

# Functional properties and Environmental sustainability of fermented yellow pea flour

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# Functional properties and Environmental sustainability of fermented yellow pea flour

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## ABSTRACT

The growing demand for sustainable and functional plant-based protein sources has positioned yellow pea (*Pisum sativum*) as a promising candidate in the development of next-generation food products. From a sustainability perspective, yellow pea fermentation represents an environmentally favorable protein source compared to animal derived alternatives. The nitrogen fixing capacity of yellow pea further contributes to sustainable agricultural practices by reducing fertilizer requirement. This study investigates the comparative environmental sustainability and functional properties of yellow pea flour subjected to solid-state fermentation (SSF) and submerged fermentation (SmF), using food-grade microbial strains; *Rhizopus oryzae* and *Lactobacillus plantarum* 299v.

Functional properties assessed, included water absorption index, water solubility index, water absorption capacity, oil absorption capacity, emulsion activity, emulsion solubility, and pasting properties. Additionally, physicochemical properties such as moisture content, color characteristics and protein content were analyzed. The properties were quantified before and after fermentation. Both SSF and SmF had effect on the functional properties of yellow pea flour, with SSF generally showing superior improvements in water solubility index and protein content. In regard of oil absorption the 48 hour fermented flours had the highest capacities for both methods. The SmF exhibited higher values for moisture content, water absorption index, water absorption capacity and pasting properties. The results reveal that while some of the treatment procedures may lead to higher qualities, some of them may contribute to lower properties of yellow pea flour.

Environmental impact was assessed using a cradle-to-gate life cycle assessment (LCA) approach, evaluating climate change during the fermentation process. There was a slight difference in environmental impact between solid state fermentation (6.56 kg CO<sub>2</sub>-Eq/1 kg) and submerged fermentation (6.41 kg CO<sub>2</sub>-Eq/1 kg). Incubation stage was identified as the hot spot of the fermentation process. SmF demonstrated a lower environmental impact than SSF due to its reduced energy consumption in anaerobic conditions. In addition, SmF offers better process control and scalability advantages. These findings support the use of yellow pea as a sustainable ingredient in functional food formulations and highlight the potential of batch mode SmF as a more environmental friendly processing method.

In conclusion, fermentation markedly enhances the functional and physiochemical profile of yellow pea, supporting its use in a wide range of food applications such as plant-based dairy, meat analogs, and baked goods. Fermentation technique emerges as a more sustainable and efficient technique, aligning with the principles of green processing and circular bioeconomy. These findings underscore the potential of fermented yellow pea as a functional, eco-friendly ingredient for the food industry.

## POPULAR SCIENCE SUMMARY

As the global population grows and concerns about climate change and resource use increase, there's a significant movement towards more sustainable and nutritious food sources. One such promising ingredient is the yellow pea. Naturally high in protein and requiring fewer resources to grow, than many animal-based or even other plant-based foods, yellow peas are attracting attention from both researchers and food producers. Fermentation is a natural process where microbes break down food which can make yellow pea even better for both human health and the planet. Two types of fermentation methods were investigated: solid-state fermentation (SSF), which mimics traditional processes like fermenting soybeans; and submerged fermentation (SmF), which is common in large-scale food production.

Life Cycle Assessment (LCA) is a systematic methodology for assessing the environmental impact of a product, process, or service across the life cycle. This encompasses all steps, from raw material extraction, manufacture, and consumption to disposal or recycling, referred to as a "cradle-to-grave" approach. In terms of climate change, LCA assists to produce more sustainable products, enhance energy efficiency, and contribute to global climate goals like carbon neutrality and reducing global warming potential. The research revealed that the SSF has higher environmental impact than the SmF in the fermentation process. The causes for these results are the use of water for the soaking and cooking phases in SSF, as well as the lack of electricity for the fermentation apparatus in the SmF process, which was carried out under anaerobic circumstances.

Both methods improved yellow pea flour in several ways. It boosted functional properties like how well the pea proteins dissolve, hold water and oil. These improvements make fermented yellow peas more useful as ingredients in products like plant-based burgers, dairy alternatives, baked goods, and high-protein snacks. This research shows that fermented yellow peas are not only functional and more versatile as food ingredients, but they can also be processed in a way that is gentle on the environment. With these characteristics, fermented yellow peas may play a key role in the development of more adaptable and sustainable food systems in the future.

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## **LIST OF ABBREVIATIONS**

WAI	Water Absorption Index
WSI	Water Solubility Index
OAC	Oil Absorption Capacity
WAC	Water Absorption Capacity
EA	Emulsion Activity
ES	Emulsion Solubility
RVA	Rapid Visco Analyzer
LCA	Life Cycle Assessment
LCI	Life cycle inventory
LCIA	Life cycle impact assessment
GHG	Green House Gas
VOC	Volatile Organic Compounds

## 1. INTRODUCTION

The existing food systems encounter various sustainability concerns. One major issue is the environmental impact of food production, which contributes to climate change, deforestation, and biodiversity loss (Poore and Nemecek, 2018). Additionally, present food systems depend on finite resources, such as water and fossil fuels, which are becoming increasingly scarce. Moreover, the current food systems have a considerable negative influence on human health, particularly the increasing incidence of chronic diseases connected to the overconsumption of animal products (Willett, 2013). Furthermore, present food systems are inequitable because they frequently fail to offer enough access to food for marginalized people, contributing to social and economic inequality (UN, 2019). As a result, it is critical to create sustainable food systems capable of providing nutritious food to a growing population while reducing negative environmental and social consequences.

Numerous sustainability concerns related to the current food systems may be resolved by alternative proteins, such as plant-based options. For instance, the amount of land and water used in the production of food has been greatly decreased by substituting plant-based products for animal-based products (Poore and Nemecek, 2018). Pulses, specially yellow peas (*Pisum sativum*), have become a popular option because of their high protein content, adaptability, and environmental advantages. In addition to improving soil fertility and lowering the demand for synthetic fertilizers, as yellow peas are nitrogen-fixing legumes with minimal water footprint. They provide a more sustainable agricultural alternative to a number of animal-based and even some plant-based protein sources (Abi-Ghanen et al., 2011; Tulbek et al., 2024).

In addition to their environmental advantages, yellow peas serve as a valuable raw material for functional food development. One promising approach to further enhance their nutritional and functional properties is through fermentation. Fermentation is a traditional bioprocess that uses microorganisms to transform food substrates, leading to improved nutritional quality, extended shelf life, and enhanced functional behavior (Siddiqui et al., 2023; Vurro et al., 2024). Laura Moraes (Lund University), through her master thesis has showed that fermentation improved protein digestibility and reduced antinutritional compounds such as phytic acid which can decrease the bioavailability of minerals and raffinose-family oligosaccharides (RFOs), molecules that may cause flatulence and other gastrointestinal issues upon consumption. This was the foundation of this project where the investigation was required on the functional properties of the fermented yellow peas, as the nutritional superiority was previously proven.

Combining environmental sustainability with enhanced functional potential, fermented yellow pea flour represents a strategic ingredient for developing sustainable, and innovative food products. However, a limited number of studies have investigated on the environmental significance of the life cycle of yellow pea and the impact of fermentation on the physicochemical and functional properties of yellow pea flour, highlighting its role in promoting sustainable food systems. Also the investigation on LCA on yellow pea, specially in the fermentation process is vital as there is a marginal gap of studies regarding the area.

## 1.1 AIM AND SPECIFIC OBJECTIVES

This master's thesis aims to have an integrated approach to the functional and environmental impact of two fermentation methods and compare the results of each other. Both the functional properties and environmental impact are interconnected in the future development of plant based food products to ensure functional qualities while utilizing minimum natural resources in the production process. The specific objectives are;

- To evaluate Functional properties with centered on Moisture content, Water Absorption Index (WAI), Water Solubility Index (WSI), Water Absorption Capacity (WAC), Oil Absorption Capacity (OAC), Emulsion Activity (EA), Emulsion Stability (ES), Pasting Property, Color and Protein Analysis.
- To conduct a Life Cycle Assessment based on climate change to evaluate the environmental impact of the fermentation process of yellow pea.

## 2. THEORETICAL BACKGROUND

### 2.1 Family Fabaceae

Scientifically referred to as Leguminosae or Fabaceae, the pea family is one of the largest and most significant plant families globally in terms of economic impact. With over 650 genera and 18,000 species of flowering plants, it is ranging from trees and climbers to herbs and shrubs (Lock, 2005). Following the orchid family (Orchidaceae) and the daisy family (Asteraceae), the fabaceae is the third largest family of flowering plants. In addition, no family possibly with the exception of the Poaceae has a more extensive geographic spread across a larger variety of environments. Except for Antarctica, legumes can be found on every continent and in nearly every type of environment, from the freshwater lakes of Amazonia to the tropical and subtropical forests of the New and Old Worlds, to the Central Asian deserts and the arctic-alpine vegetation of the temperate zone. (Harris, 2004)

Fabaceae are distinguished by its fruit, which is usually a pod (also known as a legume) that splits open on both sides to release the seeds. Peas (*Pisum sativum*), Beans (*Phaseolus spp.*), Lentils (*Lens culinaris*), Soybeans (*Glycine max*), Peanuts (*Arachis hypogaea*), Clover, alfalfa, and acacia trees are typical examples (Ayers, 2013). Members of the plant family Fabaceae are remarkable in that they have developed a symbiotic connection with rhizobia. Rhizobia infect and create root nodules on their host plants before developing into bacteroids, a symbiotic form of rhizobia. This complex relationship involves the supply of C4-dicarboxylate and phosphate by the host plants to the microsymbionts that use them in the energy-intensive process of fixing atmospheric nitrogen into ammonium, which is then made available to the host plants as a source of nitrogen, a macronutrient for growth (Liu et al., 2018).

Yellow peas are members of the legume family. These whole yellow peas are around 1/4 inch broad and light yellow to beige in color. They have a moderate, somewhat sweet taste with a soft, granular texture. Yellow peas are high in vegan protein, fiber, iron, and key vitamins and minerals such as B1 (Thiamine), B3 (Niacin), B9 (Folate), Beta Carotene, Zinc, and Selenium. They also aid digestion, lower cholesterol levels, and reduce the risk of heart disease. Pea protein, a major element in the production of meat replacements, is high in lysine but relatively low in tryptophan and sulfur-containing amino acids, such as methionine and cysteine (Leterme et al., 1990; Pilorge et al., 2021). Yellow peas are quite environmentally friendly. They thrive with less water than other crops and contribute to soil health by fixing nitrogen. This implies that plants organically replenish the soil, which reduces the demand for artificial fertilizers. Yellow peas are especially important in crop rotation, as they reduce carbon footprints and improve soil health (Nuverta. n.d.; Tulbek et al., 2017)

### 2.2 Environmental Sustainability

Since the 1970s, the concept of sustainability has been increasingly related to human sustainability on planet Earth, resulting in the most generally referenced definition. The United Nations' World Commission on Environment and Development (Brundtland, 1987) defined sustainable development as "development that meets current needs without diminishing future

generations' ability to meet their own needs." The definition of sustainability development includes three interconnected goals: environmental, economic, and social (Redclift, 1992).

The environment is a critical component of human existence and well-being. The environment provides mankind with the resources we need to live healthy lives. These resources consist of food, water, and shelter. However, humans' exploitation of the natural resources has increased exponentially. This has resulted in a variety of environmental issues, including rapid climate change, pollution, biodiversity loss, and deforestation (Vos, 2007).

Environmental sustainability is a worldwide issue that requires contributions and collaboration from a variety of parties, including corporations, individuals, and, in the grand scheme of things, governments. It includes a wide range of operations such as water treatment, waste reduction, pollution control, resource conservation using renewable means, and so on. This objective of sustainability can be realized by transitioning from the present system of unrestricted and unmonitored growth, consumption, and waste to one of monitored usage, savings, waste, and other aspects without sacrificing growth for a brighter future. (Asha et al., 2023)

### 2.3 Life cycle assessment

LCA is a tool that evaluates the potential environmental impacts and resources used throughout a product's life cycle (cradle to grave or cradle to gate), which includes raw material acquisition, production, use phases, waste management, and recycling ( Hunt et al., 1996; Guinee et al., 2011). It originates from Coca-Cola's study on beverage bottle selection in 1969, although it did not become widely used until the late 1980s (Jensen et al., 1997). The Society of Environmental Toxicology and Chemistry (SETAC) proposed the concept of LCA in 1990, which was then adopted into the International Standard Organization's (ISO) 14000 Environmental Management Standards in 1993. The ISO defines LCA as a method for gathering and analyzing a product system's inputs, outputs, and possible environmental consequences over its entire life cycle (ISO 14040, 2006). LCA is now widely utilized in environmental management, industrial production, transportation, energy, and a variety of other industries, and it has gained international recognition as a key tool for environmental management, ecological design, and sustainable development.

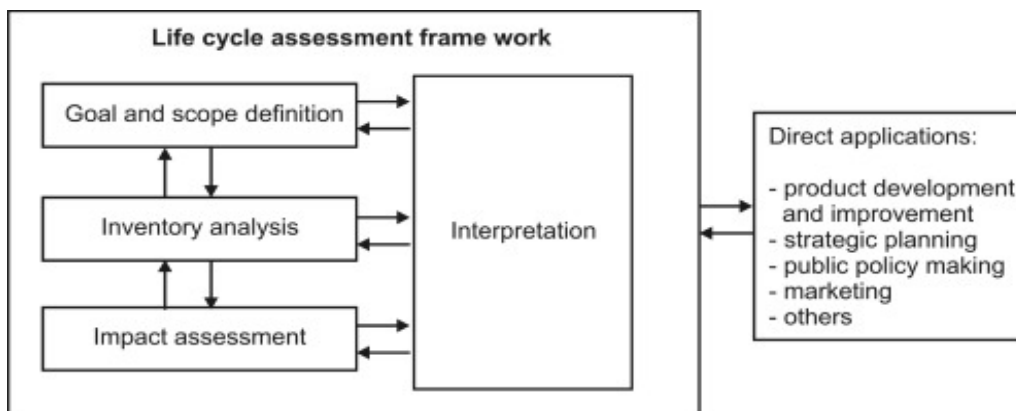


Figure 1. Stages of an LCA according EN ISO 14040.

## 2.4.1 LCA phases

### Phase 1. Goal and scope

Goal definition means developing questions or hypotheses, which essentially determine the suitable scope and LCA methodologies for the individual investigation. There are typically two types of LCA methods: attributional LCA and consequential LCA. Attributional LCA is distinguished by its emphasis on defining ecologically relevant physical fluxes into and out of a life cycle and its subsystems. Consequential LCA is defined by its goal of describing how these flows may change in response to potential actions (Finnveden & Potting, 2014; Muralikrishna & Manickam, 2017).

### Phase 2. Life cycle inventory analysis

In LCI, the product systems' inputs and outputs are collected and processed. This phase begins with identifying relevant processes in the product system, followed by data collection for quantification of inputs and outputs per functional unit for each of these processes. The data must be associated with the functional unit specified in the aim and scope specification. Data can be displayed in tables, and some conclusions can be drawn at this point. The inventory yields an LCI that contains information about all inputs and outputs in the form of elementary flow to and from the environment from all of the unit processes involved in the study (Finnveden & Potting, 2014; Muralikrishna & Manickam, 2017).

### Phase 3. Life cycle impact assessment

The goal of LCIA is to transform the LCI results into probable environmental impacts in order to better comprehend their environmental relevance. LCIA turns inventory results into prospective contributions to a number of specified impact categories, which are sometimes further modeled into what is known as the LCIA's 'areas of protection'. These domains of protection, which reflect the entities that society wishes to protect using the LCA, are frequently identified as human health, natural environment, and natural resources (Finnveden & Potting, 2014; Muralikrishna & Manickam, 2017).

### Phase 4. Interpretation

Life cycle interpretation is the fourth and last part of life cycle assessment. To begin the interpretation phase, ISO 14044:2006 recommends identifying significant issues; the implications of the methods used and assumptions made on the results (e.g., allocation rules, system boundaries, and impact categories, models and indicators used, value choices); significant impact categories; and significant contributions from life cycle stages to LCI and LCIA results (ISO, 2006b). Significant concerns are identified iteratively and evaluated, with three separate checks performed: completeness, sensitivity, and consistency (ISO, 2006b). Following the iterative process of identifying key concerns and doing evaluation checks, the case study's conclusions, limitations, and suggestions are drawn (Finnveden & Potting, 2014; Laurent et al., 2020).

## 2.4.2 Fermentation

Fermentation is said to have originated in the Fertile Crescent, and since then, nearly every culture has incorporated fermented foods into their diets. Fermentation was originally employed to preserve foods, but is now utilized to improve their physicochemical, sensory, nutritional, and safety properties. Fermented dairy, alcoholic beverages such as wine and beer, fermented vegetables, fruits, and meats are all extremely beneficial due to their improved storage stability, lower risk of food poisoning, and enhanced flavor (Siddique et al., 2023).

Over time, scientific study has linked the consumption of fermented goods to improved health state. Fermentation helps to break down chemicals into more digestible forms. It also helps to lessen the presence of toxins and pathogens in food. Fermented foods also include probiotics, which are healthy bacteria that aid digestion and nutritional absorption. Noncommunicable diseases include cardiovascular disease, type 2 diabetes, cancer, and allergies have become more common in recent years. In this regard, research studies have shown that switching to a fermented-food diet can lower the risk of noncommunicable diseases (Şanlıer et al., 2019; Abbaspour, 2024). Furthermore, during the last decade, there has been an increasing interest in fermentation technologies for converting food waste into valuable byproducts. The fermentation of various food wastes has successfully produced important byproducts such as enzymes, pigments, and biofuels (Chenebault et al., 2022).

## 2.5.1 Fermentation of plant-based products

Fermentation of plant-derived products has been used for many decades. During the Neolithic period (8500-400 BC), fermentation accidentally started with plant-derived products, such as grain, to produce wine-like beverages, which can preserve plant-based products longer (Lavefve et al. 2019). Soybean-based fermentations were developed in China and East Asia. Soy sauce is an ancient Chinese product whose precursor was mentioned about 3100 years ago (O'Toole, 2016). Traditional cereal grain fermentation relies on mixed cultures of numerous helpful bacteria known as probiotics. Improved understanding of the functional characteristics of these foods is linked to the interactions of bioactive live cells with the host, or indirectly to the ingestion of bioactive substances created during fermentation, such as dietary fiber, minerals, vitamins, and antioxidants. Lactic acid bacteria (LAB), yeasts, and fungus are the main microorganisms commonly found combined in the creation of beverages and fermented foods (Achi & Asamudo, 2019).

Fermented plant-related foods have emerged as a new food trend in recent decades, while their popularity has declined due to food industrialization, particularly in European countries (Giacalone et al. 2022; Michel et al. 2020; Profeta et al. 2020). The interest in plant-based products has intensified in recent decades due to the desire for healthy food products (Siddiqui et al. 2022; Bryant & Sanctorum 2021; Schiano et al. 2020). For example, lactic acid bacteria make kombucha, whereas acetic acid bacteria produce a product without alcohol, which is more acceptable to European customers.

### 2.5.2 Solid-state fermentation

Solid-state fermentation is the development of microbes on wet particles of solid materials in beds filled with a continuous gas phase (Mitchell et al., 2011). Fungi, which are better suited to high enzyme synthesis, are among the microorganisms that adapt most effectively to this form of fermentation. Amylases, proteases, xylanases, cellulases, and pectinases are examples of enzymes that can be generated by SSF. Fungi produce them on substrates such as cereals or cereal derivatives (Okafor, 2007; Krishna, 2005; Mitchell & Lonsane, 1992). SSF substrates are typically agrochemical residues or byproducts. Rice, wheat, barley, corn, soybeans, sugarcane, corn cob, wheat bran, and rice straw are some of the materials that can be biotransformed. These components are mostly constituted of cellulose, hemicellulose, lignin, starch, pectin, and proteins, making them exceedingly diverse. They serve as carbon and energy sources, as well as support for microbial growth. It should be noted that the fungus responds differently to the desired outcome depending on the raw ingredients used (Pandey, 2003; Brahmachari et al., 2017; Bon & Pereira Jr, 1999).

SSF has various advantages, including high productivity, long-term product stability, and cheap manufacturing costs. With continued advancement and the use of rational engineering processes, SSF will reach better levels of standardization and reproducibility in the future. This could make SSF the preferred approach for certain applications such as enzyme and food synthesis. The ecological (environmental) benefits of SSF result from the fact that the activities are carried out in the absence of a free aqueous phase. This results in minimum water consumption and consequently little effluent water output by the process. Additionally, it is capable of using agricultural waste as both a carbon and energy source. (Hölker & Lenz, 2005).

### 2.5.3 *Rhizopus oryzae*

*Rhizopus oryzae* is the most researched species, with its whole genome sequenced in 2004–2005. The genetic sequences displayed have mostly been utilized in phylogenetic research to assess the relationship between various fungus species. *Rhizopus* is probably the most well-known genus of Zygomycetes fungi, which are typically found on dead and decaying plant material. These fungus have a complicated metabolism and create a number of enzymes, allowing them to use a wide range of nutrients. Several different *Rhizopus* strains are employed to produce fermented foods and beverages, mainly in Southeast Asia. Furthermore, *Rhizopus* has various commercial applications in the production of enzymes such as amylases, pectinases, cellulases, proteases, and phytases, as well as metabolites like ethanol, lactic, and fumaric acids. The cell mass of these fungi has also found use in the food and feed industries (Lennartsson et al., 2014).

Several features make this genus appealing for widespread use in the biotechnology industry, including a large growth temperature range (from 25 to 45 °C), a broad growth and survival pH range (at least from 4.5 to 7.5), numerous fermentative substrates, and a variety of by-products produced. *Rhizopus oryzae* can grow on pentose sugars and agricultural wastes like cassava, potato pulp, potato starch, corn straw, apple industry waste, rice bran, orange peel, dairy industry wastes, wheat gluten, wheat bran, barley bran, walnut wastes, soybeans, xylose, and lignocellulosic materials (Ibarruri et al., 2018).

#### 2.5.4 Submerged fermentation

Submerged fermentation is a method for growing microorganisms in liquid nutrient media. For industrial production, this entails growing the selected microorganism in closed vessels known as bioreactors, which contain nutrient broths. The bioreactor system design provides the provision of oxygen as required for aerobic bacteria, as well as the capacity to monitor and adjust several parameters such as pH, temperature, viscosity, dissolved oxygen, foam formation, biomass formation, substrate utilization, and desired product formation (Liu *et al.*, 2019; Xiong *et al.*, 2012). The entire process is carried out in a fermenter that is continually run, and the resulting biomass is harvested using a variety of processes. The final product is centrifuged or filtered, and then dried. Aeration is also vital throughout the cultivation process since heat is generated and needs to be removed over time by an appropriate cooling device. Different microorganisms require different means of recovery, such as centrifugation for bacteria and filtering for filamentous fungi. Recovery of maximum water content is desirable because it contains important soluble nutrients, which become available after drying (Nasseri *et al.*, 2011).

SmF processes have three major modes: batch, continuous, and fed-batch. Batch mode refers to the fermentation of sterilized nutrient solution in a closed vessel by a suitable microbial culture. The benefits of such a method are minimal costs and a simple infrastructure for process control. However, the main disadvantages are limited productivity and feed-back inhibition. Continuous mode uses a carefully controlled, highly productive bioreactor system (chemostat or turbidostat) in which the sterile substrate and other medium components are fed at a specific rate and an equivalent amount of product is harvested in a time-dependent manner. Fed-batch fermentation is characterized by intermittent feeding and maintenance of optimum concentrations of needed growth substrates, with continuous product harvesting (Keshavarz, 2014)).

#### 2.5.5 *Lactobacillus plantarum*

*Lactobacillus plantarum* is a common and adaptable lactic acid bacteria. It is found in the microbiota of many foods and feeds, including dairy, meat, fish, vegetable fermented products and silage; it is also a natural inhabitant of human and animal mucosa. The exceptional ecological adaptability of *L. plantarum* to these diverse ecological niches is a result of its capacity to use a wide variety of carbohydrates, the primary end metabolite of which is lactic acid (Mayo *et al.*, 2021).

*L. plantarum*, which is taken from the environment or utilized in controlled fermentations, is widely associated with favorable qualities in many fermented foods and is added to a number of products to improve their quality or related health benefits (Corsetti & Valmorri, 2011). *L. plantarum* has been utilized as a starter culture in a variety of food fermentation procedures, enhancing the organoleptic characteristics, flavor, and texture. The use of LAB in dairy products is one of the oldest and most conventional applications and they have been used for centuries to prepare yoghurt, fermented milk products, and different types of cheese (Powell *et al.*, 2007; Danova *et al.*, 2005). In these fermentation processes, LAB are responsible for the organoleptic properties of the products that come from lactose degradation, acidification, and aromatic compound synthesis. *L. plantarum* involves to the safety of final products by producing lactic acid and other antibacterial substances (Todorov & Franco, 2010).

### 3. MATERIALS AND METHODS

#### 3.1 Yellow peas

Dried yellow peas (Lantmännen) for this project were purchased from ICA Kvantum Malmborgs, Sweden. For both solid state and submerged fermentation, yellow pea from same batch was used.

#### 3.2 Fermentation of yellow pea

Two types of fermentations were investigated in this study, fungal fermentation conducted in solid-state conditions and lactic acid fermentation which was carried out in submerged anaerobic conditions. SSF method was conducted for soaked, cooked peas and raw dry peas were used for SmF method. Each fermentation process was performed in duplicate.

##### 3.2.1 *Rhizopus oryzae* fermentation process

All the processes were conducted in duplicate. The peas were first washed and then soaked in tap water (1:3 w/v) for 18h at room temperature (~20°C), rather than under controlled temperature conditions in an incubator. Then the yellow peas were cooked for 35 minutes at 100°C in distilled water (1:2 w/v). Cooked yellow peas were put between layers of wet tissue paper.

A total of 500g of cooked whole yellow peas were produced using the previously described process. Yellow peas underwent optimization and were subjected to the fermentation procedure for tempeh production described by Ahnan-Winarno et al. (2020). The manufacturer's recommendations for the amount of starting culture fed to peas were followed, which recommended 5g of *Rhizopus oryzae* for every 600g of raw seeds. In aluminum bowls, the peas were inoculated with the starter culture and carefully mixed using metal spoons. After mixing, they were placed in 10 x 5 cm perforated polyethylene zip-lock bags spaced 1 centimeter apart and, they were placed in an incubation oven (Termaks, Sweden) at 30°C for 48 hours.

##### 3.2.2 *Lactobacillus plantarum* fermentation process

The fermentation procedure was carried out as reported by Castro-Alba, Lazarte, Perez-Rea, Carlsson, et al. (2019). In brief, 250 g of seeds were mixed for 60 seconds at maximum speed in a food processor (Laboratory mill 120, Finland). Blended peas were placed in 500mL glass bottles and incubated with 100mL of distilled water and one capsule of *Lactobacillus plantarum* 299v (7.35 Log<sub>10</sub> CFU/g dry matter) using glass rods. The bottles were fitted with plastic lids and placed in an incubation oven (Termaks, Sweden) set to 37°C for 48 hours.

#### 3.3 Sampling

During each fermentation, samples were taken at 0, 6, 12, 24, 36, and 48 hours to measure the fermentation's progress, pH, and total acidity, as well as the lactic acid concentration of each sample. Samples fermented with *R. oryzae* were removed from one plastic bag at a time. Samples of yellow peas fermented with *L. plantarum* were collected using previously sterilized metal spoons. For both cases, about 30g of each sample was stored in aluminum trays before being frozen and freeze-dried. The dried materials of SSF were ground with a mortar and both SSF and SmF samples stored in zip-lock bags at room temperature for further analysis.

### 3.4 Physiochemical property analysis

#### 3.4.1 Moisture content

The moisture content of yellow pea flour was calculated using the AOAC technique (AOAC International, 2000). 2g flour samples were dried in an oven at 105°C (Termaks, TS4057, Bergen, Norway). The samples were dried overnight, followed by 3-6 hour intervals until a consistent weight was achieved. Before being weighed, the samples were cooled in a desiccator. All analyses were performed in duplicate. The moisture content was then calculated using the following equation, with weight loss representing the quantity of water present in the samples.  $W_1$  is the sample weight before drying in grams, and  $W_2$  is the sample weight after drying in grams. The dry matter of the samples is then estimated using the information provided.

$$\text{Moisture(\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

#### 3.4.2 Protein Analysis

The protein content of the yellow pea flour samples was determined before and after fermentation using the dynamic flash combustion method (modified Dumas method) reported by Krotz et al. (2016) using the protein analyzer (Thermo Scientific™ Flash™, EA 1112 series, MA, USA). Approximately 25 mg of samples were weighed into tin capsules and placed into a carousel, which automatically delivered the samples to the combustion reactor. The calibration was carried out by making one blank tin capsule, two standards containing 25 and 50 mg of ascorbic acid, and one reference sample with known protein content. The protein content was determined by integrating the nitrogen data received after sample combustion, using a standard conversion factor of 6.25.

#### 3.4.3 Color Analysis

The color differences between yellow pea flour samples were measured in a CIELAB system with a colorimeter (CR-400 Chromameter, Konica Minolta, Japan). The device was calibrated prior to taking the readings and, a cling film was placed between the sample and the equipment to prevent external lighting from interfering with the measurement. The colorimetric characteristics were assessed as lightness ( $L^*$ ), greenness-redness ( $a^*$ ), and blueness-yellowness ( $b^*$ ).

### 3.5 Functional property analysis

#### 3.5.1 Water absorption index (WAI) and water solubility index (WSI)

The WAI and WSI of fermented and unfermented yellow pea flour samples were determined using the procedures published by Anderson (1982). 2.5g of flour were suspended in 30mL of distilled water in a pre-weighed centrifuge tube before cooked in a water bath at 70°C for 30 minutes. After cooling to ambient temperature, the mixture was centrifuged at 3000×g for 20

minutes (Eppendorf Centrifuge 5804R, Hamburg, Germany). The supernatant liquid was gently put into the tared evaporating dish. The residual gel was weighed, and the WAI was determined as grams of gel per gram of sample.

The supernatant was transferred into pre-weighed aluminum containers to determine its solid content by evaporating it in an oven at 105°C overnight. The WSI was the weight of dry solids in the supernatant from the water absorption index test, expressed as a percentage of the original weight of the sample.

$$\text{WAI (g/g)} = \frac{\text{Weight of sediment}}{\text{Weight of flour sample}}$$

$$\text{WSI (g/100g)} = \frac{\text{Weight of dissolved solid in supernatant}}{\text{Weight of flour sample}} \times 100$$

### 3.5.2 Water absorption capacity (WAC)

WAC was calculated following the method provided by Ferawati et al. (2019). In a pre-weighed centrifuge tube, 3g of sample was mixed with 25mL of distilled water and stirred every 5 minutes for 30 minutes. The tube was then centrifuged at 3000×g for 25 minutes using an Eppendorf Centrifuge 5804R (Hamburg). Following centrifugation, the supernatant was decanted, and excess moisture was removed by drying the samples in an oven (Termaks, TS4057, Bergen, Norway) at 50°C for 25 minutes. The tube was then reweighed, and the WAC was calculated as grams of water bound per gram of sample on a dry weight basis.

### 3.5.3 Oil absorption capacity (OAC)

The OAC was measured using the method published by Kaur & Singh (2005). In a pre-weighed centrifuge tube, 0.5g of sample was mixed with 6mL rapeseed oil and stirred for 1 minute. After 30 minutes, the tube was centrifuged at 3000×g for 25 minutes (Eppendorf Centrifuge 5804R, Hamburg, Germany). After centrifugation, the oil layer was removed, and the tube was inverted for 25 minutes to drain excess oil before being weighed again. The OAC was calculated as grams of oil bound per gram of material on a dry weight basis.

### 3.5.4 Emulsion activity (EA) and Emulsion stability (ES)

Emulsifying characteristics of flours were determined using Kaur and Singh's (2005) method. A flour sample (3.5 g) was homogenized for 30 seconds in 50 mL of water using a homogenizer () set at high. Rapeseed oil (25 mL) was added, and the mixture was homogenized again for 30 seconds. The mixture was then homogenized for 90 seconds with an additional 25 mL of rapeseed oil added. The emulsion was evenly divided into two 50 mL centrifuge tubes and centrifuged at 1100 g for 5 minutes. The emulsifying activity was measured as a percentage by dividing the volume of the emulsified layer by the volume of the emulsion before centrifugation.

The emulsion stability was measured using samples obtained for measuring emulsifying activity. They were heated to 85 °C for 15 minutes, then cooled and centrifuged at 1100 g for 5 minutes. The emulsion stability was calculated as the percentage of emulsifying activity persisting after heating.

$$\text{Emulsion activity and stability (EA, ES\%)} = \frac{\text{Volume of emulsified layer}}{\text{Total volume of emulsion}} \times 100$$

### 3.5.5 Pasting Properties

The pasting qualities of treated and untreated yellow pea flours were investigated using a rapid visco analyzer (RVA, Perten 4,500, Stockholm, Sweden). A suspension of 3.5g flour in 25g of distilled water was made and adjusted to account 14% moisture basis correction for the sample. The measuring methodology consisted of 1 minute of mixing, stirring, and warming to 50°C at 160 rpm, followed by 222 seconds of heating to 91°C, 150 seconds of holding at 91°C, and 228 seconds of cooling back down to 50°C at the same rate as heating. The pasting curve was used to determine the pasting temperature, peak viscosity, trough viscosity, breakdown value, final viscosity, and setback value.

### 3.6 Life cycle assessment study

The LCA study based on climate change was conducted using ISO 14040:2006 and ISO 14044:2006 standards (ISO 14044:2006; ISO 14040:2006). The Open LCA software (version 2.4.1 GreenDelta; Berlin, Germany) was utilized to analyze the data, while the Ecoinvent (version 3.11) served as background source. The LCIA method used was Environmental Footprint 3.0 and the foreground data was then analyzed.

#### 3.6.1 Defining the goal and scope

This LCA aims to evaluate and compare the environmental impact of GHG emissions of the fermentation process of yellow pea followed two fermentation methods; solid state and submerged fermentation. This provides insight into the environmental impact of fermentation methods and guides future development of the commercial scale. The functional unit used was 1000 grams of fermented yellow pea. The system boundary (Figures A, B) was established using a cradle-to-factory gate method, starting with whole yellow pea to fermented yellow pea.

For solid state fermentation, the fermentation process had 3 stages. Stage 01 was soaking followed by cooking stage and incubation stage. In the soaking stage the whole yellow pea were soaked in tap water (1:3 w/v) for 18h at room temperature (~20°C). In the cooking stage the yellow peas were cooked for 35 minutes at 100°C in distilled water (1:2 w/v). In the incubation stage they were placed in an incubation oven (Termaks, Sweden) at 30°C for 48 hours.

For submerged fermentation, the fermentation process had 3 stages. Stage 01 was blending stage followed by preparation of incubation apparatus and incubation stage. In the first stage the yellow pea were blended. In the second stage blended peas were placed in 500mL glass bottles

and mixed with 100mL of distilled water and one capsule of *Lactobacillus plantarum* 299v. In the third stage the apparatus were placed in an incubation oven (Termaks, Sweden) set to 37°C for 48 hours.

The resources which were used; microbial cultures, water and electricity were included to each stage. Waste from the blender, soaking bowl were eliminated due to a lack of information. The possible outputs such as biogenic CO<sub>2</sub>, heat, volatile organic compounds (VOC), waste gases or odors were removed as the effects were considered negligible. Biogenic CO<sub>2</sub> emissions include those associated with the natural carbon cycle, as well as those produced by the combustion, harvest, combustion, digestion, fermentation, decomposition, or processing of biologically based materials (Environmental protection agency, 2019)). In environmental analysis tools (such as SimaPro and GaBi) typically do not include the biogenic CO<sub>2</sub> emission and even treat it as a negative impact since they assume that biomass combustion has no ability to cause climate change (Foster, 2001; Frischknecht et al., 2007; Pachauri & Reisinger, 2007).

### 3.6.2 System Boundary

Below is the system boundary for SSF with all the inputs and outputs.

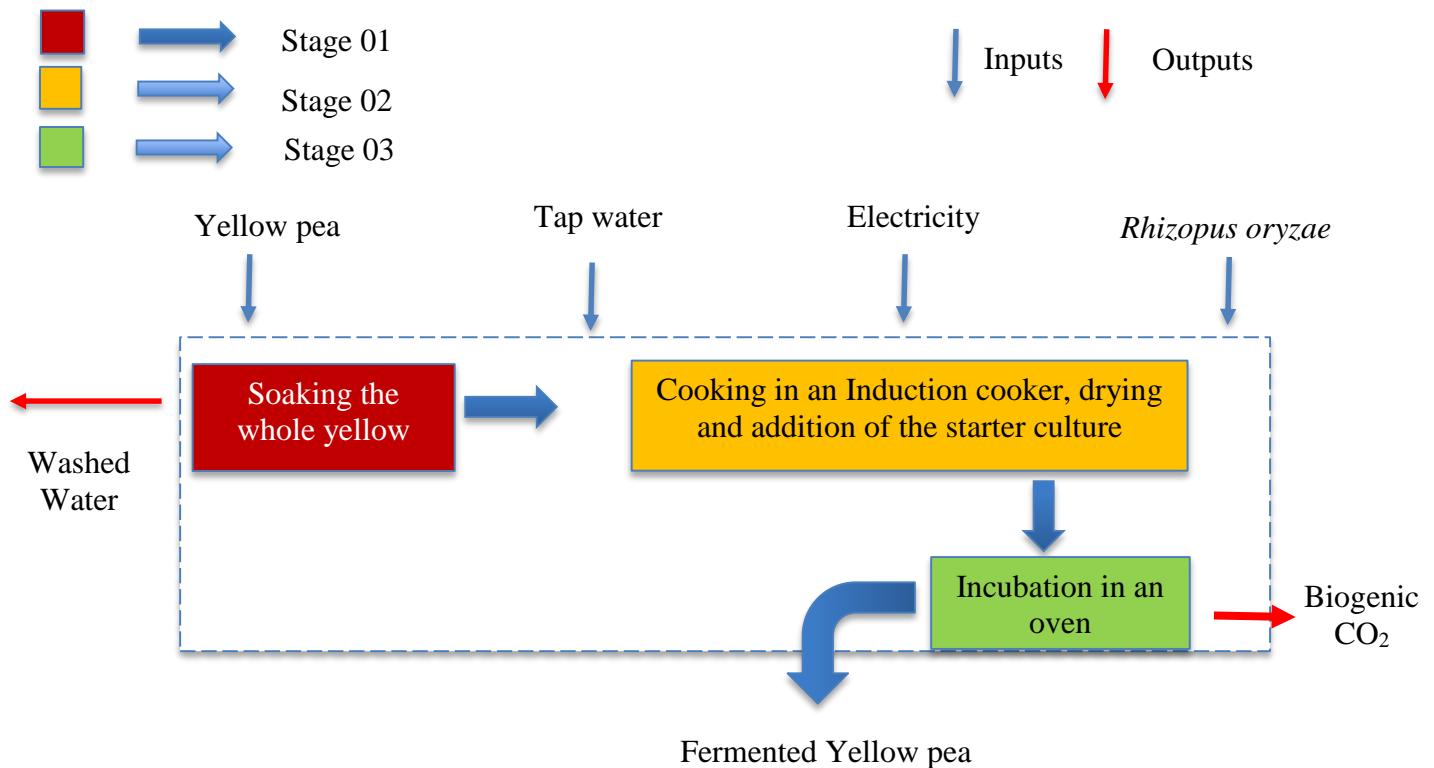


Figure A; System Boundary for Solid State Fermentation

Below is the system boundary for SmF with all the inputs and outputs.

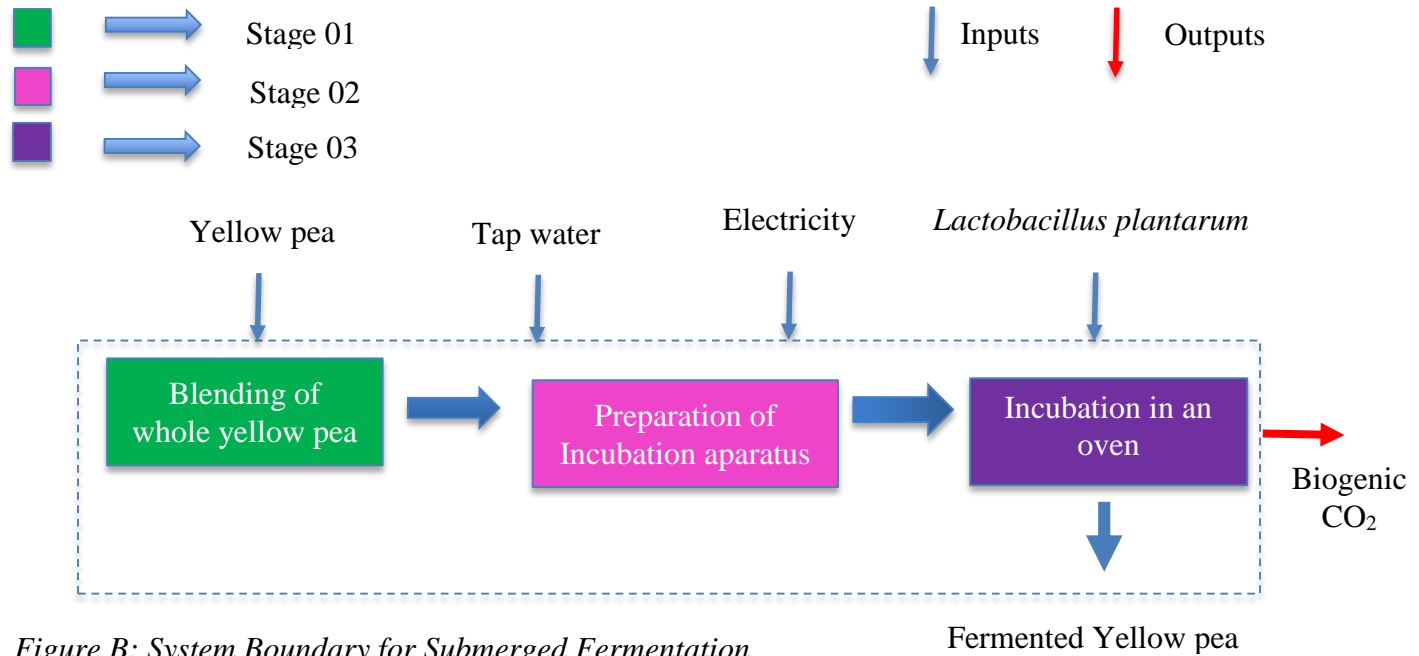


Figure B; System Boundary for Submerged Fermentation

### 3.6.3 Life cycle inventory analysis (LCI)

The dataset was based on primary data from laboratories of the Department of Process and Life Science Engineering and secondary data from literature reviews. The guidelines for the literature review included searching within the stage scale and ensuring the material was not prior to 2014, unless no other relevant sources were located. The relevance of the publication and journal was also considered in the requirements. Applicability refers to how closely the paper's procedure matches the actual process. The sources and details of equipments used for blending, cooking, and incubation of the yellow pea, are shown in Appendix B. Assumptions and limitations are stated below the Life cycle inventory tables.

Table 1. Life cycle inventories for 1 000 g of solid state fermented yellow pea divided into stages in the system boundary (Stages 1-3).

Stage	Inputs	Quantity	Unit	Processes
1	Yellow pea Water	1000 3000	g ml	Market for pea, Switzerland (CH), (Ecoinvent database) 0.389 kg CO <sub>2</sub> -Eq
2	Water Electricity Starter culture	2000 3.5 8.34	ml kWh g	Tap water production, conventional treatment. Europe without Switzerland (Ecoinvent database) 2.21e-4 kg CO <sub>2</sub> -Eq
3	Electricity	137.28	kWh	Market for electricity, low voltage, Sweden (SE), (Ecoinvent database) 0.0427 kg CO <sub>2</sub> -Eq

Table 2. Life cycle inventories for 1 000 g of submerged fermented yellow pea divided into stages in the system boundary (Stages 1-3).

Stage	Inputs	Quantity	Unit	Processes
1	Yellow pea Electricity	1000 0.05	g kWh	Market for pea, Switzerland (CH), (Ecoinvent database) 0.389 kg CO <sub>2</sub> -Eq
2	Water Starter culture	2000 0.5	ml g	Tap water production, conventional treatment. Europe without Switzerland (Ecoinvent database) 2.21e-4 kg CO <sub>2</sub> -Eq
3	Electricity	137.28	kWh	Market for electricity, low voltage, Sweden (SE), (Ecoinvent database) 0.0427 kg CO <sub>2</sub> -Eq

### 3.6.4 Assumptions and limitations

The power was considered to be of Swedish origin throughout the process. In the laboratory, the voltage was considered low, while on the pilot/industrial scales, it considered medium. No waste was generated during the fermentation process, and no residues remained in the aluminum containers. The fermented yellow peas were discarded in municipal waste instead of reused or recycled. The potential outputs of the incubation stage, such as biogenic CO<sub>2</sub>, heat, volatile organic compounds (VOC), waste gases, or odors caused by microbial activity, were neglected. Tap water was used instead of distilled water because it tends to be utilized in commercial production. Washed water in solid-state fermentation was not subject to purification, and the impact was negligible. The impact of the production of starter culture was not in literature and in relevant sources.

## 4. RESULTS & DISCUSSION

### 4.1 Physiochemical property analysis

#### 4.1.1 Moisture and protein content

The results from the moisture content as well as the protein content determination are shown in Table 3. Food manufacturers are concerned with the moisture content of their products for a number of reasons. Moisture is a critical determinant in food quality, preservation, and resistance to spoilage. Moisture content must also be determined in order to compute the uniform content of other dietary elements (Nielsen, 2010).

The moisture content of the flours ranged from 0.90 to 1.90% in the solid state fermentation and in the submerged fermentation from 1.02 to 4.40 %. There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ . Both fermented samples had increased moisture content with fermentation time and there was a marginal increment in the submerged fermented sample.

*Table 3- Moisture and protein content of yellow pea flours resulted from raw and fermented<sup>†</sup>*

Parameter	SSF – T0	SmF – T0	SSF – T48	SmF – T48
Moisture (%)	0.90 ± 0.32 <sup>aA</sup>	1.02 ± 0.20 <sup>aA</sup>	1.90 ± 0.15 <sup>cC</sup>	4.40 ± 0.59 <sup>cC</sup>
Protein (%)	22.44 ± 0.65 <sup>abA</sup>	21.32 ± 0.76 <sup>aA</sup>	24.08 ± 1.19 <sup>bA</sup>	23.01 ± 0.25 <sup>aA</sup>

*Note:* Means within columns with different letters are significantly different (Tukey HSD test,  $p < 0.05$ )

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

Standardized analytical procedures are required to assess the amount of protein present in food. There are several methods used in the food industry to quantify protein content, including the Kjeldahl, Dumas, Lowry, Bradford, and total amino acid content methods (Hayes, 2020; Shea & Watts, 1939). The protein level in flour determines the texture of baked goods; higher protein flours create chewy textures (e.g. bread), while lower protein flours produce tender products (e.g. cakes) (Wang & Flores, 2000).

The results show that the protein content of flours ranged from 22.44 to 24.08 %, in solid state and 21.32 to 23.01 % in submerged fermentation. There was no significant difference between raw (T0) and fermented (T48) flour samples and it was  $P = 0.076$ . There were slight increments of protein content when compare raw and fermented samples in both methods. According to Igbabul et al. (2014), the increased in protein content of fermented samples may be due to an increase in microbial mass during the fermentation process, which contributes to extensive breakdown of protein molecules into amino acids and smaller peptides.

#### 4.1.2 Color characteristics

Color is an important quality attribute in the food and bioprocess industries, and it influences consumer's choice and preferences. Food color is governed by the chemical, biochemical, microbial, and physical changes that occur during growth, maturation, postharvest handling, and processing. Color measurement of food products has been used as an indirect measure of other quality attributes such as flavor and pigment content because it is simpler, faster, and more accurate (Pathare, 2013).

Color and color changes can be quantified using color organization systems like the CIELAB color space, which is established by the Commission Internationale de l'Eclairage (CIE). The CIELAB expresses color lightness as L\*, red/green intensity as a\*, and yellow/blue intensity as b\*. L\* ranges from 0 (black) to 100 (white). The a\* value represents the red-green component of a color, with +a\* (positive) and -a\* (negative) representing red and green values, respectively. The yellow and blue components are represented on the b\* as +b\* (positive) and -b\* (negative), respectively (Ly et al., 2020). CIELAB color values (L\*, a\*, b\*) of solid state and submerged fermented flours are shown in Table 4.

Table 4 – CIEAB color values of raw and fermented yellow pea flours<sup>†</sup>

Parameter	SSF – T0	SmF – T0	SSF – T48	SmF – T48
L*	83.67 ± 0.24 <sup>aA</sup>	86.31 ± 0.59 <sup>abA</sup>	82.37 ± 0.59 <sup>aA</sup>	86.32 ± 0.04 <sup>abA</sup>
a*	3.15 ± 0.22 <sup>bcB</sup>	2.44 ± 0.17 <sup>bB</sup>	0.45 ± 0.11 <sup>aA</sup>	0.47 ± 0.50 <sup>aA</sup>
b*	29.67 ± 0.28 <sup>cAB</sup>	22.86 ± 0.15 <sup>aAB</sup>	20.44 ± 1.65 <sup>aA</sup>	28.56 ± 0.81 <sup>bA</sup>

Note: Means within columns with different letters are significantly different (Tukey HSD test, p < 0.05)

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

The L\* value ranges from 82.37 to 83.67 in solid state and for submerged from 86.31 to 86.32. The a\* value ranges from 0.45 to 3.15 in solid state and for submerged 0.47 to 2.44. The b\* value of all the flours was positive which is the indication of a yellow color, ranging from 20.44 to 29.67 in solid state and for submerged 22.86 to 28.56. The results indicated that for L\* value there was no significant difference between raw (T0) and fermented (T48) flour samples and it was P = 0.46. In SSF the L\* value has decreased while in SmF it has remain the same. There was a significant difference between raw (T0) and fermented (T48) and in terms of a\* and it was P < 0.001. In both fermented samples the a\* value has a significant decrease which means the redness has shifted towards greenness. In SSF the b\* value has decreased whereas in SmF the b\* value conveyed an increase. There were no significant difference between raw (T0) and fermented (T48) flour samples and it was P = 0.348.

The highest L\* values indicates that they are lighter than other flour samples and the lowest values of L\* indicate of the dark color of the flour compared to the other flour samples. All of the samples showed a positive a\* value which means that all of the samples have a red color tint with the fermented (48 hr) flour having the lowest value. In solid state fermented samples both

a\* and b\* values increased till 12 hours and showed a decrease thereafter. In submerged fermented samples both values were decreased with the fermentation time (after 6 hr). (The results for these time intervals have presented in Appendix A)

## 4.2 Functional property analysis

### 4.2.1 Water absorption index (WAI) and water solubility index (WSI)

The results for the WAI and WSI of the two fermentation methods are shown in Table 5. Determination of the water absorption index (WAI) and the water solubility index (WSI) is a descriptive characteristic used for the processing of legumes and cereals. The water absorption index and water solubility index measure the hydration capabilities of flour. The WAI is a measurement of the ability to absorb water and swell, resulting in desired consistency and body in a food system (Choi et al., 2012). It determines the volume occupied by granules or starch polymers after swelling in excess water (Yousf et al., 2017).

The WAI value for raw and fermented flour samples ranges from 4.07 to 4.67 (g / g) in solid state and from 2.56 to 3.87 (g / 100g) in the SmF. There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P = 0.03$ . The WAI showed a decrease in SSF and increase in SmF.

*Table 5 - Water absorption index (WAI) and water solubility index (WSI) of yellow pea flours resulted from raw and fermented†*

<b>Parameter</b>	<b>SSF – T0</b>	<b>SmF – T0</b>	<b>SSF – T48</b>	<b>SmF – T48</b>
<b>WAI (g / g)</b>	4.67 ± 0.08 <sup>cA</sup>	2.56 ± 0.05 <sup>aA</sup>	4.07 ± 0.03 <sup>aBCD</sup>	3.87 ± 0.14 <sup>dBCD</sup>
<b>WSI (g / 100g)</b>	6.88 ± 0.42 <sup>aC</sup>	19.62 ± 1.43 <sup>cC</sup>	23.45 ± 1.64 <sup>dE</sup>	11.66 ± 1.24 <sup>aE</sup>

*Note:* Means within columns with different letters are significantly different (Tukey HSD test,  $p < 0.05$ )

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

The WSI determines the amount of polysaccharides that are released from the granule when additional water is added. A high WSI indicates good digestibility of starch (Yousf et al., 2017). The WSI values of flour samples ranges from 6.88 to 23.45 (g / 100g) in solid state and from 11.66 to 19.62 (g / 100g) in the submerged fermentation. There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ . In solid state fermentation there was an increase in WSI with the fermentation time. According to Onweluzo & Nwabugwu (2009), the fermentation process can hydrolyze high molecular weight proteins and carbohydrates into smaller more soluble components. But in SmF a significant decrease in the WSI was observed in fermented samples.

#### 4.2.2 Water absorption capacity (WAC) and Oil absorption capacity (OAC)

The values for the WAC and OAC of fermented and unfermented yellow pea flours are shown in Table 6. The term "water absorption capacity," also known as "hydration capacity," "water binding capacity," or "water holding capacity," refers to a protein's ability to hold onto or absorb water per gram. This critical parameter determines how proteins behave in terms of gelation, swelling, and water retention. It is classified into two types: volume water absorption (Wv) and mass water absorption (Ww) (Shevkani et al., 2015; Boye et al., 2010).

The WAC value ranges from 2.17 to 2.79 (g water / g DM) in solid state and for submerged from 1.27 to 1.40 (g water / g DM). There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ . Higher WAC values can be observed in submerged fermented yellow pea samples than the raw samples. On the other hand significant decrease of WAC values can be seen in solid state fermented samples when compared to raw samples. The increase in WAC could be attributed to the degradation of macromolecules like polysaccharides and fiber, as well as changes in protein quantity and quality, which increase hydrophilic sites and trap more water within the flour matrix (Azeez et al., 2022). In contrast, a decrease in WAC could be due to less hydrophilic groups that bind water molecules during fermentation (Afoakwa et al., 2007), implying that they might be used to produce thin gruels in food compositions.

Table 6 - Water absorption capacity (WAC) and oil absorption capacity (OAC) of raw and fermented yellow pea Flours<sup>†</sup>

Parameter	SSF – T0	SmF – T0	SSF – T48	SmF – T48
<b>WAC (g water / g DM)</b>	2.79 ± 0.06 <sup>dB</sup>	1.27 ± 0.02 <sup>bD</sup>	2.17 ± 0.06 <sup>aA</sup>	1.40 ± 0.02 <sup>cdA</sup>
<b>OAC (g oil / g DM)</b>	1.37 ± 0.26 <sup>aA</sup>	0.73 ± 0.02 <sup>aA</sup>	1.72 ± 0.09 <sup>bC</sup>	1.71 ± 0.14 <sup>bC</sup>

Note: Means within columns with different letters are significantly different (Tukey HSD test,  $p < 0.05$ )

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

Oil absorption capacity is the ability of a substance to absorb and retain oil, which is often represented in terms of the amount of oil absorbed per gram of material. The oil absorption capacity of proteins and pulse flours is an important functional property. OAC influences taste, mouth feel, texture, and product yield. (Seena & Sridhar, 2005). The OAC of solid state ranged from 1.37 to 1.72 g oil/g DM, and the submerged fermented flours ranged from 0.73 to 1.71 g oil/g DM, with the 48 h fermented flours having the highest capacity for both methods. There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ .

The observed increase in OAC in the fermented yellow pea is most likely due to the unfolding of proteins and the degradation of starch during fermentation processes, which lead to higher hydrophobic amino acids and lipophilic substances and hence enhance the OAC of the flour (Adebisi et al., 2016). An increase in OAC was noted in studies by Elkhailifa & Bernhardt (2010), Xiao et al. (2015), and Chawla et al. (2017) for the fermentation of sorghum flour,

chickpeas, and black-eyed peas respectively. According to Sobowale et al., (2024) the oil absorption capacity of fermented flour samples declined until 24 hours of fermentation, after which it increased. OAC changes could be induced by variances in the degree of breakdown during fermentation (Oyeyinka et al., 2020).

The higher WAC and OAC of the fermented yellow pea flour could improve flavor retention and mouth feel and indicate the potential applications in the food industry. In agreement with the findings, increases in WAC and OAC were reported in fermented and germinated finger millet (Adebiyi et al., 2016), and fermented African yam bean flour (Chinma et al., 2020).

#### 4.2.3 Emulsion activity (EA) and Emulsion stability (ES)

Emulsifying properties refer to the capability of certain substances, particularly surface-active agents (surfactants), proteins, and polysaccharides, to facilitate the formation and stabilization of emulsions, mixtures of two immiscible liquids such as oil and water. Emulsions are thermodynamically unstable systems where one phase (dispersed phase) is distributed in another (continuous phase) in the form of droplets. Without stabilizers or emulsifying agents, these droplets tend to coalesce, leading to phase separation (Lam & Nickerson, 2013). In many natural and industrial processes, emulsifying agents are essential for reducing interfacial tension between the immiscible liquids and for forming a mechanical or electrostatic barrier around the dispersed droplets, thereby improving emulsion stability. The efficiency of an emulsifying agent is characterized by several key parameters, including emulsifying activity (EA), and emulsion stability (ES). Emulsifying activity is defined as the highest quantity of oil that can be emulsified by a fixed amount of the protein, and stability of the emulsion is defined as the rate of phase separation in water and oil during storage of the emulsion (Pearce & Kinsella, 1978).

Emulsion activity and stability results of solid state and submerged fermented flours are shown in Table 7. There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ . Fermented flours (48 hour) had the lowest value for the EA, whereas 4.40 and 4.95 for solid state and submerged fermented flour, respectively. Emulsifying activity is a critical protein-related functional feature (Hu et al. 2018) and it depends on the pH and the acidity of the medium. In this study pH decreased and acidity increased with the fermentation time (The detailed table is in Appendix A).

*Table 7 - Emulsion activity (EA) and emulsion stability (ES) of raw and fermented yellow pea Flours<sup>†</sup>*

<b>Parameter</b>	<b>SSF – T0</b>	<b>SmF – T0</b>	<b>SSF – T48</b>	<b>SmF – T48</b>
<b>EA (%)</b>	53.30 ± 0.92 <sup>cC</sup>	41.20 ± 1.16 <sup>cC</sup>	4.40 ± 0.30 <sup>aA</sup>	4.95 ± 0.93 <sup>aA</sup>
<b>ES (%)</b>	69.85 ± 1.53 <sup>cC</sup>	49.70 ± 0.82 <sup>cC</sup>	4.90 ± 0.24 <sup>aA</sup>	5.18 ± 0.78 <sup>aA</sup>

*Note:* Means within columns with different letters are significantly different (Tukey HSD test,  $p < 0.05$ )

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

Emulsifying agents function through various mechanisms, such as forming viscoelastic interfacial films, providing steric hindrance, or generating electrostatic repulsion. Their effectiveness can depend on factors like molecular structure, concentration, pH, ionic strength, and temperature (Lam & Nickerson, 2013; Patrascu et al., 2017). These properties are crucial in a wide range of applications across multiple industries. In the food industry, emulsifiers contribute to the texture, appearance, and shelf life of products like mayonnaise, dressings, and dairy items.

The type and amount of surfactants affect the stability of the emulsion. These surfactants improve the stability by creating films around water drops at water/oil interfaces. Film formation improves emulsion stability by increasing interfacial viscosity while decreasing interfacial tension (Sullivan & Kilpatrick, 2002). When comparing raw (T0) and fermented (T48) flour samples, it was  $P < 0.001$  and 48 hour fermented flours had significantly lower ES, making them the poorest sample in terms of ES among the other samples.

Low EA and ES in the fermented samples may be due to the enhanced hydrophobicity, which has influenced the protein's capacity to move to the oil-water interface to lower interfacial tension, facilitate emulsion formation, and possibly favor the aggregation of released peptides and unhydrolyzed proteins (Liang & Tang, 2013). This observation is consistent with the pH-dependent protein solubility profiles of the proteins, demonstrating that protein solubility is critical for their emulsifying properties, while 100% solubility is not required (Damodaran, 1997). Similar results have been found for pea globulins in terms of emulsion capacity (Koyoro & Powers, 1987) and pea protein isolates in terms of emulsion activity index (Barac et al., 2010).

#### 4.2.4 Pasting properties

Pasting qualities are the changes that occur in a food when heat is applied while water is present. These changes affect the end use, texture, and digestion of the food product, which affects flour mixes used for pasting (Adebowale et al., 2009). The pasting characteristics of raw and fermented yellow pea flours, such as pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity, and setback, were investigated using a rapid visco analyzer (RVA). The results of the RVA of the solid state and submerged fermented flours are shown in Table 8.

Pasting temperature is the point at which viscosity begins to rise during the heating process. Both the concentration of the starch chosen in the formulation and the choice of starches for food processing are influenced by pasting temperature, since certain starches can undergo significant changes at various ratios during processing (Mauro et al., 2023). There was a significant difference between raw (T0) and fermented (T48) flour samples and it was  $P < 0.001$ . The raw flour indicated higher values than fermented samples. The high pasting temperature of starches indicates a higher capacity to swelling and rupture (Kumar & Khatkar, 2017).

Table 8 – Pasting properties of raw and fermented yellow pea Flours<sup>†</sup>

Parameter	SSF – T0	SmF – T0	SSF – T48	SmF – T48
<b>Pasting temperature (°C)</b>	74.15 ± 2.33 <sup>bB</sup>	76.8 ± 1.41 <sup>cB</sup>	54.85 ± 3.32 <sup>aA</sup>	52.3 ± 1.27 <sup>aA</sup>
<b>Peak viscosity (cP)</b>	1008.5 ± 7.78 <sup>dF</sup>	899.5 ± 6.36 <sup>eF</sup>	281 ± 2.83 <sup>aA</sup>	303 ± 7.07 <sup>aA</sup>
<b>Trough viscosity (cP)</b>	979 ± 1.41 <sup>eF</sup>	878 ± 2.83 <sup>fF</sup>	223 ± 4.24 <sup>aA</sup>	225.5 ± 4.95 <sup>aA</sup>
<b>Breakdown (cP)</b>	29.5 ± 6.36 <sup>aA</sup>	21.5 ± 3.54 <sup>aA</sup>	58 ± 7.07 <sup>aD</sup>	77.5 ± 2.12 <sup>cD</sup>
<b>Final viscosity (cP)</b>	1527.5 ± 3.54 <sup>fF</sup>	1389.5 ± 7.78 <sup>fF</sup>	421 ± 1.41 <sup>aA</sup>	452.5 ± 3.54 <sup>aA</sup>
<b>Setback (cP)</b>	548.5 ± 4.95 <sup>dA</sup>	511.5 ± 4.95 <sup>dA</sup>	198 ± 2.83 <sup>aE</sup>	227 ± 1.41 <sup>aE</sup>

Note: Means within columns with different letters are significantly different (Tukey HSD test,  $p < 0.05$ )

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

SSF: Solid State Fermentation, SmF: Submerged Fermentation

The peak viscosity (PV) is the maximum viscosity achieved while heating or pasting. This occurs at the end of the heating stage, when the large quantity of inflated starch granules causes pasting (Thomas & Atwell, 1999). Peak viscosities of flours were significantly affected by time interval, fermentation method, and there was a significant interaction between above factors on peak viscosity. In solid state fermentation, peak viscosity ranged from 281 to 1008.5 cP, whereas in submerged fermentation from 303 to 899.5 cP, with the raw flour and 48 h fermented flour having the highest and lowest values respectively. According to Oloyede et al. (2016), higher peak viscosity in flour correlates with increased thickening power.

Trough viscosity is the minimal viscosity reached during heating or cooling processes. It assesses the ability of the paste to tolerate breakdown when cooling (Iwe et al., 2016). Trough viscosities of flours were significantly affected by time interval, fermentation method, and there was a significant interaction between above factors on trough viscosity. In solid state fermentation, trough viscosity ranged from 223 to 979 cP, where as in submerged fermentation from 225.5 to 878 cP, with the raw flour and 48 h fermented flour having the highest and lowest values respectively. The ability of a sample to withstand such high temperatures and shear stress is regarded a crucial factor in many processes (Perten, n.d.).

Breakdown value is defined as the difference between peak and trough viscosity values. As a result, breakdown is used to determine the degree of granule disintegration or paste stability (Kumar & Khatkar, 2017). Breakdown value of flours were significantly affected by time interval, fermentation method, and there were a significant interaction between above factors on breakdown value. In solid state fermentation, breakdown value ranged from 29.5 to 58 cP, where as in submerged fermentation from 21.5 to 77.5 cP, with the raw flour and 48 h fermented flour having the lowest and highest values respectively. Thus the higher breakdown viscosity of fermented flour indicated higher stability compared to raw flour.

Final viscosity is the most widely used criterion for determining the quality of a sample since it represents the material's ability to form a thick paste or gel after cooking and cooling (RVA Method Brochure). Final viscosities of flours were significantly affected by the time interval, but not by the fermentation method, and there was a significant interaction between the above factors on final viscosity. In solid state fermentation, final viscosity ranged from 421 to 1527.5 cP, whereas in submerged fermentation from 452.5 to 1389.5 cP, with the raw flour and 48 h fermented flour having the highest and lowest values respectively. According to Oloyede et al. (2016), flour with higher amylose quantity has a higher viscosity since final viscosity is mostly correlated with amylose content. The reduction in pH and the resulting acidic environment may be associated to the decrease in the final viscosity of fermented samples (Oyeyinka et al., 2020).

The setback viscosity (difference between final and peak viscosity) is displayed due to recrystallization of amylose molecules in the gel, which is a measure of the gelling capacity or retrogradation ability of starches (Hung & Morita 2005). Setback viscosities of flours were significantly affected by time interval, fermentation method, and there was a significant interaction between above factors on Setback viscosity. In solid state fermentation, Setback viscosity ranged from 198 to 548.5 cP, whereas in submerged fermentation from 227 to 511.5 cP, with the raw flour and 48h fermented flour having the highest and lowest values, respectively. A setback occurs when amylopectin retrogrades and amylose crystallizes during the cooling process of cooked flour paste (Xu et al., 2019; Kaur & Singh, 2005). The lowest value was observed for fermented whole flour, indicating less tendency to retrograde, whereas the highest value was recorded for raw flour samples. Lower retrograde tendencies are advantageous in the formulation of food products such as soups and sauces, which may encounter viscosity loss and precipitation as a result of retrogradation (Kaur & Singh 2005).

The functional properties of fermented yellow pea flour, are significantly improved compared to the non-fermented counterpart. These attributes make it suitable for a wide range of food applications, including bakery products, meat alternatives, and dairy substitutes, specially in the context of gluten-free (Jones, 2017) and plant-based diets (Craig et al., 2021). The study by Drakula et al., 2021, indicates the importance of utilizing sourdough fermentation with a carefully selected starter when adding pea flour to gluten-free bread to ensure significant antioxidant potential. Furthermore, other food components (lipids, carbohydrates, salts, etc.) and the varied structures of food matrices in the food system influence the digestibility and flavor of pea protein, which serves as the foundation for the creation and structural design of new pea protein-based products (Lao et al., 2024). Improved functional characteristics of yellow pea allow them to replace animal proteins in applications such as plant-based meat and dairy alternatives. This minimizes the environmental impact of food production (Poore & Nemecek, 2018). Yellow pea can be utilized as a natural additive alternative in clean-label products to serve as natural emulsifiers and stabilizers, substituting synthetic or animal-derived additives while meeting clean-label requirements (Lam et al., 2018; Stone et al., 2015). Improved water/oil retention and gelation help to stabilize food matrices and reduce syneresis, resulting in extended shelf life and lower spoiling rates (Boye et al., 2010). Improved protein solubility also increases component usage throughout processing. Through these reduction in food waste and process losses can be ensured. Solid-state or submerged fermentation techniques can naturally improve these functional qualities by altering protein structures and lowering antinutritional components (Schindler et al., 2012; Zhen et al., 2023).

### 4.3 LCA results and interpretation

Life Cycle Assessment (LCA) studies focusing specifically on pea products are currently limited as well as evaluation of other impact categories such as eutrophication potential, acidification potential, land use and water use. There are research focused on the climate change impact category, quantified as Global Warming Potential (GWP) over a 100-year horizon (GWP100), expressed in kg CO<sub>2</sub> equivalents (Rabani et al., 2021). The carbon footprint is a measure of total greenhouse gas (GHG) emissions generated directly and indirectly by a product, process, person, or organization. It is commonly stated in terms of carbon dioxide equivalents (kg CO<sub>2</sub>-eq). Carbon footprint focuses on GHGs while Climate change is a broader impact category (Shaoqing & Jianhua, 2021).

#### 4.3.1 Comparison of fermentation process and raw yellow pea

Contributions from the different stages of the fermentation process in terms of total greenhouse gas emissions (GHG) were evaluated for the functional unit of 1000 g fermented flour. Figure below shows the individual stages contribution to the total GHG emissions, kg CO<sub>2</sub>-eq/1000 g for SSF and SmF fermentation methods. The information which can be depicted from the Figure is the highest impact fermentation type, the hot spot of the process, and the high and low contributing stages.

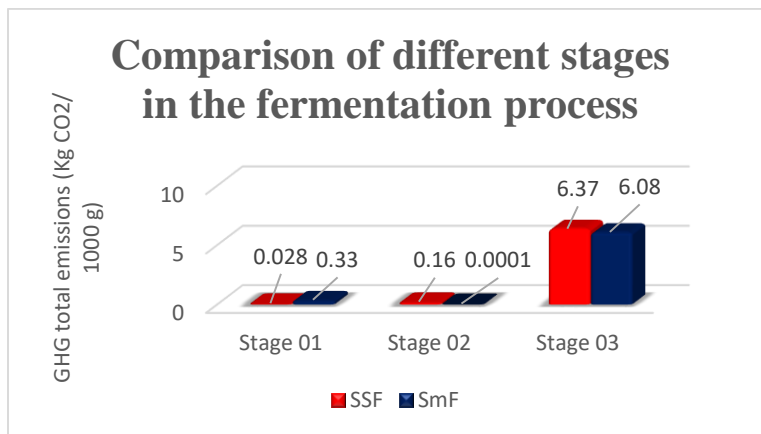


Figure 1; The total GHG emissions (kg CO<sub>2</sub>-eq/1000 g) for fermentation types are shown in the graph. The order of stages is 1-3 from left to right, with colors indicating which fermentation type is in the graph. SSF: Solid State Fermentation, SmF: Submerged Fermentation

The SSF has a total of 6.56 kg CO<sub>2</sub>-Eq/1 kg and SmF has 6.41 kg CO<sub>2</sub>-Eq/1 kg of the fermented flour. When analyzing the highest contribution from particular phases to the overall GHG emissions, stage 3 has the highest impact for both fermentation processes. So the hot spot of this process is the incubation stage. The consumption of electricity has a significant impact to the total process. The geographical coverage includes Sweden, which has a low-impact energy system. As a result, the outcomes of the research are highly dependent on the energy mix used (Brancoli et al., 2021).

Data indicate that stages 1 and 2 have minimal impact (less than 0.4 kg CO<sub>2</sub>-eq/1000 g) for both methods. Since lower electricity usage for cooking and blending of raw pea, when compared to incubation may be the reason for the lower impact value. The study of Chaudhary et al., (2018), revealed that the hotspots were the stages of wheat and yellow pea cultivation and the electricity required in the manufacturing stage.

The impact category results are shown in the table. In this study it revealed that Solid state fermentation has slightly higher environmental impact than the submerged fermentation. The reasons for this deviation are the usage of water for soaking and cooking stages in the solid state while no usage of electricity for the fermentation apparatus in the submerged fermentation process as it was conducted in anaerobic conditions.

#### 4.3.2 Impact analysis of raw and fermented yellow pea flour

*Table 9 - Impact category Results on Climate Change*

Impact category	Results (kg CO <sub>2</sub> -Eq)/ 1 kg of pea		
	SSF	SmF	Raw
Climate change	6.560748	6.409199	0.403156812
Climate change: biogenic	0.108597	0.105944	0.000445561
Climate change: fossil	5.926034	5.790022	0.402272317
Climate change: land use and land use change	0.526116	0.513232	0.000438934

SSF: Solid State Fermentation, SmF: Submerged Fermentation

The climate change can be divided into 3 parts such as biogenic, fossil, land use and land use change. The climate change regarding fossil has the highest contribution while the biogenic category has the lowest. Tidåker et al. (2021) assessed the environmental impact of five Swedish pulse crops, including yellow peas, from cultivation to factory gate. The study indicated that standard yellow pea production has a global warming potential (GWP) of 0.18 kg CO<sub>2</sub>-eq per kilogram of dry product, indicating a modest environmental impact compared to other pulses. Zhang et al. (2021) investigation on the physicochemical features and aroma differences in yellow pea flour fermented by different lactic acid bacteria strains discovered that fermentation enhanced amino acid content, decreased fat levels, and improved pasting qualities, all of which could have an impact on the environmental profile of the final product by potentially decreasing the need for additional processing or additives.

Several related studies provide insights into the environmental impacts of pea cultivation and processing, as well as the effects of fermentation when combined with other ingredients. In the study by Juliette et al., (2023) a LCA was conducted to evaluate the environmental performance of four innovative fermented food products that combine animal (milk) and plant (pea) protein sources in varying proportions (100% pea, 75% pea-25% milk, 50% pea-50% milk, 25% pea-75% milk). The system perimeter ranges from the agricultural production of ingredients to ready-to-eat products. Environmental impact results which obtained for 1 kg of ready-to-eat product were, highest impact in 25% pea product and lowest impact in 100% pea product.

Huguet et al., (2023) investigated environmental implications from agricultural production to the manufacturing of ready-to-eat products. While not limited to yellow peas, the study gives useful information on the environmental performance of fermented products containing pea proteins. While direct LCA studies on fermented yellow pea products are rare, available research suggests that adding yellow peas into food items can improve nutritional value while lowering environmental effect. According to Chaudhary et al., (2018), when compared to conventional products made solely with wheat flour, items reformulated with yellow pea flour have a lower carbon footprint.

The impact of raw and fermented yellow pea on climate change was assessed based on total greenhouse gas emissions (GHG) for the 1000 g functional unit. The table conveys the contribution of GHG emissions (kg CO<sub>2</sub>-eq/1000 g) for climate change on fermentation processes and raw pea.

Table 10 - Impact assessment results on Climate Change

Climate Change	Impact assessment results (kg CO <sub>2</sub> -Eq)		
	SSF	SmF	Raw
<b>Carbon dioxide, fossil<sup>1</sup></b>	3.067772	2.995644	0.143533578
<b>Carbon dioxide, fossil<sup>2</sup></b>	0.91645	0.895392	0.058875121
<b>Carbon dioxide, fossil<sup>3</sup></b>	0.678769	0.66452	0.100305264
<b>Carbon dioxide, from soil or biomass stock</b>	0.525663	0.512787	0.00044
<b>Sulfur hexafluoride</b>	0.476104	0.464441	-
<b>Methane, fossil</b>	0.351693	0.343834	0.033110312
<b>Dinitrogen monoxide<sup>2</sup></b>	0.243629	0.237669	-
<b>Methane, non-fossil</b>	0.105602	0.103023	0.00045
<b>Dinitrogen monoxide<sup>3</sup></b>	0.077494	0.075792	0.00808963
<b>Dinitrogen monoxide<sup>1</sup></b>	0.068696	0.06832	0.053443684

1 Emission to air, Low population density      SSF: Solid State Fermentation, SmF: Submerged Fermentation  
 2 Emission to air, unspecified  
 3 Emission to air, High population density

Carbon dioxide, fossil<sup>1</sup> shows the highest emissions across all categories. Solid state generally shows the highest emission values. Submerged conditions typically show slightly lower values than solid state. Raw yellow pea consistently shows the lowest emission values. Emission to air low population density trends to have higher impact values. Emission to air, unspecified have moderate impact values. Emission to air, higher population density have generally lower impact values. Fossil fuel derived emissions (carbon dioxide, methane) significantly overweigh non fossil sources. Sulfur hexafluoride shows moderate but consistent impact across solid and submerged states. Nitrogen oxide (dinitrogen monoxide variants) show relatively lower but measurable impacts.

The results suggest that fossil fuel combustion, particularly in solid and submerged states represents the most significant climate impacts which aligns with current scientific understanding of the primary drivers of climate change. Plant-based substitutes for animal products have greatly decreased greenhouse gas emissions, land use, and water consumption related to food production (Poore & Nemecek, 2018). Furthermore, alternative proteins could be beneficial to food security by lowering reliance on limited resources and enhancing the resilience of food systems (Sexton, Garnett & Lorimer, 2019).

In commercial scale the results will be differ than the research findings. For climate change mitigation and adaptation, Solid-State Fermentation offers several advantages over Submerged Fermentation, specially in terms of lower water and energy use, reduced GHG emissions, compatibility with circular bio economy principles. However, SmF may still be preferred where high production yields and consistent quality are priorities, specially in highly industrialized settings with renewable energy integration.

#### 4.3.3 Cradle to Gate Perspective of Fermented Yellow Pea Flour

The cradle-to-gate perspective in LCA considers all environmental impacts from raw material extraction (the "cradle") to the point at which the product exits the manufacturing facility (the "gate") before being supplied to customers (González-García et al., 2013). This method provides a targeted evaluation of production-related consequences while omitting use-phase and end-of-life processes, which might vary, depending on the application of the product, geographic location, and consumer behavior. According to Rebitzer et al. (2004), the cradle-to-gate approach enables quicker assessments when the goal is to improve manufacturing efficiency or compare production systems.

The cradle phase of fermented yellow pea flour covers agricultural cultivation, post-harvest handling and transportation, primary processing, drying, packaging and fermentation. The gate-to-grave phase consists of distribution and retail, consumer use, and end-of-life. According to LCA research, pea protein isolates and flour range between 0.4 - 1.2 kilogram CO<sub>2</sub>-eq/kg, which is much less than the emissions of soy (1.0 - 2.0 kg CO<sub>2</sub>-eq/kg) and meat (27 kg CO<sub>2</sub>-eq/kg) (Poore & Nemecek, 2018). The cradle-to-gate LCA for fermented yellow pea flour considers the environmental effects of agricultural and processing phases. This boundary excludes subsequent procedures including distribution, cooking, and disposal. This approach allows for a controlled and fair comparison of various fermentation techniques, energy sources, and substrate inputs, as well as hotspot analysis and sustainable design recommendations (Paraskevi et al., 2017; Jamekhorshid & Azin, 2023).

Cradle to gate perspective of fermented yellow pea flour may assist food makers and researchers identify low-impact processing technologies that are consistent with environmental and nutritional sustainability goals.

#### 4.4 Commercial and Entrepreneurial Dimensions of the Study

The research findings will have an impact on ingredient innovation and yellow pea niche market penetration. Functionalized pea flour can be used to create specialty ingredients for vegan, allergen-free, or gluten-free applications, all of which have high development potential (MarketsandMarkets, 2023; Grand View Research, 2024). Startups can develop high-value bioactive components by fermenting underutilized or non-premium pea fractions, contributing to the circular economy and attracting investors who prioritize sustainability (Zhou et al., 2021).

In terms of economic upscaling of local crops, regions that cultivate peas (e.g., Canada, India, and the EU) might use integrated pea processing to reduce protein import dependence (e.g., soy), hence enhancing food independence and value-added processing locally (FAO, 2016). Refined pea components are consistent with consumer preferences for environmental sustainability, high protein intake, and non-GMO plant-based diets. Brands can utilize these criteria to justify premium pricing and ESG (Environmental, Social, and Governance) positioning (Nielsen, 2021). The results of the research will be a platform for biotech and agri-food innovation, whereas the methods developed (e.g., fermentation, enhanced protein functionality) can be applied to other legumes and pulses, resulting in an expanded agri-biotechnology innovation platform (Oliveira et al., 2022).

## 5. CONCLUSION

Research on environmental sustainability and the functional properties of fermented yellow pea flour is essential for advancing sustainable food systems. Both the solid-state and submerged fermentation procedures effect the flour's functional characteristics. The results revealed that the water solubility index has higher values, in fermented samples with solid-state fermentation, exhibiting a slight increase in protein content. Furthermore, both treatments have same values in oil absorption capacity. Submerged fermented samples have higher property values in water absorption capability, water absorption index, emulsion activity and stability while having higher final viscosity when compared to solid state fermentation. Fermentation resulted in a notable variation among the samples in each time interval, making the flours lighter, less red, and more yellowish in color.

Life Cycle Assessment (LCA) provides a comprehensive evaluation of the environmental impacts associated with the yellow pea fermentation process. Solid state fermentation has a slightly higher environmental impact (6.56 kg CO<sub>2</sub>-Eq/1 kg) than submerged fermentation (6.41 kg CO<sub>2</sub>-Eq/1 kg). The incubation stage was identified as the hot spot of the fermentation process. The findings indicate that fossil fuel burning, particularly in solid and submerged fermentation process, has the greatest climate influence, which is consistent with current scientific understanding of the fundamental causes of climate change. However, fermentation could have an impact on the environmental profile of the final product by potentially decreasing the need for additional processing or additives.

Fermentation improves the functional properties of yellow pea flour, enhancing its value as a versatile, plant-based ingredient. Together, these research areas, along with prior research on the nutritional qualities of fermented yellow pea, support the development of nutritional, eco-friendly, functional quality food products and contribute to global goals in sustainability, climate change mitigation, and food security. The results highlight the importance of science based treatments, such as microbial fermentation, in facilitating the transition to more sustainable and equitable food systems. This study emphasizes the key concepts and lays the groundwork for future environmental stewardship, food innovation, and socioeconomic resilience.

## 6. FUTURE PERSPECTIVES

Future research on fermented yellow pea flour should enhance the nutritional and functional benefits by optimizing fermentation parameters such as temperature, moisture content, time, and microbial strains. Evaluating energy consumption, water consumption, and microbial inoculants, as well as assessing feasibility, economic viability, and environmental impact in practical production scenarios, will require comparative studies of industrial-scale solid-state and submerged fermentation.

Advanced microbial biotechnology may make it possible to create customized starter cultures that enhance particular attributes like flavor profile, antioxidant activity, or protein bioavailability. The functional qualities of flour for application in innovative plant-based food products may also be improved by combining fermentation with other sustainable processing techniques (such as extrusion or enzymatic treatment).

Life cycle assessments should be conducted to determine the environmental benefits of fermented yellow pea flour over conventional protein sources, including animal proteins, synthetic or cultured protein, and other legumes (soybeans, chickpeas, and lentils). Pea cultivation using regenerative agriculture or organic farming practices may drastically change LCA outcomes. Products made using fermented yellow pea flour must also undergo sensory testing and customer acceptability before they can be successfully commercialized. More specific LCA study is needed to fully evaluate the effects of fermentation processes on the physicochemical characteristics of yellow peas, which may have additional sustainability implications.

Overall, there is a significant potential for fermented yellow pea flour in terms of functional product innovation and sustainable food systems, which will encourage more interdisciplinary research and industry cooperation.

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## 8. APPENDIX

### *Appendix A. Physicochemical and Functional property analysis*

*Table 1. P<sup>H</sup> and Acidity according to the fermentation method and time interval*

Sample	pH	Weight (g)	NaOH Volume(μL)	Acidity (%)
T0 – Submerged	6.44	10.02	95	0.0085
T0 - Solid State	6.65	10.09	20	0.0018
T6 - Submerged	6.01	10.30	105	0.0092
T6 - Solid State	6.65	9.61	20	0.0019
T12 - Submerged	4.26	10.41	240	0.0208
T12 - Solid State	6.61	10.12	20	0.0018
T24 - Submerged	3.93	10.12	380	0.0338
T24 - Solid State	6.58	10.12	50.5	0.0045
T36 - Submerged	3.90	10.44	420	0.0363
T36 - Solid State	5.95	9.89	80	0.0073
T48 - Submerged	3.93	10.37	440	0.0382
T48 - Solid State	6.23	10.11	100	0.0089

#### *Acidity Standard Formula*

$$\text{Acidity} = \frac{(V \times N \times \text{Acid Factor})}{W}$$

- V* - Volume of NaOH used  
*N* - Normality of NaOH  
*Acid Factor* - 0.09 for lactic acid  
*W* - Weight of the sample

Table 2 - Moisture and protein content of yellow pea flours resulted from raw and fermented<sup>†</sup>

Time*Fermentation method	Moisture (%)	Protein (%)
T0 – SSF	0.90 ± 0.32 <sup>a</sup>	22.44 ± 0.65 <sup>ab</sup>
T0 - SmF	1.02 ± 0.20 <sup>a</sup>	21.32 ± 0.76 <sup>a</sup>
T6 - SSF	1.39 ± 0.18 <sup>b</sup>	23.01 ± 1.24 <sup>ab</sup>
T6 - SmF	3.19 ± 0.59 <sup>b</sup>	22.15 ± 0.33 <sup>a</sup>
T12 - SSF	1.37 ± 0.15 <sup>b</sup>	21.78 ± 0.89 <sup>a</sup>
T12 - SmF	3.09 ± 0.23 <sup>b</sup>	22.24 ± 1.92 <sup>a</sup>
T24 - SSF	1.40 ± 0.14 <sup>b</sup>	22.67 ± 1.01 <sup>ab</sup>
T24 - SmF	4.43 ± 0.55 <sup>c</sup>	22.46 ± 0.29 <sup>a</sup>
T36 - SSF	1.58 ± 0.20 <sup>bc</sup>	23.31 ± 0.52 <sup>ab</sup>
T36 - SmF	4.40 ± 0.33 <sup>c</sup>	22.69 ± 1.18 <sup>a</sup>
T48 - SSF	1.90 ± 0.15 <sup>c</sup>	24.08 ± 1.19 <sup>b</sup>
T48 - SmF	4.40 ± 0.59 <sup>c</sup>	23.01 ± 0.25 <sup>a</sup>

Note: Means within columns with different letters are significantly different (Duncan test, p < 0.05)

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

Table 3 – CIEAB color values of raw and fermented yellow pea Flours<sup>†</sup>

Time*Fermentation method	CIELAB color values		
	L*	a*	b*
T0 – SSF	83.67 ± 0.24 <sup>a</sup>	3.15 ± 0.22 <sup>bc</sup>	29.67 ± 0.28 <sup>c</sup>
T0 - SmF	86.31 ± 0.59 <sup>ab</sup>	2.44 ± 0.17 <sup>b</sup>	22.86 ± 0.15 <sup>a</sup>
T6 - SSF	81.48 ± 0.22 <sup>a</sup>	3.76 ± 0.20 <sup>c</sup>	33.41 ± 0.47 <sup>d</sup>
T6 - SmF	82.82 ± 0.86 <sup>a</sup>	2.29 ± 0.28 <sup>b</sup>	32.12 ± 0.91 <sup>c</sup>
T12 - SSF	82.00 ± 1.39 <sup>a</sup>	4.00 ± 1.15 <sup>c</sup>	35.44 ± 0.33 <sup>d</sup>
T12 - SmF	86.00 ± 0.72 <sup>ab</sup>	2.06 ± 0.21 <sup>b</sup>	29.29 ± 0.32 <sup>b</sup>
T24 - SSF	83.79 ± 0.01 <sup>a</sup>	2.85 ± 0.20 <sup>bc</sup>	28.78 ± 0.42 <sup>c</sup>
T24 - SmF	85.03 ± 0.18 <sup>ab</sup>	1.19 ± 0.00 <sup>ab</sup>	29.28 ± 0.72 <sup>b</sup>
T36 - SSF	81.98 ± 0.13 <sup>a</sup>	1.23 ± 0.37 <sup>ab</sup>	24.44 ± 1.13 <sup>b</sup>
T36 - SmF	86.68 ± 1.74 <sup>b</sup>	0.61 ± 0.62 <sup>a</sup>	28.84 ± 0.11 <sup>b</sup>
T48 - SSF	82.37 ± 0.59 <sup>a</sup>	0.45 ± 0.11 <sup>a</sup>	20.44 ± 1.65 <sup>a</sup>
T48 - SmF	86.32 ± 0.04 <sup>ab</sup>	0.47 ± 0.50 <sup>a</sup>	28.56 ± 0.81 <sup>b</sup>

Note: Means within columns with different letters are significantly different (Duncan test, p < 0.05)

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

Table 4 - Water absorption index (WAI) and water solubility index (WSI) of yellow pea flours resulted from raw and fermented†

Time*Fermentation method	WAI (g / g)	WSI (g / 100g)
T0 – SSF	4.67 ± 0.08 <sup>c</sup>	6.88 ± 0.42 <sup>a</sup>
T0 - SmF	2.56 ± 0.05 <sup>a</sup>	19.62 ± 1.43 <sup>c</sup>
T6 - SSF	4.67 ± 0.08 <sup>c</sup>	7.12 ± 0.16 <sup>a</sup>
T6 - SmF	2.80 ± 0.05 <sup>b</sup>	15.29 ± 0.33 <sup>b</sup>
T12 - SSF	4.72 ± 0.07 <sup>c</sup>	7.31 ± 0.19 <sup>a</sup>
T12 - SmF	3.63 ± 0.08 <sup>c</sup>	11.76 ± 0.65 <sup>a</sup>
T24 - SSF	4.28 ± 0.16 <sup>b</sup>	11.79 ± 0.79 <sup>b</sup>
T24 - SmF	3.76 ± 0.12 <sup>cd</sup>	11.55 ± 0.96 <sup>a</sup>
T36 - SSF	4.01 ± 0.07 <sup>a</sup>	19.25 ± 1.45 <sup>c</sup>
T36 - SmF	3.76 ± 0.12 <sup>cd</sup>	12.04 ± 0.64 <sup>a</sup>
T48 - SSF	4.07 ± 0.03 <sup>a</sup>	23.45 ± 1.64 <sup>d</sup>
T48 - SmF	3.87 ± 0.14 <sup>d</sup>	11.66 ± 1.24 <sup>a</sup>

Note: Means within columns with different letters are significantly different (Duncan test, p < 0.05)

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

Table 5 - Water absorption capacity (WAC) and oil absorption capacity (OAC) of raw and fermented yellow pea Flours†

Time*Fermentation method	WAC (g water / g DM)	OAC (g oil / g DM)
T0 – SSF	2.79 ± 0.06 <sup>d</sup>	1.37 ± 0.26 <sup>a</sup>
T0 - SmF	1.27 ± 0.02 <sup>b</sup>	0.73 ± 0.02 <sup>a</sup>
T6 - SSF	2.69 ± 0.09 <sup>cd</sup>	1.20 ± 0.08 <sup>a</sup>
T6 - SmF	0.94 ± 0.06 <sup>a</sup>	1.76 ± 0.12 <sup>b</sup>
T12 - SSF	2.53 ± 0.08 <sup>bc</sup>	1.20 ± 0.09 <sup>a</sup>
T12 - SmF	1.33 ± 0.04 <sup>bc</sup>	1.65 ± 0.10 <sup>b</sup>
T24 - SSF	2.43 ± 0.08 <sup>b</sup>	1.22 ± 0.10 <sup>a</sup>
T24 - SmF	1.40 ± 0.03 <sup>cd</sup>	1.68 ± 0.08 <sup>b</sup>
T36 - SSF	2.23 ± 0.09 <sup>a</sup>	1.45 ± 0.05 <sup>ab</sup>
T36 - SmF	1.42 ± 0.01 <sup>d</sup>	1.73 ± 0.09 <sup>b</sup>
T48 - SSF	2.17 ± 0.06 <sup>a</sup>	1.72 ± 0.09 <sup>b</sup>
T48 - SmF	1.40 ± 0.02 <sup>cd</sup>	1.71 ± 0.14 <sup>b</sup>

Note: Means within columns with different letters are significantly different (Duncan test, p < 0.05)

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

Table 6 - Emulsion activity (EA) and emulsion stability (ES) of raw and fermented yellow pea Flours<sup>†</sup>

Time*Fermentation method	EA (%)	ES (%)
T0 – SSF	53.30 ± 0.92 <sup>c</sup>	69.85 ± 1.53 <sup>c</sup>
T0 - SmF	41.20 ± 1.16 <sup>c</sup>	49.70 ± 0.82 <sup>c</sup>
T6 - SSF	62.93 ± 1.41 <sup>d</sup>	72.45 ± 1.48 <sup>d</sup>
T6 - SmF	42.63 ± 0.89 <sup>c</sup>	55.95 ± 1.72 <sup>d</sup>
T12 - SSF	66.23 ± 0.55 <sup>e</sup>	76.00 ± 1.50 <sup>e</sup>
T12 - SmF	45.13 ± 1.01 <sup>d</sup>	60.90 ± 1.25 <sup>e</sup>
T24 - SSF	7.25 ± 1.30 <sup>b</sup>	7.75 ± 0.58 <sup>b</sup>
T24 - SmF	7.23 ± 0.13 <sup>b</sup>	8.20 ± 0.14 <sup>b</sup>
T36 - SSF	5.08 ± 0.13 <sup>a</sup>	6.63 ± 0.13 <sup>ab</sup>
T36 - SmF	6.18 ± 0.13 <sup>ab</sup>	7.20 ± 0.24 <sup>ab</sup>
T48 - SSF	4.40 ± 0.30 <sup>a</sup>	4.90 ± 0.24 <sup>a</sup>
T48 - SmF	4.95 ± 0.93 <sup>a</sup>	5.18 ± 0.78 <sup>a</sup>

Note: Means within columns with different letters are significantly different (Duncan test,  $p < 0.05$ )

<sup>†</sup> All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

Table 7 - Pasting properties resulted from raw and fermented yellow pea Flour<sup>†</sup>

Time*Fermentation method	Pasting temperature (°C)	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
T0 – Solid State	74.15 ± 2.33 <sup>b</sup>	1008.5 ± 7.78 <sup>d</sup>	979 ± 1.41 <sup>e</sup>	29.5 ± 6.36 <sup>a</sup>	1527.5 ± 3.54 <sup>f</sup>	548.5 ± 4.95 <sup>d</sup>
T0 - Submerged	76.8 ± 1.41 <sup>c</sup>	899.5 ± 6.36 <sup>e</sup>	878 ± 2.83 <sup>f</sup>	21.5 ± 3.54 <sup>a</sup>	1389.5 ± 7.78 <sup>f</sup>	511.5 ± 4.95 <sup>d</sup>
T6 - Solid State	72.95 ± 1.63 <sup>b</sup>	996.5 ± 0.70 <sup>d</sup>	962 ± 8.49 <sup>de</sup>	34.5 ± 7.78 <sup>a</sup>	1420.5 ± 0.71 <sup>e</sup>	458.5 ± 9.19 <sup>c</sup>
T6 - Submerged	76.2 ± 1.41 <sup>c</sup>	870 ± 7.07 <sup>d</sup>	839 ± 1.41 <sup>e</sup>	31 ± 5.66 <sup>ab</sup>	1341.5 ± 9.19 <sup>e</sup>	502.5 ± 7.78 <sup>d</sup>
T12 - Solid State	68.95 ± 5.02 <sup>ab</sup>	983.5 ± 4.95 <sup>d</sup>	940 ± 14.14 <sup>d</sup>	43.5 ± 9.19 <sup>a</sup>	1116.5 ± 4.95 <sup>d</sup>	226.5 ± 9.19 <sup>b</sup>
T12 - Submerged	75.55 ± 1.34 <sup>c</sup>	840 ± 0.00 <sup>c</sup>	796 ± 8.49 <sup>d</sup>	44 ± 8.49 <sup>b</sup>	1235 ± 7.07 <sup>d</sup>	439 ± 1.41 <sup>c</sup>

T24 - Solid State	61.95 ± 4.74 <sup>ab</sup>	926.5 ± 9.19 <sup>c</sup>	880 ± 0.00 <sup>c</sup>	46.5 ± 9.19 <sup>a</sup>	1100.5 ± 6.36 <sup>c</sup>	220.5 ± 6.36 <sup>ab</sup>
T24 - Submerged	64.8 ± 1.84 <sup>b</sup>	825.5 ± 9.19 <sup>c</sup>	758.5 ± 9.19 <sup>c</sup>	67 ± 0.00 <sup>c</sup>	1165.5 ± 3.54 <sup>c</sup>	407 ± 5.66 <sup>b</sup>
T36 - Solid State	56.75 ± 3.32 <sup>a</sup>	546.5 ± 9.19 <sup>b</sup>	495.5 ± 0.71 <sup>b</sup>	51 ± 9.90 <sup>a</sup>	706 ± 5.66 <sup>b</sup>	210.5 ± 6.36 <sup>ab</sup>
T36 - Submerged	53.85 ± 2.19 <sup>a</sup>	626.5 ± 0.71 <sup>b</sup>	551.5 ± 2.12 <sup>b</sup>	75 ± 2.83 <sup>c</sup>	782 ± 2.83 <sup>b</sup>	230.5 ± 0.71 <sup>a</sup>
T48 - Solid State	54.85 ± 3.32 <sup>a</sup>	281 ± 2.83 <sup>a</sup>	223 ± 4.24 <sup>a</sup>	58 ± 7.07 <sup>a</sup>	421 ± 1.41 <sup>a</sup>	198 ± 2.83 <sup>a</sup>
T48 - Submerged	52.3 ± 1.27 <sup>a</sup>	303 ± 7.07 <sup>a</sup>	225.5 ± 4.95 <sup>a</sup>	77.5 ± 2.12 <sup>c</sup>	452.5 ± 3.54 <sup>a</sup>	227 ± 1.41 <sup>a</sup>

*Note:* Means within columns with different letters are significantly different (Duncan test,  $p < 0.05$ )

† All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD

**Appendix B. Inventory data Tables**

*Table 1. Impact categories and the values according to the fermentation method.*

<b>Impact categories</b>	<b>Unit</b>	<b>SSF</b>	<b>SmF</b>
Acidification	mol H <sup>+</sup> -Eq	0.06651	0.06496
Climate change	kg CO <sub>2</sub> -Eq	6.56075	6.40920
Climate change: biogenic	kg CO <sub>2</sub> -Eq	0.10860	0.10594
Climate change: fossil	kg CO <sub>2</sub> -Eq	5.92603	5.79002
Climate change:			
Land use and land use change:	kg CO <sub>2</sub> -Eq	0.52612	0.51323
Ecotoxicity: freshwater	CTUe	723.28947	707.54604
Ecotoxicity: freshwater, inorganics	CTUe	53.53353	52.31706
Ecotoxicity: freshwater, metals	CTUe	664.77628	650.25973
Ecotoxicity: freshwater, organics	CTUe	4.97966	4.96925
Energy resources:			
Non-renewable	MJ, net calorific value	670.10575	653.78200
Eutrophication: freshwater	kg P-Eq	0.00480	0.00469
Eutrophication: marine	kg N-Eq	0.01598	0.01574
Eutrophication: terrestrial	mol N-Eq	0.11756	0.11498
Human toxicity: carcinogenic	CTUh	8.48788E-9	8.28387E-9
Human toxicity: carcinogenic, inorganics	CTUh	0.00000	0.00000
Human toxicity: carcinogenic, metals	CTUh	5.10959E-9	4.98673E-9
Human toxicity: carcinogenic, organics	CTUh	3.37829E-9	3.29714E-9
Human toxicity: non-carcinogenic	CTUh	5.47734E-7	5.34801E-7
Human toxicity: non-carcinogenic, inorganics	CTUh	3.39718E-8	3.32062E-8
Human toxicity: non-carcinogenic, metals	CTUh	4.87180E-7	4.75657E-7
Human toxicity: non-carcinogenic, organics	CTUh	2.70603E-8	2.64055E-8
Ionising radiation: human health	kBq U235-Eq	47.30682	46.14757
Land use	dimensionless	276.28304	271.99511
Material resources: metals/minerals	kg Sb-Eq	0.00057	0.00056
Ozone depletion	kg CFC-11-Eq	1.94677E-7	1.90144E-7
Particulate matter formation	disease incidence	5.69726E-7	5.56481E-7
Photochemical oxidant formation:			
Human health	kg NMVOC-Eq	0.02927	0.02861
Water use	m <sup>3</sup> world Eq deprived	30.89769	30.14582

*Table 2. Equipments for blending, cooking, and incubation of the yellow pea.*

<b>Equipment</b>	<b>V</b>	<b>Hz</b>	<b>A</b>	<b>kW</b>	<b>Time</b>	<b>Sources for specification</b>
Induction Cooker (GWM, Type CK 21)	230	50	-	3.5	35 min	<a href="https://www.kitchen-arena.com.my/coo-table-top-induction-cooker-2-heating-zone-ck-tt-2b350.html">https://www.kitchen-arena.com.my/coo-table-top-induction-cooker-2-heating-zone-ck-tt-2b350.html</a>
Blender (Laboratory Mill 120, Perten Instrument AB)	220-240	50	6	0.75	250g/1 min	<a href="https://www.koneteollisuus.fi/product-category/laboratory-mills/">https://www.koneteollisuus.fi/product-category/laboratory-mills/</a>
Incubation Oven (Termaks 4/5, Type TS 8136)	230	50	-	1.43	48 hrs.	<a href="https://nordiclabtech.com/termaks/">https://nordiclabtech.com/termaks/</a>