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Life Cycle Assessment of Burger Patties Produced with Extruded Meat Substitutes

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Abstract

Extruded meat substitutes, due to their high protein content, meat-like texture and meat processing compatibility, are very popular as the main ingredient of plant-based burger patties. The extrusion of plant-based proteins can be performed by two technologies: high moisture extrudates (HME) and low moisture texturized vegetable proteins (TVP). The largest difference between the technologies relates to the moisture content prevailing inside the extrusion barrel. The extrusion processes also vary in their throughput, and yields. Life cycle assessment (LCA) was performed to compare the environmental performance of the two extrusion technologies applied to two plant-based raw materials (soymeal and pumpkin seed flour). Additionally, the study compared plant-based burger patties to meat burger patties (beef, pork and chicken). The impact of plant-based burger patties was at least ten-fold lower than meat burger patties. TVP-production exhibited a higher environmental impact compared to HME (20-40% higher depending on the raw material). The best performing plant burger patties were HME-soy-patties, in contrast with the worst-performing plant TVP-soy patties. TVP-pumpkin seed patties presented lower impacts compared to TVP-soy ones.

Keywords: burger patties, high moisture extrusion, Life cycle assessment, meat substitutes, pumpkin seed flour, texturized vegetable protein

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Table of abbreviations

1,4-DB eq	1,4-dichlorobenzene equivalent
AP	Acidification potential
Bq	Becquerel
CMC	Carboxymethylcellulose
CV	Coefficient of variation
DIL	Deutsches Institut für Lebensmitteltechnik e.V.
EP	Eutrophication potential
Eq	Equivalent
Fe	Iron
FU	Functional unit
GWP	Global warming potential
HME	High moisture extrusion
ISO	The International Organization for Standardization
LCA	Life cycle assessment
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
LU	Land use
mPt	Millipoint
NMVOC	Non-methane volatile compounds
NPK	Nitrogen (N), phosphate (P) and potassium (K)
OD	Ozone depletion
PM	Particulate matter
PO	Photochemical oxidation or ozone formation
PS	Pumpkin seed
Pt	Point (ecopoint)
SD	Standard deviation
TVP	Texturized vegetable protein

1 INTRODUCTION

Currently, the world population counts over 7.5 billion people. It will likely reach 8.5 billion in 2030 and rise to 10 billion people in 2050 (United Nations, 2019). This is an increase of one third of the world population in a time span of merely 20 years. Already at this point, we are witnessing the overexploitation of natural resources and degradation of the environment. This is best visible in the "Earth Overshoot Day", which is a term used to represent the date on which humanity's resource consumption exceeds the earth's capacity to regenerate those resources (Global Footprint Network, 2020). Such a date indicates the problems of sufficient global food supply on one hand and increasing environmental impact of food overconsumption on the other hand. One of the most polluting activities in the current food system is the production of animal proteins (Poore and Nemecek, 2018). Moreover, developing countries demonstrate an increased meat consumption in absolute and relative rates over the past few years trying to "catch" with the Western lifestyle (Popkin et al., 2012). That points towards the arising problem of a high environmental impact associated with animal-derived food products. The search for more sustainable meat alternatives is envisioned as a potential solution for both supplying the growing demand in meat and reducing the environmental impact of protein sources (de Bakker and Dagevos, 2012).

Nowadays, meat substitutes gain an increased interest as alternative protein sources, and especially those produced with application of texturization technologies. Alternative products assembled from plant (Osen et al., 2014; Sá et al., 2020), insects (Smetana et al., 2018, 2019a), microalgae (Caporgno et al., 2020; Grahl et al., 2018) and many other sources (Kumar et al., 2017) are aiming for biting properties and texture of meat products such as poultry meat (Cavitt et al., 2004; Meullenet et al., 2005), pork (Olsson et al., 2003) and beef (Hansen et al., 2006). Moreover, many products based on extruded plant-based meat substitutes including burger patties are already available in supermarkets of many countries. Previous Life Cycle Assessment (LCA) studies of plant-based burger patties only include beef patties for the comparison (Heller and Keoleian, 2018; Khan et al., 2019). Furthermore, available studies rarely include the variations in scale or processing technologies of meat analogues.

Previous LCA studies on burger patties produced with extruded meat substitutes only focussed on global warming potential (greenhouse gas emissions), land occupation, water consumption, energy consumption and aquatic eutrophication potential (Heller and Keoleian, 2018; Khan et al., 2019). Complete comparison that included multiple midpoint and endpoint categories are not available to the best knowledge of the authors. It can be expected that the impact categories will gain a higher score for meat products than the plant-based products. Global warming potential for beef is highlighted to reach 24.0 kg CO_2-eq. per kg produced beef meat (Zervas and Tsiplakou, 2016). At the same time, the highest emission for the cultivation of soy is merely 0.7 kg CO_2-eq. per kg cultivated soybeans (Dalgaard et al., 2008). This means that large differences between meat and plant-based burger patties can be expected concerning the climate change impact (global warming potential). On the other hand, the possibility exists that plant-based patties are characterized by a high environmental impact in other midpoint categories, which would level down potentially beneficial performance of plant-based patties.

Extrusion is a process where a raw material is fed into a horizontal barrel with one or more screws. There, it is subjected to a high temperature and pressure, and forced through a shaped die, with the aid of screws inside the barrel, to form the desired product. A well-defined setting of process parameters such as flow rate, the die geometry, barrel temperature and system parameters (e.g. pressure profile) are crucial to generate products with targeted characteristics (Alam et al., 2016). Varying the fraction of water added can result in different types of end-products using the same raw materials.

Extrusion of protein-based formulations at high moisture content in the barrel in combination with a cooling die at the end of the barrel results in a fibrillar-like texture imitating meat. This type of die allows to preserve the moisture in the product and to form the fibrous meat-like texture (Fig 1.) when the protein-water matrix flows through the die and is cooled down. The texture is formed because, while cooling, layers of protein molecules flow to each other, polymerise, cross-link and reorient into the typical fibrous structure (Guy, 2001). Fibrous structure formation depends on the material properties and the flow profile inside the die, mainly determined by the die design and heat transfer. Typically, this technology is referred to as high moisture extrusion (HME) and produces extrudates with a high moisture content (more than 50% water content).

Applying a low moisture content in the barrel, in combination with a pelletizer (rotating knife), creates dry extrudates that can be stored at ambient temperatures for a long period (up to 6 months). In this case, the drier protein matrix is subjected to pressures up to 50 bar inside the barrel. When the protein matrix is pushed through the expansion die, the pressure decreases instantly. This sudden drop causes moisture to rapidly evaporate and create bubbles in the agglomerated structure. These bubbles leave voids inside the structure, creating the typical texturized vegetable protein (TVP) texture (Guy, 2001). If the required moisture content (< 10%) is not reached, then a fluidised bed or conveyor belt dryer can be installed for further drying. The technology to produce TVP is often referred to as cooking extrusion. After storage, the TVP extrudates should be remoistened to be processed into burger patties.

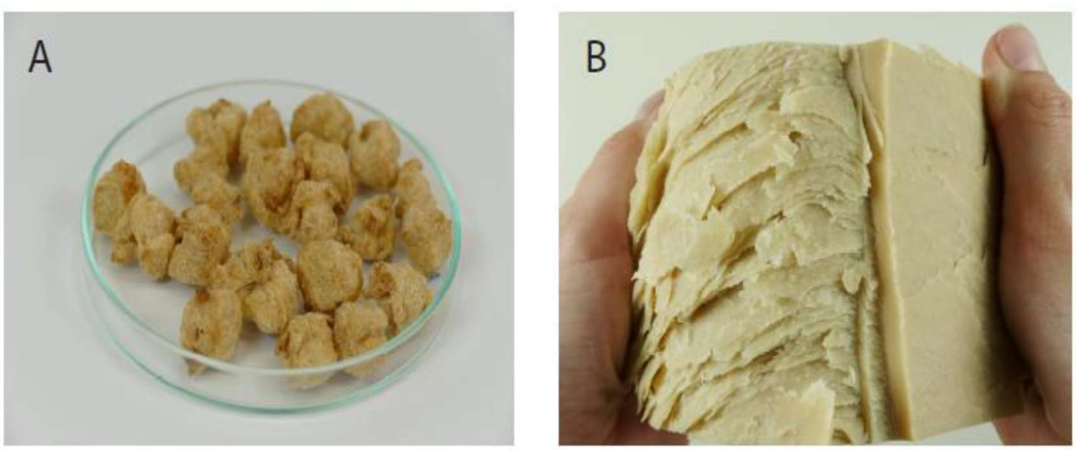

Fig. 1 Extrusion intermediate products made from soy concentrate: (A) TVP balls; (B) fibrillar texture of HME (authors figure)

Due to the fibrillar-like structure, it is possible to further process the HME and TVP extrudates with traditional meat processing technologies into burger patties, sausages, nuggets, etc. This is the reason why not only consumers, but also meat processing companies have an increased interest in these types of products.

Currently, soybean protein concentrates and isolates are the benchmark raw materials to produce extruded meat substitutes. Substantial research is performed on soymeal as a raw material (Lin et al., 2002; Wu et al., 2018). Today, three countries account for over 80% of the total soybean cultivation (Karuga, 2018): the United States of America, Brazil and Argentina. Hence, nearly all soy or soymeal processed in Europe is imported from the American continent(s). Such global supply chains are always in risk of disturbances due to various factors. Locally-produced and supplied raw materials are more beneficial from this perspective. That is why our study relied on a locally produced raw material source, suitable for extrusion. Moreover, current study aims for a comparison of the environmental impact of high moisture extrusion and low moisture, cooking extrusion incorporated on the same processing scale. Also, it includes more meat types than previous studies, such as chicken and pork as comparison benchmarks. Those meat types are characterised by much lower impacts compared to beef (Kalhor et al., 2016; McAuliffe et al., 2016; Zervas and Tsiplakou, 2016). Results of this study should indicate whether plant-based burger patties produced with different extrusion processing technologies are indeed more environmentally friendly than meat burger patties.

The objective of the study is to compare burger patties produced from plant sources (soy protein concentrate and pumpkin seed flour) produced with different extrusion technologies. A secondary aim is set for the comparison of produced plant-based burger patties with meat burger patties (beef, chicken and pork), considering the supply chain starting from raw material extraction (cradle) and ending at production (gate). Soymeal concentrate is used as benchmark vegetable protein and pumpkin seed flour is considered as a local alternative source. The packaging process and material of the burger patties are not included as they are assumed to be similar for all products. Hence, the functional unit in this study is one kilogram of fresh, ready-to-pack burger patties. In this way, differentiations in the production and processing of meat and meat substitutes are included in the study. The packaging material, packaging process and method of distribution were assumed to be similar for all types of burger patties included. Uncertainty analysis is performed to investigate the robustness of the results. Scenario analysis tests if an alternative pumpkin seed harvesting method affects the results for the local alternative. Also, it is investigated if a prolonged, frozen storage of the high moisture extrusion intermediates affects the results. Lastly, the sensitivity analysis also includes a verification of the main study results with an alternative characterization method (IMPACT2002+).

2 MATERIALS AND METHODS

2.1 Plant materials

Extruded soymeal protein concentrate with 67% protein in dry matter (Solae Europe Sa, Switzerland) is used as a benchmark. Extruded pumpkin seed flour with 61% protein in dry matter (Fandler, Austria) is used as a European, locally produced alternative raw material.

2.2 Extrusion process

This study focuses on the extrusion of plant-based proteins to produce extruded meat substitutes, further used for burger patties production. Extrusion was performed with the aid of a production scale twin-screw, co-rotating, water-cooled extruder (ZSK 43 Mv, Coperion®, Stuttgart, Germany). This is a cooking extruder that can be used for high moisture extrusion (HME) as well as to produce texturized vegetable protein (TVP). The two technologies differ in the water fraction of the formulation inside the extruder barrel (Table 1). For HME, the moisture content is typically above 50%. For TVP, it is around 20%. Consequently, the difference in moisture content leads to differences in product temperatures, pressure profiles and mechanical and thermal energy input. The process parameters used in this study are presented in Table 1.

Table 1 Extrusion parameters for high moisture extrusion (HME) and texturized vegetable protein (TVP) based on soymeal concentrate (Soy) and pumpkin seed flour (PS)

Product	Product influent (kg/h)	Water influent (L/h)	Total rate (kg/h)	Screw speed (rpm)	Barrel temperature [a] (°C)	Product temperature [b] (°C)	Pressure [c] (bar)
HME Soy	58	112	170	1300	120-160	124	28
HME PS	40	43	83	1000	120-140	116	17
TVP Soy	72	18	90	700	130-140	147	44
TVP PS	70	20	90	600	140-150	122	45

[a] Set temperature profile in the heating zone.
[b] Product temperature at the die
[c] Pressure at the end of the barrel, just before entering the die.

The configuration at the end of the extruder barrel also affects the properties of the resulting intermediate product(s). HME products were made using a ‘cooling die’ with a rectangular design (FKD-2100, DIL e.V., Quakenbrück, Germany). This cooling die consists of four segments. Each segment has a length, height, and width of respectively 800 mm, 12 mm and 147 mm (Fig. 2). TVP are produced differently, relying on an expansion die, composed of 4 holes with a diameter of 4 mm each, in combination with a pelletizer (ZGF 70, Coperion, Stuttgart, Germany) to cut the extrudate strands (Fig. 2). After extrusion, the desired moisture content (< 10%) was reached with the aid of a conveyor belt dryer.

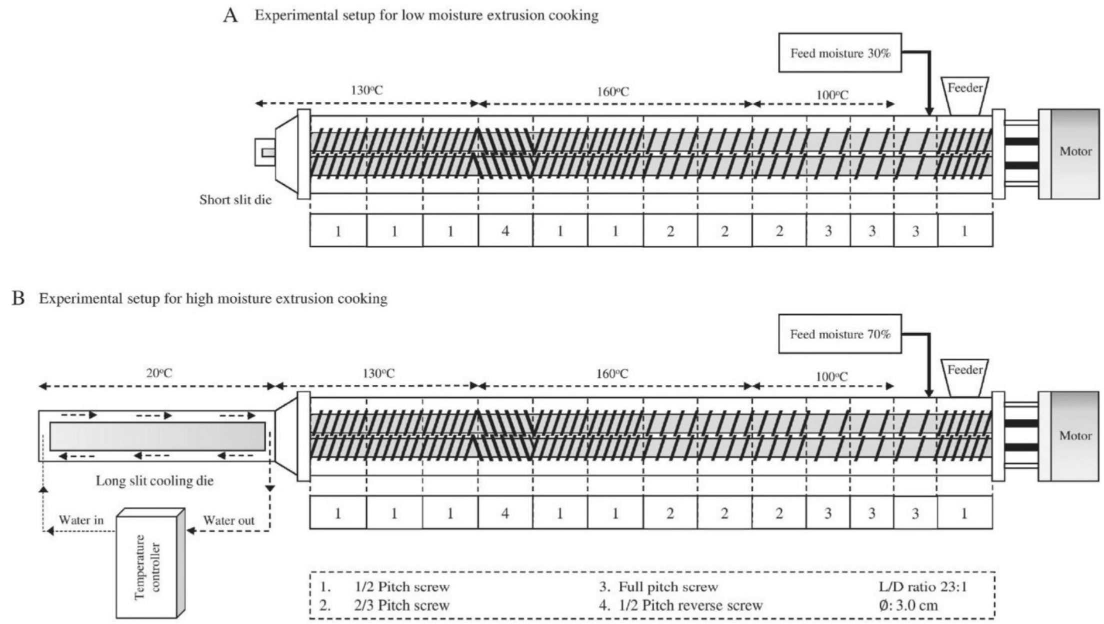

Fig. 2 Cross-section scheme of extruder for Texturized Vegetable Proteins production (A) and High Moisture Extrusion (B), License number 4953100605833 (Samard et al., 2019)

2.3 Life cycle assessment of burger patties

The LCA in this study was performed following the international standards (ISO 14040, 2006; ISO 14044, 2006). The study relied on the attributional approach because the consequences in the surrounding market were not investigated. Attributional modelling allows for data allocation if the impacts of two or more products from the same production are investigated. Here, all impacts of every input and output of all used unit processes are summed and linked to the chosen functional unit (Ekvall et al., 2016).

2.3.1 Goal

The goal of this study was two-sided. First, the environmental impacts of plant-based burger patties, made from extruded meat substitutes, are compared to the impacts of meat burger patties. The results should indicate whether (1) the local alternative is more sustainable than the benchmark plant-based raw material and whether (2) meat burger patties, other than beef burger patties, also present a higher environmental impact compared to plant-based patties. Second, it was investigated if the type of extrusion technology (HME *versus* TVP) affects the overall results. This study does not address taste, texture, colour and hence consumers acceptance of the burger patties. Nevertheless, the plant-based patties were produced to be as similar as possible to plant-based patties found in the supermarket.

2.3.2 Investigated products

In total seven different types of burger patties were analysed in this study. Four of them were produced via extrusion: (1) the HME soy burger patty, (2) the HME pumpkin seed burger patty, (3) the TVP soy burger patty, (4) the TVP pumpkin seed burger patty; and three were used as comparison benchmark products: (5) the beef burger patty, (6) the chicken burger patty and (7) the pork burger patty.

2.3.3 Functional unit and system boundary

The chosen functional unit (FU) was “one kilogram of fresh, ready-to-pack burger patties”. The generalized system boundary of this study (Fig. 3) visualizes all processes that are involved and contribute to the environmental impact. This study involved a “cradle to gate” approach starting from raw materials production to the production of raw burger patties. Consequently, the study covered the production and acquisition of raw materials and ended up at the “factory gate”, i.e. just before transport to the distributors. Hence, packaging, distribution to the supermarket and from there to the consumer, consumption and waste treatment after consumption are not included in the scope of the study.

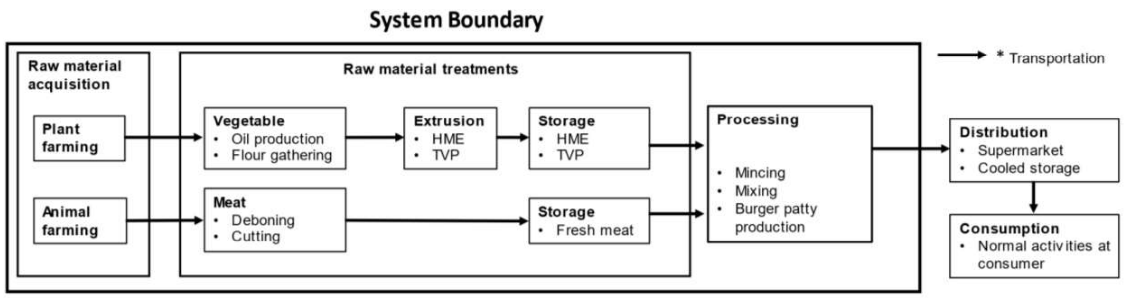

Fig. 3 Generalized scheme of system boundary of the study

Both for plant-based and meat burger patties, the systems started with the acquisition or extraction of raw materials. For soy and pumpkin cultivation, this included sowing, growth and harvesting of the crops. In this phase, typical factors that contribute to the environmental impact are tillage (ploughing, hoeing, earthing-up, etc.), fertilizers (ammonium nitrate, phosphate, potassium, liquid manure, etc.), land occupation and irrigation (water consumption). For beef, chicken, and pork, the study relied on the models of LCA Food DK database, from which the inventory for farming and slaughtering was used. The major environmental burden for beef is allocated at the farm level, where the cattle emits a considerable amount of greenhouse gasses (Mogensen et al., 2015). In literature, chicken is stated to be the most environmentally friendly type of all meat products (Rodic et al., 2011; Roy et al., 2009). The actual emissions from chickens on the farms only have a small impact (Kalhor et al., 2016). The highest impact of broiler rearing is related to feed and electricity consumption. Also, for pork production, the highest impact is identified to be the animal feed (McAuliffe et al., 2016).

The next phase in the system was pre-treating the raw materials to obtain suitable products for production of burger patties. For beef, chicken, and pork, this included slaughtering, deboning, cutting and storage of the meat. These steps are more energy- and resource-efficient compared to the rearing step (Mogensen et al., 2015).

Meat-based burger patties production was modelled based on the inventory data available in databases for raw materials: beef minced meat; chicken, fresh, from slaughterhouse; and pork minced meat as initial raw material input from LCA Food DK database (Nielsen et al., 2003). The production and processing (grinding, mincing, mixing and forming into burger patties) of other components of burger patties such as potato starch (4%), water (4-7%) and salt (1-2%) were modelled based on the similar processing inputs for electricity applied to plant-based burger patties.

For the plant-based burger patties, this included extrusion and storage of the extrudates until production of the burger patties. The main ingredients for plant-based burger patties considered in this study were extruded meat substitutes produced with soymeal concentrate and pumpkin seed flour. These types of meals and flours are by-products of vegetable oil production.

Like meat, HME extrudates have a high moisture content (> 50%) and require storage at reduced temperatures. In this study, the extrudates were stored in a freezer (-18°C) for two weeks and thawed for two days in a refrigerator (4-7°C). In contrast, TVP extrudates have a low moisture content (< 10%) and can be stored at room temperature and in a low relative humidity. To produce the plant-based burger patties, TVP intermediates should be remoistened and HME intermediates should be thawed. Afterwards, all subsequent steps to process the extrudates into plant-based burger patties are similar to that of meat burger patties. These steps include mincing with a 2.5 cm die (fd mincer fd-70 CE, Gilde, Frankfurt am Main, Germany), mixing of all ingredients (KVL6320S Kenwood, Hampshire, the United Kingdom) and pressing of the burger patties (50 g, 5 cm diameter, 1.5 cm thickness).

To produce plant-based patties, extrudates need binders and a source of fat. The combination of the protein-rich extrudate matrix, binding agent and a source of fat preserves the moisture during the post-production frying step. This is necessary in order to imitate the juiciness of a meat burger patty and to improve the overall acceptability of the plant-based burger patty (Khalafalla et al., 2010). For plant-based patties, the study relied on a carboxymethyl cellulose (CMC) solution as a binding agent and a fat emulsion, produced with pea protein and rape oil, as source of fat. The production of the CMC solution and fat emulsion was also included in the system boundary. The comparability of burger patties was assured through colour and physical properties (compressing and cutting) (Supplementary materials).

2.3.4 Life cycle inventory analysis (LCI)

2.3.4.1 Software

The software used to perform the LCA was SimaPro 8.0 (PRé Consultants B.V, Amersfoort, NL, 2009) with integrated databases such as “ecoinvent v2.2” (Zurich, Switzerland) and “LCA Food DK” (Nielsen, et al., 2003). The considered characterization method was ReCiPe v1.08 (Goedkoop et al., 2009), since this method considers as well midpoint (climate change, ozone layer depletion, human toxicity, ecotoxicity, acidification, land occupation, metal and fossil depletion) as endpoint (damages to human health, ecosystems and resource availability) categories. Furthermore, this method allows an overall single score product comparison. This study made use of the “20% percent rule” (Matthews et al., 2014) during the single score and scenario analysis. This implies that a difference is substantial when results differ at least 20% from each other. This rule was not used for the comparison of midpoint impact results which led to the conclusions. Midpoint impact results were analysed for the uncertainty with Monte Carlo simulation (see part 2.3.6).

2.3.4.2 Data and sources

This study relied on data collected from multiple sources. All data considering the processing of burger patties (extrusion, mincing, mixing, and pressing) were acquired from pilot scale production facility of DIL (DIL Deutsches Institut für Lebensmitteltechnik e.V., Quakenbrück, Germany) (Table 2). Further, the data for soymeal production were derived from the ecoinvent v2.2 database (Zurich, Switzerland). They covered growth and harvesting of the soybean and the extraction of oil. The model for the cultivation and harvesting of pumpkins was based on the model for zucchini, found in the ecoinvent v2.2 database, because edible pumpkins and zucchini are both part of the *Cucurbitaceae* family. To convert the information found on zucchini to pumpkin and pumpkin seeds harvesting, internet sources, i.e. secondary data, was used (Devi and Palmei, 2018; Hacıseferogulları and Acaroglu, 2012; Minderhoud and Troost, 2008) and Table 2.

Table 2 Data and sources used for pumpkin cultivation modelling

Pumpkin yield	**Seed yield**	**Land use**	**Fertilizer**	**Irrigation**
Ton pumpkin/ha	% seed/kg pumpkin	m^2a [a]	kg NPK/kg pumpkin	m^3/kg pumpkin
(Statistisches Bundesamt (Destatis), 2019)	(Poštić et al., 2018)	(Groww, 2016)	(van Wijk and Stilma, 2011)	(van Wijk and Stilma, 2011)
18	3.90	0.139	0.01	0.0039

[a] area*time unit expressed as square metres-year: 13 weeks (0.25 years) of land occupation for 0.556 m^2 per kg pumpkin

To finally obtain the pumpkin seed flour, four main unit processes were considered. The first unit process included sowing, growth and harvesting of whole pumpkins. Afterwards, the pumpkins were transported to a seed processing plant where seeds were separated from the skin and flesh. The skin is not useful for further processing and was returned to the fields as peat. The pumpkin flesh was further processed in the food industry. Hence, this unit process had more than one output. Therefore, economic revenue allocation based on the bulk prices of the output products was opted. After the seeds were collected, they were roasted as a preparatory step for the actual seed pressing. Roasting reduced the moisture content (longer shelf life), added aroma and flavour and loosens the husk from the seed, which facilitated the seed pressing. The fourth unit process was the seed pressing. This process had two output products, being oil and flour. For this reason, economic revenue allocation based on the bulk prices of these output products was used.

Models for extrusion (HME and TVP) were, as discussed earlier, based on data derived from own trials on a production scale twin-screw, co-rotating, water-cooled extruder. Primary data for electricity and water consumption were obtained during the production trials (Table 3). Also, primary data for electricity consumption were acquired during the production of patties (mincing, mixing, pressing). During extrusion, flow meters (Zenner water meter type ETK QN = 2.5, Saarbrücken, Germany) were installed for the measurement of water consumption during cooling of the motor and barrel and for product incorporation. Electricity consumption of motor, heating barrel and pelletizer (only for TVP) was measured with Fluke 325 true RMS clamp meters (Fluke, Washington, USA). This device measured the live Ampère-values recorded with a camera (EOS M10, Canon, Tokyo, Japan) and data were collected by taking values every five seconds of these recordings. Afterwards, these collected ampere values were calculated into total electricity consumption for a three-phase electric circuit with equation (1):

$$W = I * U * PF * \sqrt{3} \quad (1)$$

where:

- W = Power (Watt)
- I = Current (Amperes)
- U = Voltage (V) (400V)
- PF = Power factor (0,85)

The electricity consumption of the hopper and the water pump (or a drier for TVP) was measured with a WM5-96 network analyser (Carlo Gavazzi, Steinhausen, Switzerland) which allowed to calculate the power used during the process.

2.3.4.3 Primary data

Table 3 summarizes the results for electricity and water consumption during extrusion. This table is based on a weekly production cycle of four days in which the four different extrudates are produced throughout the whole day (8 hours) on different days. There was a difference between the product yield, due to different process parameters and different product properties. Waste produced during the extrusion cycles (heating phase materials, fallen scraps) was treated as biowaste. After the extrusion, the intermediates were stored, as explained in paragraph 2.3.3.

Table 3 Electricity and water consumption for each type of extrudate during one cycle (8 hours) of actual extrusion. High moisture extrusion (HME) of soymeal produced 1439.12 kg useful product and 48.01 kg waste, HME pumpkin seed flour (PS) produced 708.48 kg useful product and 27.20 kg waste. Texturized vegetable protein (TVP) production of soy produced 640.00 kg useful product and 48.66 kg waste, TVP PS production produced 577.80 kg useful product and 43.10 kg waste.

Product	Phase	Time (min)	Power (kWh)	Water (L)	Energy use (kWh/kg)	Water use (L/kg)
HME Soy	Warm-up	30	7.07	377	0.01	0.52
	Start-up	12	5.2	404	0.007	0.57
	Extrusion	480	190	15800	0.27	22.3
	Total	522	203	16600	0.29	23.4
HME PS	Warm-up	30	7.07	377	0.01	0.52
	Start-up	12	5.2	404	0.007	0.57
	Extrusion	480	190	15800	0.27	22.3
	Total	522	203	16600	0.29	23.4
TVP Soy	Warm-up	30	6.72	232	0.01	0.36
	Start-up	33	10.0	589	0.016	0.92
	Extrusion	480	147	10400	0.23	16.2
	Total	543	164	11200	0.26	17.5
TVP PS	Warm-up	30	5.93	185	0.01	0.35
	Start-up	33	7.41	274	0.01	0.48
	Extrusion	480	99.6	7010	0.17	12.1
	Total	543	113	7470	0.2	13.0

Energy consumption during mincing, mixing and pressing of the plant-based and meat burger patties were in a range of 0.0213 to 0.0225 kWh/kg burger patties. This indicated the similarity in processing conditions between extruded meat-substitutes and actual meat.

2.3.5 Sensitivity and scenario analyses

Sensitivity and scenario analyses were performed to determine how assumptions, calculations and/or uncertainties affect the reliability of results and conclusions. Three major assumptions taken during modelling could possibly influence the total impacts:

1. **Pumpkin cultivation:** Pumpkin seeds are harvested mainly by two methods. The method considered in this study relied on harvesting whole pumpkins and separating the seeds from the flesh and skin afterwards. The alternative way is by harvesting through a rough separation of seeds from the pumpkins by heavy machinery on the field (Moty, 2019). The alternative scenario for the cultivation of pumpkins and harvesting of the seeds with heavy machinery was evaluated and compared with the model in the main study. The results were then investigated to identify the impact of the pumpkin cultivation method.
2. **Extrudate storage**: In the chosen model, a two-week frozen storage period followed by two days thawing under refrigerated conditions was opted for HME extrudates. For TVP extrudates, a storage of two weeks at room temperature was chosen. During the scenario analysis, a more prolonged storage was compared to that of the chosen model. It was tested whether storage of one, two, four and eight months of frozen storage for HME and storage on room temperature for TVP affected the results.
3. **Alternative LCIA method**: The main study results were generated with ReCiPe v1.08 (Goedkoop et al., 2009). An alternative characterization method was applied during a sensitivity analysis to verify the main study results. IMPACT2002+ (Jolliet et al., 2003).

2.3.6 Uncertainty analysis

Since a large part of the models in the LCI-phase are based on secondary data and LCI models available in the databases, the results had certain level of uncertainty. To investigate how robust the results were, an uncertainty analysis was performed in SimaPro 8.0 (PRé Consultants B.V, Amersfoort, NL) with 1000 simulation runs of the "Monte Carlo analysis". This test uses a pedigree matrix (Ciroth et al., 2016) and tests the uncertainty of all midpoint impact categories. Important results of this analysis are the obtained mean, standard deviation (SD) and coefficient of variation (CV). The CV represents the relative magnitude of the uncertainty (Ciroth et al., 2016). If a high CV is obtained, then no robust conclusions were made for that specific midpoint category.

3 RESULTS AND DISCUSSION

3.1 Midpoint characterization factors

Differences were observed in environmental impact between meat burger patties and plant-based patties at midpoint categories (Table 4). Beef burger patties had the highest overall environmental impact. They also were leading in impact of the most categories (12 out of 17), except for terrestrial and freshwater ecotoxicity (chicken burger patties), urban land occupation (TVP soy burger patties), natural land transformation (chicken burger patties) and metal depletion (TVP PS burger patties). Chicken and pork burger patties also showed high relative impacts in most midpoint impact categories. TVP PS were the highest contributor in metal depletion and TVP soy patties were the most influential in urban land occupation. HME soy patties had lower environmental impact in most categories than PS-based burger patties which makes HME soy burger patty with the least environmental impact at midpoint level. These results provide an initial indication that patties made from vegetable protein might be not "environmentally friendly" in every midpoint impact category. However, they require further analysis in terms of significance, sensitivity, and comparability with other studies.

Table 4 Midpoint impact category results expressed per functional unit (one kilogram of fresh, ready-to pack burger patties). The highest impacts are indicated bold and underlined. The method used was ReCiPe Midpoint (H) V1.08 (World Recipe H). The abbreviations used were high moisture extrusion (HME), texturized vegetable proteins (TVP) and pumpkin seed flour (PS)

Impact category	Unit	Beef burger patties	Chicken burger patties	Pork burger patties	HME soy burger patties	HME PS burger patties	TVP soy burger patties	TVP PS burger patties
Climate change Human Health	kg CO_2 eq	**26.6**	6.05	5.83	0.53	0.75	0.87	0.94
Ozone depletion	mg CFC-11 eq	**0.088**	0.074	0.072	0.033	0.041	0.043	0.05
Human toxicity	kg 1,4-DB eq	**0.9**	0.15	0.2	0.03	0.05	0.03	0.06
Photochemical oxidant formation	kg NMVOC	**0.07**	0.007	0.008	0.002	0.002	0.003	0.003
Particulate matter formation	kg PM_{10} eq	**0.1**	0.01	0.01	0.001	0.002	0.002	0.002
Ionising radiation	kBq U235 eq	**0.1**	0.1	0.1	0.06	0.1	0.07	0.1
Climate change Ecosystems	kg CO_2 eq	**22.4**	5.1	4.9	0.5	0.6	0.7	0.8
Terrestrial acidification	kg SO_2 eq	**0.7**	0.07	0.09	0.003	0.007	0.004	0.01
Freshwater eutrophication	kg P eq	**0.006**	0.0006	0.0006	0.0002	0.001	0.0003	0.002
Terrestrial ecotoxicity	kg 1,4-DB eq	0.11	**0.14**	0.11	0.005	0.005	0.006	0.005
Freshwater ecotoxicity	kg 1,4-DB eq	0.026	**0.033**	0.03	0.001	0.001	0.001	0.001
Marine ecotoxicity	kg 1,4-DB eq	**0.006**	0.004	0.004	0.0005	0.0008	0.0008	0.001
Agricultural land occupation	m^2a	**5.91**	4.28	5.24	0.79	0.49	1.39	0.59
Urban land occupation	m^2a	0.002	0.002	0.002	0.01	0.01	**0.015**	0.01
Natural land transformation	m^2	0.05	**0.06**	0.03	0.0007	0.0001	0.0015	0.0001
Metal depletion	kg Fe eq	0.03	0.01	0.01	0.02	0.03	0.03	**0.04**
Fossil depletion	kg oil eq	**1.87**	0.5	0.5	0.1	0.2	0.1	0.2

The comparison of some midpoint impact categories with other studies indicated that the results are mostly in the range of those indicated for both meat and plant-based products (Table 5). However, land use impacts of the current study were in the lower range for the most products, which might relate to the LCIA methodology used. Similarly, the energy use impact for plant-based products were lower than those available for pea-based burger patty (Heller and Keoleian, 2018), which might be explained with a more extended system boundary and more advanced recipe.

Table 5 Environmental impact of meat and meat substitutes produced: global warming potential (GWP), energy use (EU) and land use (LU)

Protein source	System/ study case	Country [a]	Functional unit	GWP kg CO_2 eq.	LU m^2/year	EU MJ	Source
Beef	Conventional [b]	GR	kg meat	24.0	-	-	(Zervas and Tsiplakou, 2016)
	Conventional	UK-S	kg meat	15.6-25.3	22.8-38.5	26.8-40.7	(de Vries and de Boer, 2010)
	Conventional	DK	kg meat	11.1-19.4	-	7.5-30.2	(Mogensen et al., 2015)
	Conventional	GLO	kg meat*	65-190	84-623	-	(Poore and Nemecek, 2018)
	Conventional	USA	kg burger patty	35.6	33.4	100.3	(Heller and Keoleian, 2018)
	Conventional	USA	kg burger patty	25.3-37.5	37.0-102.5	-	(Khan et al., 2019)
	Conventional	**DK-DE**	**kg burger patty**	**26.6**	**5.9**	**78.3**	**Current study**
Poultry	Conventional	GR	kg meat	5.0	-	-	(Zervas and Tsiplakou, 2016)
	Conventional	UK	kg meat	4.6-5.5	6.4-7.3	12.0-14.5	(de Vries and de Boer, 2010)
	Conventional	IR	kg meat	2.9-5.4	-	-	(Kalhor et al., 2016)
	Conventional	**DK-DE**	**kg burger patty**	**6.1**	**4.3**	**19.8**	**Current study**
Pork	Conventional	GR	kg meat	7.50	-	-	(Zervas and Tsiplakou, 2016)
	Conventional	UK-S-NL	kg meat	3.7-6.4	7.3-15	15.5-22.0	(de Vries and de Boer, 2010)
	Conventional	IE	kg meat	4.08-4.8	-	-	(McAuliffe et al., 2016)
	Conventional	**DK-DE**	**kg burger patty**	**5.8**	**5.2**	**21.2**	**Current study**
Soy	Consequential [e]	ART	kg soy	0.73	3.0-3.6	-	(Dalgaard et al., 2008)
	Conventional	CA	kg soymeal	0.3	-	3.5	(Pelletier et al., 2008)
	Mass based allocation	BR	kg soy	0.39	0.68	-	(Lehuger et al., 2009)
	Mass based allocation	BR	kg soymeal	6.52	-	25.5	(Silva et al., 2017)
Pumpkins	Conventional	NL	kg pumpkin	0.24	0.6	1.7	(van Wijk and Stilma, 2011)
Plant-based processed foods	Conventional	USA	kg burger patty	3.52	2.7	53.7	(Heller and Keoleian, 2018)
	Conventional	USA	kg burger patty	3.1-4.0	1.6-3.7	-	(Khan et al., 2019)
	Conventional on SPP	GLO	kg ready to eat product	2.7	1.1-1.4	27.8-36.9	(Smetana et al., 2015)
	Conventional on SPP	**GLO-DE**	**kg burger patty**	**0.53-0.87**	**0.79-1.4**	**4.2-5.9**	**Current study**
	Conventional on PS	**NL-DE**	**kg burger patty**	**0.75-0.94**	**0.48-0.59**	**6.4-7.5**	**Current study**

*- recalculated value; PS – pumpkin seed flour; SPP – soy protein concentrate

[a] Country abbreviations: Greece (GR), United Kingdom (UK), Denmark (DK), Iran (IR) Greece (GR), Ireland (IE) Argentina (ART), Canada (CA), Brazil (BR), the Netherlands (NL), the United Nations of America (USA), Sweden (S), Global (GLO), Germany (DE).

[b] Conventional means normal farming methods and attributional, economic revenue allocation was applied.

[d] Non-methane volatile organic compounds: acetone, benzene, ethanol, formaldehyde, etc.

[e] Consequential modelling was used.

3.2 Endpoint characterization factors

The endpoint categories indicated the potential damages related to areas of protection which were human health, ecosystems and resource scarcity, and were linked with midpoint categories through "damage pathways" (Goedkoop et al., 2009). It was observed that plant-based patties had lower impact than meat burger patties in every endpoint category (Fig. 4). This result was in line with our expectations and literature results (Table 5). Furthermore, beef burger patties had almost 95% higher impact in every endpoint category compared to all plant-based patties, regardless of the extrusion technology used. Hence, plant-based patties not only have a considerably low CO_2-eq. impact, but they scored better in every endpoint category. Overall, plant-based patties were more environmentally sustainable than meat burger patties.

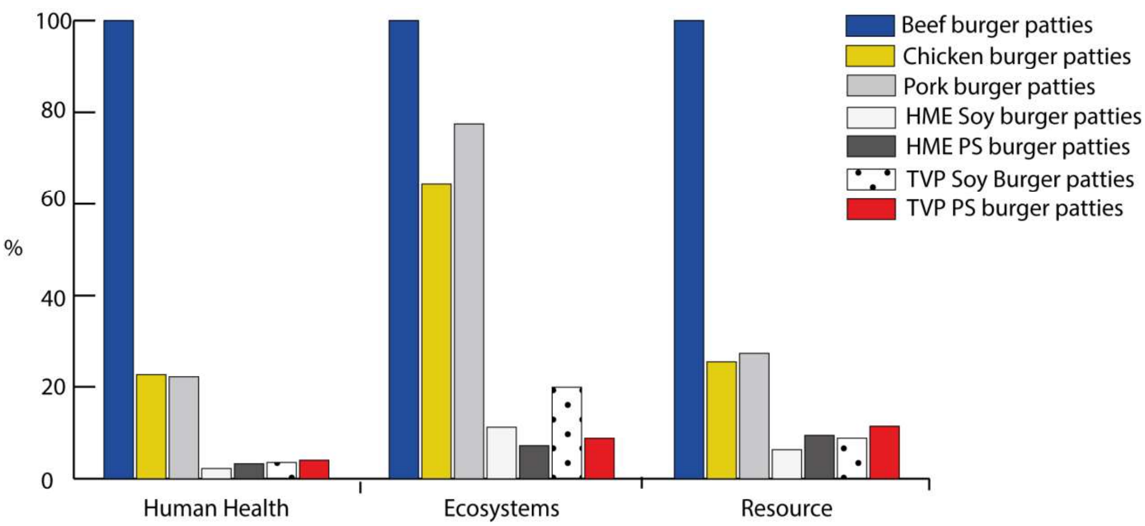

Fig. 4 Relative environmental impact of compared products with the highest impact set at 100%; endpoint impact categories; functional unit: 1 kilogram of fresh, ready-to-pack burger patties; ReCiPe Endpoint (H) v1.08 (World Recipe H) method

3.3 Single score product comparison

A single score product comparison allowed an integrated analysis of which midpoint categories had the highest weight on the total impact in their respective endpoint category. Furthermore, it gave a visual representation of how much the total cumulative burden differs between all products.

Fig. 5 represents the integrated environmental impact results. Beef burger patties clearly had the highest potential burden (2.431 Pt) followed by chicken burger patties (0.908 Pt) and pork burger patties (0.878 Pt). According to the "20%-rule", the total impact of beef burger patties was considerably higher than that of all other burger types. However, appropriate uncertainty analysis is presented further. Chicken, and pork burger patties had lower impact than beef, which corresponds well to literature studies (Table 5). Importantly, all types of plant-based patties, regardless of the extrusion technology used, showed a lower environmental impact than all meat burger patties included in this study. Such a conclusion corresponds well to the results presented in other burger and meat substitute studies (Heller and Keoleian, 2018; Khan et al., 2019; Smetana et al., 2015).

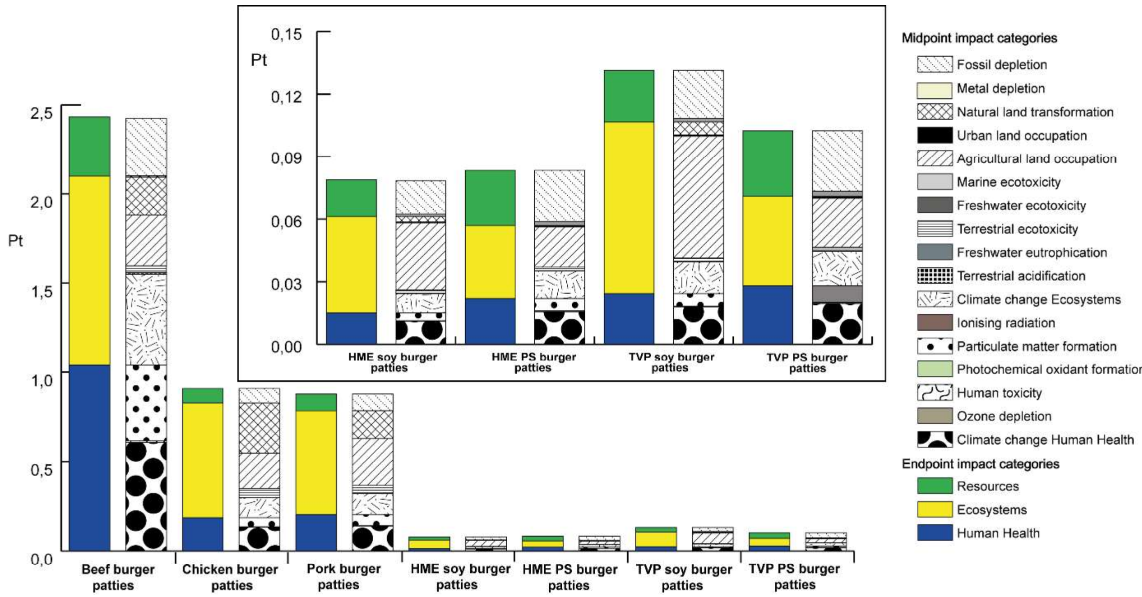

Fig. 5 Integrated environmental impact of compared products; single score product comparison functional unit: 1 kilogram of fresh, ready-to-pack burger patties; ReCiPe Endpoint (H) v1.08 (World ReCiPe H) Single score method; HME - high moisture extrusion, TVP - texturized vegetable protein, PS - pumpkin seeds, Pt - point

For all types of meat burger patties, there were seven midpoint categories that contributed the most to the total burden. These were climate change, human health, particulate matter formation, climate change ecosystems, terrestrial ecotoxicity, agricultural land occupation, natural land transformation and fossil depletion. All other midpoint impact categories were characterized by such a low score that they could be neglected.

The total impact of all plant-based patties varied in the range of 5-10% of impact compared to the meat burger patties (Fig. 5). The integrated single-score impact of plant-based patties indicated that TVP soy patties (131.3 mPt) had the highest potential impact followed by TVP PS patties (102.6 mPt), HME PS patties (83.4 mPt) and HME soy patties (78.8 mPt).

Therefore, TVP soy patties were the most environmentally impacting among plant-based patties, which was contrasting to the results for HME soy patties, showing the lowest impact. Soy patties gained the lowest (HME) as well as the highest (TVP) score among all plant-based patties, which emphasized the importance of the processing technology.

Potential explanation in more beneficial results of soy-based patties relates to the higher agriculture yield rates, more efficient processing, and well-established extrusion process (Beck et al., 2017; Samard et al., 2019; Smetana et al., 2019b). During the trials it was observed that the HME technology generated a considerably higher amount of useful end-product (with moisture differences accounted for), hence resulting in overall higher efficiency of the process. HME of soymeal yielded 1439.12 kg and HME of pumpkin seed flour delivered 708.48 kg useful product. TVP of soymeal resulted in 640.00 kg useful product, while TVP of pumpkin seed flour provided 577.80 kg useful product.

Burger patties produced with TVP pumpkin seed flour (102.6 mPt) had a lower overall impact compared to TVP soy patties (131.3 mPt). This is in contrast with HME-based patties, where soymeal patties (78.8 mPt) demonstrated a lower impact compared to pumpkin seed flour patties (83.4 mPt). An explanation can lay in the ratio of throughputs between pumpkin seed flour and soymeal during both extrusion technologies. The ratio between both raw materials is considerably lower for low moisture extrusion compared to the ratio for high moisture extrusion.

Moreover, soymeal concentrate appeared to be the most efficient raw material for both extrusion technologies. This was expected because soymeal was used as a benchmark in this study. A lot of research has been performed on soymeal as a raw material, resulting in favourable processability and high throughputs (Lin et al., 2002; Wu et al., 2018). Pumpkin seed flour as a raw material is rather new and its processability is not optimized yet. If further research on pumpkin seed flour as a raw material for extrusion can increase its throughput, then it has the potential to become more sustainable than soymeal concentrate.

3.4 Sensitivity and scenario analyses

Sensitivity and scenario analyses were conducted to investigate the reliability of the study. Here, it was done by testing the model made for the pumpkin seed harvesting, the model for the storage of the extrusion intermediates and the chosen characterization method.

3.4.1 Alternative pumpkin harvesting method

The analysis of potential environmental impact of two harvesting methods of pumpkin seeds (Fig. 6) indicated that application of an alternative seed harvesting technology (on-field seed harvesting) resulted in a high increase of the impact, with 197.740 mPt for HME and 274.091 mPt for TVP, compared to the initial scenarios of harvesting pumpkins and separating them in a seed processing plant. The higher impact is directly linked to more intensive use of heavy agricultural machinery, which contributes to the emissions of greenhouse gasses and depletion of fossil resources.

As discussed earlier (Table 2), all information used for these two models is derived from literature and therefore should be further analysed based on primary data. Further research in which primary data can be collected during the cultivation on pumpkins and harvesting of pumpkin seeds may confirm that on-field seed harvesting is less environmentally friendly method.

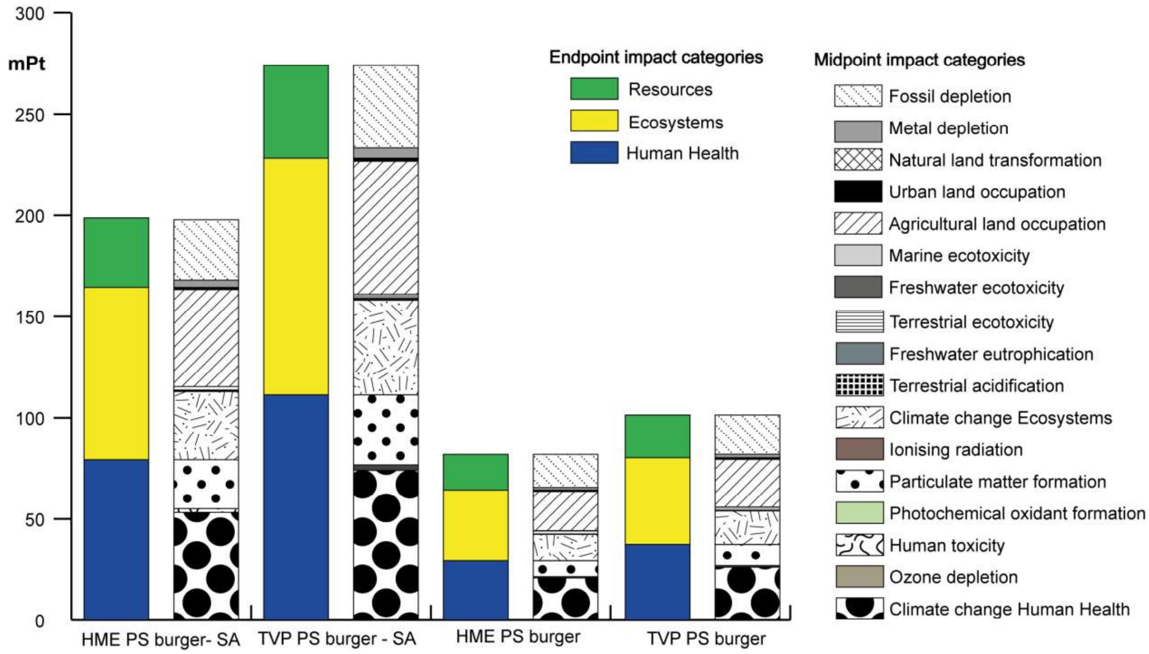

Fig. 6 Environmental impact changes due to the changes in agricultural harvesting method and extrusion processing method (for pumpkin seed only); functional unit: 1 kilogram of fresh, ready-to-pack burger patties; ReCiPe Endpoint (H) v1.08 (World ReCiPe H/A) Single score method; SA – scenario analysis (on-field seed harvesting with the harvesting of whole pumpkins); HME - high moisture extrusion, TVP - texturized vegetable protein, PS - pumpkin seeds, mPt - millipoints

3.4.2 Prolonged extrusion intermediate storage

HME soy patties (102.3 mPt) and HME PS patties (108.9 mPt) stored for eight months obtained a higher score compared to two weeks storage (resp. 78.8 and 83.4 mPt). Therefore, a prolonged intermediate storage involves a higher potential impact. Storage of eight months for TVP soy patties (139.1 mPt) and TVP PS patties (111.1 mPt) presented only a minor increase compared to two weeks storage (131.3 and 102.6 mPt, respectively). Nevertheless, even for eight months storage, TVP-patties were still the least environment friendly.

3.4.3 Alternative characterization method

Application of different characterisation methods may sometimes influence the results and even conclusions (Owsianiak et al., 2014). Therefore, it was necessary to analyse the outcomes with an alternative method. Fig. 7 visualizes the differences in potential environmental impact between all types of burger patties, generated with the IMPACT2002+ characterization method. Beef burger patties still showed the highest potential impact (18.5 mPt) and differed considerably from all other types of burger patties (>2%). Pork burger patties had a higher score (3.0 mPt) than chicken burger patties (2.3 mPt). This deviated from the previous results in this study, where chicken burger patties obtained a higher score than pork burger patties. There was, however, no considerable difference between the two types of meat burger patties (< 20%).

The plant-based patties also differed considerably (1-15% of impact) from all types of meat patties in the alternative characterization method (Fig. 7), which is also in line with the previous study results and other studies (Heller and Keoleian, 2018; Khan et al., 2019). Accordingly, low moisture extrusion (TVP) gained a higher impact score compared to high moisture extrusion. This can be considered as a confirmation of the fact that low moisture extrusion is indeed the least environmentally friendly extrusion technology. Furthermore, HME soy patties had the lowest score (0.2 mPt) followed by HME PS patties (0.3 mPt), TVP PS patties (0.3 mPt) and TVP soy patties (0.32 mPt). TVP soy patties still received the highest score and differed considerably (> 20%) from HME soy patties. The alternative LCIA methods confirmed the results of the current study and indicated them to be not sensitive to the choice of the LCIA method.

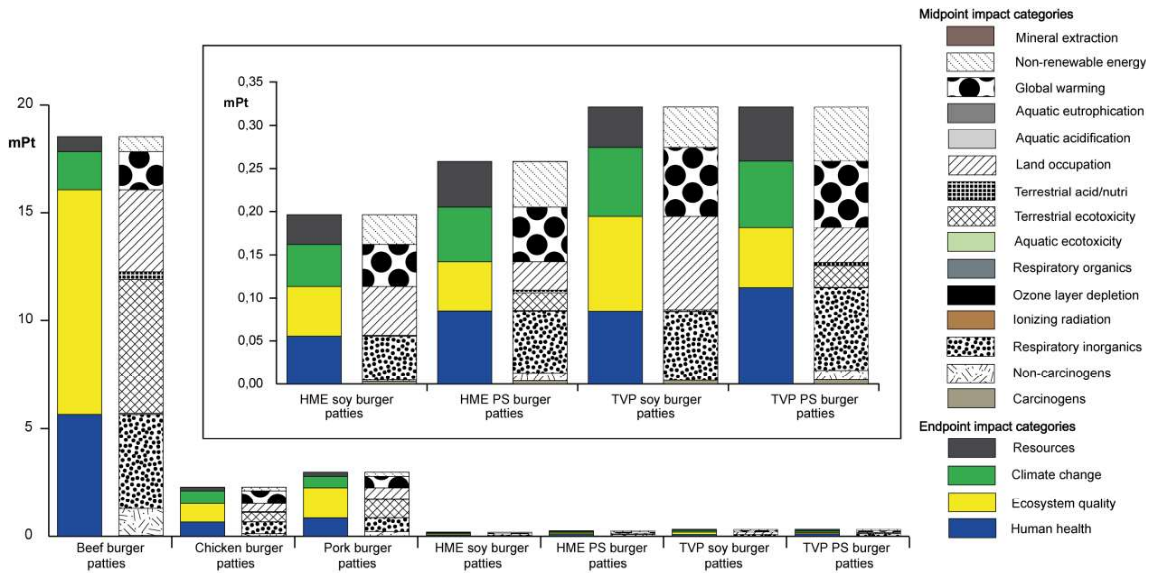

Fig. 7 Environmental impact of burger patties analysed with an alternative characterisation method; functional unit: 1 kilogram of fresh, ready-to-pack burger patties; IMPACT2002+ v2.14 (IMPACT2002+) single score method; HME - high moisture extrusion, TVP - texturized vegetable protein, PS - pumpkin seeds, mPt - millipoints

3.5 Uncertainty analysis

The Monte Carlo sampling technique was applied to assess the robustness of the LCIA data. The uncertainty analysis was conducted using a 1000-run Monte Carlo analysis in the SimaPro software for all types of burger patties. High results for coefficient of variation are linked to high uncertainty. Table 6 presents the obtained results from the Monte Carlo simulation. Marine ecotoxicity, freshwater ecotoxicity, terrestrial ecotoxicity, terrestrial acidification, photochemical oxidant formation, human toxicity metal depletion, freshwater eutrophication, ionizing radiation and ozone depletion were excluded from presentation as categories with relatively low impact (Fig. 5). Uncertainty levels were moderate in most cases and allowed to draw specific conclusions. Uncertainty analysis in categories of fossil depletion, climate change, natural land transformation, agricultural and urban land occupation, and particulate matter formation despite relatively high variations (CV) in land transformation indicated that

HME technology is significantly more environmentally beneficial than TVP for both raw materials (Table 6). Application of soy concentrate had significantly lower impact in categories of fossil depletion, natural land transformation, climate change and particulate matter formation than pumpkin seed flour in both cases (HME and TVP). Application of pumpkin seed flour had the same impact in urban land occupation and was more beneficial in agricultural land occupation than soy protein concentrate.

Table 6 Uncertainty analysis results for high moisture (HME) and texturized vegetable protein (TVP) soymeal and pumpkin seed flour (PS) patties, calculated with 1000-run Monte Carlo analysis in ReCiPe. The coefficient of variation (CV) is the ratio of the standard deviation (SD) to the mean. High CV results are linked to high uncertainties.

Impact category	HME soy burger patties			HME PS burger patties			TVP soy burger patties			TVP PS burger patties		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Fossil depletion	0.1	0.0085	7%	0.15	0.01	8%	0.14	0.01	9%	0.18	0.017	9%
Natural land transformation	0.0007	0.0001	21%	0.00009	0.00004	48%	0.001	0.0003	21%	0.0001	0.00006	47%
Urban land occupation	0.01	0.002	19%	0.01	0.002	18%	0.01	0.0035	24%	0.01	0.003	22%
Agricultural land occupation	0.8	0.06	8%	0.5	0.04	9%	1.4	0.14	10%	0.6	0.06	10%
Climate change	0.5	0.05	7%	0.75	0.06	7%	0.7	1.09	16%	0.94	0.08	8%
Particulate matter formation	0.001	0.0002	15%	0.0015	0.0001	7%	0.002	0.0004	22%	0.002	0.00016	8%

4 CONCLUSIONS AND FUTURE PROSPECTS

The environmental performance of four different types of plant-based patties and three types of meat burger patties was evaluated by means of LCA. Two different types of raw materials (soymeal and locally grown pumpkin seed flour) were processed with two different types of extrusion technologies (HME and TVP) to get intermediates further used to produce four variants of plant-based burger patties.

Plant-based patties had a lower overall environmental impact than meat-based (for all compared meat types). Meat-based burger patties had at least five times higher total environmental impact than plant-based burger patties (despite raw material and extrusion technology used). Plant-based patties produced with the high moisture extrusion (HME) technology had a significantly lower environmental impact compared to patties based on low moisture texturized vegetable proteins (TVP) in categories of fossil depletion, climate change, natural land transformation, agricultural and urban land occupation, and particulate matter formation, which were responsible for the highest share of relative impact. This implies that the overall impact was dependent on the extrusion technology used. Further optimisation of pumpkin seed flour production and processing is needed to be environmentally competitive to soy protein concentrate.

The results of the scenario analysis showed that harvesting pumpkin seeds on the field had a greater impact than whole pumpkin harvesting. This was mostly related to the intensity of heavy machinery use. Furthermore, a prolonged storage of extrudate intermediates resulted in a higher impact. Lastly, the sensitivity analysis of the characterization method proved the validity of the life cycle inventory analysis (LCIA) results.

The results indicated that HME technology with the application of soymeal concentrate could be considered as the most environmentally viable option to produce meat substitutes among the considered options. This technology can be recommended for more sustainable meat substitutes production. Processing of protein biomass into TVP with further rehydration, on the other hand, should be avoided if no long storage of produced extrudates is required.

Even though the study relied on the comparison of relatively similar products processed in a similar way, there is a need to perfume more studies, which would consider diverse alternative proteins emerging on the market and further system consequences of their emergence. Also, future LCA results based on different functional units used (energy content, protein content, nutritional value, amino acid profile, etc.), additional assessment categories (biodiversity) and higher production scales should confirm the results of the study. Moreover, there is a need in higher level studies presenting the higher-level complex model of the food system and alternative protein sources in that model.

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