nose and sniff three times, 15sec inter stimulus interval
O_algae	weakly perceivable – strongly perceivable	sample 1132 (rather weakly perceivable); spirulina powder (strongly perceivable)	
O_musty	not perceivable – strongly perceivable	sample 1132 (rather not perceivable); old grass, 4 days on compost (strong)	
A_color	green – black	solution of 0,04g of a mix of yellow and blue color (green); solution of 0,098g of a mix of yellow and blue color (black)[6]	visually, standard daylight in the booths
A_layered	not perceivable – strongly perceivable	sample 2213 (not perceivable); surimi (strongly perceivable)	extent to which single layers are visible
A_moist	dry – moist	sample 3321 (dry); sample 2213 (moist)	gloss of the surface and wet stain on the plate
TX_elastic	not elastic – strongly elastic	sample 2213 (not elastic); sample 1132 (rather strongly elastic)	ability of the sample to relax from applied pressure, pressed with finger
TX_firm	soft – firm	sample 2213 (soft); sample 3321 (rather firm)	coherence of the sample, assessed by sectioning off a piece and evaluating the force needed
TX_brittle	not brittle – strongly brittle	sample 2213 (not brittle); sample 3321 (rather not brittle)	assessment of the edge after sectioning a piece, a brittle edge is uneven
TX_fibrous	not fibrous – strongly fibrous	sample 2213 (not fibrous); chicken meat, cooked for 20min (strongly fibrous)	breaking a strip and assessment of the fiber formation
F_overall	weak – strong	sample 1132 (rather weak); sample 3321 (strong)	flavor intensity overall after 5 chews
F_chicken	not perceivable – strongly perceivable	sample 2213 (rather not perceivable); chicken meat, cooked for 20min (strongly perceivable)	flavor intensity after 5 chews
F_earthy	not perceivable – strongly perceivable	sample 1132 (rather not perceivable); fresh champignon (strongly perceivable)	flavor intensity after 5 chews
M_juicy	dry – juicy	chicken meat, cooked for 20min (dry); sample 2213 (juicy)	ability to release moisture during chewing, assessed after 5 chews
M_soft	not perceivable – strongly perceivable	sample 3321 (rather not perceivable); sample 2213 (strongly perceivable)	ease of chewing, assessed after 5 chews
M_crumbly	not perceivable – strongly perceivable	sample 2213 (rather not perceivable); sample 3321 (rather strongly perceivable)	the extent to which the sample breaks into many small pieces while chewing, assessed after 5 chews
AT_overall	weak – strong	sample 1132 (weak); sample 3321 (strong)	aftertaste intensity overall 5sec after swallowing
AT_umami	not perceivable – strongly perceivable	sample 1132 (rather weak); solution of 3.6g/l MSG (strongly perceivable)	aftertaste intensity of umami 5sec after swallowing

[4] "Rather" refers to a fixation at 0.25 or 0.75 scale points, respectively.
[5] For comprehensive understanding of the sample names, the reader is referred to the example in Appendix A of this paper
[6] According to DIN 10961 (DIN, 1996) by the German Institute for Standardization, which is based on ISO 8586-1 (ISO, 1993).

Test protocol

After production, the samples were cut and vacuum packed in portions of 300 g to serve 12 panelists with one package per session. Samples were kept frozen (-18 °C) until two days before a panel session. To defreeze them, they were put in the refrigerator overnight at 4 °C. The next day, the samples were cooked since this is a necessary step when processing products derived from high moisture extrusion cooking (Lin et al., 2002); the heat during cooking minimizes the risk of contamination during further processing. Prior to cooking, the extruded sample was cut into strips of 10 g each. One liter of water was set to the boil and then to simmer (~95 °C) on an induction stove. The strips of one package were put into the simmering water and boiled for 10 min. Three out of four samples with a moisture content of 77 % had to be cooked for just 5 min; a longer cooking duration would have destroyed the compact structure completely. Since all samples had to be presented to the panel in a comparable way and the DoE was dependent on the inclusion of all samples to predict the sensory profile of the entire product set, these samples could not be discarded. After cooking the samples cooled down at room temperature (~21 °C) in aluminum bowls. Then they were tightly covered with cling film and kept in the fridge at 4 °C until the next day. To prepare the panel session, the samples were put on small white plates and assigned with the corresponding 3-digit code. One serving per panelist consisted of two pieces à 10 g (20 g in total) at room temperature (Figure 5.1).

Figure 5.1: To the left, the extruder is shown, while one of the spirulina-soy extrudates is produced. This "endless" string of sample was cut into blocks of 300 g from which the strips of 10 g (right picture) were cut prior to cooking for sensory evaluation.

Panelists were instructed to use one of the pieces for tasting and the second one for assessment of texture attributes. They were provided with water and white bread to neutralize their sense of taste and coffee beans to neutralize the sense of smell. When measuring the product attributes, the inter-attribute interval was set to be 15 seconds while the inter-product interval was set to two minutes before the next sample was served.

Instrumental texture analysis

A texture profile analysis (TPA) and a Meullenet-Owens razor shear force analysis (MORS) of the 16 samples were conducted with a texture analyzer (TA.XT plus, Stable Micro Systems). Particularly the first is a common technique investigating the texture of extruded products (Ahirwar et al., 2015; Kitcharoenthawornchai & Harnsilawat, 2015; Li et al., 2005; Lin et al., 2000). The latter was included since it is more convenient yet as reliable as other standard methods (e.g., Warner Bratzler shear) to measure tenderness of poultry meat (Lee et al., 2008).The elimination of the sample cutting steps needed for Warner Bratzler shear, but not for MORS speeds up the measurement preparation.

Before analyses the samples were handled identically as for the sensory analyses regarding cooking, cooling and storage. Each measurement was repeated five times per sample and the average of these values was the basis for statistical analyses.

Texture profile analysis (TPA)

The TPA was done according to a modified version of the method of Lin et al. (2000). Five cylindrical pieces (25 mm diameter) per sample were punched from the center of squared 5 cm x 5 cm pieces. Each cylindrical piece was compressed twice to 50 % of the original height by a compression probe (P/75) at a trigger force of 5 g. The load cell used was 50 kg and the crosshead speed during the measurement was set at 0.5 mm/s. The time between the two compressions was set at 0.01 sec. The measurements were analyzed using the preinstalled macro “simplified TPA”. The parameters hardness [N], springiness, chewiness [N] and cohesiveness were recorded as is customarily done in texture profile analysis.

Shear force with Meullenet-Owens razor shear (MORS)

Tenderness is one of the most relevant texture attributes of meat when it comes to consumer perception (Owens et al., 2004; Xiong et al., 2006). MORS was the method of choice to measure this attribute. Even though the panel agreed not to include tenderness or its opponent toughness (Szczesniak et al., 1963) into the list of attributes for sensory profiling, it still appears worthwhile to instrumentally investigate tenderness which is also a widely used measure to characterize meat in the industry (Lee et al., 2008).

Regardless the fact that MORS does not require homogeneous sample dimensions (Lee et al., 2008), a 5 cm x 5 cm piece per sample was penetrated five times with the razor blade to gather both shear force and energy. The fiber orientation was vertical towards the horizontal blade. It was made sure that the cuts were located at least 0.5 cm from the edge of the sample. The crosshead speed was 1 mm/s before, 2 mm/s during and 10 mm/s after the test, respectively; the load cell was 50 kg. The trigger force was set at 5 g and the razor blade (height 24 mm; width 8 mm) moved 15 mm through the sample at a sample height of 10 mm. The blades were exchanged after 100 tests to ensure sharpness.

The maximum shear force [N] recorded is the highest peak of the curve and can be interpreted as the highest resistance of the sample to shearing (Ruiz De Huidobro et al., 2005), while the shear energy [N*mm] was recorded as the area under the force-deformation curve.

Statistical analysis

Panel performance was checked using the reliability measure Cronbach's alpha (CA) which reflects the consensus of the panelist for a given attribute across the 16 samples. CA of 0.7 or better imply consensus of the panel as an "analytical instrument" (Pinto et al., 2014). The so called corrected item-total correlation, which in this setting is the panelist-panel correlation, assesses whether a panelist's evaluation correlates with the rest of the panel. That way, panelists with low consensus might be discarded.

In order to determine the effects of the process parameters (spirulina content, temperature, screw speed, moisture and batch) on product perception linear mixed models (SPSS procedure GLMM with normal distribution and identity link settings) were calculated per attribute. Fixed effects were afore mentioned process parameters as continuous factors at level 1 while random effects were panelists at level 2 and replicates nested in panelists at level 1.

The resulting fixed effects' coefficients indicate how the sensory attributes will change if the extrusion parameters are increased by one unit and whether this impact is significant

(p < .05). Based on the coefficients from the GLMM of the set of 16 samples, the sensory profiles for all 162 products were estimated. As a final step, a Principal Component Analysis (PCA) was calculated across the 162 estimated products to visualize the sensory space of products and attributes. The process parameters are passively included in the map.

To study the relationship between the textural parameters with one another and with the sensory parameters, the textural parameters of 162 products were estimated by model coefficients from the fixed effects of the five process parameters in a two-level linear mixed model with the five replicate assessments nested in the 16 samples. Pearson correlation coefficients were calculated amongst the estimations across the 162 products.

All analyses were done using IBM SPSS Statistics 23 (IBM Corp., 2015) and Microsoft Office Excel 2010.

Panel performance check

Cronbach's alpha resulted at 0.7 except for earthy flavor (F_earthy, α = 0.6); and for the brittle texture (TX_brittle), Cronbach's alpha is even negative. That implies that the panel is not a reliable tool to measure the attribute brittle adequately, be it because the references were not sufficient for the panelists to learn what to look for when evaluating it, because more training would have been needed or because the samples actually did not vary in terms of brittleness. For the 16 other attributes the panel measurements were reliable.

Besides, the panelist-panel correlation per panelist and descriptor was assessed to determine the panel performance. A correlation value less than 0.3 indicates that the corresponding measurement of the panelist did not correlate very well with the scale of the panel in general (Field, 2009). The already known disagreement regarding the attribute TX_brittle is reflected by 10 out of 12 correlations below 0.3. A tendency of discordance for F_earthy is shown by six panelist-panel correlations lower than 0.3.

Correlations below 0.3 are reported for four panelists for chicken flavor (F_chicken) and for three panelists for both elastic texture (TX_elastic) and crumbly mouthfeel (M_crumbly). It is essential to note that on average all corrected panelist-panel correlations exceed the score of 0.3, except for TX_brittle. Based on this result, TX_brittle is discarded from further analysis.

Results and discussion

Estimation of sensory properties of all possible products

As documented in Table 5.3, spirulina content significantly affected all sensory descriptors (p < .05). Odor, flavor and aftertaste got more intense with increasing spirulina content, while texture became less elastic and fibrous but softer. Furthermore, the color got darker and the moist and layered appearance was less visible.

The level of moisture during extrusion affected 16 attributes (p < .05), all except the earthy flavor (F_earthy). Higher moisture increased moist appearance and a soft and juicy mouthfeel. All other attributes - connected to odor, flavor, texture or aftertaste - were less pronounced with increased moisture.

Increased temperature during extrusion modified the constitution of the sample towards higher firmness and fibrousness due to an increased level of protein denaturation. The study by Hirata et al. (2000) investigated, whether antioxidant activity is affected by heating and found that denatured protein is as active as intact protein. However, they do not state the temperature at which they spray-dried spirulina so the implications for extruded spirulina need to be further investigated. The products were significantly less soft and more layered. Maybe less important for further processing but still with a significant impact was temperature regarding increased moist appearance. A high screw speed level increased firmness and layered appearance but made products less soft, less juicy and less moist in appearance. The batch had a main effect on appearance and texture only. The products fabricated on the second day turned out to be less layered and moist in appearance as well as less firm, fibrous and elastic.

Table 5.3: Coefficients (*b*) and p-values (*p*) of sensory attributes and extrusion parameters.

		Spirulina		Temperature		Screw speed		Moisture		Batch	
Attributes	**Constant**	***b***	***p***	***b***	***p***	***b***	***P***	***b***	***p***	***b***	***p***
O_overall	6.18	1.69	***	0.13	n.s.	0.04	n.s.	-0.53	***	0.06	n.s.
O_algae	5.75	1.45	***	0.03	n.s.	0.02	n.s.	-0.30	*	0.07	n.s.
O_musty	3.36	0.78	***	0.08	n.s.	-0.22	n.s.	-0.25	*	0.06	n.s.
A_color	6.93	2.94	***	-0.06	n.s.	0.00	n.s.	-0.66	***	-0.13	n.s.
A_layered	5.57	-0.36	**	0.81	***	0.49	***	-1.64	***	-0.71	***
A_moist	4.79	-0.51	***	0.29	*	-0.36	*	2.04	***	-0.31	**
TX_elastic	3.95	-1.02	***	-0.13	n.s.	0.12	n.s.	-0.38	*	-0.29	*
TX_firm	4.67	-0.46	***	0.54	***	0.63	***	-2.83	***	-0.40	***
TX_fibrous	4.35	-1.30	***	0.60	***	0.24	n.s.	-1.58	***	-0.45	***
F_overall	5.71	1.70	***	0.13	n.s.	0.24	n.s.	-0.69	***	0.10	n.s.
F_chicken	4.63	0.86	***	0.22	n.s.	0.39	**	-0.86	***	-0.03	n.s.
F_earthy	3.99	0.73	***	-0.03	n.s.	-0.11	n.s.	-0.23	n.s.	0.04	n.s.
M_juicy	4.93	-0.59	***	0.19	n.s.	-0.44	**	2.28	***	-0.16	n.s.
M_soft	6.01	0.43	***	-0.25	*	-0.55	***	2.70	***	-0.01	n.s.
M_crumbly	4.94	0.45	**	-0.07	n.s.	0.24	n.s.	-1.31	***	-0.04	n.s.
AT_overall	4.89	2.14	***	-0.13	n.s.	0.14	n.s.	-0.55	***	0.05	n.s.
AT_umami	4.31	2.16	***	-0.04	n.s.	0.08	n.s.	-0.28	*	0.00	n.s.

*** $p < .001$; ** $p < .01$; * $p < .05$; *n.s.* $p \geq .05$

High spirulina content and high levels of screw speed intensified the flavor of chicken while higher moisture diminished it. Temperature and batch had no significant effects. Even though comprehensive knowledge on the thermally induced formation of flavor in meat exists (Mottram, 1998), such insights are lacking for meat alternatives in general. The Maillard reaction is usually discussed in the flavor development. Various studies show that Maillard reaction occurs during extrusion (Arêas, 1992; Yaylayan et al., 1992; Žilić et al., 2014) and it is reasonable to assume that, since spirulina is a complete biomass containing sugars and proteins, the chicken flavor stems from Maillard products. An instrumental aroma compound analysis would help to clarify this and to better understand what spirulina and soy-based extrusion products and chicken meat have in common to provoke this association. Such an analysis would also shed light on the compounds provoking the earthy flavor, which was rather difficult for the panel to measure objectively.

Based on the model obtained from 16 DoE-based samples, the sensory attributes for all 162 possible factor combinations were estimated by applying the coefficients from Table 5.3. With the estimated sensory profiles of the 162 products, a PCA of the 17 attributes was conducted. It clearly revealed two dimensions (96 % of variance explained, whereas the third Eigenvalue was below 1.0), presumably due to the common sources of the estimations.

The PCA map is displayed in Figure 5.2. The location of the dependent variable (sensory attributes ♦) and passively added independent variables (process parameters ★) in the plot mark the direction of the correlational vector, i.e. of respective high intensities. The respective low intensity pole locates in the opposite region of the plot. For example, products low in moisture are positioned in the upper right part of the plot while products low in spirulina are located in the lower left part of the plot. Generally, it can be noted that products located close to each other are more similar than products located on opposite areas of the plot. The closer a sample is located in the same direction as an attribute the higher the intensity of the respective attribute.

The first dimension in Figure 5.2 describes products according to their spirulina content. Products located at the lower right part of the plot contained 50 % spirulina whereas products at the upper left part consisted of 10 % spirulina and 90 % soy. High spirulina content resulted in black color (A_color). With its intensive aroma profile with earthy notes and a musty odor, the microalga was influential regarding overall flavor intensity (F_overall), algae and musty odor (O_musty, O_musty) and the aftertaste of the products turned out more pronounced.

Products produced with only small amounts of spirulina were estimated to be elastic, fibrous and firm due to the advantageous properties of soy during extrusion (Arêas, 1992; Liu & Hsieh, 2008). Also Wild et al. (2014) and Kumar et al. (2015) underlined the good textural properties of soy during extrusion.

As can be seen at the bottom part of Figure 5.2, high moisture resulted in moist appearance (A_moist) and products had a distinct soft and juicy mouthfeel (M_soft, M_juicy). The negative relation of moisture content with TX_firm and TX_fibrous qualified these products as less firm and less fibrous, which implies to keep moisture low to support fiber formation. Products in the lower left part of the plot contained 77 % moisture while the products to the upper right were produced with 57 % moisture. Products with medium to high moisture and high spirulina content were to be found at the bottom right of the plot.

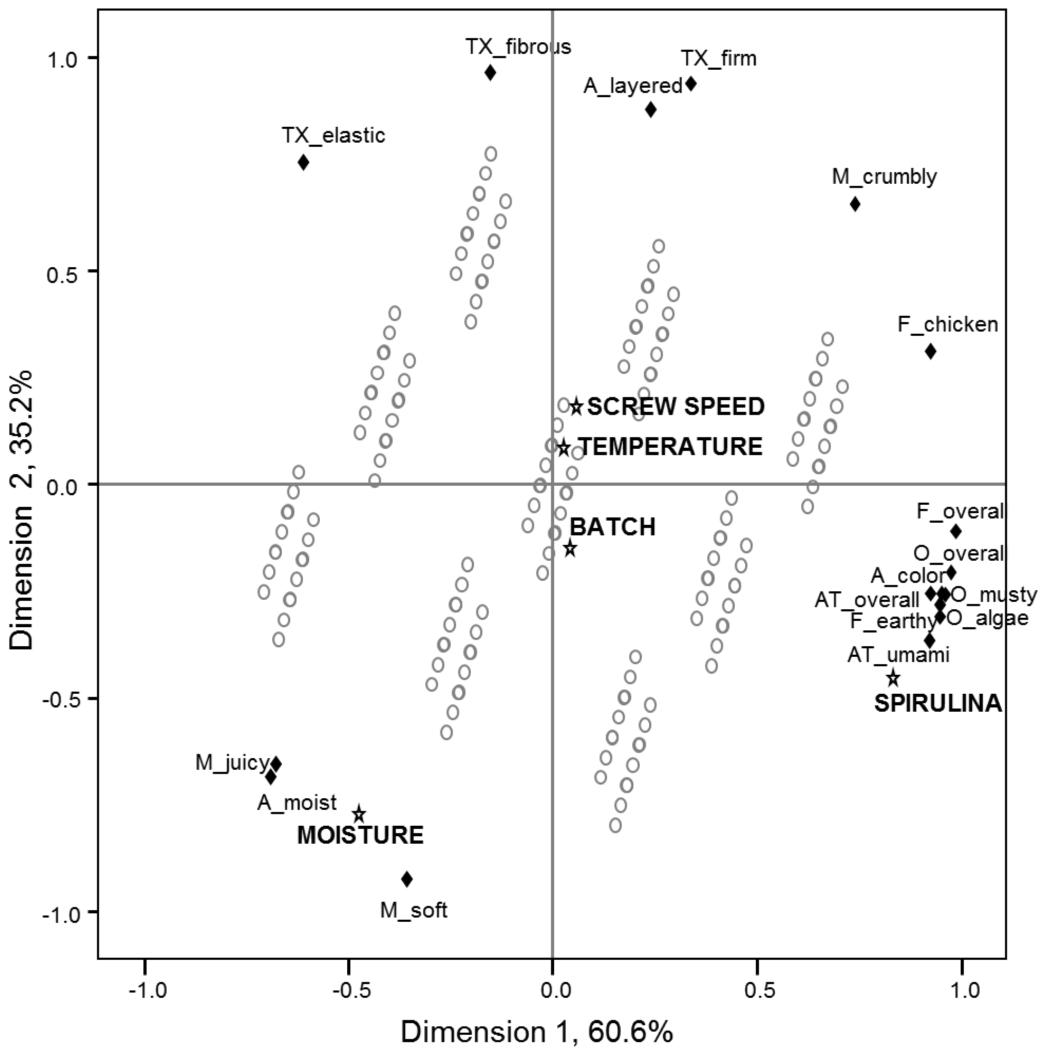

Figure 5.2 : Bi-Plot of a Principal Components Analysis (PCA) of the estimated profiles for all 162 possible products given the four process factors. Symbols: ○ = products, ♦ = attributes, ★ = process parameters (passive).

Within each moisture by spirulina cluster, products manufactured with high screw speed and high temperature level are oriented to the top right while products produced with low levels of these process parameters are oriented to the lower left. Screw speed and temperature should influence texture positively due to an increase in total energy input (Osen et al., 2014). Indeed products that were made with a high screw speed and high temperature were obviously more firm (TX_firm) and appeared more layered (A_layered). Products produced at high temperature appear to be more fibrous (TX_fibrous). However, in dry extrusion, a positive interaction effect of temperature and screw speed on expansion and the water solubility index was found by Joshi et al. (2014). Rehrah et al. (2009) found the same interaction on expansion in peanut-based meat alternatives derived by high moisture extrusion cooking. Interaction effects, however, cannot be studied based on the present DoE which only allows analyzing the main effects.

Besides, there appeared a small batch effect (Figure 5.2 and last column of Table 5.3). It implies that the product condition is subject to minor day-to-day changes. Based on this finding, the source of this effect needs to be identified to secure consistent product quality. As Li et al. (2005) described it, water is injected and then the premix of soy and spirulina powder is introduced. Usually, no defined amount of water is used but the moisture is recalculated based on first samples and then adjusted accordingly. This is assumed to be the explanation for changes in batch constitution.

As can be summarized based on the PCA (Figure 5.2), spirulina content and moisture overruled the screw speed and temperature levels in terms of sensory discrimination. When keeping the moisture low while screw speed and temperature are high, it is possible to obtain firm and fibrous spirulina products with a distinct algae flavor.

Relationship between sensory perception, process parameters and texture analysis

Moisture level during extrusion had a significant effect on analytically measured texture attributes (Table 5.4). This underlines the aforementioned suggestion to decrease moisture during production to support intended texture formation. Products with higher moisture content during extrusion were less hard, less springy, less chewy and less cohesive. A similar finding could be noted regarding shear force and shear energy: the more moisture during extrusion, the less force or energy to break the structure was needed. Higher moisture content is thought to cause incomplete texturization (Lin et al., 2002) which might be the explanation of a measurable decrease regarding textural properties. In turn, it is noted that with 57 % moisture, the shear force lies between 4.9 and 16.6 N (mean value 8.7 N), while the force to cut chicken meat is app. 1.5 N higher. The shear energy lies between 37 and 117.3 N*mm (mean value 79.1 N*mm) which is reported to be app. 50 N*mm higher for chicken breast (Cavitt et al., 2005, 2004; Lee et al., 2008; Xiong et al., 2006).The spirulina content, however, only affected springiness. It is likely that soy lent the products the capacity to return to original size/ shape after compression; springiness has been attributed to meat analogs since their early phase of development (Elzerman et al., 2013).

Table 5.4: Coefficients (*b*) and p-values (*p*) of texture attributes and extrusion parameters.

		Spirulina		Temperature		Screw speed		Moisture		Batch	
Attributes	**Constant**	***b***	***p***	***b***	***p***	***b***	***p***	***b***	***p***	***b***	***p***
Shear force [N]	5.13	0.54	n.s.	0.20	n.s.	-0.52	n.s.	-3.36	***	0.18	n.s.
Shear energy [N*mm]	49.81	5.85	n.s.	2.99	n.s.	-5.24	n.s.	-28.68	***	0.77	n.s.
TPA_Hardness [N]	6009.87	571.23	n.s.	128.60	n.s.	51.39	n.s.	-4397.23	***	869.88	n.s.
TPA_Cohesion	0.59	-0.02	n.s.	-0.01	n.s.	0.01	n.s.	-0.04	**	0.02	n.s.
TPA_Springiness	68.87	-4.71	**	-1.92	n.s.	0.00	n.s.	-4.86	**	0.17	n.s.
TPA_Chewiness [N]	2711.77	-78.77	n.s.	-46.49	n.s.	214.24	n.s.	-2398.61	***	411.15	n.s.

*** $p < .001$; ** $p < .01$; * $p < .05$; *n.s.* $p \geq .05$

Some significant correlations between texture analysis and sensory attributes are reported in Table 5.5. Products perceived as firm in texture needed higher shear force (*r* = 0.85, *p* ≤ .01) and energy (*r* = 0.84, *p* ≤ .01) to be cut. Sensory elasticity correlated strongly with instrumental springiness (*r* = 0.84, *p* ≤ .01) since both assessments are based on the extent to which the products return to their original size/ shape after compression (Meullenet et al., 1998). In some publications (Civille & Liska, 1975; Drake et al., 1999; Szczesniak, 1963), the two terms are even used synonymic. The fibrous texture described by the panel correlates strongly with all analytically measured texture parameters.

Table 5.5: Pearson correlation coefficients (r) between texture analysis parameters and sensory attributes (n = 162 estimated products).

	Sensory parameters					
	TX_elastic		**TX_firm**		**TX_fibrous**	
Texture analysis	***r***	***p***	***r***	***p***	***r***	***p***
Shear force	.14	n.s.	.85	**	.58	**
Shear energy	.10	n.s.	.84	**	.57	**
TPA_Hardness	.13	n.s.	.85	**	.56	**
TPA_Cohesion	.49	**	.79	**	.69	**
TPA_Springiness	.84	**	.70	**	.80	**
TPA_Chewiness	.30	**	.90	**	.67	**

** $p < .01$; * $p < .05$; *n.s.* $p \geq .05$

Chumngoen and Tan (2015) explain that instruments only measure the physicochemical properties of products that provide stimuli to human senses while (only) sensory analysis can directly measure human perception. Preferences based on human sensation are what food producers need to respond to and since 'instrumental texture measurements are empirical and do not necessarily "translate" across food products' (Lawless & Heymann, 2010), the two assessments are not interchangeable. Nevertheless, the instrumental texture analysis serves as a valuable validation of the panel data and could be used in future studies to predict the sensory properties of extruded meat alternatives.

Prospective environmental sustainability

Although not investigated directly in this study, environmental sustainability, primarily LCA analyses, is an important aspect for determining the overall benefit gleaned through the production and subsequent consumption of spirulina-based meat alternatives. Di Paola et al. (2017) found that the substitution of animal-based protein with plant protein resulted in reduced resource requirements both for land and water and decimated release of greenhouse gases. This might very well apply for plant-like proteins as from spirulina, too. With spirulina, Smetana et al. (2017) calculated LCA based on different European conditions and showed that depending on the production system, spirulina can have a comparably high impact on the environment, particularly due to heat and energy use for autotrophic cultivation. This is a status quo of the progress of algae cultivation to date. However, research in algae cultivation is a very young field with a history of 65 years (Chaumont, 1993) while the history of conventional agriculture dates back thousands of years. Therefore it stands to reason that the microalgae production chain will develop towards more sustainable approaches in the future. Many cultivation trials are being done, comparing autotrophic cultivation in photo bioreactors, open raceway ponds and heterotrophic cultivation in fermenters (Smetana et al., 2017). Open raceway ponds are the most common way to source spirulina worldwide (Small, 2011), even though these ponds are prone to evaporation and contamination which in turn minimize the chance of sustainable production. To prevent that, canopies or greenhouses (including heating) would be a reasonable solution to prevent from excessive water use[7]. Spirulina cultivation in photo-bioreactors would become more sustainable, if run based on renewable resources or biogas to limit energy expenditure. The extensive research activities in the field indicate that ways will be established to improve the spirulina production and define appropriate conditions. Cultivation will become less resource demanding, e.g. regarding water expenditure and heating but these changes in energy/ production systems take time. According to Greene et al. (2017), microalgae require comparably less land to produce an equivalent

[7] As demonstrated by Dr. Eberhard Bioenergie GmbH & Co. KG in Neustadt-Glewe (Mecklenburg-Western Pomerania), Germany.

amount of yield. And LCA studies will guide efforts to reduce environmental impacts further. After all, sustainability benefits that are currently emerging will be realized for the benefit of our planet in the years to come.

Conclusions

The microalga spirulina is suitable to partially substitute soy in extruded meat alternatives. The content of spirulina (10 to 50 %) and the amount of moisture used during extrusion (57 to 77 %) had the strongest impact on the sensory profile, as well as on the instrumentally measured texture attributes. The temperature of key extruder processing zones (four to six) and screw speed (600 to 1200 r/min) had a minor effect on the constitution of the products.

The results support the hypothesis that a higher content of spirulina biomass leads to visually darker products and a pronounced aroma profile. The hypotheses that lower moisture would promote fiber formation and lead to more springy and cohesive products was proven. The temperature during extrusion had no significant effect on elasticity but supported the production of fibrous and firm products. Lastly, the analytical measurements confirmed the sensory evaluation of the texture.

Based on the results, it has become clear that the well understood properties of soy during extrusion persisted even when adding spirulina as an entire biomass. Amounts between 30 and 50 % spirulina still led to distinctively fibrous, elastic, firm and layered products, if moisture was low. The screw speed and temperature should be at high levels to support texture formation. However, future research needs to take energy expenditure into account as to not reverse the reduced energy input needed by spirulina with higher energy usage for the processing of the soy-spirulina mix. Furthermore, it remains to be shown in future projects how the aroma and texture profile is influenced by further processing of the intermediate products, e.g. by frying or deep frying. As Small, (2011) states, "Spirulina's taste does not justify serving it as a stand-alone item" which would in turn explain why already available products contain only small amounts of the microalga. Even though high spirulina contents are a novelty in the food sector, further product development at this early stage is promising and the flavor profile and potential matching seasoning needs to be further investigated. The sustainability goal of the food development is to replace meat with something that is meat like so that a large share of the western population would be willing to (partly) substituting out meat. As soon as it is possible to reach out to many people who are considering a step towards more sustainable food choices, production system growth and according market growth will additionally support a transition towards a more sustainable food chain.

Conflicts of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Appendix A

DoE Table of 16 different samples. The sample name reflects the corresponding levels (1,2,3) of spirulina content, temperature, screw speed and moisture, respectively. Reading Example: Sample 1123 contains 10 % spirulina (1), was produced at a temperature of 140 °C (1), a screw speed of 900 r/min (2) and with a moisture content of 77 % (3).

	Spirulina [%]	**Temperature [°C]**	**Screw speed [r/min]**	**Moisture [%]**	**Day1 / Day2 of Production**
1111D1	10	140	600	57	Day 1
1111D2	10	140	600	57	Day 2
1221	10	160	900	57	Day 1
1231	10	160	1200	57	Day 2
1132	10	140	1200	67	Day 1
1312	10	180	600	67	Day 1
1123	10	140	900	77	Day 2
1313	10	180	600	77	Day 2
2111	30	140	600	57	Day 1
2331	30	180	1200	57	Day 2
2122	30	140	900	67	Day 2
2213	30	160	600	77	Day 1
3111	50	140	600	57	Day 2
3321	50	180	900	57	Day 1
3212	50	160	600	67	Day 2
3133	50	140	1200	77	Day 1

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Paper II

6 Paper II: Consumer-oriented product development: The conceptualization of novel food products based on spirulina (*Arthrospira platensis*) and resulting consumer expectations

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Abstract

The world population is steadily growing and the demand for protein increases along with it, yet our planetary resources are finite. Spirulina (*Arthrospira platensis*) is an underutilized protein source suitable for human nutrition, and little is known about the use of spirulina as a food and the associated consumer opinion. New product development (NPD) requires early and active participation of consumers for the success of new products; therefore, a mixed method approach was applied to conceptualize (sensory profiling of spirulina extrudates and expert interviews) and then evaluate consumer's willingness to try (consumer survey) three innovative products: pasta filled with spirulina, maki-sushi filled with spirulina, and spirulina jerky. To evaluate the consumer orientation towards novel, spirulina-based products, 1035 consumers from three countries (GER n = 348; FR n = 337; NL n = 350) were surveyed regarding their hedonic opinion about these concepts. A photo of each product was systematically accompanied by a benefit description covering health, sustainability, or innovation. Each consumer sequentially evaluated three combinations thereof (Latin square design). A multilevel model was used to analyze consumers' responses regarding novelty, interest, overall liking, and expected flavor liking. Overall, spirulina-filled pasta was identified as the most preferred product. Mediation analysis revealed that this could be partly explained by familiarity with products in that category (i.e., pasta more than sushi and jerky). In conclusion, all spirulina product concepts would work equally well, if pasta, sushi, and jerky were similarly familiar to the target consumer population. All tested benefits were equally accepted with each product, with the exception that spirulina jerky would have to be marketed as being innovative. Country differences can be neglected.

Introduction

The future protein supply is of major concern, as according to predictions, the world population will reach 8.9 billion in 2050 and peak at 9.2 billion by 2075 (United Nations (UN), 2004). Simultaneously, the worldwide middle class is expanding accompanied by increased living standards which results in a high demand for animal-based protein (Boland et al., 2013). The production of conventional protein, particularly the conversion from plant into animal protein, puts a lot of pressure on land and freshwater resources (Aiking, 2011). Therefore, new approaches to tackle the future protein supply need to be implemented.

The microalga spirulina (*Arthrospira platensis*), with its protein content of 63% dry matter (Becker, 2007), is a promising food ingredient when it comes to enlarging the repertoire of protein sources available for human consumption. While spirulina already plays a role in human nutrition in Africa (Ak et al., 2016; Carcea et al., 2015) and is widely used in parts of Asia due to its health benefits (Vaz et al., 2016), European consumers are not yet familiar with the microalga on a broad scale. Even though spirulina was identified as a "source of nonconventional protein of considerable interest" (Ciferri, 1983) 35 years ago, product prototypes based on new protein sources, like spirulina, rarely exist (Boland et al., 2013). One reason may be the limited knowledge regarding application in recipes and the rarity of convenience products in supermarkets. Most often in western countries, spirulina is used as a food supplement, additive, or dye and is marketed as powder, pills, or capsules (Vaz et al., 2016), while actual foods are less widespread. To promote a broader use of spirulina, the shift from supplement to food ingredient needs to be pushed forward and innovative novel products need to be developed.

Studies show that novel food products are most successful when they are developed on the basis of consumer orientation (Costa & Jongen, 2006; Moskowitz & Hartmann, 2008). In order to create potentially successful consumer-oriented products, novel product concepts incorporating spirulina extrudates were developed based on sensory data since extrusion was shown to be suitable to texturize protein utilizing spirulina (Grahl et al., 2018). Expert interviews were conducted and followed by an online study designed to confront consumers with these concepts and incite their reaction towards these new products. In addition, consumers do not value products intrinsically, but much of the value is derived from the benefits a product delivers through certain product characteristics (Grunert & van Trijp, 2014). Even more so, successful novel food products are hinged on the degree to which they provide consumer-sought benefits (Barrena & Sánchez, 2012). Novel products that carry concrete and relevant benefits, but are not substantially changed from already available products, are likely to be well accepted by consumers (Guerrero et al., 2009). Spirulina inherits various beneficial traits that were of interest in this study, namely, health, sustainability, and innovation.

Spirulina's health benefits relate to plant-derived proteins and polyunsaturated fatty acids, while the protein source is cholesterol-free and also low in calories. Minerals and antioxidants complete the "healthy" nature of spirulina (Tokusoglu & Ünal, 2003). The substantial content of γ-linolenic acid (18:3 ω-6) deserves attention because spirulina is one of the best known sources (Falquet, n.d.). Polyunsaturated fatty acids (PUFAs), of which γ-linolenic acid is one, have been shown to have anti-inflammatory effects (Fan & Chapkin, 1998; Sergeant et al., 2016). Spirulina protein contains all essential amino acids (Gutiérrez-Salmeán et al., 2015) and therefore can contribute to a health-oriented diet.

The sustainability benefits are associated with a localized production potential using marginal amounts of land for production in tanks on land,[8] allowing for increased independence from soy imports that require high land occupation (Meier et al., 2014) and cause greenhouse gas emissions through long transportation routes (Prudêncio da Silva et al., 2010). To date, the technology is still in its infancy stages and the sustainability remains dependent on production conditions (Smetana et al., 2017), but improvements to production technology can be implemented to make microalgae more sustainable in the future (e.g., usage of renewable energy to operate photo bioreactors) (Taelman et al., 2015).

Spirulina as a mostly unknown ingredient in European food products brings about its innovative characteristics. Therefore, products containing spirulina as a main ingredient (not just a supplement) could be framed as a trendy snack with a special taste, an edible innovation, or a culinary highlight. Various publications deal with the incorporation of spirulina in food applications, for example, bread (Ak et al., 2016; Selmo & Salas-Mellado, 2014), cereals and biscuits (Joshi et al., 2014; Singh et al., 2015; Tańska et al., 2017), pasta (Özyurt et al., 2015; Zouari et al., 2011), or probiotic milk drinks (Beheshtipour et al., 2013). However, such food products are seldom commercially marketed, partly because spirulina was shown to have a negative effect on sensory characteristics (Ak et al., 2016; Beheshtipour et al., 2013).

[8] As demonstrated by various companies in Western Europe which are producing spirulina (e.g., Roquette-Klötze in Klötze (Saxony-Anhalt), Germany).

Finally, although the protein gap problem is Europe-wide; there remain culturally and regionally dependent food product perceptions and consumption attitudes and behaviors (Askegaard & Madsen, 1995; Guerrero et al., 2009). A standardized novel food product could be marketed throughout the continent or different products may appeal to different countries. Therefore, a cross-national approach was applied herein to allow for a country-comparison of whether the spirulina products fulfill similar expectations across different consumer bases or not. Studies confirm cross-cultural differences regarding vegetarianism (Ruby et al., 2013), protein diets (de Boer et al., 2006), sensory appeal, and convenience of food (Januszewska et al., 2011), which are pertinent points regarding consumer expectations to spirulina-based novel food products. This study investigates consumer expectations when products are presented with corresponding beneficial traits to anticipate which products (if any) should be pursued for prototype development and sensory testing. However, testing all three products directly in a consumer test including degustation in a sensory lab (be it in the context of a meal or without) would have been costly and time-consuming regarding product development and testing time. Therefore, to reduce resource expenditure to a necessary level, this current concept study provided initial insight into the most promising product category for integrating the microalga spirulina into European dietary habits.

Altogether, the study answers four research questions:

I) Which of the actual products is the most popular according to consumers, when spirulina is included as a main ingredient?

II) Does familiarity with the product category explain overall liking of the new spirulina products?

III) Which of the benefits, when presented in connection with a product, has the highest impact on overall liking?

IV) Are the answers to the questions I-III country-dependent?

Materials and methods

Expert interviews and product conceptualization

Due to restricted product experience, consumers are generally a poor source of inspiration and creativity (Lilien et al., 2002), and fixation on existing products may hinder "thinking out of the box" (van Kleef et al., 2005). Thus, prior to factoring in consumer input, it was decided to conduct in-depth interviews with culinary experts active in their field to initiate product conceptualization. Four expert interviews were conducted in 2017, where an already developed spirulina extrudate (Grahl et al., 2018) (the basis for the products used in this study) was presented to sample as an instigator. During the interviews, a chef, an aroma researcher, an algae retailer, and a celebrity food-expert were introduced to various product ideas like pasta, sushi, salad, nuggets, crispy bites, polenta with spirulina, stuffed puff pastry, and jerky to hear about their opinion regarding potential development in these directions.

Based on the interviews, the three most convincing and promising product ideas were determined to be spirulina-filled pasta, spirulina-filled maki-sushi, and spirulina jerky as a vegan alternative to beef jerky (Figure 6.1). As McGee (2004a) states, the key to pasta's appeal are its moist, yet substantial texture and its neutral flavor. Particularly, the latter renders pasta dough into a suitable partner for a broad range of ingredients, and the interviewed experts came to the same conclusion. Maki-sushi was described as a promising concept because consumers know conventional sushi contains nori algae, which is dark green to black, similar to spirulina. It was assumed that consumers' knowledge and expectations of conventional sushi made with nori could be transferred to the microalga spirulina with its dark coloring and could provoke an expected color-flavor fit. Finally, the idea to produce a vegan version of a dried meat snack stems from the initial use of extrudates based on high moisture extrusion cooking, namely, meat analogues. It was assumed that extrudates could lend the spirulina jerky the chewiness and bite-texture expected while enjoying regular beef jerky. In addition, beef jerky has usually a dark color due to the drying process of the meat. The experts' opinion was that a vegan dried snack makes sense for consumers as it extends the offer of dried "on-the-go" snacks to a product that is convenient, savory, and rich in protein. Therefore, the 3 chosen products cover a broad range of novelty (i.e., well-known pasta to trending jerky) but are not completely unknown, therefore making them optimal to confront consumers with. After the products were identified, various prototypes were produced manually. The products of choice were finally photographed in a professional photo studio.

Consumer questionnaire

The aim of the questionnaire was to determine which product was most accepted and which benefits affected the acceptability. In order to ensure that participants had adequate background information, prior to evaluating product concepts, the participants were primed with general information about spirulina as follows:

"The microalga spirulina is characterized by its high protein content and is therefore suitable to be eaten as an alternative to other protein-containing foods such as meat or dairy products. That is why we are researching to expand the food supply with spirulina. Our mission is supported by the benefits of spirulina in terms of the environment and a healthy diet. In addition, spirulina products are a novelty to the food market, helping us to make the future of food production more sustainable and to set new trends."

Next to the text, a picture of a small pile of spirulina powder was shown. Participants were asked to rate how relevant they found the information from "not relevant at all" (1) to "very relevant" (9).

Then, the consumers were exposed to three spirulina-based products (filled pasta, sushi, and jerky; Figure 6.1) and three benefit orientations (environmentally sustainable, healthy, or innovative). The products were shown on photos and accompanied by a brief text explaining a benefit orientation. A Latin square combinatorics allowed each benefit and each product to be presented to a single consumer only once. The participants evaluated three product-benefit combinations consecutively, where the product was visually shown in a photo and the benefit was contextualized adjacently (APPENDIX I). Using 9-point hedonic rating scales, participants answered questions regarding novelty ("not at all novel" (1) to "very novel" (9)), interest ("not at all interesting" (1) to "very interesting" (9)), overall liking ("dislike extremely" (1) to "like extremely" (9)), and expected flavor liking ("very bad" (1) to "very good" (9)).

Finally, participants were asked whether they had known of the microalga prior to the survey, and whether they had already tasted it. Participants were also asked how often they eat conventional filled pasta, sushi, and beef jerky (6-point scale: 1 = several times a week, 2 = once per week, 3 = every second week, 4 = once a month, 5 = less often than once per month, and 6 = never). Familiarity with the product categories was derived from this question of frequency of consumption. The answers were clustered into three categories and effect coded: +1 = several times a month, 0 = on a monthly basis or less often, and -1 = never. At the end, demographic data (city size and education) were collected.

Figure 6.1: Pictures of spirulina-filled pasta, spirulina-filled sushi, and spirulina jerky (from left to right) as they were shown to the consumers. The verbal benefits can be found in Appendix I.

The questionnaire was written in German and translated into French and Dutch. It was than retranslated from two independent native speakers back to German and checked for consistency. The study protocol was approved by the Ethics Committee of Georg-August-University, Goettingen.

Recruitment of consumer participants

An online survey was distributed through a commercial, established survey company (Research Now SSI, Frankfurt, Germany) in September 2017. A sample of 1227 surveys in total was collected across three countries. Samples respectively representative of the German, French, and Dutch populations, were recruited via e-mail from the online panelist pool using set quotas regarding age and gender. Potential participants were screened out if they did not eat meat. After a data quality check, 1035 valid interviews remained: Germany (n = 348), France (n = 337), and the Netherlands (n = 350). Characteristics of the final sample are shown in Table 6.1.

Table 6.1: Sample characteristics (n = 1035).

Variable	Levels	GER [%] n = 348		FR [%] n = 337		NL [%] n = 350	
		sample	population	sample	population	sample	population
Gender	Female	53	51	52	51	53	50
	Male	47	49	48	49	47	50
Age	18-29	18	21	19	24	24	24
	30-49	42	41	45	43	44	41
	50-64	40	38	36	33	32	34
Education[a],*	Low	23		4		20	
	Medium	48		50		20	
	High	29		45		60	
	Not (yet) graduated	-		2		1	
City size*	village (up to 5,000 inhab.)	18		30		19	
	small town (up to 20,000 inhab.)	20		19		23	
	medium-sized city (20,000 to 100,000 inhab.)	23		29		32	
	big city (100,000 inhab. and more)	39		22		25	

* no quota regarding education or city size sample
[a] Education levels: *Low:* up to pre-vocational education; *Medium:* secondary vocational education and pre-university education; *High:* university degree
inhab. = inhabitants

Data quality and analysis

In the first step, data quality was checked and mischievous respondents were removed. The questionnaire included scales on usage and attitudes which are beyond the scope of this paper but were used to clean the data by eliminating participants who were crisscrossing (11 %) or failing to answer a trap question correctly (10 %). The final sample included 1035 respondents. An ANOVA (post hoc test to differentiate between countries: Duncan) was used to compare the perceived relevance across consumers of the three countries.

Because product-benefit combinations were nested within individual participants, multilevel modelling was applied. At level L1 the dependent variables (DV) novelty, interest, overall liking, and expected flavor liking as well as familiarity in mediation analysis were located. The fixed experimental factors product and benefit were also located at L1. The individual participant represented by a categorical ID was included as a random factor and built level L2. At L2, predictors representing individual differences (e.g., country) were included in the model. Parameters were estimated by the generalized linear mixed model (glmm) procedure in SPSS IBM 24.0 (rating DV probability distribution: normal, link function: identity), and least significant difference (LSD) was used to compare estimated marginal means. Models were compared using the Bayesian information criterion (BIC); the smaller the BIC value, the better the fit of the model. Model M0 without fixed factors revealed a large share of between-participants variance, and the intraclass correlation (ICC) showed that participants used the scale differently. The multilevel approach is specifically suited for that scenario by including the random intercept in all models. M1 introduced the experimental factors product, benefit, and their interaction, while M3 considered country moderations as well.

Model M2 tried to explain the product effect through the inclusion of familiarity with the product category on the within-participant level. It was assumed that liking would be mediated by familiarity. Mediation analysis followed the approach of Baron & Kenny (1986). To investigate mediation of the product main effect (c), models tested path (a) and (b) in Figure 6.2 and observed, whether (c') declines to nonsignificance if (b) is included.

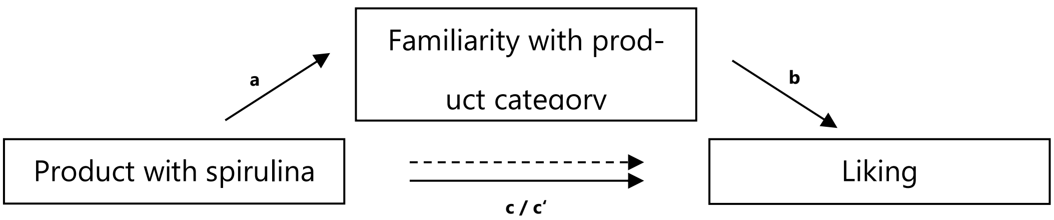

Figure 6.2: Model of a new product's acceptance mediated by the familiarity with the product category.

Results and discussion

Relevance and foreknowledge of spirulina

National differences in perceived relevance of spirulina were identified (F (2, 1032) = 76.7; p = .001). French consumers found spirulina to be more relevant as a food ingredient (mean = 6.9^{a}, SD = 1.6) than German (mean = 5.4^{b}, SD = 2.4) and Dutch (mean = 5.0^{c}, SD = 2.2) consumers (means with different superscript letters differ significantly). This might be explained by the result that 59 % of the French consumers heard about microalgae before this study while 46 % of the German and only 33 % of the Dutch consumers did so. Foreknowledge of microalgae generally enhanced perceived relevance of spirulina, F (1, 1033) = 76.8; p = .001. However, the country effect did not disappear, when foreknowledge was included in the ANOVA, thus foreknowledge only partly mediated the country effect. Additionally, the low Dutch foreknowledge is rather surprising since the National Institute for Public Health and Environment of the Dutch Ministry of Health, Welfare and

Sports published a report on the replacement of meat and dairy by more sustainable protein sources several years ago (Tijhuis et al., 2011). Consequently, alternative protein-based convenience food products, like a spirulina burger and insect meatballs, are already commercially available in the Netherlands (House, 2016). Of the French consumers who knew about microalgae in general, 80 % knew of spirulina in particular, while only 74 % of the German and 67 % of the Dutch consumers who knew about microalgae in general were acquainted with spirulina in particular. In case spirulina was known, approximately 1/3 of the consumers in France and Germany had already tried it (28 % and 30 %, resp.). Only 20 % of the Dutch consumers who stated that they knew spirulina had already eaten it. According to these numbers, there is still a need to increase the prominence of microalgae in general, and for spirulina in particular, as relevant food ingredient. In order to identify possibilities to increase consumer awareness regarding consequences of meat consumption and the advantages of alternative protein sources, Hartmann and Siegrist (2017) reviewed several studies and found that strategies to motivate environmentally friendly protein consumption behavior are lacking. They came to the conclusion that nudging strategies towards reduced meat consumption need to be established. Part of these nudging strategies could be convenient algae food products, served in a familiar context, as presented in this study.

Consumer acceptance

Main effects of product - benefit combinations on consumer ratings

Model M1 introduced the experimental factors product, benefit, and their interaction and controlled for the position of the product-benefit combination in the order design and for a country main effect in scale usage. Significant differences in interest, overall liking, and expected flavor liking were due to both factors, that is, products and benefits, whereas the product * benefit interaction was only significant regarding interest (Table 6.2).

Table 6.2: Novelty, interest, overall liking, and expected flavor liking as a function of experimental factors product and benefits controlled for position (presentation order) and country (M1).

		Novelty		Interest		Overall Liking		Expected Flavor Liking	
Fixed effects	***df*, 3093**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Position	1	37.03	.001	15.69	.001	18.67	.001	5.12	.024
Country	2	49.19	.001	7.14	.001	2.66	.070	0.24	.786
Product	2	34.98	.001	134.30	.001	135.50	.001	130.93	.001
Benefit	2	2.00	.136	5.24	.005	6.99	.001	7.53	.001
Product * Benefit	4	2.15	.072	3.71	.005	1.74	.138	1.90	.108

Figure 6.3 visualizes the marginal means for M1. Across all DV, pasta came out on top. Regarding overall liking, consumers preferred pasta ($M = 5.47^a$) clearly over sushi ($M = 4.87^b$) and jerky ($M = 4.34^c$) (means with different superscript letters differ significantly). Concerning overall liking of the benefits, healthy spirulina ($M = 4.99^a$) is preferred over sustainable spirulina ($M = 4.75^b$). This finding coincides with the results of Tobler, Visschers, & Siegrist (2011) who found that health or taste claims have a stronger impact on consumption behavior than environmental motives. Consequently, marketing strategies should not focus solely on sustainability morals in order to successfully induce consumption pattern changes. A main effect of the benefit was not found regarding novelty; that is, none of the benefits of spirulina were perceived as more novel than any other. However, a small product effect rendered novelty of sushi with spirulina as a bit less novel than pasta or jerky with spirulina, which confirms the flavor pairing strategy intended with the sushi concept.

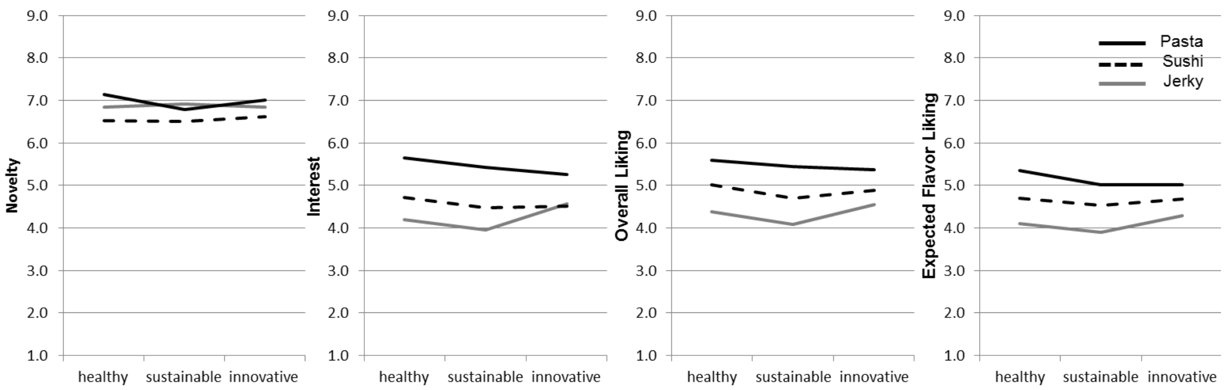

Figure 6.3: Novelty, interest, overall liking and expected flavor liking measured on a 9-point scale as a function of product and benefit. Scales: novelty ("not at all novel" (1) to "very novel" (9)), interest ("not at all interesting" (1) to "very interesting" (9)), overall liking ("dislike extremely" (1) to "like extremely" (9)) and expected flavor liking ("very bad" (1) to "very good" (9)). Values depicted are marginal means derived from M1.

Interest is the only DV where a product * benefit interaction reached significance (F (4, 3093) = 3.71; p = .005). It was highest for healthy pasta and lowest for a sustainable jerky. Jerky is generally of lowest interest and least liked across all scales; however, if presented with the innovative benefit, it was viewed as more acceptable and interesting than with the other benefits. This is rather surprising, considering the fact that beef jerky itself is a product of an ancient preservation technique that is based on dehydration through drying (McGee, 2004b). Nevertheless, beef jerky is experiencing a revival and is nowadays marketed as a trendy savory protein snack with concentrated flavor and chewy texture that appears innovative and novel to younger generations.

Familiarity as a mediator for product liking

Pasta turned out to be more familiar compared with sushi and jerky. It is a staple food with a long tradition in countries across Europe. It is flexible when it comes to combining it with other dishes and sauces, is easy to prepare, and therefore makes it a familiar food product.

Consequently, it turned out that product liking was partly mediated by familiarity with the product category. Familiarity with the product categories (ICC 30 %) varies strongly between the three products (F (2, 3102) = 544.80; p = .001; full model not shown) and pasta turned out to be most familiar. It was on average consumed on a monthly basis (M = -.03[a]). Sushi was less often consumed (M = -.39[b]) and jerky (M = -.76[c]) was never tasted by a majority of the consumers. Table 6.3 reports model M2 for overall liking (BIC = 13033, smaller than for M1 = 13298) which extends M1 by familiarity and its interactions with the experimental factors. Since familiarity strongly predicts consumer evaluations (F (1, 3084) = 268.95; p < .001), the product main effect on evaluations partly diminished (F (2, 3084) = 2.90; p = .055).

Table 6.3: Overall liking as a function of the experimental factors product and benefits controlled for country and position (presentation order), extended by familiarity and its interactions (M2).

		Overall Liking	
Fixed effects	***df*, 3084**	***F***	***p***
Position	1	20.91	.001
Country	2	1.35	.258
Product	2	2.90	.055
Benefit	2	0.79	.455
Product * Benefit	4	1.00	.407
Familiarity	1	268.95	.001
Familiarity * Product	2	15.89	.001
Familiarity * Benefit	2	1.87	.155
Familiarity * Product * Benefit	4	0.56	.689

Figure 6.4 illustrates path (a) and (b) of the mediation. Overall liking of the spirulina products was shown to depend on familiarity with the product category (path (b)). However, the familiarity slope was not as strong when pasta was concerned. As already mentioned, the product categories themselves possess different levels of average familiarity (path (a), depicted by stars in Figure 6.4).

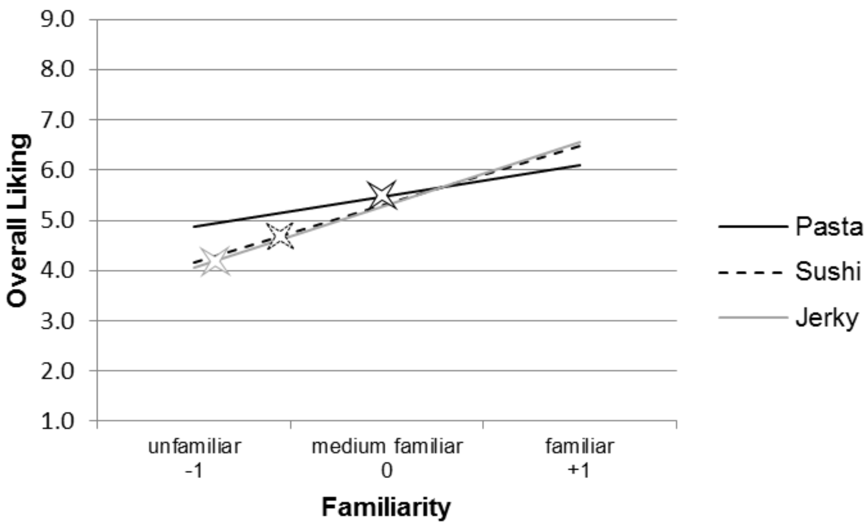

Figure 6.4: Overall liking of spirulina as a function of familiarity with product category (stars mark the average familiarity of each product category).

Thus, the spirulina products evoked an overall liking according to mere familiarity with the product category. In turn, it implies that, if the product categories were equal in familiarity (e.g., all consumed regularly), the spirulina products would have received more or less the same liking. It can be concluded that different product categories are conceivable with spirulina as long as the category is sufficiently familiar for the targeted consumers. Similar findings can be reported for soy products, where hedonic responses have been shown to be more positive when familiar than when perceived as unfamiliar by consumers (Fenko et al., 2015). Familiarity with the product category has an impact on general acceptability as well as the willingness to try a new variant of that category (Martins & Pliner, 2005; Wansink, 2002), which will be of importance in sensory testing of spirulina-filled pasta and help to overcome potential food neophobia.

Examining moderation by country

Although M3 fitted better than M1 (e.g., for overall liking BIC M3 = 13293 smaller than BIC M1 = 13298), country moderations of experimental factors were small (Table 6.4). Main effects were more or less pronounced, for example, German consumers clearly liked spirulina pasta (marginal means 5.70[a], 4.94[b], and 4.60[b] for pasta, sushi, and jerky, resp.) while French consumers particularly dislike spirulina jerky (marginal means 5.47[a], 4.95[b], and 4.18[c] for pasta, sushi, and jerky, resp.).

Table 6.4: Experimental factors product, benefit, country and their interaction as moderators for the dependent variables novelty, interest, overall liking and expected flavor liking (M3).

		Novelty		**Interest**		**Overall Liking**		**Expected Flavor Liking**	
Fixed effects	***df1*, 3077**	***F***	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Position	1	36.48	.001	14.76	.001	18.11	.001	4.39	.036
Country	2	49.24	.001	7.29	.001	2.57	.077	0.22	.800
Product	2	37.22	.001	135.93	.001	136.55	.001	131.10	.001
Benefit	2	1.98	.138	4.84	.008	6.66	.001	7.14	.001
Product * Benefit	4	2.29	.058	3.74	.005	1.76	.134	1.98	.095
Country * Product	4	10.97	.001	2.46	.043	2.14	.073	1.92	.104
Country * Benefit	4	1.11	.348	0.79	.533	1.08	.367	0.46	.764
Country * Product * Benefit	8	2.77	.005	0.85	.561	0.85	.559	1.28	.249

Country interactions reached significance only for novelty and interest evaluations. Regarding novelty (Figure 6.5), French consumers placed sustainable spirulina jerky over sustainable spirulina pasta; Germany and France were of different opinion concerning innovative spirulina sushi, and for the Dutch healthy spirulina jerky seems least novel. Regarding interest, German consumers do not differentiate much between their interest in sushi or jerky (marginal means 5.49[a], 4.30[b], and 4.20[b] for pasta, sushi, and jerky), whereas French (marginal means 5.78[a], 5.04[b], and 4.52[c]) and Dutch (marginal means 5.09[a], 4.38[b], and 3.98[c]) consumers do so (Figure 6.6).

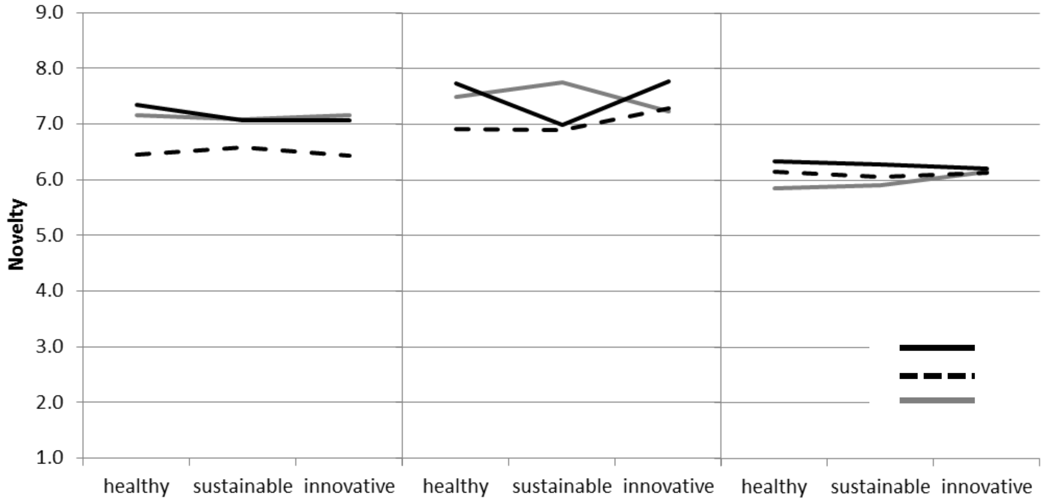

Figure 6.5: Novelty (9-point scale, ("not at all novel" (1) to "very novel" (9)) as a function of product, benefit, and country (marginal means from M3).

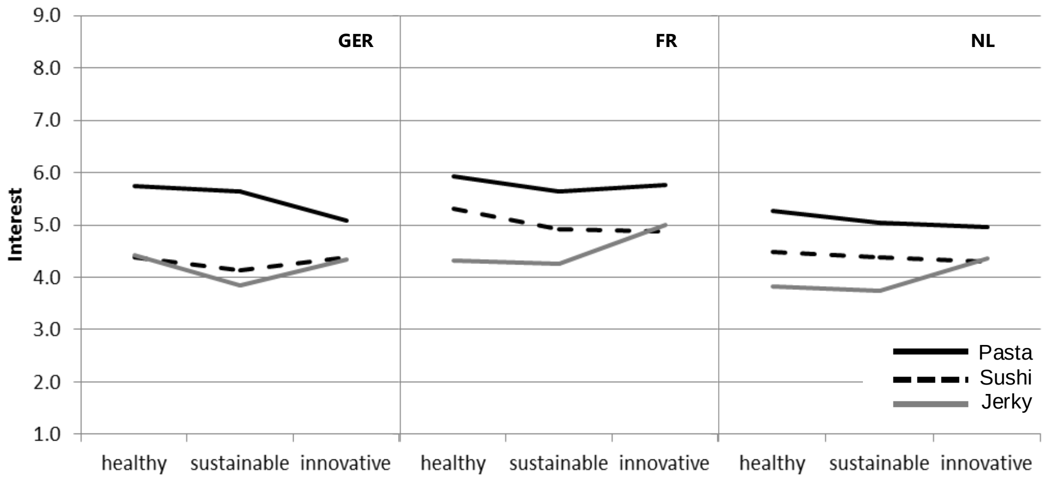

Figure 6.6: Interest (9-point scale, ("not at all interesting" (1) to "very interesting" (9)) as a function of product, benefit, and country (marginal means from M3).

However, cross-country similarities were larger than differences. Thøgersen (2017) recently clustered 10 European countries and identified three food-related lifestyle regions; Germany, France, and the Netherlands were grouped together with the UK. He found that the cluster in itself is not homogeneous, and segments are differently distributed. Nevertheless, the segment pattern is shared across countries within one region which might be an explanation why country differences in the present study are only found to be small.

It should be noted that the consumer evaluations in the current study are based on visual stimuli only; experiencing the taste of food might overrule the effect of visual components (Zellner et al., 2010). Also the acceptance of potential side dishes of the main meal item of interest might be of relevance for consumers to holistically evaluate a product in a survey (Jimenez et al., 2015). The comparably low overall liking scores throughout the study can be explained by the fact that sensory experience is decisive when it comes to repeating a trial purchase (Grunert & van Trijp, 2014). Consequently, more information is needed both from a consumer and research perspective, since a product with bad taste will not be bought again, even if healthy or sustainable (Boland et al., 2013).

Conclusions

The acceptance of three different products based on the microalga spirulina was investigated across three European countries. Familiarity with product categories, which was deduced from the frequency of consumption of the categories, proved to be a mediator. That is, different product categories are conceivable with spirulina as long as the category is sufficiently familiar for the targeted consumers. Overall, pasta was most liked across countries, with country differences being minor. This indicates that product marketing does not need to be culture-specific. Yet, there is still a need to increase the prominence of microalgae in general, and for spirulina in particular, as relevant food ingredient. Future research will focus on the development of appealing flavors for different pasta fillings so that consumers can give a fully informed opinion about the products, and product optimization can commence.

Conflicts of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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APPENDIX I

	Pasta	Sushi	Jerky
Health	**Spirulina pasta: a healthy alternative to ravioli with meat filling** A healthy pasta thanks to spirulina: plenty of protein and polyunsaturated fatty acids, cholesterol-free and low in calories. Because of the minerals and antioxidants, spirulina pasta is good for you.	**Spirulina sushi: a healthy alternative to sushi with fish filling** Spirulina sushi is healthy and contains a lot of protein, polyunsaturated fatty acids, minerals and antioxidants. Unlike some fish frequently used for sushi, spirulina does not contain any mercury, which is harmful to your health. Spirulina sushi is good for you.	**Spirulina-Jerky: a healthy alternative to beef jerky** Jerky made from dried beef is a typical snack, but Spirulina-Jerky is also healthy. In addition to its high protein content to satisfy your hunger, spirulina contains polyunsaturated fatty acids, the snack is cholesterol-free, low in calories and rich in minerals and antioxidants. Spirulina-Jerky is good for you.
Sustainability	**Spirulina pasta: a sustainable alternative to ravioli with meat filling** Spirulina is a more sustainable protein source than meat, whose production consumes a lot of land and causes climate-damaging CO_2. The micro-algae can be cultivated on a small area of land in tanks or in ponds and therefore is locally produced, just like the durum wheat in the dough. Spirulina pasta: a delicious meal with a clear conscience.	**Spirulina sushi: a sustainable alternative to sushi with fish filling** Spirulina is a more sustainable source of protein than fish, as fish farms pollute the environment and overfishing leads to social conflicts. The micro-algae can be cultivated regionally on a small area of land in tanks or in ponds, which saves on transport and gives the sushi a regional flair. Spirulina sushi is a high-quality treat with a clear conscience.	**Spirulina-Jerky: a sustainable alternative to beef jerky** Spirulina is more sustainable than dried Jerky beef, whose production consumes a lot of land and causes climate-damaging CO_2. The micro-algae can be regionally cultivated on a small area of land in tanks or in ponds, which saves on mileage and gives the snack a regional flare. With recycled packaging Spirulina-Jerky becomes a high-quality treat with a clear conscience.
Innovation	**Spirulina pasta: new alternative to ravioli with meat filling** Conventional ravioli are a thing of the past! Spirulina is the basis for the pasta with a unique filling. Algae are trendy and bring their own flavor. The recipe was developed with a celebrity chef: it's star-suspicious. Spirulina pasta is an edible innovation and a culinary highlight at the same time.	**Spirulina sushi: a new alternative to sushi with fish filling** Spirulina sushi is the food of the future: everything from the sea, but without the animal-ethical problems of fish farming. Yet, it is rich in protein, trendy, has a characteristic taste and satisfies your hunger. All in black and white and without compromise: Spirulina sushi is an edible innovation and a culinary highlight at the same time.	**Spirulina-Jerky: new alternative to beef jerky** Spirulina-Jerky, unlike jerky made from dried beef, is the food of the future: always in your pocket, durable & dry, rich in protein and satisfying. A trendy snack with its special taste. Unlike cattle, spirulina breeding is conceivable in the city (Urban farming in tanks on building facades) and perfects self-sufficiency. Spirulina-Jerky is an edible innovation and a culinary highlight at the same time.

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Paper III

7 Paper III: Alternative protein sources in Western diets: Food product development and consumer acceptance of spirulina-filled pasta

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Abstract

Spirulina (*Arthrospira platensis*) is currently being increasingly researched for its usability and suitability in human nutrition. The inclusion of alternative protein sources, such as spirulina, in familiar products is a possible strategy to nudge consumers towards dietary change in Western societies. Filled pasta variants with three levels of spirulina-soy-extrudate in the filling (10 %, 30 % or 50 %) were developed and investigated for their sensory characteristics and consumer liking. Recipe development was based on the concepts of flavor-flavor learning (beet-ginger or tomato) and masking (lemon-basil) to account for the musty and earthy notes of the alga. Consumer tests were conducted in Germany (n = 139), the Netherlands (n = 137) and France (n = 144), and conventional sensory profiling was accomplished with trained panelists (n = 12). Consumers preferred the lemon-basil flavor over tomato and beet-ginger flavored fillings. As expected, liking decreased with increased extrudate content, regardless of the flavor. For all flavors expected liking was higher than liking after product exposure; for the lemon-basil flavor the gap was the smallest. It was shown that low general food neophobia and familiarity with spirulina promoted consumer liking. The sensory profiling revealed that the spirulina-soy-extrudate content affected all sensory attributes identified across all flavors, but for the tomato flavor the most. Overall, this study confirms that it is important to consider differences in consumer motivation as well as the product's conceptual and intrinsic sensory characteristics in new product development.

Introduction

The attitude and perception of consumers towards new products is crucial for innovations (Grunert & van Trijp, 2014). Early involvement of consumers in the process of new product development is important to facilitate the design of relevant new products (van Kleef et al., 2005). Beyond that, consumer-oriented product development is a success factor in the food industry (Costa & Jongen, 2006; Grunert & van Trijp, 2014) because early insights into consumer needs and desires allow for indication of market opportunities, reveal technology acceptance and support product optimization through prototype testing (Grunert et al., 2011). Thereby, the potential of failure of new food products can be reduced.

Food neophobia, i.e. the reluctance to eat unknown foods, is an obstacle when it comes to the establishment of food innovation and can be the reason why some new food products fail (Barrena & Sánchez, 2012). Food neophobia is a widespread phenomenon originating from fear of potentially harmful foods (van Trijp & van Kleef, 2008), and when characteristically strong, shifts the balance between interest in and rejection of novel foods towards rejection. To some extent, it can help explain the conservatism towards innovative foods (Rozin & Vollmecke, 1986). There is ample evidence of consumers' caution towards unknown foods (Damsbo-Svendsen et al., 2017) and food technologies (Cox & Evans, 2008), which has to be considered when novel product ideas are developed. Increased familiarity (Siegrist et al., 2013), adequate benefit communication (Knight et al., 2008), or repeated exposure (Pliner, Pelchat, & Grabski, 1993; Tuorila, Meiselman, Bell, Cardello, & Johnson, 1994) are some strategies to overcome food neophobia. Finally, the social context related to food acceptance (Hobden & Pliner, 1995) should not be overlooked.

Society and culture influence the perception of food and affect what is perceived as appropriate or unacceptable to eat (Prescott, Young, O’Neill, Yau, & Stevens, 2002). Algae and insects might cause disgust in Western Europe while they are an established part of Asian food culture. Innovative ingredients like these do not fit easily into dishes of the Western European cuisine (de Boer & Aiking, 2019). Nevertheless, algae as well as insects play a major role in the approaching field of alternative protein sources to ensure protein supply of the future (Henchion et al., 2017).

Meat-based diets require more energy, land and water than plant-based diets and are therefore considered less sustainable (Pimentel & Pimentel, 2003; Sabaté & Soret, 2014). An increase in Western society's consumption of alternative proteins aims to shift diets in the name of sustainability; therefore consumer-oriented pathways have been explored as a strategy to nudge consumers towards dietary change (Schösler et al., 2012). Research has been conducted to better understand how to convince consumers to partially (de Boer et al., 2014) or fully substitute meat with plant-based protein foods (Hoek et al., 2011; Schösler et al., 2012). Particularly, substitution has been found to be difficult, since textural properties and flavor of meat substitutes do not achieve consumer's expectations (Elzerman et al., 2013; Hartmann & Siegrist, 2017). Meanwhile, products for vegetarians that focus on dissimilarity compared to meat can be distinguished from those that are specifically targeted at meat eaters by attempting to accurately mimic the characteristics of a meat burger, e.g. Impossible Foods or Beyond Meat (Keefe, 2018). Due to the characteristics of algae, it is obvious that meat substitution through the consumption of algae will not be accomplished with an algae schnitzel meant for meat lovers, but rather by using algae as the protein source in a one bowl meal.

The microalga spirulina (*Arthrospira platensis*) has a protein content of 63 % dry matter (Becker, 2007) and contains all essential amino acids (Gutiérrez-Salmeán et al., 2015). Consequently, it is a promising food ingredient enlarging the repertoire of protein sources for human consumption. High moisture extrusion cooking was shown to be one alternative to process spirulina to be suitable for meat alternative products (Grahl, Palanisamy, et al., 2018).

To find out which food concept utilizing the spirulina-soy-extrudate is most promising from a consumer preference viewpoint, an online survey showed that a staple food (spirulina-filled past) proved most promising against a flavor- and color-paired food (maki sushi) and a convenient snack (spirulina jerky) (Grahl, Strack, et al., 2018). The present study is based on this finding and intends to test real spirulina-filled pasta products for consumer acceptance.

However, earlier findings suggest that spirulina has a negative effect on sensory characteristics (Ak et al., 2016; Beheshtipour et al., 2013), which is likely related to its musty-earthy flavor profile (Grahl, Palanisamy, et al., 2018). To date, no research investigates strategies to overcome these characteristics (in filled pasta). Recipe development is therefore based on the concepts of flavor-flavor learning (beet-ginger or tomato) (Yeomans, Leitch, et al., 2008) and masking (lemon-basil) in order to investigate different approaches to incorporating spirulina. Finally, filled pasta with three levels of spirulina-soy-extrudate in the filling (10 %, 30 % or 50 % extrudate content) are served during central location tests (CLT) in Germany, France and the Netherlands with the primary aim to identify consumer preferences for novel pasta filled with spirulina in the three different countries. To gain a deeper understanding of consumer preferences, the study takes food neophobia, familiarity with spirulina, and the effect of expectations on liking into account. Parallel to consumer testing, a trained sensory panel evaluated the pasta using conventional profiling in order to test the expected masking effect, i.e. a decreased intensity of the algae flavor. Our study answers five research questions:

- Which pasta-flavor in combination with spirulina is liked by consumers best?
- What is the accepted level of spirulina-soy-extrudate content in filled pasta?
- Is acceptance mediated by expectation of concept or sensory properties, or both?
- Does food neophobia or familiarity with spirulina influence acceptance?
- Do there exist cross-country differences amongst neighboring Western countries?

Materials and methods

Pasta production

The samples consisted of pasta stuffed with a spirulina-soy-extrudate filling using semolina-based dough.

Grahl, Palanisamy, et al. (2018) found that a replacement of 50 % soy protein concentrate by 50 % spirulina in high moisture cooking extrusion is possible. Consequently, the spirulina-soy-extrudate for the filling consisted of 50 % spirulina powder (Spirulina premium II procured from the Institute for Food and Environmental Research (ILU e.V.) in Nuthetal, Germany) and of 50 % functional soy protein concentrate of the brand ALPHA 8 IP (obtained from Solae, LLC, St. Louis, Missouri., U.S.A.). The extrusion parameters were identified to be optimal at a temperature of 160 °C, moisture of 55 % and a screw speed of 600 rpm (Grahl, Palanisamy, et al., 2018) and the extrudate for the filling was produced accordingly. After extrusion, the extrudates were vacuum-packed and stored at -18 °C. Prior to pasta production, the deep-frozen extrudate was cut into irregular pieces of app. 5 mm with a 30 liter vacuum cutter (5000 Express, Kilia, United Kingdom) normally used for sausage production, vacuum-packed again and deep-frozen (10 days) until the pasta production commenced.

The pure flavor of spirulina does not justify it to be used as a single ingredient (Small, 2011). Pasta fillings were therefore based on various recipe concepts which, together with the flavor of spirulina, were expected to match. Two flavors, i.e. "tomato-spirulina" and "beet-ginger-spirulina", were developed based on the concept of flavor-flavor learning (Yeomans, Leitch, et al., 2008). The concept implies that pairing an unfamiliar flavor with a liked or disliked flavor results in liking changes for the unfamiliar flavor (Brunstrom, 2005). Tomato and spirulina have comparably high contents of glutamic acid in common (Dewi et al., 2016; Yamaguchi & Ninomiya, 2000), an amino acid that contributes to umami taste. The fifth basic taste is associated with a rich and savory taste experience and was previously shown to increase liking if paired with novel flavors (Prescott, 2004; Yeomans, Gould, et al., 2008). The flavor "beet-ginger-spirulina" originated from one of the expert interviews conducted prior to the online survey to identify the preferred product category with spirulina (Grahl, Strack, et al., 2018). It has been suggested that the earthy flavor of spirulina (Grahl, Palanisamy, et al., 2018) could go well with the familiar earthy flavor of beet that stems from a compound called geosmin, known for its earthy aroma (Lu et al., 2003). To enhance the desirable earthy flavor and to ensure a trigeminal complexity, the filling was complemented with the warm spiciness of ginger.

Spirulina is a cyanobacterium and these microorganisms are generally suspected to be producers of the compounds geosmin and 2-methylisoborneol (MIB) which cause an earthy and musty odor (Selli et al., 2006). Geosmin converts into the odorless product argosmin under acid conditions (Liato & Aïder, 2017) so that the earthy aroma diminishes. Although spirulina has not been shown to contain geosmin (Milovanović et al., 2015), spirulina provokes a sensation similar to this compound. Assuming that lemon in combination with spirulina would have a masking effect, lemon-basil-spirulina was included in the study as the third flavor.

The pasta was produced together with a local pasta producer (Casaluko GbR, Schauenburg, Germany). Each flavor described above was prepared and immediately mixed with three different proportions of the defrosted extrudate pieces, in order to realize 10 %, 30 % or 50 % extrudate content in the entire filling. In consequence, the more extrudate content, the less flavor filling was incorporated in a piece of pasta. The nine different fillings were stuffed between two layers of dough using the customized pasta machine of Casaluko GbR (Figure 7.1). After production, the pasta was stored at -18 °C until use within half a year after production.

Figure 7.1: Filled pasta products as produced in cooperation with Casaluko GbR (left) and a close-up of a pasta sample with a filling that contains 50 % of the spirulina-soy extrudate (right).

Pasta preparation

Both for the CLT with consumers and the sensory panel sessions, the pasta was prepared in the same way. Per piece of pasta (10 to 12 g), 100 ml of water and 0.5 g of iodine free salt were used. A certain amount of water (depending on the number of panelists or consumers per session) was set to the boil and then to simmer (~95 °C) on an induction stove. Finally, salt and the frozen pasta was added into the simmering water and boiled for 5 minutes. Pasta was served intact on white plates assigned with a corresponding 3-digit code. Every consumer or panelist received two pieces of pasta per serving; each pasta piece had a core temperature of 65 to 70 °C when served.

Conventional sensory profiling

Conventional profiling was conducted to describe the samples qualitatively while also quantifying the intensities and magnitude of the sensations of the novel spirulina pasta. The sensory panel consisted of 12 panelists who were selected according to ISO 8586-1 (ISO, 1993). To perform the descriptive analysis, they were trained compliant with DIN 10967-1 (DIN, 1999). The panel leader structured and guided the panel sessions with the purpose to select the descriptive vocabulary. The panelists generated lists of descriptors through consensus to ensure agreement upon the attributes. Each of the pasta flavors was described by a separate set of attributes which contained up to 24 attributes, depending on the flavor. During product development, the panel acquired extensive experience regarding spirulina as an alternative protein and the specific products; a first batch of prototypes of the pasta was used to establish pasta profiles. Due to their extensive previous experience with spirulina-filled pasta, the sensory panel only required three training sessions per flavor.

The intensity perception of spirulina-related attributes shows the expected effects of flavors (e.g., masking spirulina characteristics by lemon in the lemon-basil flavor). Therefore, only the 13 attributes recorded in all three flavor variants were considered in the joint analysis (Table 7.1). The list of descriptors comprised odor (O_), appearance (AP_), taste (T_), mouthfeel (M_) and aftertaste (AT_) attributes. Various foods, sniffing strips and tasting solutions were served to exemplify stimuli for the consensus language development. Panelists were provided with water and unsalted crackers to neutralize their sense of taste and coffee beans to neutralize their sense of smell. When measuring the product attributes, the inter-attribute interval was set to be 15 sec while the inter-product interval was set to 2 min.

One session for data collection per flavor followed where the three varying extrudate content samples were assessed by each panelist in a randomized order and blinded by three-digit codes. Due to limited availability of raw materials, the samples were assessed in duplicate for each combination of flavor with extrudate content. During measurement sessions, each panelist sat isolated in individual booths in the sensory lab, constructed in concordance with ISO 8589 (ISO, 2007). Both training and measurement took place in the sensory lab of the Georg-August-University in Goettingen where the panel met twice a week for 90 to 120 min.

Table 7.1: Overview of attributes assessed for all three flavor variants incl. scales, reference products, and the assessment of the attributes.

Attribute	Scale (Anchors)	Reference [9]	Assessment
O_overall	weak – strong	pasta with 50 % extrudate content (rather weak); pasta with 10 % extrudate content (strong)	
O_algae	weakly perceivable – strongly perceivable	cube of algae extrudate (strongly perceivable)	*One odor attribute after the other:* cut one piece of pasta into two parts, holding one part 2 cm under the nose and sniff three times, 15sec inter stimulus interval
O_earthy	not perceivable – strongly perceivable	solution of geosmin in propylene glycol ($C_3H_8O_2$) applied on sniffing strips Lemon-Basil and Tomato: 0.187 % (rather not perceivable); 0.375 % (strongly perceivable) Beet-Ginger: 0.75 % (rather not perceivable); 1.5 % (strongly perceivable)	
AP_black	weakly perceivable – strongly perceivable	solution of 0.098 g of a mix of yellow and blue color in 1 l of water (strongly perceivable)[10]	black part of the filling visually assessed, standard daylight in the booths
M_firm	soft – firm	pasta with 10 % extrudate content (soft); pasta with 50 % extrudate content (firm)	noticeable resistance to deformation while chewing, assessed after 3 chews
M_moist	not perceivable – strongly perceivable	pasta with 50 % extrudate content (rather not perceivable); pasta with 10 % extrudate content (strongly perceivable)	ability to release moisture during chewing, assessed after 3 chews
T_overall	weak – strong	pasta with 50 % extrudate content (rather weak); pasta with 10 % extrudate content (strong)	
T_algae	weakly perceivable – strongly perceivable	cube of algae extrudate (rather strongly perceivable)	
T_salty	weakly perceivable – strongly perceivable	solution of sodium chloride in 1 l of water Lemon-Basil and Tomato: 1.3 g/l (weakly perceivable); 1.87 g/l (strongly perceivable) Beet-Ginger: 1.04 g/l (weakly perceivable); 1.3 g/l (strongly perceivable)	
T_sour	not perceivable – strongly perceivable	solution of citric acid in 1 l of water Lemon-Basil and Tomato: 0.32 g/l (weakly perceivable); 0.40 g/l (strongly perceivable) Beet-Ginger: 0.26 g/l (weakly perceivable); 0.32 g/l (strongly perceivable)	*One taste attribute after another:* taste intensity after 7 chews
T_sweet	not perceivable – strongly perceivable	solution of sucrose in 1 l of water Lemon-Basil and Tomato: 7.2 g/l (weakly perceivable); 8.64 g/l (strongly perceivable) Beet-Ginger: 8.64 g/l (weakly perceivable); 10.37 g/l (strongly perceivable)	
T_umami	not perceivable – strongly perceivable	solution of monosodium glutamate in 1 l of water Lemon-Basil, Tomato and Beet-Ginger: 0.4 g/l (weakly perceivable); 0.48 g/l (strongly perceivable)	
AT_overall	weak – strong	sample with 50 % extrudate content (rather weak); sample with 10 % extrudate content (strong)	aftertaste intensity overall 5sec after swallowing

Abbreviations: O_ = odor, AP_ = appearance, T_ = taste, M_ = mouthfeel, AT_ = aftertaste

[9] "Rather" refers to a fixation at 0.25 or 0.75 scale points, respectively. Where applicable, attributes had two references.

[10] According to DIN 10961 (DIN, 1996) by the German Institute for Standardization, which is based on ISO 8586-1 (ISO, 1993).

Consumer study

A consumer study was conducted to determine consumer preferences of pasta-filling flavor and extrudate content. The sample comprised consumers from Germany (Göttingen; n = 139), France (Toulouse; n = 144) and the Netherlands (Utrecht; n = 137) and was balanced according to gender and age groups (18 to 35 years, 36 to 50, and 51 to 65 years). Consumers were screened and included in the study if they would not reject to eat filled pasta, in general, and algae as a part of pasta fillings, in particular. According to an incomplete block design, each consumer tasted two out of three flavors and was assigned to a respective session. Oral informed consent was obtained from all consumers, who were registered participants of a consumer panel and an incentive was given to compensate for their participation. The publication of the data according to the study protocol was approved by the Ethics Committee of Georg-August-University, Goettingen.

Study design

Two main factors varied within consumers per session: flavor and extrudate content. In order to avoid mistakes and to guarantee feasibility of pasta preparation in the sensory laboratory, all consumers of a given session received an identical order of samples. Since odor, taste and aftertaste have been shown to be more intense with increasing extrudate and therefore spirulina content (Grahl, Palanisamy, et al., 2018), it was important to present extrudate contents in a systematic order (i.e. consumers received either 30 %-10 %-50 % or 30 %-50 %-10 %). Similarly, first-order effects were also expected, both for the first sample in the session and for the first sample of the second flavor, further justifying the need to serve the medium extrudate content sample (30 % extrudate content) first. Thus, the first-order effect was not confounded with the linear continuous factor of extrudate content; the design precludes modeling a squared relation. Due to three possible flavor combinations (lemon-basil & beet-ginger, lemon-basil & tomato or beet-ginger & tomato) in two possible sequences and two possible rotations of extrudate content, 12 test cells exist. The order of the 12 cells was randomly allocated while controlling for no correlation with flavor. Taken together, three order factors were controlled for: first order effect, extrudate content order, and flavor order (see Table 7.2).

Assessment

At the beginning of the test, consumers were informed that they were about to taste six pasta samples with algae filling (spirulina) and that samples would be served plain and without any sauce. The reasoning being that the main interest was in the pasta flavor and this should not be covered or confounded by sauce. Before the first product-related question was asked, consumers received general information regarding the microalga spirulina. The information was phrased as follows: "The microalga spirulina is characterized by high protein content and is therefore suitable to be eaten as an alternative to other protein foods, such as meat or dairy products. Spirulina pasta is not only advantageous in terms of the environment and a healthy diet but also a novelty in the food market that helps us to shape the future of food production sustainably and set new trends. That is why we are researching into spirulina as part of pasta fillings combined with different flavors." They were then told the upcoming flavor and asked for their expected flavor liking (9 - point hedonic scale; "would dislike extremely" (1) to "would like extremely" (9)). Then, the pasta was served and consumers were asked about their overall liking, followed by liking of appearance, odor, texture, flavor and aftertaste (9 - point hedonic scale; "dislike extremely" (1) to "like extremely" (9)). The questions were repeated per extrudate content and then repeated for the second flavor. All questions regarding consumption behavior and attitudes as well as food neophobia and familiarity with spirulina were posed at the end of the questionnaire. Neophobia towards food was of particular interest, because the new ingredient spirulina was incorporated in otherwise well-known pasta. It was uncertain whether food neophobia would have an impact on liking, if a familiar product category is involved. The food neophobia scale used in this study was developed by Pliner & Hobden (1992) and is usually measured on a 7 - point scale; however, a 5 - point scale (1 = disagree strongly, 5 = agree strongly) was used, as previously done by Henriques, King, & Meiselman (2009) and Fenko, Backhaus, & van Hoof (2015). This was done to ensure that the questionnaire only contained 5 - point scales and 9 - point scales throughout the study. Regarding familiarity with spirulina, consumers had to choose between four ordered answers: 1 = "I have consumed it more than once", 2 = "I have tried it once", 3 = "I have heard about it, but never tried it" or 4 = "I never heard about it before". Each session took approx. 45min for the consumers to evaluate the six samples.

Data Analysis

All statistical analyses and modeling were performed using the software R, version 3.4.3 (R Core Team, 2017) with the following packages: lmerTest (Kuznetsova et al., 2017), emmeans (Lenth, 2018), SensoMineR (Husson et al., 2017) and FactoMineR (Le et al., 2008).

Analysis of sensory evaluations

First of all, panel performance was checked according to Grahl, Palanisamy, et al. (2018). Panelist replicability, i.e. the correlation of the two assessments across the 9 products, was positive in 94 % of the panelist-attribute-combinations. The so-called corrected item-total correlation was calculated with each panelist being an item and the panel without him/her being the corrected total. This assessed the magnitude of a panelist's evaluation correlated with the rest of the panel. The item-total correlation was also positive in 94 % of the panelist-attribute-combinations and the failures dispersed over attributes and panelists. Consequently, all 12 panelists remained in further analyses. Cronbach's Alpha assessed the consensus of the panel for a given attribute across the 9 samples and reached 0.85 for the 13 attributes of interest. Five fixed effects (flavor, extrudate content coded -1, 0, +1 for a linear and +0.5, -1, +0.5 for a squared relation and their interaction with flavor) and random effects of panelists, replication and their interaction were estimated. Attributes with significant fixed effects were reduced by a principal components analysis (PCA), mainly to illustrate which flavor could mitigate effects of extrudate content.

Linear mixed effects models to explain overall liking, expected liking and the expectation-experience gap

Linear mixed effects regression models were used in order to explain overall liking, expected liking and the expectation-experience gap. Prior to model calculation, the independent variables were transformed through effect coding, mean centering, or assigned as categorical factors (Table 7.2).

Even though consumers were asked to state their overall liking followed by liking of appearance, odor, texture, flavor and aftertaste, only the modeling of overall liking is reported because no additional information regarding the research questions was gained from analyzing the latter variables.

Before analyzing the food neophobia scale, five items needed to be reversed to correctly measure food neophobia (Cronbach's Alpha = 0.74). The range of theoretical food neophobia scores (= sum of all answers per person) was between 10 (extremely food neophilic) and 50 (extremely food neophobic). For the data analysis, the food neophobia score was mean-centered per country. Familiarity data were aggregated into two categories: "I don't know spirulina" for those consumers who had never heard about spirulina and "I know spirulina" for those who at least had heard about it before the time of the study. This factor was included to test whether basic knowledge, or "familiarity", with spirulina could make a difference in the dependent variable, at least to some extent.

To explain overall liking, extrudate content, flavor, country, gender, age, food neophobia and familiarity with spirulina were included as fixed factors. The model controlled for first order, flavor order and extrudate content order random effects. A random intercept and a random slope for the extrudate content nested within consumers were included. The degrees of freedom approximation applied was Satterthwaite. Categorical fixed factor effects were evaluated using *F*-statistics. When a fixed factor effect was significant ($p \leq 0.05$), a *post hoc* comparison of estimated least square means (LSmeans) was performed using the Tukey adjustment.

Table 7.2: Overview of the independent variables and their respective levels as considered in the mixed effects models.

Factors	Levels	Coding
Extrudate content	10 %	-1
	30 %	0
	50 %	1
First order effect	30 %	1
	10 %	-0.5
	50 %	-0.5
Extrudate content order	30 % - 10 % - 50 %	-1
	30 % - 50 % - 10 %	1
Flavor	lemon-basil, beet-ginger or tomato	categorical
Flavor order	first flavor	-1
	second flavor	1
Country	GER, NL or FR	categorical
Gender	Female	-1
	Male	1
Familiarity with Spirulina	I know or I don't know it	categorical
Age	ranging from 18 to 65 years	continuous, mean-centered per country
Food neophobia score	value between 10 (extremely food neophilic) and 50 (extremely food neophobic)	continuous, mean-centered per country

A significant effect of e.g. flavor or familiarity with spirulina may follow either the impact of the concept (conceptual pathway), of the sensory perception (sensory pathway), or both. In order to disentangle the two paths, a mediation analysis was carried out. Effects based on the conceptual path alone should be fully mediated by expected liking. Effects based on the sensory path, i.e. based on actual product experience, will not (or only partly) be mediated by the expected liking and thus re-appear in the expectation-experience gap models.

Expected liking prior to tasting was also modeled. Expectations were only elicited for flavor liking, thus the model includes flavor, flavor order and all the consumer characteristics from Table 7.2. For effects performing similar in both models, a conceptual pathway can be assumed. If the variation in liking was of conceptual, but not sensory, origin the effect in question would no longer be significant in the expectation-experience gap model; where the dependent variable is the gap between the expected and experienced product.

To assess the gap between expected and experienced product liking and in consideration of the criticism of difference scores (e.g., Edwards, 2001), a regression approach was conducted. To do so, an expectation-centered liking score was built by subtracting the average expected liking per country from the overall liking scores and set as dependent variable. Consequently, the intercept of the model directly estimated the magnitude of the expectation-experience gap. As explanatory variables, the expectation-centered liking score and all previously mentioned independent variables (Table 7.2) were included. Thus, the expectation-experience gap model estimated scores reflecting effects of the product experience as if expectations had been equal. Due to the mean-centring per country, LSmeans resembled the mere difference between expected and overall liking at the country mean level, but other estimates did not. Modeled significant effects occurring in the expectation-experience model are attributable to the sensory pathway.

Results

Conventional sensory profiling

All 13 attributes were significantly affected by either flavor, extrudate content (linear and squared), or their interactions; however saltiness was not influenced by extrudate content nor its interaction with flavor, and flavor did not affect firmness nor its interaction with extrudate content (Table 7.3).

Table 7.3: *p*-values of *F*-statistics with 2 degrees of freedom for flavor and its interactions and 1 degree of freedom for linear and squared extrudate content main effects.

	Flavor	Extrudate content		Flavor*Extrudate content	
		linear	squared	linear	squared
O_overall	**	**	n.s.	n.s.	***
O_earthy	***	***	n.s.	***	*
O_algae	n.s.	***	***	**	***
AP_black	***	***	*	***	***
M_moist	***	***	*	***	***
M_firm	n.s.	*	n.s.	n.s.	n.s.
T_overall	***	***	n.s.	**	n.s.
T_sweet	***	***	n.s.	***	*
T_sour	***	***	*	***	*
T_salty	***	n.s.	n.s.	n.s.	n.s.
T_umami	***	***	n.s.	***	***
T_algea	*	***	**	***	***
AT_overall	n.s.	***	n.s.	***	***

*=p<0.05, **=p<0.01, ***=p<0.001, n.s.= not significant

In order to show how flavors moderate the extrudate content effects in 11 of the sensory attributes and to test the expected masking of spirulina characteristics with lemon in the lemon-basil flavor, the sensory profiling data was reduced by a PCA (Figure 7.2): the first and the second principal component (dimensions) accounted for 70 % and 21 % of variance, respectively. The first dimension (Dim 1) describes overall intensity of odor and of taste versus spirulina attributes, i.e. black appearance, algae odor and taste, earthy odor as well as firm mouthfeel. The second dimension (Dim 2) merely contrasts a savory taste, i.e. salty versus sour taste of the flavors, and thus separates the lemon-basil pasta from the other two flavors. Products of each flavor are distributed between the right and left side of the plot due to their extrudate content. This spread differs across flavors and is largest for the tomato flavor and smallest for the lemon-basil flavor, therefore reflecting the linear extrudate content*flavor interaction. This illustrates the masking effect of the lemon flavor. Another interesting insight is the nonlinearity of spirulina related intensities despite equidistant extrudate content: 30 % extrudate content with tomato flavor evokes almost as low spirulina related intensities as 10 % extrudate content with tomato. Thus, at low extrudate content levels, spirulina perception may resemble the one of tomato. Additionally, the overall intensity of the aromas decreased with increasing spirulina-extrudate content. Consequently, a more intense algae flavor was confounded with a weaker aroma of the flavor base (i.e. the spices).

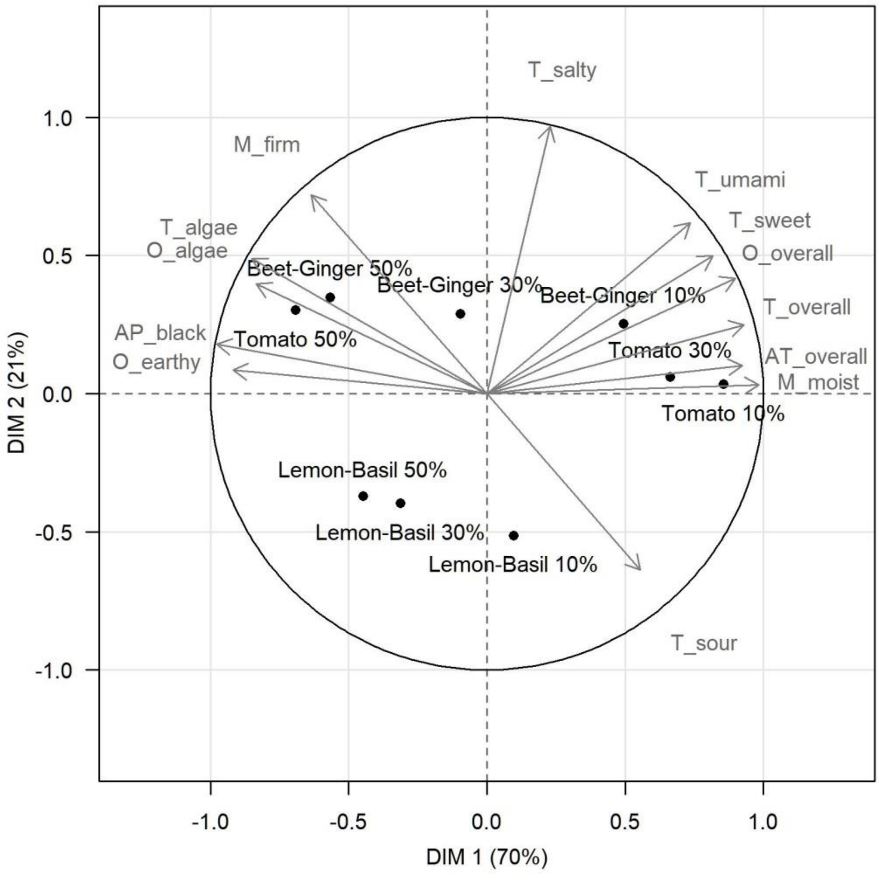

Figure 7.2: Principal component analysis (PCA) of conventional profiling for 9 pasta samples, including 13 attributes. AP_= appearance, O_= odor, T_= taste, M_= mouthfeel, AT_= after-taste. The percentage behind each sample refers to the extrudate content in the filling.

Consumer overall liking based on flavor and extrudate content

Table 7.4 exhibits the consumer sample per country as characterized according to age, gender, food neophobia and familiarity with spirulina . No substantial differences between countries exist.

Table 7.4: Participants' characteristics per country. Absolute numbers (n) followed by percentages or means followed by SD.

	Country			Total
	Germany	**The Netherlands**	**France**	
Mean Age ± *SD*	42.8 ± 13.6	42.9 ± 14.3	41.6 ± 13.9	42.4 ± 13.9
Gender				
Female	75 (54 %)	75 (55 %)	71 (49 %)	221 (53 %)
Male	64 (46 %)	62 (45 %)	73 (51 %)	199 (47 %)
Food neophobia Score ± *SD*	22.2 ± 6.6	21.0 ± 6.2	19.5 ± 5.8	20.9 ± 6.3
Familiarity with Spirulina				
I don't know it	37 (27 %)	30 (22 %)	36 (25 %)	103 (25 %)
I know it	102 (73 %)	107 (78 %)	108 (75 %)	317 (75 %)
Total	139	137	144	420

SD = Standard Deviation.

Generally, pasta was accepted with an overall mean of 5.9 on a 9-point hedonic scale. The liking scores were influenced by the pasta-related variables of flavor and extrudate content, as well as country of residence, food neophobia and familiarity with spirulina (Table 7.5). Age of the consumers had no significant effect on overall liking (F (1, 420.49) = 0.85; p = 0.36); for gender a non-significant trend of higher scores for women appeared (F (1, 420.63) = 3.39; p = 0.07).

Table 7.5: Mixed effects model of overall liking with fixed factors flavor, extrudate content, country, gender, age, food neophobia, familiarity with spirulina and a priori selected interactions; controlled for order. Random factor: consumer.

				Overall Liking
	F	***df1***	***df2***	***p***
First order	33.89	1	1678.82	<0.001
Flavor order	4.86	1	1678.86	0.03
Spirulina order	1.59	1	419.58	0.21
Flavor	50.64	2	1907.36	<0.001
Extrudate content (linear)	265.68	1	419.52	<0.001
Extrudate content * Flavor	11.32	2	2094.73	<0.001
Country	12.79	2	419.33	<0.001
Gender	3.39	1	420.63	0.07
Age	0.85	1	420.49	0.36
Food neophobia	9.96	1	420.65	0.002
Familiarity with spirulina	15.44	1	420.01	<0.001
Flavor * Country	0.81	4	1907.90	0.52
Extrudate content * Country	5.05	2	419.34	0.01
Extrudate content * Flavor * Country	0.10	4	2094.78	0.98
Extrudate content * Food neophobia	1.59	1	422.46	0.21
Extrudate content * Familiarity with spirulina	2.48	1	420.37	0.12

Numerator (df1) and denominator (df2) degrees of freedom, as well as F and p-values for the fixed factors and interactions.

As can be seen from Figure 7.3 a, the lemon-basil flavor was most liked, while the tomato and beet-ginger flavor were less liked (F (2, 1907.36) = 50.64; p < 0.001). This holds true across all three countries, given that the interaction between flavor and country was not significant (F (4, 1907.90) = 0.81; p = 0.52). Furthermore, there was a significant interaction of flavor with extrudate content (F (2, 2094.73) = 11.32; p < 0.001). As can be seen from Figure 7.3 b, the extrudate content slope was steeper for the tomato flavor compared to the other flavors. This implies that the tomato flavor was least suitable to accompany the characteristics of spirulina with increasing extrudate contents. Irrespective of the flavor, an extrudate content of 10 % was always more liked than a content of 50 % (F (1, 419.52) = 265.68; p < 0.001)).

Overall, the pasta performed differently across countries: German and Dutch consumers liked the pasta better than the French (F (2, 419.33) = 12.79; p < 0.001). Additionally, an interaction effect between country and extrudate content (F (2, 419.34) = 5.05; p = 0.01) shows that German consumers despite giving generally high scores penalized 50 % extrudate content more than consumers in the other two countries (Figure 7.3 c).

As expected, both food neophobia and familiarity with spirulina affected liking (Table 7.5). With increasing food neophobia the pasta was less liked (b = -0.03, t(420.70) = -3.16, p = 0.002). However, the slopes for extrudate contents were not affected by food neophobia (F (1, 422.46) = 1.59; p = 0.21). As shown in Figure 7.3 a, consumers who knew about spirulina before the study liked the pasta significantly more (F (1, 420.01) = 15.44; p < 0.001), regardless of the extrudate content (F (1, 420.37) = 2.48; p = 0.12).

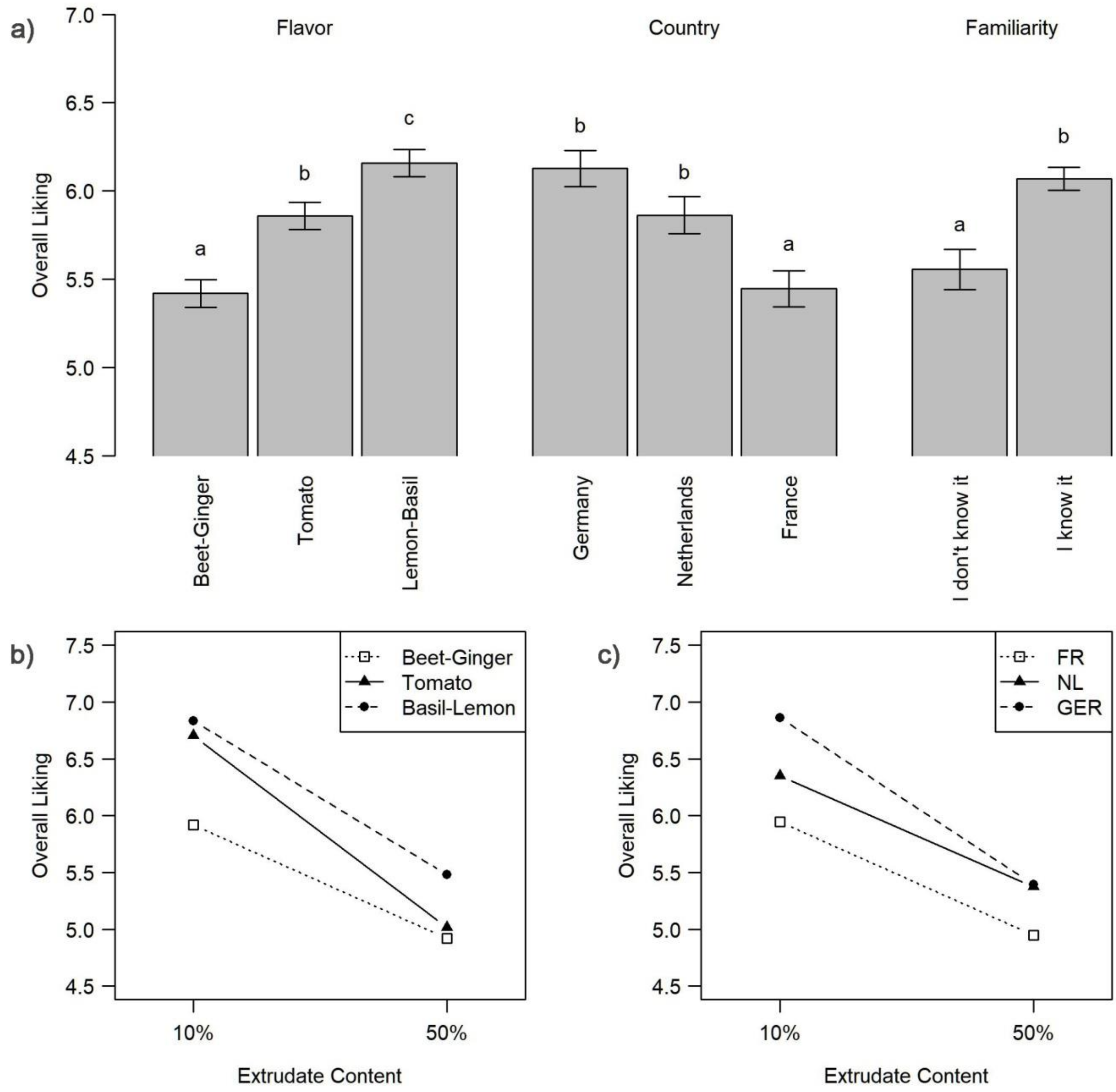

Figure 7.3: LSmeans (± 1.96*SE) for overall liking as affected by flavor, extrudate content, country and familiarity with spirulina. N = 420 consumers. 9-point scale from 1 (dislike extremely) to 9 (like extremely). [a,b,c] Same superscripts indicate non-significant differences. Post-hoc comparison of LSmeans with Tukey's test at p = 0.05.

Decoding the conceptual and the sensory pathways

After consumers received verbal information about spirulina and the statement that they would be receiving three consecutive samples of a pasta flavor, they were asked about their expected flavor liking. It was shown by the expected liking model that expected liking was influenced by pasta flavor, country, age of participants, food neophobia, and familiarity with spirulina (Table 7.6). Food neophobia had a negative impact on expectation (b = -0.05, $t(420.96)$ = -5.29, p < 0.001), while familiarity with spirulina had a positive impact (Figure 7.4). These effects on expected liking can be attributed to the conceptual pathway.

With increasing age, consumers had higher expectations (*b* = 0.01, *t*(420.55) = 2.41, *p* = 0.02) and, even if insignificant, female consumers tended to have higher expectations than male (*b* = -0.12, *t*(420.78) = -1.88, *p* = 0.06). The gender trend thus can be attributed to the conceptual pathway, too; whereas the conceptual age effect seemed to be compensated by an inverted sensory effect, because it was no longer evident in overall liking (Table 7.5).

Table 7.6: Mixed effects model of expected liking with fixed factors flavor, country, gender, age, food neophobia, familiarity with spirulina; controlled for flavor order. Random factor: consumer.

			Expected Liking	
	F	***df1***	***df2***	***p***
Flavor order	0.09	1	419.15	0.77
Flavor	23.43	2	529.60	<0.001
Country	3.92	2	419.17	0.02
Gender	3.53	1	420.78	0.06
Age	5.83	1	420.55	0.02
Food neophobia	28.03	1	420.96	<0.001
Familiarity with spirulina	14.99	1	420.01	<0.001
Flavor * Country	0.25	4	529.88	0.91

Numerator (*df1*) and denominator (*df2*) degrees of freedom, as well as *F* and *p*-values are given for the fixed factors and interaction.

The LSmeans of expected liking are shown in Figure 7.4. Consumers expected to like the tomato and the lemon-basil pasta best. Thus, the lower overall liking of the beet-ginger flavor can be attributed to the conceptual pathway. German consumers expected to like the pasta generally more than Dutch consumers and expectations were higher if spirulina was familiar. Familiarity can be considered closely related with the conceptual pathway.

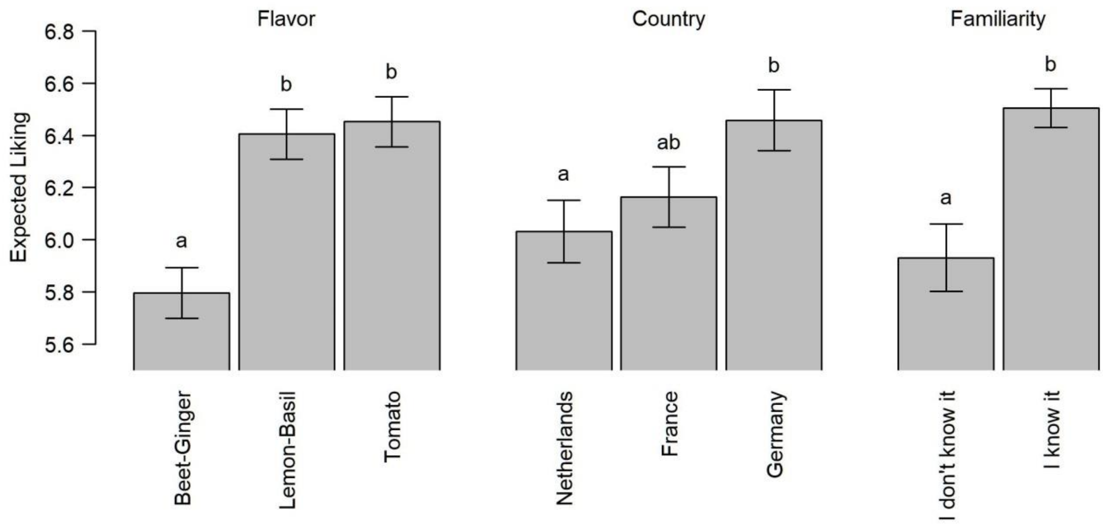

Figure 7.4: Expected liking (± 1.96*SE) for categorical factors flavor, country, and familiarity with spirulina. N = 420 consumers. 9-point scale from 1 (would dislike extremely) to 9 (would like extremely). [a,b] Same superscripts indicate non-significant differences. Post-hoc comparison of LSmeans with Tukey's test at p = 0.05.

The expectations based on pasta flavor descriptions were not fully met by the later experience; the total mean of the expectation-experience gap was -0.51 (*SE*= 0.04). Figure 7.5 a illustrates disappointment as interpreted by bar height. The expectation-experience-gap was highest for the beet-ginger flavor; French consumers on average were most disappointed (Figure 7.5 a). Even though expectation regarding tomato and lemon-basil were similarly high prior to tasting (Figure 7.4), the latter did not disappoint as much as the tomato flavor after tasting. That implies that tomato disappoints consumers via the sensory pathway, yet conceptually the product is liked. A similar finding can be reported regarding country. While the expectation of Dutch and French consumers was comparable before tasting, the disappointment of the French consumers via the sensory pathway was significantly higher. For the Dutch, their experience most closely fits their expectation; for Germans a slight disappointment emerged via the conceptual pathway. Age did not affect the expectation-experience gap. However, if consumers were familiar with spirulina, they were less disappointed by the actual taste (Figure 7.5 a).

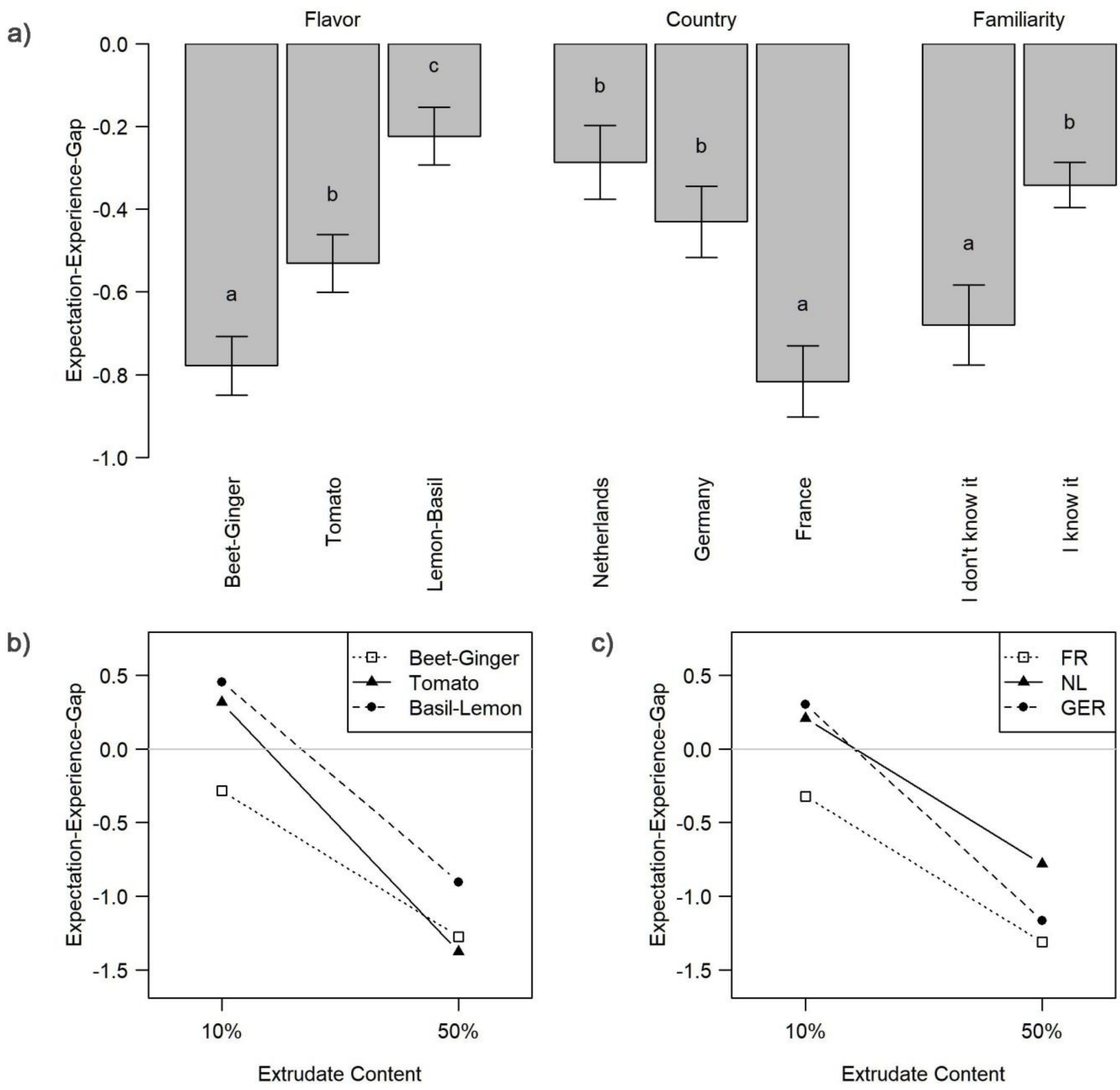

Figure 7.5: LSmeans (± 1.96*SE) of the experienced liking controlled for the expected liking as affected by flavor, country, gender and familiarity with spirulina. N = 420 consumers. Higher bars illustrate higher disappointment. For analysis, overall liking was mean-centered by the expectation mean of the country and controlled by the expectation centered with the country mean. Thus, only country LSmeans resemble the difference between expected and overall liking. LSmeans estimate effects of the product experience as if expectations within country had been equal. [a,b] Same superscripts indicate non-significant differences. Post-hoc comparison of LSmeans with Tukey's test at p = 0.05.

The food neophobia effect and the gender trend disappeared regarding the expectation-experience gap. The variables were already mediated by expectations, i.e. they operate only via the conceptual pathway. The disappointment (= gap) however, was still different across flavors as well as countries and familiarity with spirulina (Table 7.7).

Table 7.7: Mixed model of the expectation – experience gap (overall liking, centered for expectation per country controlled for expectations centered with country means) with fixed factors flavor, extrudate content, country, gender, age, food neophobia, familiarity with spirulina, a priori selected interactions and controlled for order. Random factor: consumer.

	Overall Liking, expectation centered			
	F	***df1***	***df2***	***p***
First order	33.07	1	1623.68	<0.001
Flavor order	4.20	1	1623.90	0.04
Spirulina order	1.83	1	370.23	0.18
Expected Liking	153.37	1	1385.08	<0.001
Flavor	27.49	2	1967.18	<0.001
Extrudate content	261.69	1	414.22	<0.001
Extrudate content * Flavor	11.03	2	2037.65	<0.001
Country	11.23	2	375.17	<0.001
Gender	2.22	1	373.08	0.14
Age	0.01	1	373.95	0.93
Food neophobia	2.40	1	389.22	0.12
Familiarity with spirulina	9.18	1	382.79	0.003
Flavor * Country	0.64	4	1951.46	0.63
Extrudate content * Country	4.97	2	414.03	0.01
Extrudate content * Flavor * Country	0.09	4	2037.66	0.99
Extrudate content * Food neophobia	1.56	1	417.12	0.21
Extrudate content * Familiarity with spirulina	2.44	1	415.06	0.12

Numerator (*df1*) and denominator (*df2*) degrees of freedom, as well as *F* and *p*-values are given for the fixed factors and interaction.

Since expectation was not asked separately, the effect of the extrudate content and its interactions with flavor, country, food neophobia and familiarity with spirulina cannot be mediated; all effects at the bottom of Table 7.7 resemble Table 7.5 and are attributable to the sensory pathway. The interaction plots (Figure 7.5 b & Figure 7.5 c) demonstrate that in the case of lemon-basil and tomato flavored pasta, the actual tasting experience for 10 % extrudate content was not lower than anticipated, according to expectation scores, i.e. these pasta flavors exceeded expectations. In the case of the beet-ginger pasta, none of the variants fulfilled the low expectations. Obviously, consumers were most disappointed by the tomato pasta with 50 % extrudate content via the sensory pathway. French consumers were generally disappointed via the sensory pathway, no matter the extrudate content. There was no general difference regarding the expectation-experience gap between Dutch and German consumers (Figure 7.5 a), but German consumers are more disappointed by the 50 % variants than Dutch (Figure 7.5 c).

Discussion

The study aimed at identifying a flavor that either masked or appropriately complemented the musty and earthy notes of spirulina (Grahl, Palanisamy, et al., 2018) when used in a pasta filling. Lemon-basil with spirulina was preferred, partly due to the concept (compared with beet-ginger), but mainly due to sensory perception (compared with tomato flavor). For the lemon-basil flavor, liking also declined less with increasing extrudate content compared with the tomato flavor. When incorporating spirulina in food, masking the musty taste with lemon thus appears more promising than adding similar flavors. This is in line with the results of the sensory profiling which showed that spirulina-related attributes are less pronounced in the lemon-basil pasta. Even though there are no reports of 2-methylisoborneol (MIB) or geosmin in spirulina (Cuellar-Bermúdez et al., 2017; Milovanović et al., 2015), the acidity of lemon still appeared to mask the intensities of the musty-earthy notes. Protein and lipid degradation are likely involved in the off-flavors of spirulina (Cuellar-Bermúdez et al., 2017), but the mechanism behind the reduction of off-flavors by acidity remains unknown. The beet-ginger flavor added more mustiness and it is likely that this partly explains why this pasta flavor, additionally to the conceptual pathway, was also least accepted via the sensory pathway. The pairing of tomato with spirulina, based on them having glutamic acid in common, was foreseen to be more favorable than beet-ginger. However, the tomato flavor experienced the steepest decrease in liking, which was only attributable to the sensory pathway. On the whole, the acidity of the lemon-basil flavor was most preferred by consumers and therefore would likely be most successful if taken to market.

This study shows that the intensification of spirulina flavor through an increase in spirulina-soy-extrudate has a negative effect on liking, overall. It implies that pasta filling recipes have to be adjusted if higher amounts of spirulina should be processed. Extrudate content appears to be a key factor in liking and plays a significant role in increasing liking when reduced. By investigating differing extrudate contents, insights were gained into the perception and acceptance of the recipes, in general - and spirulina, in particular. The preferred flavor concept identified (lemon-basil) also acts as a foundation for future studies to focus on product optimization. In this study, only pasta flavors with 10 % of spirulina-soy-extrudate were accepted by consumers. In addition, liking for the pasta with lemon-basil or tomato flavor was higher than expectations, at least in Germany and the Netherlands. Future products incorporating spirulina should consider pre-processing steps of spirulina biomass in order to reduce the musty-earthy flavor. Fermentation of spirulina has been shown to be promising in this regard (Bao et al., 2018).

The negative effect on liking of increased spirulina content was observed across all investigated countries. Nevertheless, German consumers had the highest acceptance, especially with low extrudate contents. It is likely that this finding is related to the fact that the pasta was developed and produced in Germany. For example, expert interviews were conducted with German habitants to generate flavor ideas. Consequently, the differences in liking justify the approach of cross-cultural studies, even if the three countries under investigation in this study were geographically and culturally close, and therefore differences in liking were assumed to be subtle. As the mediation analysis revealed, differences between Germany and the Netherlands can be attributed to the conceptual pathway. Differences between France and the other two countries can be attributed to the sensory pathway. Consequently, long standing traditions, food culture (Pettinger et al., 2006) and cross-cultural differences in quality perception (Grunert, 1997) should not be neglected in novel food product development.

Gender and age did not have a significant effect on overall liking. However, age had a positive effect on expectations. Thus a positive conceptual pathway and a negative sensory pathway from age towards overall liking could be compensating one another. Gender only showed a tendency to influence liking and expectation (no significant effect was observed), where female consumers expected more from the pasta than men. It is likely that theses expectation were influenced by the provision of information about spirulina prior to tasting. Health benefits of the microalga were mentioned and female consumers have been shown to react positive to health food claims (Ares & Gámbaro, 2007). A review by Verain et al. (2012) about consumers of sustainable food did not identify a clear association between age and consumption; yet the study did find a tendency for women to eat "green" and exhibit more environmental concern compared to men.

As expected, food neophobia had a negative effect on pasta liking, while familiarity with spirulina had a positive effect. Neophobic consumers are not only known for their reluctance to try novel food, but also for their lower appreciation of novel and unfamiliar flavors (Siegrist et al., 2013). Familiarity with spirulina promoted acceptance and it is therefore important to consider the degree of familiarity with the products of interest, also in cross-cultural studies (Torrico et al., 2019). However, both effects are identified to operate via the conceptual pathway only.

While comparing the expected liking with the experienced liking of the pasta, it was noted that the pasta flavors sounded appealing, but that the expectations outstripped eating quality. If the pasta would be marketed without optimization, consumers might buy and try it once, but repeated purchases would be unlikely as consumers would likely be disappointed by the taste. For example, the tomato flavor was conceptually well liked (high expected liking); yet through the sensory pathway (overall liking) consumers were left disappointed (negative expectation-experience gap). Being a popular dish in Europe, many consumers have often eaten pasta with tomatoes, thereby evoking specific expectations. These expectations were obviously not met by the combination of tomato and a distinct taste of spirulina, likely leaving consumers disappointed. Generally, product development should always aim to match expectations created through marketing and packaging (credence quality dimensions) with experience attributes (Cardello, 2003).

Disappointment among French consumers was higher than among Dutch consumers even though their expectations before tasting were comparable. This was attributed to the sensory pathway. This hints at the aforementioned culinary traditions for which France is famous for (Pettinger et al., 2006). On the other hand, Dutch consumers' expectations fit most closely with their experienced liking of such alternative protein foods. This could be due to consumer exposure, given that Dutch retailers already at the time of the study offered spirulina burger and insect meatballs as alternative protein food (House, 2016). Mere exposure proved to be promising in order to increase acceptance, provided that the first contact with a new product has a positive connotation (Hoek et al., 2013). Hence, all sources of disliking which were attributed to the conceptual pathway only, i.e. gender-trend, food neophobia, low familiarity with spirulina, could disappear with positive marketing and exposure.

The negative evaluation of spirulina taste at higher extrudate content levels is underlined by the combined assessment of consumer and sensory data. Pasta samples that were strongly associated with algae-related attributes were least liked. Samples with lemon-basil or beet-ginger flavor were evenly distributed across the PCA; nevertheless, a lower liking of the beet-ginger flavor was attributed to both, the conceptual and the sensory pathway. The sensory aspects may be due to higher sweet, umami and salty intensities. The tomato pasta samples were separated between the "better" (10 % and 30 % extrudate content) and "less good" side (50 % extrudate content) of the PCA (Figure 7.2). Together with a steeper decrease in consumer liking compared to the lemon-basil pasta, it is assumed that tomato fits least with higher amounts of algae.

Limitations of the study

One limitation of the consumer study was the decision to confound the first-in-order effect with the samples containing 30 % of extrudate. It was a conscious decision to serve the pasta with 30 % extrudate first in order to not confound the first-order effect with the linear continuous factor of extrudate content. In consequence the extrudate content was modelled only linearly and estimates for the 30 % content were no longer informative. Looking at the observed non-linearity in the sensory data, a nonlinear modelling of spirulina extrudate on liking seems worthy to follow up on with a fully randomized sample order. Furthermore, sensory data revealed that the more intense algae flavor was confounded with a weaker aroma of the flavoring ingredients, which can be seen as another limitation of this study. Designing a product to include pasta with different soy vs. spirulina contents in the extrudate as well as equal amounts of spices could lead to an even more comprehensive understanding of appropriate recipe development. Additionally, only German habitants were considered for the expert interviews that were the basis of the whole development process. Cross-country expert interviews might have led to more diverse opinions and finally differentiated product ideas.

Conclusions

This study investigated the pasta fillings tomato-spirulina, beet-ginger-spirulina and lemon-basil-spirulina with different spirulina-extrudate contents in order to evaluate consumer acceptance for novel food products. It was shown that lemon-basil is the most liked flavor and that the flavor of spirulina is only accepted in small amounts, so that the microalgae taste is not too distinct. Consequently, recipe optimization is necessary if higher proportions of the spirulina-extrudate are to be processed into an acceptable product. Masking the distinct spirulina taste appears to be more promising than enhancing it.

Familiarity with spirulina was shown to promote acceptance, while food neophobia hindered liking, but only via the conceptual pathway. Thus, an increase in the prominence of microalgae in general, and of spirulina in particular, would be helpful in order to foster the consumption of alternative protein sources. However, taste must meet expectations so that consumers are not disappointed; otherwise threatening a lasting success on the food market. With an optimized recipe, neophilic consumers who are open-minded to novel products may be convinced to repeatedly consume such products. Overall, it is important to consider individual differences as well as intrinsic product quality characteristics when designing a new product. Based on this study, microalgae pasta should be optimized further and marketing strategies on the basis of the optimization approaches can be developed.

Author contributions

Conceived the sensory study: SG, MS and DM. Organized sample production and performed the sensory experiments: SG. Analyzed the data: AM, SG and MS. Wrote the manuscript: SG. Involved in the discussion of the manuscript: SG, MS, AM and DM. All authors have read and approved the manuscript.

Declarations of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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8 General discussion and future challenges

The goal of the dissertation was to develop food products based on the microalga spirulina taking perception and acceptance of Western European consumers into account. It was aimed to figure out how spirulina can be integrated into a Western European food context. Another aim was to understand how food must be constituted so that consumers are curious and willing to eat it if novel ingredients such as spirulina are incorporated. Foods containing spirulina can serve to replace conventional animal-based dietary protein sources. Changing consumption patterns is a slow cultural process which takes years, so that humankind is advised to have solutions ready for the looming problems, i.e. increasing world population, unequal distribution of food and the Westernization of dietary habits in developing countries (Guyomard et al., 2012). Since the substitution of established foods is more or less impossible on a broad scale, this research aims to broaden available food sources considered healthy, environmentally friendly and palatable more than actual trying to substitute animal-derived food by algae. The work of this dissertation can only be considered a small step towards a potentially meat reduced diet of the West; therefore some challenges for future studies remain.

Environmental impact of cultivation and processing of spirulina

Germany, the Netherlands and France are characterized by intensive livestock farming and high stocking densities. Consequently, animal welfare and the environmental impact of meat production and consumption are pertinent topics. Microalgae are of high interest because they are assumed to have the potential to promote sustainability, particularly in agro-intensive regions (Taelman et al., 2015). As a part of the *Sustainability Transitions* project, the environmental impact of high-moisture extruded meat substitutes based on different plant and plant-like ingredients was investigated through life cycle assessment (LCA) (Smetana et al., 2017). It was shown that microalgae had a higher impact on the environment than traditional protein concentrates, for instance from soybean or pea. While traditional crops have a global warming potential (kg CO_2 equivalents) below one, it was reported to be between 14.7 and 245.1 for microalgae, depending on species and the cultivation system. Similar discrepancies can be reported for energy demand: being about 5 MJ for soy or pea concentrate, values are tremendously higher for microalgae (217.1 to 4181.3 MJ). As opposed to meat alternatives based on soy and autotrophic spirulina, meat alternatives with the heterotrophically produced microalgae *Chlorella vulgaris* had lower environmental impacts than pork and chicken meat. That implies that the cultivation of specific species of microalgae is decisive when evaluating the environmental impact. However, it is not possible to cultivate spirulina in a heterotrophic manner like in a fermenter. It should be noted that the LCA study by Smetana et al. (2017) put a focus on spirulina grown in a PBR or open pond operating in Germany. Knowing that spirulina requires temperatures of around 35 °C to grow (chapter 3.2.2), much energy is required for heating in a temperate climate. In warmer climate zones, e.g. in Spain, less energy would be needed and the effect on the environment would be comparatively lower, provided that water requirements do not rise due to higher evaporation. Considering the time spent in optimizing meat production in comparison to the rather young sector of algae cultivation, one can surely assume that the environmental impact will decrease over time, even if soy-spirulina-extrudates are not yet more environmentally friendly than beef, pork, chicken or soy. The cultivation of spirulina on agro-industrial waste and wastewater may be one way to increase production sustainability, while decreasing production costs, as previous research shows (Ljubic et al., 2018; Markou & Georgakakis, 2011). However, the biomass originating from such production is to date useful in biofuels but not in foods due to strong variation in composition of cultivating media (Markou & Georgakakis, 2011). Because it is important to locate algae production in clean waters to prevent the accumulation of water-borne contaminants and pathogens (Parodi et al., 2018), the production with waste water would be a tremendous challenge with regard to food hygiene. Therefore, more focus should be put to the use of renewable energy sources and a decrease of the energy expenditure (Taelman et al., 2015) to facilitate production sustainability of food grade microalgae.

It would be conceivable to cultivate the algae in connection with biogas as a source of carbon to mitigate CO_2 emissions (Mann et al., 2019; Wang et al., 2008) or photovoltaic energy generation (Smetana et al., 2017). Even the usage of waste heat from power plants to extend growing seasons of microalgae has been shown to be technologically feasible (Mohler et al., 2019) but the authors explain that the purchase of heat integration equipment increases the production costs of biomass by a factor of three which is extremely uneconomical. Research on more efficient microalgae cultivation does not stop with the use of resources; the locations where PBR can be established are now also the focus of architecture. Algae cultivation could contribute to bringing fresh food production into urban centers. First attempts to install PBR on the facades of houses have been initiated (Elrayies, 2018) and could contribute to solve the problem of transportation of natural produce in ever growing cities. Even though somewhat visionary, vertical farming and aquaponic systems could be concepts for sustainable microalgae cultivation, even in cities. However, the cultivation of spirulina on a large scale is still hampered by production costs, technical difficulties with extraction and refining related to scalability and cell disruption treatment as well as poor sensory palatability (Bleakley & Hayes, 2017; Henchion et al., 2017).

Clarification of the spirulina aroma

Ciferri and Orsola (1985) questioned whether there would even be a future for the direct consumption of spirulina, except during emergency conditions like malnutrition. They argue that the potential of the microalga seemed to have reached its limits, particularly due to its flavor. Future research should further exploit the potential of spirulina by clarifying the aroma of the microalga to gain a better understanding of the characteristic aroma substances. While some reports claim that spirulina has a rather mild flavor (Kay & Barton, 1991) spirulina's flavor is often described as musty or earthy off-odor, off-flavor or fish-like (Aguero et al., 2003; Becker, 2007; Lupatini et al., 2017; Stanic-Vucinic et al., 2018). The distinct algae flavor of spirulina as a result of the sensory analysis in **Paper I** played an integral role in establishing the further course of the scientific work. Based on potential off-flavors, the research generally aimed at masking or complementing spirulina's unique flavor because it was assumed that this was necessary to develop acceptable products. Even the idea to incorporate spirulina in sushi (**Paper II**) makes partly use of the typical algae flavor. In **Paper III**, it was shown that the "lemon-basil-spirulina" pasta was liked best by consumers. The idea evolved from the fact that fish is often served with lemon to reduce potential earthy-musty sensations. Geosmin, which provokes such a sensation, converts into the odorless product argosmin under acid conditions (Liato & Aïder, 2017) so that the earthy aroma diminishes. Even though spirulina has not been shown to contain geosmin, the concept still worked but no chemical explanation can be given why masking with lemon is better accepted than flavor pairing approaches.

Despite the general awareness of off-aroma, limited research has been published that sheds light on the respective compounds responsible for the sensation (Cuellar-Bermúdez et al., 2017; Milovanović et al., 2015). No aroma compounds have been analyzed in the extrudates or pasta either so that to date there is no clear answer to the question of what actually constitutes the spirulina flavor and whether it is attributed to changes during storage. Subsequently, future research should investigate changes of the aroma during storage that might occur due to protein- and lipid degradation to determine factors affecting flavor. At the same time, processing through fermentation needs to be pursued more consequently and combined with decent sensory analysis. Even though Bao et al. (2018) found fermentation to be promising related to flavor development, their sensory analysis using a 4 cm desirability scale to measure odor and color as unspecific attributes should be improved. Next to sensory properties, fermentation of spirulina caused an improvement of the nutritional profile because it enhanced phenolic compounds and antioxidant capacity (de Marco Castro et al., 2019).

Based on previous research, it is assumed that aromatic compounds in cyanobacteria develop in response to the habitat (freshwater, marine or terrestrial cultivation systems) (Watson, 2003). Different geographical locations of cultivation also affect the volatile compound composition (de Jesus et al., 2018). Nevertheless, the findings in the specific case of spirulina are not very concrete and the comparative investigation of spirulina from different environments and different geographical locations within Europe is advisable.

Another path that could be pursued related to increased flavor acceptance over time would be mere exposure studies with consumers. Repeated consumption of novel food might increase consumer acceptance (Pliner, 1982; Zandstra et al., 2004), while other studies report stability of liking independent of the number of exposures, in particular for familiar products like yoghurt (Frøst, 2006). Focusing on a familiar product like pasta incorporating a novel ingredient like spirulina, these ambivalent insights would make it an interesting task to investigate potential changes in acceptance for spirulina pasta over time.

If aroma research reveals that spirulina is not easily applicable in food production, protein extraction would be a remaining alternative, even if not yet competitive as compared to established protein-rich food (Bleakley & Hayes, 2017). The extraction removes the flavoring substances of the protein concentrate (Lupatini et al., 2017), and protein concentrates bear more diverse application possibilities. Spirulina protein concentrate would possibly give the chance for HMEC to be done without soy. Since consumer acceptance is largely dependent on taste, aroma constitution and changes need to be better understood. Looking at the advances of research related to soy, it can be assumed that ways will be identified to reduce the undesirable aroma of spirulina. Soy was for a long time deemed being not palatable due to its beany flavor which through improvements in product development and research has been noticeable reduced (MacLeod & Ames, 1988).

Product and recipe development

Sustainable practices in human nutrition are conceivable if, on the one hand, sustainable production of raw materials is warranted and, on the other hand, consumer acceptance is given. In this dissertation, consumer-oriented product development based on analytical and hedonic sensory evaluations was the approach of choice because it was aimed to produce spirulina food that would attract consumers' attention from the beginning.

The results of the sensory results of **Paper I** may appear straight forward. Basically it was found that extrudates with higher moisture were more moist and juicy, while higher algae content led to a more pronounced algae taste. At this point it should be mentioned that research in the field of sensory analysis of meat alternatives is scarce and studies such as the "meat universe" by Rødbotten et al. (2004) have not yet been carried out for meat alternatives. However, such an investigation would contribute to a better understanding of the requirements for meat alternatives that have to be met to enable imitation of certain meat dishes. As mentioned in chapter 3.3.1, the main focus for the analysis of intermediate meat substitutes in research is mostly on physical or chemical analysis and less on sensory analysis. In this respect, the study conducted in **Paper I** is one of few of its kind and a first step towards a better understanding of the relationships between process parameters and sensory quality for meat alternatives.

Consumer acceptance of novel, innovative microalgae-based products can be increased by involving consumers at an early stage of product development (Costa & Jongen, 2006; Moskowitz & Hartmann, 2008). The present consumer studies were conducted in Germany, the Netherlands and France to elicit potentially different perceptions and acceptance of the products investigated. In the future, it would be desirable to conduct cross-cultural studies involving countries with different cultural exposures to algae as food. In contrast to the EU, Asia has a historical tradition for algae consumption (Vigani et al., 2015). To derive consumer insights related to general perception, individual experiences and culture, it would be worthwhile to compare European consumers with Asian consumers, as was done by Hartmann et al. (2015) to investigate insects as food. One can assume that perception of the product ideas in **Paper II** would have turned out differently, depending on different cultural and dietary background, particularly because familiarity with each of the concepts presented is likely to be different. Comparing consumers within Europe showed that differences in perception and acceptance exist (e.g., regarding novelty perception and interest in the product ideas) but that these can be neglected since similarities prevail. Especially the agreement on the acceptance of pasta over sushi and jerky was decisive for further development. Consumers across Europe liked the flavor "lemon-basil-spirulina" (**Paper III**) and the benefit of a healthy food (**Paper II**). Marketing strategies and flavor selection for spirulina pasta do not have to be different in order to launch such products in either one of the investigated countries which is a beneficial insight for the food industry to safe resources that would otherwise be invested in marketing and further consumer research. Concluding, country differences within Western Europe are deemed irrelevant but cross-continental research would be needed to assess the global market potential.

In the consumer study (**Paper III**) it could be shown that an increase of the spirulina content led to a decrease of the overall liking, whereby low algae contents (10 % extrudate content consisting of 50 % spirulina) were generally accepted. This is a relevant aspect for further development of the pasta because it implies that recipe adjustments are necessary if larger quantities of algae are to be processed. Sensory appeal is key to willingness to try and if consumers' expectations do not match with experience, products will not last long on the market (Steptoe et al., 1995). Other than flavor, health is a central aspect shaping consumer food choice (Hoek et al., 2017). Even though health was investigated as a promising benefit in theory since only photos were shown (**Paper II**), future research should test actual foods together with benefits to identify suitable marketing strategies. Consumer acceptance might increase if health benefits would be specifically considered while tasting an unconventional product like algae pasta. A nutritional evaluation of the final product is still lacking, but will be decisive in evaluating health benefits. Additionally, it would be worthwhile knowing how much of spirulina-soy-extrudate is actually necessary to make the pasta a product that does not exceed protein contents recommended by dietary guidelines. More than answering the question of "How far can we go?" this project should have been also looking at "How much is actually necessary?". It cannot be ruled out that pasta with a comparatively low algae content, which was already well liked by consumers, will provide sufficient protein. In this regard, direct comparisons with conventional pasta would be useful but have not been drawn, yet.

Nutritional impairment through the heat of extrusion was shown to destruct amino acids (e.g., lysine) and consequently decrease bioavailability in soy (Žilić et al., 2014). Milder conditions of extrusion (no more than 140 °C) would be favorable to limit the risk of thermal impact on nutritional quality. In other words: nutritional evaluations are all the more necessary, because extrusion of spirulina was done at 160 °C. Palanisamy et al. (2019) investigated whether HMEC influences the antioxidant capacity induced through phenolic compounds and flavonoids contained in spirulina and found an increase after extrusion due to a disintegration of the cell walls during the process. An extrusion process at 160 °C with 50 % moisture and a screw speed of about 800 rpm including 50 % of spirulina seemed to be favorable. These settings are similar to those chosen for the extrudates used in the production of the pasta (160 °C, 55 % moisture and a screw speed of 600 rpm). Overall, the extrusion process influences the composition of extrudates compared with the raw material and more research is needed to derive optimal settings related to health, even if it means to compromise related to macro- and micronutrients.

Even though it was intended to use HMEC derived extrudates as meat alternative, the concept of replacing a whole muscle meat was set aside due to flavor and color of spirulina. Here, the opinion of the interviewed experts was decisive because it was generally assumed to be worthwhile not to hide the spirulina flavor and at the same time use considerable quantities of the algae in products that are rather unlike meat in the classical sense. If HMEC is further followed up upon, future research could focus on the innovative category jerky, as it is a dark colored dried meat snack and makes use of the bite that is created through texturization. Since color plays an important role in the perception of food (Spence et al., 2010) and hiding the color in spirulina jerky is not possible, the extent to which black foods are accepted and which associations consumers articulate should also be further investigated. The production of jerky is really the only product where it is necessary to use extrusion processing; other recipes for pasta or sushi could be created using fresh or dry spirulina. Sometimes product ideas are ahead of their time and might fit better with tomorrow’s lifestyle, provided products are more familiar (Valdovinos, 2009). This may be the case for spirulina jerky.

Instead of sensory testing in a sterile environment like a sensory laboratory, future investigations should consider home use testing as a more valid measure of product acceptability (Meiselman, 2009) or virtual reality to mimic a consumption setting closer to actual reality (Ung et al., 2018). In this very early stage of spirulina product development it was decided in favor of central location test (**Paper III**) because it was still very new to the market and difficult to compare with. First insights needed to be gathered related to algae content and match of flavor pairing and to do so, testing in the sensory laboratory is suitable. With the favored flavor "lemon-basil-spirulina" at hand, more flexibility is given related to future study conceptualization. For instance, a meal context study would be worthwhile considering. Meal studies have been shown to increase acceptability (King et al., 2004). Even a combination of different meal contexts combined with several exposures would be conceivable as was done by Hoek et al. (2013). Such a study would not only consider consumer acceptance but also the influence and interaction with the person, the environment and changes over time. It should not go unmentioned that the decision in favor of a certain test setup is also always connected to the decision about higher internal or external validity of the results (Roe & Just, 2009). While the first refers to the ability of an experiment to explain causal relations, the latter refers to the ability of the results of a given experiment to be generalized to other situations (Plaza et al., 2019). Therefore, the step of first testing in the sensory laboratory appears comprehensible.

9 Project limitations

A major limitation related to the product development is the strong and sole focus on HMEC. The idea to incorporate spirulina in the extrusion process originated from the protein content. However, HMEC products solely serve to actually substitute meat which is obviously difficult in case of a pronounced algae flavor and a dark color. Such products would consequently, if at all, only serve as an alternative to meat but never as an analog or substitute. The scope of product ideas was therefore broadened to be more flexible and investigate in which context the extrudate could be of use. This resulted in the production of filled pasta where the extrudate is used as part of the filling. In order to use it as a filling, the extrudate was cut into small irregular pieces that were possible to squeeze through the ravioli machine. To a certain extent, the energy-intensive texturization using HMEC is contradictory to the subsequent comminution. It was not until the consumer survey (**Paper II**) that it became apparent which application of the extrudate had the greatest potential for success. At this point, the investigation of spirulina by extrusion had already been decided. Otherwise, one could easily think of spirulina-filled pasta containing the spirulina powder only. Extrusion leads to higher prices of the processed biomass and also complicates the production logistics.

Additionally, the project assumed algae and insects to be more sustainable than soy imports as they would help Europe to become more independent from soy imports. It turned out that sustainability assumptions related to microalgae cultivation are still somewhat visionary, at least with production occurring in Europe. The vast amounts of microalgae used in the project for feed and food were imported from Myanmar and China, two of the main producing countries. This contradicts itself with the approach of the possible avoidance of imports from overseas. However, it can be explained by the fact that it was not possible to procure about 2000 kg of the raw material at reasonable prices, at least four years ago. Furthermore, soy still needs to be blended with spirulina to produce extrudates to secure texturization. Using the complete biomass of spirulina required the addition of soy concentrate.

There still exist numerous gaps in defining which diet is more sustainable than another and maybe the moderate consumption of meat is as good as a vegan or vegetarian diet. To identify alternatives that harbor promising innovations to improve or change unsustainable systems in order to create opportunities for a transition process, research has to start somewhere, even if it is ahead of time.

10 Conclusions

The research in this dissertation demonstrates that spirulina can be incorporated in the establishment of food production processes like HMEC without having to compromise on textural properties. Furthermore it elucidates that a novel ingredient like spirulina should be incorporated in a product category that is familiar to consumers to ease product launch. Consumers can best imagine a food when they know the context in which it should be eaten. A new food in an unfamiliar context would only create a large obstacle in acceptance, like shown in the online survey. A familiar context implies that consumers have the opportunity to make the new food part of their dietary habit without having to make any significant changes. Related to marketing, it was shown that health-promoting properties of novel foods appeal most to consumers and should be followed up for marketing purposes.

Overall, sensory appeal is one of the most important factors related to food choice and therefore most decisive regarding success of a product. The results of the consumer test led to the conclusion that small amounts of spirulina (10 % of spirulina-soy-extrudate corresponding to 5 % of spirulina powder in the total filling) are acceptable and that higher proportions of spirulina (50 % of spirulina-soy-extrudate corresponding to 25 % of spirulina powder in the total filling) necessitate recipe optimization. In order to create acceptable recipes, a clear understanding of the flavor of the alga including potential impact throughout storage is needed. With an optimized recipe, early adopters, who are presumably neophilic, could be attracted to the product created as a part of this research. Such consumers will also determine if the spirulina-based food has a chance to establish in the market of meat alternatives or not. It would be advisable to set a focus on adventurous consumers instead of the general population right away. Once initial products become more well-known, the range of products can be increased. The growing awareness of the quality and benefits of the microalga spirulina in a food will trigger interest and willingness to try this increased selection. Thus, a meat-reduced, if not plant-based, diet is possible and can be supplemented by new taste experiences.

At this very moment, microalgae cultivation and processing is still in its early stages and remains comparably expensive as well as energy demanding. A large part of the research conducted will benefit the optimization of price and sustainability aspects in short time so that the best opportunities based on microalgae are yet to come.

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Summary

Sustainability in food production has become a dominant social issue, as the environmental impact of food production is inevitable. The focus is on animal foods, as their production requires many resources. In addition, current food production will reach planetary limits if the demand for food of a constantly growing world population has to be met. Since a sole increase in the productivity of existing systems is not sufficient, alternative food sources must be established and their suitability for human consumption must be investigated in order to secure food production in the future.

Using the microalga spirulina (*Arthrospira platensis*) as an example, this dissertation shows how consumer-oriented product development can contribute to a sustainability transition, i.e. a change towards sustainable food production and nutrition. The microalga can be produced in Germany independent of arable land available and it is a promising food ingredient due to its nutrient content. Therefore, this PhD project aimed to investigate the suitability of spirulina for innovative foods that promote a meat-reduced diet using a mix of methods from qualitative and quantitative sensory research.

The first study investigated the applicability of spirulina in extrusion by means of descriptive analysis using a trained sensory panel. According to a Design of Experiment (DoE), systematically varied extrusion parameters were examined with regard to their effects on the sensory properties of spirulina-soy-extrudates. The spirulina content in the extrudate, the screw speed of the extruder, the moisture content in the extrudate and the process temperature were investigated. It was found that high moisture resulted in a corresponding appearance; moist products produced a juicy and soft mouthfeel. High spirulina content resulted in black color, intense taste with earthy notes and a musty algae odor. Products with low spirulina content were elastic, fibrous and firm. As a result, possibilities for controlling the desired texture of future spirulina extrudates were derived: low moisture, high screw speed and temperature lead to firm and fibrous products with a distinct algae aroma.

In a second step, expert interviews were conducted to identify promising product ideas which were consequently tested in an online survey. Preferences of consumers for three different product categories with the extrudate were investigated and the marketing opportunities based on the benefits sustainability, health and innovation were explored. A staple food (pasta), a product following the principle of "flavor pairing" (sushi) and a savory snack (jerky) were shown to 1035 consumers in Germany, the Netherlands and France on photos. Spirulina-filled pasta was preferred over the other two categories as pasta is generally very familiar. The data analysis showed a mediating effect of familiarity. This means that all three product categories are conceivable with spirulina, provided they would be equally well-known by consumers. In addition, health-promoting properties were appreciated most by consumers.

Based on the results of the second study, various pasta variants in three flavors (lemon-basil, beet-ginger or tomato) with different contents of spirulina-soy-extrudate (10 %, 30 % or 50 %) were developed and examined in a sensory consumer test. 420 participants from Germany, the Netherlands and France were each served six out of a total of nine pasta variants to determine acceptance. The lemon-basil flavor was most accepted by consumers, followed by tomato and beet-ginger. It was also shown that an increase of the amount of spirulina-soy-extrudate in the filling led to a drop in acceptance. This implies that an increase in spirulina content causes a more pronounced algae flavor and requires further recipe adjustment. In order to derive recommendations for the recipe adjustment, sensory profiling of the pasta variants was done and the objective evaluation was linked to consumer liking. It turned out that the sensory perception caused by spirulina (algal smell and taste, earthy smell and black appearance of the filling) should be reduced to increase consumer acceptance.

In summary, the research in this dissertation shows that spirulina is suitable to contribute to a meat-reduced diet. Familiar product categories make it easier for consumers to accept novel products. If higher contents of the spirulina-soy-extrudate are to be processed, recipe optimization is necessary. The present dissertation offers sufficient starting points for product and recipe improvement.

Zusammenfassung

Nachhaltigkeit in Bezug auf die Lebensmittelproduktion ist mittlerweile ein dominierendes gesellschaftliches Thema, da der Umwelteinfluss der Herstellung von Lebensmitteln vielfach nachgewiesen wurde. Dabei stehen vor allem tierische Lebensmittel im Fokus, da ihre Erzeugung sehr ressourcenintensiv ist. Hinzu kommt, dass die derzeitige Lebensmittelproduktion an planetarische Grenzen stoßen wird, wenn der Lebensmittelbedarf einer stetig wachsenden Weltbevölkerung erfüllt werden muss. Da eine Produktivitätssteigerung bestehender Systeme allein nicht ausreicht, müssen alternative Lebensmittelquellen erschlossen und auf ihre Eignung für die menschliche Ernährung untersucht werden, um die Nahrungsmittelproduktion der Zukunft zu sichern.

Die vorliegende Dissertation zeigt am Beispiel der Mikroalge Spirulina (*Arthrospira platensis*), wie konsumentenorientierte Produktentwicklung zur *sustainability transition*, d.h. einem Wandel hin zu einer nachhaltigen Nahrungsproduktion und Ernährungsweise, beitragen kann. Die Mikroalge kann unabhängig von zur Verfügung stehender Fläche in Deutschland produziert werden und ist wegen ihres Nährstoffgehalts eine vielversprechende Lebensmittelzutat. Ziel des Promotionsvorhabens ist es daher, mittels Methoden-Mix aus qualitativer und quantitativer Sensorik-Forschung die Eignung von Spirulina für innovative Lebensmittel zu untersuchen, die eine fleischreduzierte Ernährung fördern.

Die erste Studie untersuchte mit Hilfe deskriptiver Analyse durch ein trainiertes Sensorik-Panel zunächst die Handhabbarkeit von Spirulina in der Extrusion. Mittels eines *Design of Experiment* wurden systematisch variierte Extrusionsparameter hinsichtlich ihrer Effekte auf die sensorischen Eigenschaften der Spirulina-Soja-Extrudate geprüft. Hierbei wurden der Spirulinaanteil im Extrudat, die Drehzahl der Extruderschnecken, der Feuchtigkeitsanteil im Extrudat sowie die Prozesstemperatur untersucht. Es zeigte sich, dass ein hoher Feuchtigkeitsgehalt in einem entsprechenden Aussehen resultierte; feuchtere Produkte riefen ein saftiges und weiches Mundgefühl hervor. Ein hoher Spirulinaanteil führte zu schwarzer Farbe, intensivem Geschmack mit erdigen Noten und einem muffigen Algengeruch. Produkte mit geringem Spirulinaanteil waren elastisch, faserig und fest. Schließlich wurden Möglichkeiten zur Steuerung der gewünschten Textur zukünftiger Spirulina-Soja-Extrudate abgeleitet: geringe Feuchtigkeit, hohe Drehzahlen der Extruderschnecken und Temperatur ermöglichen die Herstellung fester und faseriger Produkte mit ausgeprägtem Algengeschmack.

In einem zweiten Schritt wurden Experteninterviews zur Eingrenzung entwickelter Produktideen durchgeführt. Die hierbei als vielversprechend herausgearbeiteten Lebensmittelkategorien wurden in einer Online-Befragung getestet. Die Präferenzen der Konsumenten für drei verschiedene Lebensmittelkategorien mit dem Extrudat wurden erhoben und die Marketing-Chancen basierend auf den Benefits Nachhaltigkeit, Gesundheit und Innovation ausgelotet. Ein Grundnahrungsmittel (Pasta), ein Produkt das dem Prinzip des *„Flavor Pairing"* folgt (Sushi) und ein praktischer Snack (Jerky) wurden 1035 Konsumenten in Deutschland, den Niederlanden und Frankreich auf Fotos gezeigt. Spirulinagefüllte Pasta wurde gegenüber den beiden anderen Kategorien bevorzugt, da Pasta im Allgemeinen sehr vertraut ist. Die Datenanalyse ergab einen moderierenden Effekt der Bekanntheit. Das bedeutet, dass bei gleichem Bekanntheitsgrad alle drei Produktkategorien mit Spirulina denkbar sind. Zudem fanden gesundheitsfördernde Eigenschaften bei Konsumenten Gefallen.

Aufbauend auf die Ergebnisse aus der zweiten Studie wurden verschiedene Pastavarianten in drei Geschmacksrichtungen (Zitrone-Basilikum, Rote Bete-Ingwer, Tomate) mit unterschiedlichem Gehalt an Spirulina-Soja-Extrudat (10 %, 30 % oder 50 %) entwickelt und in einem sensorischen Konsumententest untersucht. 420 Teilnehmern aus Deutschland, den Niederlanden und Frankreich wurden je sechs aus insgesamt neun Pastavarianten serviert, um die Akzeptanz zu messen. Die Geschmacksrichtung Zitrone-Basilikum akzeptierten die Konsumenten am meisten, gefolgt von Tomate und Rote Bete-Ingwer. Es konnte zudem gezeigt werden, dass eine Steigerung des Spirulina-Soja-Extrudat-Anteils mit einem Abfall des Gefallens verbunden ist. Dies impliziert, dass eine Erhöhung des Spirulinaanteils u.a. mit einem deutlicheren Algengeschmack einhergeht und eine weitere Rezepturanpassung erforderlich macht. Um für die Rezepturanpassung konkrete Handlungsempfehlungen zu ermöglichen, wurde die Pasta sensorisch profiliert und die Daten mit dem Konsumentengefallen in Verbindung gebracht. Es stellte sich heraus, dass die durch den Zusatz von Mikroalgen hervorgerufenen sensorischen Eindrücke (Geruch und Geschmack nach Alge, erdiger Geruch und schwarze Füllung) reduziert werden sollten, um die Konsumentenakzeptanz zu erhöhen.

Zusammenfassend lässt sich feststellen, dass Spirulina geeignet ist um einen Beitrag zu einer fleischreduzierten Ernährung zu leisten. Vertraute Produktkategorien erleichtern Konsumenten die Akzeptanz von neuen Produkten. Sofern höhere Anteile des Spirulina-Soja-Extrudats verarbeitet werden sollen, ist eine Rezepturoptimierung empfehlenswert. Hierfür bietet die vorliegende Dissertation hinreichend Ansatzpunkte.

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Declaration

1. I hereby declare that this work has not been made available in the same or in a similar form for the purpose of meeting examination requirements.

   I further declare that I have not applied for a doctorate at any other university.

   Göttingen, ...

   ....

   (Signature)

2. I hereby declare that this dissertation was written independently and without unauthorized assistance.

   Göttingen, ...

   ....

   (Signature)

Authors' contributions

Being the first author of the three publications in this dissertation, I declare my contribution to each paper being about 90 % of the work. I was involved in every step of the process, comprising planning, conducting the experiments, data analysis and writing the manuscript. Each of the steps was initiated by me and at most further developed through feedback from the co-authors. It should be highlighted, that the extrusion of the samples investigated in Paper I was done at the German Institute for Food Technology (DIL e.V.) in Quakenbrück according to the Design of Experiment established by me under supervision of Micha Strack. I personally attended the production and accompanied the process. Based on the analysis of Paper I, I decided with which sample to proceed for the study in Paper III, backing up the decision with Megala Palanisamy, who was in charge of extrusion at DIL e.V. The recipe development for the pasta was done just by me while the production was done by me together with Casaluko GbR in Baunatal. The company isi GmbH in Rosdorf provided their partner network in order to conduct the consumer test in Germany, the Netherlands and France. I personally supervised the test at each of the locations. For Paper II, I conceived the product ideas after several visits at fairs and talking to producers, conducted the expert interviews based on my own interview guideline, and supervised the photo session with Fotostudio Wilder in Göttingen. I prepared the sushi myself while pasta and jerky were kindly provided by DIL e.V. The product concepts were written by me under supervision of Micha Strack. The performance of the sensory experiments for Paper I and Paper III were done by me. Generally, the work would not have been possible without the valuable support and advice by all the co-authors whose involvement in the different tasks is mentioned separately for each paper:

Paper I

Conceived the sensory study: SG, LMD and MS. Developed the Design of Experiment: SG and MS. Produced the samples: MP, SG and ST. Performed the sensory experiments: SG. Analyzed the data: SG and MS. Wrote the manuscript: SG. Involved in the discussion of the manuscript: SG, MP, MS, LMD, ST and DM. All authors have read and approved the manuscript.

Paper II

Conceived the study: SG, MS and RW. Analyzed the data: SG and MS. Wrote the manuscript: SG. Involved in the discussion of the manuscript: SG, MS, RW and DM. All authors have read and approved the manuscript.

Paper III

Conceived the sensory study: SG, MS and DM. Organized sample production and performed the sensory experiments: SG. Analyzed the data: AM, SG and MS. Wrote the manuscript: SG. Involved in the discussion of the manuscript: SG, MS, AM and DM. All authors have read and approved the manuscript.