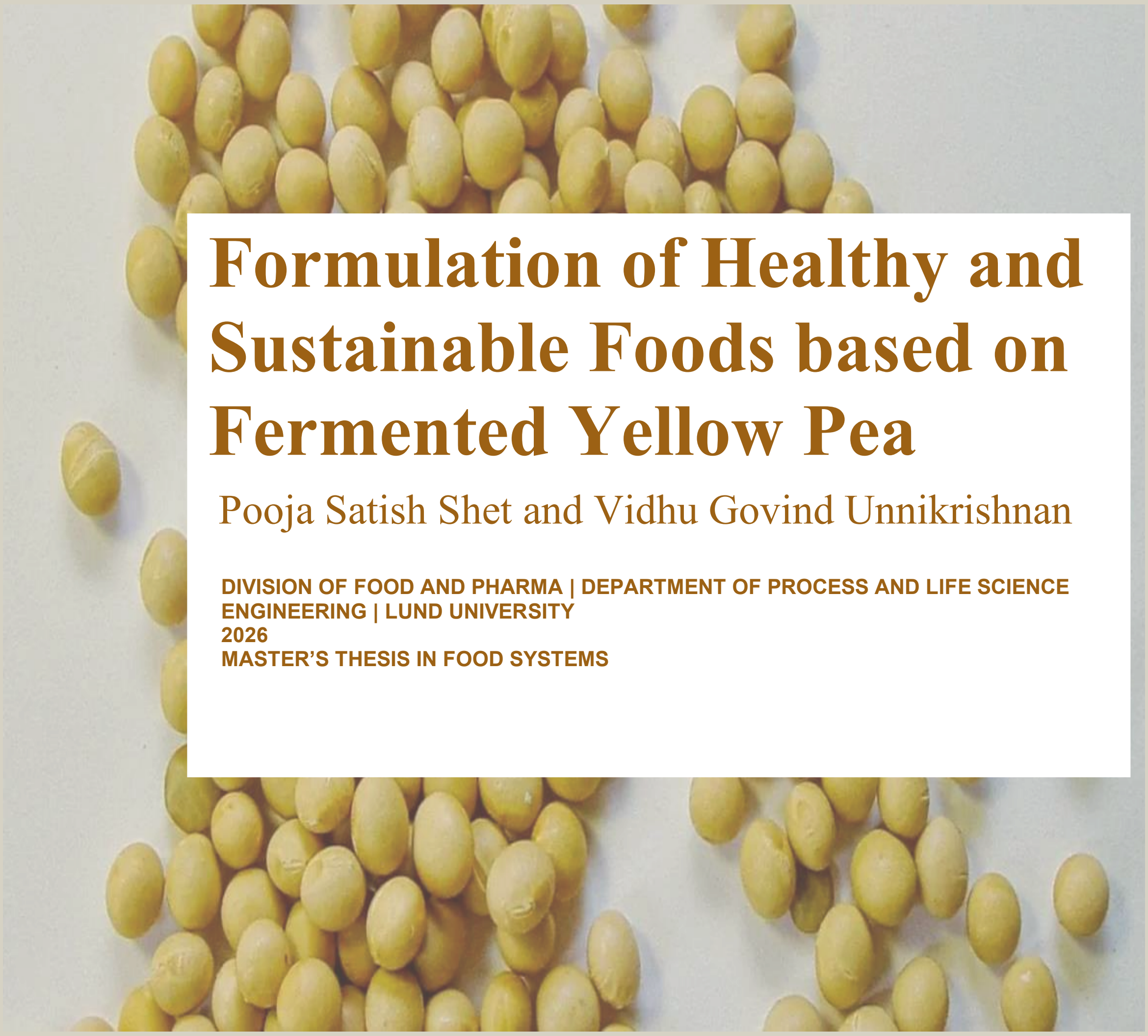

Formulation of Healthy and Sustainable Foods based on Fermented Yellow Pea

Pooja Satish Shet and Vidhu Govind Unnikrishnan

DIVISION OF FOOD AND PHARMA | DEPARTMENT OF PROCESS AND LIFE SCIENCE ENGINEERING | LUND UNIVERSITY

2026

MASTER'S THESIS IN FOOD SYSTEMS

Formulation of Healthy and Sustainable Foods based on Fermented Yellow Pea

Master's Thesis by

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Popular Summary

Switching to a plant-based diet isn't always an easy sell for consumers. Raw yellow pea flour often faces market resistance due to its distinct "beany" off-flavors and natural anti-nutritional factors. To overcome these hurdles, this Master's thesis investigates how microbial fermentation can improve the functional and sensory qualities of yellow pea flour. By utilizing *Lactiplantibacillus* bacteria for submerged fermentation (SmF) and *Rhizopus delemar* (formerly known as *Rhizopus oryzae*) fungi for Solid State Fermentation (SSF), we naturally improved the protein content and flour functional properties to transform it to food prototypes.

With a growing global population, finding climate resilient and sustainable protein sources is an urgent priority. Traditional animal farming places a heavy burden on our planet by depleting fresh water resources and driving up greenhouse gas emission. In contrast, nutrient dense pulses like the yellow pea offer a profoundly greener footprint, requiring far less water and fossil energy than livestock.

To bridge the gap between raw ingredients and final consumer options, the processed flours were tested in two food products namely, traditional Swedish crispbread and 50-50 beef to yellow pea hybrid burger patty. In the Crispbread trials, the two fermentation methods showed complementary physical and sensory strengths within the final products. The key strengths of SSF crispbread had a crunchy snap of traditional rye crispbread. Meanwhile, the key strength of the SmF crispbread formulation lie in its uniform acidification, which masked leguminous bitterness and eliminated beany notes, earning it the highest panel marks for flavor and overall sensory acceptance.

The development of the hybrid burger patty targeted reducing meat intake without eliminating the familiar texture and flavour of traditional beef products. The key strengths of the fungal SSF hybrid patty are its low cooking loss and volume retention during pan frying, as the enzymatic breakdown from fungal growth enhances water binding to minimize shrinkage and maintain structural integrity in the pan far better than unfermented control or SmF formulations.

Abstract

Yellow peas possess an excellent nutritional profile with a high protein content and fermentation, enhancing the flour's functional and physicochemical properties. This study evaluated the impact of Solid-State Fermentation (SSF) with *Rhizopus delemar* (formerly known as *Rhizopus oryzae*) and Submerged State (SmF) with *Lactiplantibacillus plantarum* on yellow pea flour, alongside its application in crispbread formulation and burger patty.

The functional properties of the flour, pH and acidity, moisture content, water adsorption capacity, oil adsorption capacity, protein content and pasting properties were measured. Alongside its application in crispbread formulation, analysis for its moisture, texture and protein and burger patty's cooking loss, reduction in diameter, moisture content, and texture analysis was performed. Prototype crispbreads were formulated by replacing 30% of the rye flour with the fermented yellow pea flours, and hybrid beef patties were made by 50% substitution with yellow pea for beef.

The formulation for crisp followed Laura (2024), and for patty, through Solina A.B.'s consultation. The sensory evaluation was done for both of the products. Finally, the Life Cycle Assessment was carried out, focused on measuring the carbon footprint of each product from Cradle to Gate. Fermentation effectively altered the flour's physicochemical profile: protein content increased from 19.00% in the unfermented control to 20.87% for the SSF sample and 23.50% for the SmF sample, while moisture content decreased, respectively. Functional properties also improved, with a slight increase in water absorption capacity despite a slight decrease in oil absorption capacity. Pasting property analysis revealed that SSF flour exhibited reductions across pasting temperature, peak, trough, and setback viscosities, whereas SmF flour demonstrated higher heat resistance with high pasting temperature but lower peak and trough, setback viscosities compared to the control. Instrumental texture analysis demonstrated that the SSF sample had the desirable crunch, also SmF is the most effective for masking leguminous bitterness.

Hybrid burger patties indicated that the unfermented control exhibited the highest diameter shrinkage, cooking loss was most pronounced in the SmF patty. Also, texture analysis and moisture content of cooked patties indicated no significant differences among the samples. However, qualitative feedback in sensory evaluations of patty across all three samples highlighted improvements, specifically the addition of spices and enhancements to the product's overall juiciness for higher overall acceptability. The LCA revealed that raw material acquisition was the primary driver of total greenhouse gas emissions, ranging from 10.45–28.88 kg CO_2e for the patties analysed (control, SmF, SSF, Commercial) and 0.66–2.50 kg CO_2e for the crispbread. This is due to the high upstream impacts of beef. During the processing stage, pan-frying and baking emerged as the dominant sources of energy consumption for the patties and crispbreads, respectively.

Keywords: Yellow pea, Fermentation, Plant-based Burger Patty, Crispbread, SSF, SmF.

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Thank you,
Pooja and Vidhu

Table of Contents

Contents

1. Introduction

The global population is projected to increase to 8.60 billion by 2050 and 11.20 billion by 2100. The growing population needs sustainable solutions and, moreover, a resilient food system to meet higher nutritional demands for mankind, such as protein. The traditional animal based foods are effective in for the protein, but it comes from a limitation of excessive land use, significant fresh water consumption, mainly greenhouse gases with cattle production alone responsible for approximately 14.5% of global anthropogenic emissions, mainly methane and nitrous oxide (Nirmal et al., 2024).Beef production requires, on average, 13 kg of grain inputs for 1 kg of meat caloric equivalency and chicken requires 2.3 kg of grain per 1 kg of meat (Sabaté and Soret 2014). The water footprint of animal production is significantly higher. In contrast, producing soy protein requires up to 26 times less water and 11 times less fossil energy than producing equivalent animal protein (Sabaté and Soret 2014). These efficiencies make plant proteins versatile and highly sustainable, with applications in numerous sectors (Nirmal et al., 2024).

Complementing these sustainability metrics, the *EAT-Lancet Commission* emphasizes how these dietary shifts also align with human health. A central feature of the planetary health diet (PHD) is the importance of nuts and legumes, including beans, pulses, and soy, as major protein sources, with small amounts of red meat and dairy foods. The far higher ratio of polyunsaturated fatty acids to saturated fatty acids in these plant foods compared with red meat and dairy products predicts lower LDL cholesterol; this prediction was confirmed in a meta-analysis of randomized trials of red meat intake. In long-term cohort studies with repeated assessments of diet, mortality was lowest when plant protein was compared with red meat, dairy foods, and poultry; associations with type 2 diabetes and coronary heart disease have been similar (Rockström et al., 2025).

Pulses are the edible seeds of leguminous plants, which are pod-bearing, such as lentils, yellow pea, beans, and chickpea, and have a low glycemic index and higher protein and fiber content. Epidemiological studies have proved that consumption of pulses reduces the risk of obesity and diabetes mellitus. Among them, yellow peas are considered very safe to consume, relatively inexpensive, and a higher-quality source of fiber and protein for consumption (Smith et al., 2012; Marinangeli et al., 2009) compared to foods made from whole yellow pea flour and whole wheat flour to identify foods with a lower glycemic index. Finally, the Life Cycle Assessment tool can be used to determine the environmental load of a product, process or activity throughout its life cycle (Roy et al., 2008). A research gap exists in the LCA regarding food products derived from fermented yellow peas. This study focuses on the LCA of plant-based patty and crisp bread product development.

1.1 Aim and Objectives

This master's thesis aims to take an integrated approach to evaluating the nutritional, functional, and sensory characteristics, along with the environmental impact of food products, such as crispbread and burger patty, developed from yellow pea flour fermented with *Lactiplantibacillus plantarum* and *Rhizopus delemar* (formerly known as *Rhizopus oryzae*). A comparison of the results will be conducted across the food product development process, from the fermented flours to the sensory evaluation of the final food prototypes. Determining both product qualities and environmental impact will aid in the future development of plant-based food products, ensuring functional qualities while utilising minimal natural resources in the production process.

- Develop the fermentation processes of yellow pea flour using *Lactiplantibacillus plantarum* through Submerged Fermentation (SmF) and *Rhizopus delemar* through Solid-State Fermentation (SSF). Then, dried fermented yellow pea flour is evaluated based on nutritional properties, such as protein content, moisture content and functional characteristics.

- Formulate food prototypes, specifically crispbread (30%) and hybrid beef-yellow pea burger patties(50%), by incorporating oven-dried fermented yellow pea flour as a main ingredient and perform functional analysis.
- Conduct a sensory analysis of the developed food products using a 7-point hedonic scale.

- Conduct a comparative Life Cycle Assessment (LCA) with a 'cradle to gate' boundary for the entire process.

2. Background

2.2. Yellow Pea

Legume pulse crops are considered a popular source of protein in tropical and subtropical countries. When considering Western nations like the USA, it's still in the growing stage. In recent years, soybeans have been the most consumed plant protein, and concerns have arisen because of allergenicity and genetic modification of the crop (Millar et al., 2019). Therefore, attention has been drawn to pulses; the most common variety of pea is the yellow cotyledon one, followed by green and others. Similar to cereals, starch is the primary component of pulse flour, which makes it a better substitute in the nutritionally enhanced breads and other bakery products (Millar et al., 2019). Also, pea seeds have high starch, protein, vitamins and are generally low in fats with varying composition depending upon the variety. Also, yellow peas contain residual antinutrient substances like protein inhibitors and natural pigments like tannins and anthocyanins, which can be reduced by fermentation (Moraes, 2024).

2.3. Fermentation

The art of fermentation was practiced by ancient societies and was dated to be as old as human civilization on earth (Marsh et al., 2014; Chojnacka et al., 2010). Over time, scientific studies led to the fermentation process to improve organoleptic properties and nutritional attributes of legume foods. Fermentation improves endogenous enzyme activity, resulting in hydrolytic reactions that lead to the partial removal of antinutritional components of pulses (Ma, Z et al., 2018). It also enhances protein digestibility by breaking larger protein particles to smaller units, such as polypeptides, peptides and oligopeptides (Liu et al., 2023). Research suggested that pea proteins are high quality plant-based proteins derived from yellow peas with 20% and 30% of protein with a digestibility of 88.38% (Karlsson et al., 2024; Al-Qaisi et al., 2024). The preparation of koji by the fungus *Aspergillus oryzae* led to the identification of various additional fermentation microbes and starter cultures, leading to development in the fermentation industry. Fermented food and its roles in enhancing the microbiome link with different aspects of human health and diseases was revealed by the human gut microbiome research (Mannaa et al., 2021).

2.4. Solid State Fermentation

Solid State fermentation is a bioprocess where in the presence of little or no free water microorganisms are grown on moist solid materials, making a convenient environment suitable for fungal cultivation and enzyme production. In this process, enzymes such as proteases, amylases and lipases are secreted to break down proteins, starches, lipids and degrade antinutritional components increasing the digestibility. Additionally, it improves the protein quality, antioxidant capacity and functional properties (Feng et al., 2023). Tempeh is a traditional SSF food in which legumes are inoculated with *Rhizopus delemar* after soaking and cooking until the mycelium packs the substrate into a cake. The process of pre-soaking and a 48h fermentation period are critical processing steps as they play an important role in the preparation of substrate for fungal colonisation and allow a sufficient period of time for biochemical changes. During this fermentation process, *Rhizopus delemar* secretes hydrolytic enzymes, thereby improving digestibility, flavor, and nutrient availability (Martin-Miguelez et al., 2024). The mycelium is fully developed with an ideal taste and texture, which leads to high protein solubility and in amino acids content (Mohammed et al., 2024).

2.5. Submerged Fermentation

Submerged fermentation represents a critical liquid- phase bioprocess extensively utilized for microbial cultivation and the subsequent biosynthesis of enzymes (Panda et al., 2008). *Lactobacillus plantarum*, a lactic acid bacteria which is commonly utilized under suitable culture conditions (Behera et al., 2018). The formation of microbial enzymes are dependent on substrate composition, temperature, pH and incubation period. Therefore, the present study used an autoclaved substrate to ensure sterility and a 48h fermentation period to allow microbial growth and biochemical transformation.

2.6. Formulation of Plant-Based Foods

From a technological standpoint, the current state of the art in the formulation of plant-based foods focus heavily on bioprocessing to meet a rapidly escalating global consumer demand. Additionally, the shift around health, environmental sustainability, and the plant-based sector transitioning away from artificial additives towards cleaner labels. Raw pulse flour is difficult to incorporate into food matrices due to inherent structural limitations (Johansson et al., 2026). To satisfy this demand without relying on chemical treatments, modern processing standardises either anaerobic SmF or SSF to initiate enzymatic hydrolysis, breaking down large proteins into smaller functional peptides while synthesizing polysaccharides and reducing pH through organic acidification. This enzymatic breakdown cleaves complex proteins into smaller, highly functional properties. This also results in improving water solubility, masking bitterness and eliminating anti-nutritional compounds. By optimising these scalable microbial pathways, food effectively bridges the gap between raw crops and functional plant proteins, establishing textural networks and sensory profiles required for competitive retail adoption (Deen et al., 2026).

2.7. Environmental Sustainability

From an environmental standpoint, optimising the utilisation of yellow pea flour in crispbread formulations offers a highly effective strategy for reducing the ecological footprint of staple baked goods. By successfully transforming this locally grown, resilient pulse into a functional baking ingredient through microbial fermentation, this approach establishes a viable pathway for producing nutrient-dense, low-impact consumer foods that align directly with climate mitigation goals (Harwatt et al., 2024). Tang et al. (2024), a study from the UK compared Plant-Based Meat Analogues (PBMAs) with traditional beef burgers, while examining 100g patties from cradle to fork, the researchers found that PBMAs significantly outperformed beef burgers in sustainability, with a 65% reduction in global warming potential and a 45% decrease in water use. Extrapolating this finding to the UK population predicted an annual saving of 3 million tons of CO_2e if consumers switch from beef to PBMA. Another study, which compared beef patty, PBMA and hybrid patty, the findings reveal that the beef burger is significantly more resource-intensive, requiring 21 times more water and emitting 13 times more greenhouse gases than the plant-based option, with the hybrid burger's impact falling in between the two (Dominguez-Lacueva et al., 2025).

2.8.LCA

There are commonly four stages in LCA. In the first, Goal and Scoping stage, statements are made which will define the purpose of the study, the product of the study, the system boundaries, units, and certain assumptions. System boundaries are represented by an input-output flow diagram of the system; all operations that take place in the life cycle of the product are included within the system boundary (Roy et al., 2008). The second stage is Life Cycle Inventory Analysis. This phase has data collection, so it's a bit more time-consuming, and it also depends on the data available. There are databases which can be used effectively with an LCA software. The data should include all inputs and outputs from the process. Input data is mostly energy; whether it's renewable or not, and raw materials which goes into the product and outputs are usually products and co-products, along with emissions to land, water and air during the process (Roy et al., 2008b). Life Cycle Impact Assessment is done as the third stage with an aim to understand and analyze the environmental impacts of the inventory, considering the goal and scope of the study(Roy et al., 2008b). Finally, in the Interpretation stage, conclusions are drawn which are used to support decisions or the study of products. In this stage, major environmental issues are identified for conclusion or improvements, which is consistent with the initial goal and scope of the study (Roy et al., 2008b).

3. Materials And Methods

3.1. Materials
Yellow peas cultivated in Sweden (Lantmännen AB), were purchased from the supermarket. Lactiplantibacillus plantarum 299v® (Probi Mage, Sweden) was bought from a pharmacy store in Lund, Sweden and Rhizopus *delemar* was purchased from Top Cultures (BV, Belgium), a starter culture containing 8 million spores per gram. Methylcellulose was received from the company Solina A.B. For the crispbread formulation, commercial rye flour, rapeseed oil, whole wheat flour, baking powder, and salt (NaCl), common to both, were purchased from the supermarket. All these ingredients were acquired from the supermarket ICA Malmborgs Tuna in Lund, Sweden. All materials were stored under recommended conditions prior to formulation. The control product consisted of unfermented, milled yellow peas.

3.2. Methods

3.2.1. Fermentation
In this study, two distinct types of fermentation processes were applied to yellow peas to assess how fermentation influences the final flour characteristics: a fungal Solid-State Fermentation (SSF) and a lactic acid bacterial Submerged Fermentation (SmF) under anaerobic conditions. The fermentation process was previously developed in the work conducted by Fernanado, 2025.

3.2.2. Solid-State Fermentation via *Rhizopus delemar*
For the SSF process, whole yellow peas were soaked in distilled water at double their weight for 18 hours. Following the soaking period, the peas were cooked in distilled water at a ratio of 1:2 for 35minutes at 100°C until achieving a soft texture, after which they were manually dehulled under hot tap water. The prepared substrates were then inoculated with a *Rhizopus* starter culture following standard portions for legume-based tempeh production (Chen et al., 2023). In accordance with the manufacturer's instructions, the starter culture was added to the batch at a ratio of 5g of *Rhizopus delemar* for every 600g of cooked peas. After inoculation, the peas were mixed thoroughly using a s

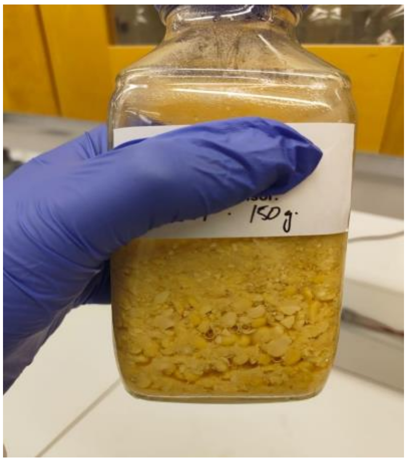
(a) (b)

Fig 1. Incubated yellow peas with *Rhizopus delemar* at 0hours (a) and 48hours (b) after fermentation at 30°C.

sterilised equipment in aluminium bowls, packed into perforated polythene zip-lock bags to allow controlled aeration, and incubated at 30°C for 48 hours to support fungal colonisation and tempeh development (Figure 1).

3.2.3. Submerged Fermentation via *Lactiplantibacillus plantarum*

The submerged fermentation of yellow peas with *Lactobacillus plantarum* was carried out under anaerobic conditions in accordance with procedures described by Cabuk et al., 2018. Approximately 150g of raw yellow pea seeds were milled and subsequently autoclaved prior to fermentation to eliminate native microbial contamination and standardize the processing equipment environment (Batbayar, 2022). The 150g of milled pea flour was then transferred into 500mL glass bottles, mixed with 300mL of distilled water(1:2), and inoculated with one capsule of *L. plantarum* (yielding and initial cell density of 7.35 Log_{10} CFU/g dry matter).The mixture was homogenized using glass stirring rods, and the bottles were incubated in an oven Termaks TS4057 Bergen maintained at 37°C for 48 hours with tightly secured plastic lids to maintain anaerobic conditions .



(a)

(b)

Fig 2. Incubated yellow peas with *L.plantarum* at 0hours (a) and 48hours (b) after fermentation at 37°C

3.3. Acid-Base Analysis

To monitor lactic acid content, the pH and titratable acidity of 2g samples were suspended in 20mL of distilled water. Later measured at T_0 (Time at 0^{th} hour)and T_{48} (Time at 48^{th} hour) a pH meter, with potentiometric titration analysis against 0.01N NaOH to an accurate endpoint of pH 8.3, serving as a safer, precise alternative to colorimetric indicators (Dunnick, 1996; Ahmed et al., 2024

3.4. Physicochemical analysis in the fermented yellow pea flours

3.4.1Moisture Content

The moisture content of the yellow pea flour, before and after fermentation, was calculated by following the AOAC technique (AOAC International, 2000). A 2g flour sample was taken and dried in an oven at 105°C (Termaks, TS4057, Bergen, Norway). Drying was carried out overnight, followed by a 3–6-hour interval, until a constant weight was obtained. Before the samples were weighed, it was cooled inside a desiccator, ensuring limited moisture absorption. All experiments were performed in duplicates. Moisture content can be determined by calculating the weight loss, which is the quantity of water present in the sample.

3.4.2. Oil Absorption Capacity

To measure the fat absorption, the method specified by Kaur and Singh (2004b) was used, where (0.5) g of the sample is taken and mixed with 6ml of rapeseed oil in pre-weighed centrifuge tubes. Then the content was stirred for 1 minute. Later, when the holding period of 30 minutes was done, it was centrifuged for 25 minutes at 3000g. Then the oil on top was removed, and the tube was inverted for 25 minutes to remove excess oil, before weighing it again. The Oil Holding Capacity was calculated on the basis of grams of oil bound per gram of the sample on a dry basis.

$$\mathbf{OAC(g/g) = (O_2\text{-}O_1)/O_0} \qquad (1)$$

O_o = weight of the sample O_1 = weight of the tube with sample. O_2 = weight of the tube, sample, and the oil absorbed

3.4.3. Water Absorption Capacity

Water Absorption Capacity was determined using a centrifugation-based procedure adapted from (Lawrence et al., 2025) study. In a pre-weighted centrifuge tube ,1g of sample was mixed with 10mL of distilled water and vigorously mixed sample by using a vortex for exactly 1 min. The suspension was centrifuged at 3000 × g for 25 min using an Eppendorf Centrifuge 5804R (Hamburg, Germany). After the decantation. The tube is reweighed and then WAC was calculated as grams of water bound per gram of sample on a dry weight basis. The WAC values were determined using the following equation;

$$\mathbf{WAC(g/g) = (W_2 - W_1) / W_0} \qquad (2)$$

. Wo = weight of the sample W1 = weight of the tube with sample W2 = weight of the tube, sample, and the water absorbed

3.4.4. Protein Analysis

The protein content of the unfermented, solid-state and submerged-state fermented samples from freeze-drier was blended into powder and analysed through dynamic flash combustion DUMAS method (Thermo Scientifics Flash TM, EA 1112 Series), following the procedure described by Krotz and Galotta, 2026. When performing the analysis, 25 mg of each sample was combusted into tin capsules and placed into a carousel. The system was calibrated using a series of control samples, including an empty tin capsule (Blank), ascorbic acid standards at 25mg and 50mg concentrations, and a protein reference sample to ensure analytical accuracy. To detect the protein content of the samples, the protein factor was set as 6.25.

3.4.5. Pasting Properties

The pasting properties of yellow pea flour, both fermented and unfermented, were measured by a rapid visco analyser (RVA, Perten 4,500, Stockholm, Sweden). A suspension of 3.5g of flour in 25g of distilled water was made and was tailored to the pre-set value of 14% moisture basis correction for the sample. The pre-set software programme 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.

3.5. Physicochemical Analysis on Formulated Products

3.5.1. Texture analysis

Texture analysis was conducted as a key quality assessment tool for both fermented crispbread and plant-based burger patties. The mechanical properties and structural fracturability of the formulated crispbread were evaluated (TA. XTplus-Texture Analyser, Stable Micro Systems, UK) with a vertical compression probe mounted over a hollow cylindrical platform supporting the crispbread sheet. In the TPA setting, a strain of 50% was set, and a diameter of 2 mm standard probe was used for measurement of hardness and brittle fracturability. While in plant-based patties, a strain of 20% was set and a 35 mm cylindrical probe was used for measurement of hardness, springiness and cohesiveness.

3.6. Sensory Evaluation

Sensory evaluation was carried out as part of the new product development stage using a 7-point hedonic scale to assess consumer acceptance of both the fermented crispbread and the plant based hybrid patty with 20 untrained panelists from LTH School of Engineering (Cardello and Jaeger, 2010).For the crispbread, a 7 -point hedonic scale was applied to compare the samples in which 30% of traditional recipe specifically containing rye flour and wheat flour was replaced with SmF flour and SSF flour and unfermented yellow pea flour. The samples were randomly coded and presented in an unbiased order to minimize panelist bias .Panellists evaluated appearance, color, aroma ,taste, crispness, aftertaste, and overall acceptability for each cracker sample .In the case of the plant-based hybrid patty, sensory assessment focused on color , aroma, texture, taste ,juiciness, off-flavour, and overall acceptability,since these attributes are key determinants of consumer liking in meat-substituted products. The samples in which 50% of the beef meat was replaced with SmF flour and SSF flour, and unfermented yellow pea flour. Scores were recorded on the hedonic scale ranging from dislike extremely (1) to like extremely (7) , allowing comparison of product liking and overall sensory quality across both product categories.

3.7.LCA Methodology

This LCA aims to evaluate the environmental impact of GHG emissions produced from Crispbread and Burger Patty. For this study, the LCA of CO_2 emissions was only considered, and the data obtained were from SAFAD and the LCA of fermented flours were taken from a previous study (Fernando, 2025).To evaluate the environmental sustainability of the plant-based and hybrid formulations, the life cycle inventory parameters were calculated using the Sustainability Assessment of Food and Diets (SAFAD) model developed by the Swedish University of Agricultural Sciences (SLU) (Röös, 2025). This web-based, open-source tool enables the integration of specific raw primary commodity data, regional transport matrices, processing energy requirements, and agricultural distribution footprints to systematically benchmark environmental indicators within the Swedish food system context. (Röös,2025). The carbon footprint of water has been obtained from Carbon Cloud (2026).

3.7.1. Goal and Scope

The functional unit was chosen as 1000 grams for all the samples. The process followed was a cradle-to-gate approach. In case of crispbread, the study evaluated three distinct formulations: a control crispbread (unfermented flour), a Solid State Fermented (SSF) yellow pea flour variant, and a Submerged Fermentation (SmF) yellow peas flour variant. Similarly, for patty, three hybrid samples with 50% substitution of control (raw yellow pea), SmF and SSF samples with a total of 3 products in comparison. This is done to understand the sustainability aspects of the substituted alternative when compared to the whole beef patty.

3.7.2. System Boundary

Crispbread

To ensure a transparent inventory, the product life cycle for the crispbread is strictly divided into two distinct manufacturing stages:

Stage 1: Raw Material Acquisition: This stage encompasses the cumulative environmental impacts of all the input ingredients prior to preparation. The carbon footprints and inclusion rates are integrated exactly from primary formulations. The emissions for the Swedish Solid-State (6.56 kg CO_2 e) and Submerged (6.41 kg kg CO_2 e) fermented yellow pea flours taken from the former master's thesis literature (Fernando, 2025) and tap water (0.01 kg CO2e) (Carbon Cloud, 2026).

Stage 2: Processing and Crispbread Preparation: This stage isolates the mechanical and thermal energy consumed during manufacturing. It includes the mixing of raw ingredients, rolling, and cutting. To focus purely on the primary energy drivers, a strict cutoff rule is applied to minor mechanical steps (mixing, rolling, cutting) as their energy contribution falls well below 1% (Mogensen et al. 2009) of the process total. Thermal processing is quantified based on baking inside a 14.3kW electric oven (Sveba Dhlen AB) for a total operational duration of 30 minutes (0.5 hours), combining pre-heating and active baking at 200 °C.

Plant-Based Hybrid Patty

The inventory of the manufacturing process was divided into 2 stages

Stage 1 is about raw material acquisition, which has all the cradle to gate impact of the input ingredients prior to patty preparation or manufacturing. The emissions for the Swedish beef cuts were sourced from the SLU SAFAD database, while the impacts of the solid-state (6.56 kg CO2e) and submerged (6.41 kg CO2e) fermented yellow pea flours taken from former master thesis literature (Fernando, 2025). Tap water (0.01 kg CO2e) (Carbon Cloud, 2026), along with salt, oil, and methylcellulose were also accounted for in this stage.

In stage 2, Processing and preparation of patties has been considered. The process involved hydrating the yellow pea flour (33%) with tap water (26.4%), and followed by the addition of salt (1%) and sunflower oil (5.6%). The hydrated plant matrix was combined with the beef cuts processed through a meat grinder (Bosch, 800W). Following the addition of methylcellulose (1%), then the mixture was hand pressed by Weber press of 100g. The patties were subsequently pan-fried (3.5 kW) until reaching a target internal temperature of 75°C. Energy consumption (kWh) was calculated based on equipment power ratings and operational duration.

3.7.3. Assumptions and Limitation

The carbon footprint of yellow pea, which is fermented and oven dried and milled into flours, has been directly taken from (Fernando, 2025). The mixing process of the patty was done by hand, so there was no emission considered in the mixing process. The Weber press for getting the patty shape was hand-pressed, which we have not considered in the study. Methylcellulose was added at 0°C but the electricity required for cold rooms was ignored in the study.

For crispbread, excluded mixing, dough rolling, and cutting stages, as these low power mechanical operations contribute less than 1% of the total manufacturing stage energy when compared (Mogensen et al. 2009). Energy from localized ventilation fans used after baking and before sealing was excluded from the processing footprint. This study also has commonly ignored other emissions on land water and soil, and only focused on carbon dioxide.

3.7.4. Statistical Analysis

Quantitative data for all evaluated components are reported on a dry basis as mean values ± standard deviation. To compare the variations within the different matrices including the fermented yellow pea flours, crispbreads, and hybrid burger patties separate one-way ANOVAs followed by Tukey's post hoc tests were applied to determine statistical significance.

4.Result and Discussion

4.1. pH & Acidity

Based on the fermentation and microbial strains utilised, the unfermented yellow pea control samples established a consistent native pH of 6.50 and a titratable acidity of 0.02%, typical for raw pulse, where organic acids remain unmetabolized (Amanipour et al., 2024). Following a 48 hour incubation, the anaerobic SmF by *L. plantarum* exhibited a chemical shift of pH falling significantly to 4.19 and the TA inversely rising to an average of 0.32% lactic acid equivalent, requiring a high titrant volume of 35.6mL of 0.01N NaOH. This drop is facilitated by the high-water activity and rapid nutrient dilution to liquid, enabling bacteria to efficiently metabolize free mono and oligosaccharides into organic acids (Amanipour et al., 2024).

Conversely, the SSF by R. *delemar* showed an intermediate acidification pathway, achieving a moderate average pH of 5.14 and a TA of 0.10% lactic acid equivalent, requiring a lower titrant volume of 11.0mL of NaOH. This metabolic variation occurs because the fungal setup generates alkaline species and proteolytic by products that act as a natural buffer partially neutralizing free hydronium ions and preventing the pH from dropping into the deeper acidic in bacterial setup (Ahumada Ochoa, 2025). From an application standpoint, the highly acidic environment produced by the Lactobacillus in SmF setup pH of 4.19 is excellent for applications requiring strong microbiological barriers, shelf-life extension in plant based dairy alternatives. On the other hand, the moderate acidity profile generated by the Rhizopus SSF setup pH of 5.14 provides a balanced profile for textural meat analogues or tempeh style foods, where extensive structural breakdown is desired without creating an overly sour taste profile (Ahumada Ochoa, 2025).

Table 1: pH and Acidity of raw and fermented yellow pea Flours.

Parameter	Control (T_0)	SSF (T_{48})	SmF (T_{48})
Acidity	19 ± 0.32^a	23.50 ± 0.59^c	20.87 ± 0.49^b
pH	6.50 ± 0.08^c	5.14 ± 0.07^b	4.19 ± 0.03^a

Note: All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. Means within the same row bearing different superscript letters (^{a,b,c}) are significantly different from one another according to One Way *ANOVA* followed by *Tukey's* HSD post- hoc test ($p<0.05$). SSF: Solid State Fermentation, SmF: Submerged Fermentation, Control; Unfermented yellow pea flour.

4.2. Analysis Of Fermented Flours

4.2.1. Moisture & Protein Content

The moisture content of yellow pea flour ranges from 1.75 to 12.25 %. For the control sample, it's 12.25, which is the raw yellow pea milled. And for SmF and SSF, after oven-drying and milling, it is 1.75%, 9.12%. Significant difference between the raw sample and the fermented is ($p < 0.001$). It can be observed as SSF samples have a higher value of moisture content than the SmF sample after fermentation. In patty development, a flour with higher moisture content can lead to a decrease in WAC and reduced binding efficacy. Flour is more effective when it's dry, as it can absorb water and bind the ingredients together (Ramatsetse et al., 2024). The higher moisture content in the flour means it will require a longer time in the baking process to get similar results when compared with a flour with lower moisture content (K. A. Millar et al., 2017). This also depends on how fiber and protein behave under higher temperature stress (K. A. Millar et al., 2017).

Regarding the protein analysis ,the results observed via the dumas method, crude protein content varied significantly across the treatments with the SmF flour demonstrating the highest concentration (23.50% ± 0.50%), followed by the SSF flour (20.87% ± 0.49%),both representing a notable increase relative to the unfermented control flour (19.00% ± 0.32%).The higher protein recovery in SmF compared to SSF may due to the highly homogenous liquid medium optimizing microbial proliferation and metabolic efficiency, resulting in a more rapid utilization of non-protein constituents and a higher relative proportion of microbial biomass.

The enhanced protein content strengthens the brittle matrix of the crispbread, preventing excessive crumbling and ensuring a desirable, snappy texture. In Burger Patty, the partially hydrolyzed, concentrated proteins function as excellent emulsifying and gelling agents, trapping both water and fat within a stable structural network.

Table 2: Moisture and Protein of raw and fermented yellow pea Flours.

Parameter	**Control (T_0)**	**SSF (T_{48})**	**SmF (T_{48})**
Moisture (%)	12.25 ± 0.28^c	9.12 ± 0.25^b	1.75 ± 0.86^a
Protein (%)	19 ± 0.32^a	20.87 ± 0.49^c	23.50 ± 0.5^b

Note: All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One-Way ANOVA.SSF: Solid State Fermented Flour, SmF; Submerged Fermentation flour and Control; Unfermented yellow pea flour.

4.2.2. Oil Absorption Capacity (OAC) and Water Absorption Capacity (WAC)

The OAC for control has been obtained as 2.81 g oil/g DB. The oven-dried and milled samples of SMF and SSF had values of 2.76 g oil/g DB and 2.39 g oil/g DB, respectively. While in the SmF sample there was no significant difference observed between the control, the SSF sample (T48) exhibited a statistically significant decrease in OAC ($p < 0.01$). During fermentation, the OAC usually increases, as observed in previous studies (Fernando, 2025). The decrease in OAC for SSF, this might be due to two reasons: one, during oven-drying, because the samples are exposed to high heat, as the internal amino acids exposed when yellow pea proteins unfold are more hydrophilic than hydrophobic.

Table 3: Oil Absorption Capacity (OAC) and Water Absorption Capacity (WAC) of raw and fermented yellow pea flour.

Parameter	Control (T_0)	SSF(T_{48})	SmF (T_{48})
OAC (g oil/g DB)	2.81 ± 0.08^b	2.39 ± 0.13^a	2.76 ± 0.19^b
WAC (g water / g DM)	2.17 ± 0.08^a	3.52 ± 0.09^c	2.85 ± 0.06^b

Note: Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One Way ANOVA. All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. SSF; Solid State Fermented flour, SmF; Submerged Fermentation flour and Control; unfermented yellow pea flour.

WAC of yellow pea flour increased after fermentation, with the SSF sample showing the highest value of 3.52 g water/g DM, followed by SmF at 2.85g water/g DM, whereas the control flour showed the lowest WAC at 2.17g water/g DM. This increase suggests that fermentation improved the water binding capacity of the flour matrix, likely due to partial hydrolysis of proteins and starch during microbial processing. A similar trend has been reported in fermented legume flours, where SSF often enhances hydration properties more than SmF because fungal growth and enzymatic activity can alter the surface structure and expose additional polar binding sites. The higher WAC observed in the SSF flour is particularly relevant for product development because improved water binding can contribute to better dough handling, softness and texture in bakery formulations. In the present study, the dough prepared with SSF flour was notably softer during rolling, which may be attributed to its greater capacity to retain water (Kaur, 2021). SSF has lower OHC than SMF due to RO, which releases extracellular enzymes like protease and lipase to digest its substrate. Over 48 hours, it hydrolyses the pea proteins, breaking them down into very small, highly soluble smaller peptides, which cannot entrap and hold on to oil when compared to LP. This can also explain why WAC is higher for SSF flour(Sukma et al., 2022; Gbogouri et al., 2004).

Since OAC is determined by the physical entrapment of oil within the protein matrix and hydrophobic interactions between nonpolar amino acid side chains and lipid hydrocarbon chains (Wang et al., 2020), which is critical for maintaining juiciness and preventing a dry mouthfeel in meat analogue formulations (Shevkani et al., 2014; Ribéreau et al., 2017). A severely reduced OAC is problematic, as it indicates the flour has suffered extreme protein degradation and lacks the basic structural integrity to form a cohesive matrix (Ribéreau et al., 2017; Shevkani et al., 2015).

4.2.3. Pasting Properties

The properties measured for raw and fermented yellow pea are pasting temperature, peak viscosity, trough viscosity, breakdown, final viscosity, and setback (Balet et al., 2019).The pasting temperatures ranged from 71.15°C to 90.75°C as seen in Table 4, there was a statistically significant difference in pasting temperature between all three sample groups ($p < 0.001$), after fermentation the values are 90.75 °C and 71.15 °C, after Submerged fermentation (SmF) sample significantly increased the pasting temperature compared to the raw control ($p < 0.001$), while solid-state fermentation (SSF) sample significantly decreased it ($p < 0.001$).

Table 4: Pasting Properties of raw and fermented yellow pea Flours.

Parameter	**Control (T_0)**	**SSF (T_{48})**	**SMF (T_{48})**
Pasting temperature (°C)	75.2 ± 0.4[b]	71.15 ± 0.4[a]	90.75 ± 0.28[c]
Peak viscosity (cP)	773 ± 20[c]	52 ± 3.8[a]	587 ± 2.44[b]
Trough viscosity (cP)	704 ± 11[c]	50.5 ± 3.31[a]	545.5 ± 0.5[b]
Breakdown (cP)	68.4 ± 9.2[c]	1.5 ± 0.57[a]	41.5 ± 0.57[b]
Final viscosity (cP)	999.25 ± 15.9[c]	61.5 ± 3.31[a]	743.66 ± 1.5[b]
Setback (cP)	294.75 ± 5[c]	11 ± 0.00[a]	196.25 ± 3.09[b]

Note: Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One Way ANOVA. All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD.SSF: Solid State Fermented flour, SmF: Submerged Fermentation flour and Control: unfermented yellow pea flour.

The peak viscosity was in a range of 52 to 773 cP. Both fermentation methods significantly reduced the peak viscosity of the samples compared to the raw control ($p < 0.001$). The highest peak viscosity is observed for raw sample; 773 cP**,** as fermentation with consecutive milling and drying has reduced the values to 587 cP for SmF and 50 cP SSF. Trough viscosity measures the ability of a paste to withstand breakdown at the time of cooling (Iwe et al., 2016). The values ranged from 704 to 50 cP, with the highest for the control and the lowest for the SSF sample. SSF with milling and drying lowers the hot paste stability, with ($p<0.001$) for before and after fermentation. Breakdown value is measured by the difference between peak viscosity and trough viscosity (Balet et al., 2019). The breakdown values ranged from 1.5 to 68.4 cP, highest for Control and lowest for SSF.

The lower breakdown, which is lower peak and trough viscosity in SSF is due to amylase activity, which breaks complex starch to simple sugars during fermentation as mentioned by similar study on soybean starch extract fermented with *R. oligosporus* (Olanipekun et al., 2009*)*. Final viscosity can be observed as the paste is cooled back to 50 °C and held for a period; the viscosity increases again to reach the final viscosity (Balet et al., 2019). The final viscosity ranged from 61.5 to 999.25 cP, highest value for control and lowest for SSF. The setback viscosity arises when a rise of viscosity occurs owing to amylose getting rearranged, the same being leached out through the swollen starch during cooling and is utilised as a measure of gelling ability (Devi et al., 2018). There was a highly significant reduction in setback viscosity across all treatments ($p < 0.001$). The setback viscosity ranged from 11 to 294.75 cP, highest for Control and lowest for SSF.

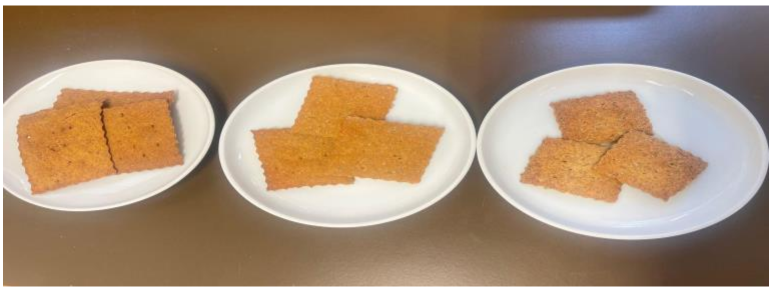
SSF appears more suitable for maintaining tenderness in a burger patty because it gelatinises at a lower pasting temperature and exhibits low setback. As established in literature, a low setback indicates a reduced tendency for starch to retrograde and firm excessively during cooling (Balet et al., 2019; Jiang et al., 2021; Olanipekun et al., 2009). By contrast, the control flour's higher final viscosity and setback indicate a stronger tendency toward retrogradation and firmer gel formation (Balet et al., 2019; Jiang et al., 2021), which could contribute to a denser and less juicy patty. SmF's higher pasting temperature suggests slower starch activation (Balet et al., 2019), which may reduce its usefulness as an early-acting binder during cooking.

For crisp bread applications, flour functionality must support dough formation while limiting excessive retrogradation that can lead to a hard, brittle texture after cooling (Balet et al., 2019). The control flour, with its higher peak viscosity and setback, would be expected to form a stronger matrix but also retrograde more strongly, increasing hardness during storage (Balet et al., 2019; Jiang et al., 2021). SmF appears to offer qualities in the middle of the other two samples as it's retaining sufficient pasting behaviour to support structure while showing lower setback than the control, a profile associated with reduced staling and a more shatterable texture (Balet et al., 2019). SSF, although resistant to retrogradation, may be too weak in structure for standalone crisp bread formulations due to the severe starch degradation typical of prolonged fermentation (Olanipekun et al., 2009).

4.3. Formulation of Products

4.3.1. Swedish Crispbread Formulation

Crispbread is a deeply rooted cultural staple in Nordic food tradition. Based on the research paper (Kovaleva, Galchenko and Kolchanov, 2021), the baking protocol for thin, crispy products, the samples were baked at a temperature of 200°C for 15 minutes. Following preliminary formulation trials with varying substitution levels, a 30 % substitution of whole wheat flour with fermented yellow pea flour was determined to be optimal for sensory assessment. The dry ingredients including the composite flours, salt and baking powder were sifted. Subsequently, wet ingredients were blended and then dough was kneaded for 5 minutes until smooth. The dough was rolled to a thickness of 1.5 mm, shaped into 3cm squares and baked for 12 minutes at 200°C (Sveba Dahlen, Sweden). After baking, the crackers were cooled and packed in a vacuum-sealed polyethene bag.



Control sample **SMF sample** **SSF sample**

Figure 3. Crispbread

***Table 5*: The formulation followed for developing the crisp bread from yellow pea**

Ingredient	**Control (g)**	**SSF flour (g)**	**SmF (g)**
Rye flour	18	18	16
Yellow pea flour	30	30	30
Whole Wheat Flour	13	13	12
Water	32	30	33
Rapeseed oil	5	7	7
Baking powder	1	1	1
Salt	1	1	1

Note: The flour has been varied based on the control sample; the raw pea milled sample, the Solid State and Submerged State Fermented oven dried sample. The samples were made in replicates (n=4) for 3 consecutive days.

4.3.2. Plant-Based Hybrid Patty

The substitution of yellow pea was limited to 50 per cent, as higher substitution rates may lead to higher off-flavours, colour changes and texture issues (Stanišić et al., 2025). Along with beef and yellow pea, water, oil, and salt were added. For binding, methylcellulose was added to it, which was added based on the recommendation provided by Solina A.B. The samples consisted of unfermented yellow pea, which was milled, oven-dried SmF milled, and oven-dried SSF milled. The aim of the formulation was to achieve a 50:50 ratio of beef to yellow pea with consistency across the three products and avoid breakdown while frying in the pan at higher temperatures, the present formulation was derived from personal trials and consultation with Solina A.B. The sequence of process followed for the preparation was then the addition of salt and yellow pea flour together after weighing and mixing it well, and then addition of water to hydrate the mixture (0.8 ratio yellow pea to water, except for raw pea flour; water content was adjusted to moisture level to prevent stickiness), later added oil to have a proper binding, and then beef cuts was added.

Figure 4. Hybrid Patty

The mixture was then added to a meat grinder by Bosch and extruded into a minced beef yellow pea mixture, then, at 0°C, methylcellulose was added, mixed well and rested for some time. Later, the mixture was pressed into the shape of a patty with a Weber press 100g (1/4th) and placed inside the heating pan and fried until the thermal centre reached 75 ° C.

***Table 6*: The formulation followed for developing the hybrid patty.

Ingredient	Control (g)	SSF (g)	SmF (g)
Minced Beef	33	33	33
Yellow pea flour	33	33	33
Water	26.4	26.4	26.4
Sunflower oil	5.6	5.6	5.6
Methylcellulose	1	1	1
Salt	1	1	1
Total	100	100	100

Note: Yellow pea flour has been varied based on -the control unfermented yellow pea, Solid State and Submerged State Fermented oven dried milled samples. The samples were made in replicates (n=4) .

4.4. Physicochemical Characteristics of Food Prototypes

4.4.1. Moisture & Protein Content of Crispbread

Overall, all three crispbread samples exhibited exceptionally low residual moisture levels, ranging from 0.5% to 0.8%. The control made with unfermented flour showed a moisture content of 0.6%. The application of SSF resulted in the lowest absolute water retention at 0.5%. Conversely, SmF yielded the highest average moisture content among the treatments at 0.8%. However, One -way ANOVA revealed that the choice of processing pathway did not introduce any statistically significant variations in the moisture content of final baked products.

The minimal moisture percentages across all treatments are highly characteristic of crispbread products, which rely on low water metrics to ensure mechanical stability, long term shelf stability, and crispness. As observed by Chandra-Hioe et al. (2016), this starch breakdown fundamentally alters the gelatinization and drying profile of legume flours during thermal processing. This intensive water loss accelerates both the Maillard reaction and crust rigidification, resulting in a drier, significantly crisper product with the dark, desirable toasted attributes noted by sensory panelists.

The quantitative determination of crude protein via the Dumas method revealed highly significant variations ($p<0.01$) across the crispbread formulations. Both fermentation pathways substantially elevated the protein profile of the yellow pea-rye composite matrices, with solid state fermentation (SSF) and submerged fermentation (SmF) reaching mean values of 12.44% and 12.29%, respectively, compared to the unfermented control sample, 8.33 %. Subsequent Tukey’s HSD post hoc analysis ($p < 0.05$)confirmed that while both fermentation significantly outpaced the control layout, the difference between SSF and SmF was statistically negligible.

Table 7: Moisture and Protein content of Crispbread formulated from unfermented and fermented yellow pea flour

Parameter	Control	SSF	SmF
Moisture	0.6 ± 0.3^{a}	0.5 ± 0.40^{a}	0.8 ± 0.3^{a}
Protein content (%)	8.33 ± 0.10^{a}	12.44 ± 0.14^{b}	12.99 ± 0.10^{b}

Note: All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. Means within the same row bearing different superscript letters (a,b) are significantly different from one another according to One Way *ANOVA* followed by *Tukey's* HSD post- hoc test (p<0.05). SSF: Solid State Fermentation, SmF: Submerged Fermentation Control: Unfermented yellow pea flour.

4.4.2. Texture Analysis of Crispbread

The instrumental texture profile analysis revealed a highly significant difference (p< 0.001) for both parameters across all treatments. Submerged fermentation (SmF) structurally reinforced the matrix to the highest degree, yielding a maximum hardness of 2521.43 g alongside a consolidated, minimal peak count of 2.00. Conversely, solid state fermentation (SSF) maximized fracturability, producing a significantly elevated crispness profile 0f 17.00 peaks while maintaining a moderate hardness threshold 1115.35g that was double the baseline Control 626.30 g. Subsequent Tukey's HSD post-hoc analysis (p< 0.05) confirmed that all three formulations differed significantly from one another across both metrics, proving that the specific fermentation pathway directly dictates the final mechanical behaviour of the product.

The peak force, representing the absolute hardness of the matrix, varied substantially across the formulations. The control sample exhibited the lowest resistance to deformation, with peak force ranging between approximately 400g and 850g. The key strengths, inclusive of a paragraph for the SmF formulations, lie in its exceptionally robust and rigid macromolecular matrix, which yielded the highest mechanical hardness, with peak forces climbing aggressively to a range of 1750g to 3250g. Conversely, the SSF formulation demonstrated intermediate texturing effects, displaying a peak force range between 900g and 1350g.

The control showed baseline jaggedness indicative of light crispness, the SSF crispbread centred on its optimised balance of structural integrity and high fracturability. On the other hand, the SMF curve was defined by a steep, unified peak, pointing toward a brittle but remarkably solid structure that snaps cleanly rather than fracturing progressively. The observed differences in hardness and fracturability can be fundamentally due to changes in the food matrix composition, specifically regarding starch gelatinization, gluten network formation, or fiber water interactions. The drastic increase in hardness observed in the SMF samples aligns with the findings of Tunick et al. (2013). The multi-pecked, highly fracturable behaviour of the SSF sample mirrors the classic crispness profiles described by Roudaut et al. (2002). Additionally, in this study, the instrumental profile strongly correlates with the sensory data, where the panel of evaluators rated the SSF sample highest in overall crispness.

Table 8: Textural parameters Hardness and Fracturability of crispbread formulations

Formulation	Hardness (g)	Fracturability	Linear Distance
Control	626.3 ± 175.71[a]	8 ± 1.83[b]	Low
SSF	1115.35 ± 170.82[b]	17 ± 2.58[c]	Medium -High
SmF	2521.43 ± 602.82[c]	2 ± 0.82[a]	High

Note: All values are mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One Way *ANOVA* followed by *Tukey's* HSD post- hoc test ($p<0.05$).SSF: Solid State Fermentation, SmF: Submerged Fermentation, Control: Unfermented yellow pea flour. Linear Distance, (g*s), g=grams, s=seconds.

4.4.3. Functional Properties of Hybrid Patty

Reduction in Diameter: When patties are cooled to room temperature after cooking. A vernier calliper (Luna) was used to measure the diameter of raw and cooked patties.

Shrinkage in diameter is highest for the control unfermented yellow pea flour-beef hybrid patty with 8.5, followed by hybrid SmF with 7.7 and lowest for SSF, aligning well with the explanation of cooking loss. There is a significant difference ($p<0.001$) between the control and SSF. This can be explained by the proteolysis and structural modification of the protein matrix, which occurred during the solid-state fermentation. These modifications can enhance water-binding affinity, as shown in the moisture content results of the flour, explaining the observed reduction in cooking loss and diameter shrinkage compared to the control patty (Elhalis et al., 2023).

Cooking loss: The cooking loss of patties was measured in percentage which is the weight difference between cooked and uncooked patties.

Cooking loss has been observed as highest for hybrid SmF patty as 12.15, followed by the unfermented control and SSF, with no significant difference between the last two, but overall significance is ($p<0.001$). This says fermentation has impacted the cooking loss, and higher loss can be related to the WAC of the samples. Then followed by control and SSF, which shows that the patty made of SSF can help hold the patty in shape without losing its water content.

Moisture Content for patty: The moisture analysis for the patty was followed similarly to the method described in the sections before, except a 10g sample was used from the patty samples for measurement and dried for 24 hours, cooled for 10 minutes, and weighed till the weight became constant.

The final moisture content of the cooked patties was evaluated to determine the residual hydration after thermal processing. Statistical analysis revealed no significant difference in final moisture content among the Control, SmF, and SSF samples ($p > 0.05$). This indicates that despite the different structural modifications induced by fermentation, the cooking process yielded products with consistent final moisture retention. While the fermentation method significantly influenced the rate of weight reduction during cooking, as evidenced by the cooking loss data, the final moisture state of the patties remained uniform across all experimental groups.

Table 9: Cooking loss and Moisture percentage of Hybrid Patty.

Parameter	Control	SSF	SmF
Shrinkage of diameter (%)	8.5 ± 0.7^{b}	3.82 ± 0.8^{a}	7.7 ± 1.1^{b}
Cooking loss (%)	6.45 ± 0.65^{a}	5.68 ± 1.16^{a}	12.15 ± 1.61^{b}
Moisture Percentage	45.2 ± 1.23^{a}	46.57 ± 2.2^{a}	48.9 ± 1.4^{a}

Note: Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One Way ANOVA.† All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. SSF: Solid State Fermented hybrid beef patty, SmF: Submerged Fermentation hybrid beef patty and Control: unfermented yellow pea flour hybrid beef patty.

4.4.4. Texture Analysis of Hybrid Patty

The texture analysis is measured using TPA, there are three parameters considered, hardness, springiness and cohesiveness. All the samples used for texture analysis were cooked and were stored in the freezer for one day, then thawed before analysis.

Hardness can be defined as the force (g) applied during the first compression (Herawati & Kamsiati, 2021). There is no significant difference between the control samples and the fermented samples p>0.05, which implies the ingredient doesn't determine the hardness and during storage in the freezer and thawing the sample for the experiment, the sample might have gone through some kind of structural damage.(Mulot et al., 2019). Cohesiveness is the strength of the internal bond in the sample (Herawati & Kamsiati, 2021). It's the ratio of the area under the second peak and the area under the first peak (Kassama et al., 2003).

Table 10: Texture analysis of patty.

Parameter	Hardness (g)	Springiness (%)	Cohesiveness
SSF	2524.72 ± 164	98.13 ± 1.51	3233.97± 190
SMF	3539.54 ± 757	100.00 ± 2.04	3985.55 ± 808
Control	2824.94 ± 994	98.66 ± 0.50	2896.64 ± 1034

Note: Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One Way ANOVA.† All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD.SSF: Solid State Fermented hybrid beef patty, SmF: Submerged Fermentation hybrid beef patty and Control: unfermented yellow pea flour hybrid beef patty.

Overall, there is no significant difference between the control and fermented samples (p>0.05) throughout the samples for cohesiveness. The springiness value is the product's ability to return to its initial position after the first compression until the second compression starts (Herawati & Kamsiati, 2021b). There wasn't any significant difference between control and fermented product (p<0.05). It can be concluded that the substitution with yellow pea flour if fermented or not doesn't influence the springiness.

4.5. Sensory Evaluation

4.5.1. Crispbread

The sensory evaluation of the yellow pea- rye composite crispbread samples indicates that fermentation significantly enhances palatability. Among the formulations for appearance, colour, aroma, or crispness, demonstrating that fermentation does not introduce olfactory defects. However, critical flavour vectors exhibited significant variations ($p < 0.05$). SmF achieved the highest score in taste (5.90), after taste (5.70), and overall acceptability (6.40), outperforming the unfermented control to a statistically significant degree ($p<0.05$) according to Tukey's HSD post hoc analysis.

The distinct sensory profiles of the samples highlight the divergent biochemical pathways of SmF and SSF. The superior taste and aftertaste scores of CB03 (SmF) are attributed to the uniform acidification facilitated by Lactic Acid Bacteria (LAB) in a submerged environment. According to Cabuk et al. (2018), LAB fermentation effectively mitigates leguminous "beany" off-flavors by degrading volatile compounds like hexanal and modifying the protein surface to mask bitterness. This uniform enzymatic diffusion leads to the clean and balanced sensory profile that garnered the highest overall acceptability.

Regarding Sample CB02, the standard performance in crispness and strong colour results from the intensified Maillard reaction characteristics of fungal SSF. During the solid-state growth, fungi produce a dense array of extracellular proteases and amylases that hydrolyse complex starches and proteins into free amino acids and reduce sugars. These molecules serve as critical precursors for the Maillard reaction during the baking phase. As noted by Chandra-Hioe et al. (2016), the high concentration of these precursors in fungal treated legume flours leads to advanced browning and the formation of brittle, aerated crust. This biochemical shift explains why CB02 achieved a robust, darker colour and superior crispness compared to the control and submerged samples.

Table 11: Mean Sensory Scores of Yellow Pea-Rye Composite Samples.

Parameter	**Appearance**	**Colour**	**Aroma**	**Taste**	**Crispness**	**After taste**	**Overall acceptability**
Control CB01	5.55 ± 1.05^{a}	5.85 ± 1.04^{a}	5.50 ± 1.19^{a}	5.15 ± 0.93^{a}	5.85 ± 1.04^{a}	5.00 ± 1.12^{a}	5.75 ± 0.72^{a}
SSF CB02	5.50 ± 1.00^{a}	5.50 ± 1.10^{a}	5.45 ± 0.89^{a}	5.55 ± 0.60^{ab}	6.20 ± 0.89^{a}	5.40 ± 0.88^{ab}	6.15 ± 0.75^{ab}
SmF CB03	5.65 ± 1.09^{a}	5.95 ± 1.10^{a}	5.50 ± 1.05^{a}	5.90 ± 0.91^{b}	6.05 ± 0.94^{a}	5.70 ± 0.92^{b}	6.40 ± 0.50^{b}

Note: All values are mean ± SD of duplicate samples from duplicate trials (N=20) ± SD. Superscript letter (^{a,b,ab}) within the same column indicates statistically significant differences ($p<0.05$) based on One-Way *ANOVA* followed by *Tukey's* HSD post- hoc test ($p<0.05$). SSF: Solid State Fermentation, SmF: Submerged Fermentation. Control: unfermented yellow pea substitute.

Ultimately, the study demonstrates that SmF is the most effective tool for flavor refinement and masking bitterness, whereas fungal SSF is superior for textural optimization and enhancing the visual “toasted” character through Maillard browning.

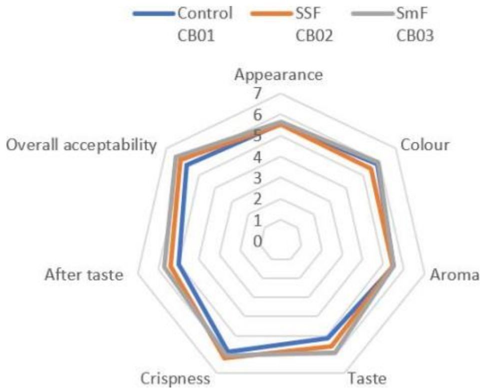


Figure 5. Sensory analysis results: 7-point hedonic scale characteristics.

The parameters considered for analysis are texture, color, aroma, taste, juiciness, off-flavor and overall acceptability (Petrat-Melin & Dam, 2023b; Asha et al., 2025). The questionnaire was prepared similar to crisp bread sensory evaluation as highlighted in the methodology section. The three samples were served in plates with three divisions, and marked with product codes. The participants had to fill the form after tasting the product, and water was given before tasting each sample.

4.5.2. Hybrid Patty

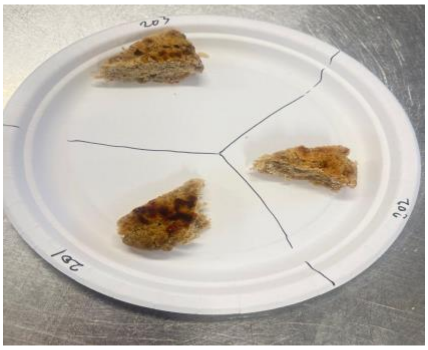


Fig 6. Example of product presentation during sensory test.

The statistical analysis revealed no significant differences ($p>0.05$), among the Control (203), SmF (202), and SSF (201) samples across all evaluated parameters. Mean scores for all formulations generally clustered around the 2.95 to 4.9 range, corresponding to the “Dislike much,” “Neither like nor dislike,” and “Like a little” categories on the 7-point scale.

Table 12: Sensory scores for Hybrid Patties

Parameter	Texture	Color	Aroma	Taste	Juiciness	Off-flavour	Overall acceptability
(201) SSF	4.09 ±0.7^{a}	4.71 ±1.3^{a}	4.57 ± 1.2^{a}	4.6 ±1.3^{a}	3.5 ±1.6^{a}	3.9 ± 1.1^{a}	4.38 ±1.2^{a}
(202) SmF	3.8 ± 1.3^{a}	4.9 ± 1.3^{a}	4.19 ± 1.4^{a}	4.19 ± 1.7^{a}	2.95 ± 1.4^{a}	3.6 ±1.3^{a}	3.95 ± 1.6^{a}
(203)Contr-ol	3.95 ± 1.4^{a}	4.14 ±1.27^{a}	4.14 ±1.27^{a}	4.28 ± 1.7^{a}	3.52 ± 1.27^{a}	3.61 ±1.6^{a}	3.95 ±1.6^{a}

Note: Means within the same row bearing different superscript letters (a,b,c) are significantly different from one another according to One-Way ANOVA. All values are the mean ± SD of duplicate samples from duplicate trials (n=4) ± SD. SSF: Solid State Fermented hybrid beef patty, SmF: Submerged Fermentation hybrid beef patty and Control: unfermented yellow pea flour hybrid beef patty.

While these baseline scores lean toward neutrality, this can be highly expected and can be considered as a positive outcome for unseasoned hybrid meat prototypes. Plant based ingredients usually have beanie off-flavor, bitterness, or astringency that typically drive hedonic scores down into the lower "Dislike" ranges (1–3). Moreover, microbial, fungal fermentation can develop off-flavors in products. Considering the fact that the score of SSF patty had overall acceptability of 4.38, and taste scoring 4.6 demonstrated higher interest from participants. It was also observed that juiciness for all three patties was ver; low, this is due to the limitation to add water, which will disturb the overall structural stability of the patty.

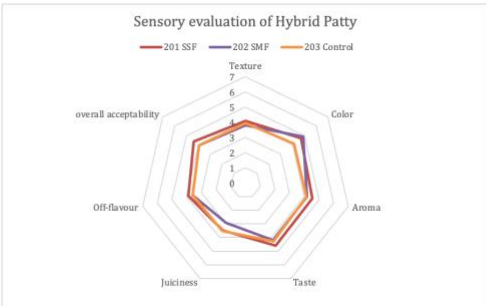


Figure 7. Sensory analysis of patty with 7-point hedonic scale characteristics.

Panellist feedback can be analysed for the product improvement, almost the majority agreed with adding more salt, chilli to make it spicy and oil. Some suggested adding rosmarin to enhance the aroma. Almost all participants commented on the strong raw flavour of the unfermented pea, which was less likely acceptable, and the product could have been juicier. When asked to pick a product separately among the three, 77.3% of the participants selected 201 SSF patties, followed by 202 SmF patties and Control, reaffirming its higher potential to be a commercial product. This finding also aligns well with the functional properties exhibited by SSF flour.

4.6.1 Life Cycle Inventory

Table 13: For Stage 1: The carbon footprint obtained for the ingredients for crisp bread from SLU SAFAD, along with the carbon footprint adjusted according to the recipe for 1000g are listed in the table below.

Parameter	**Carbon Footprint/kg (kg CO2 e)**	**Ingredient emission for 1kg of Control (kg CO2 e)**	**Ingredient emission for 1kg of SSF (kg CO2 e)**	**Ingredient emission for 1kg of SmF (kg CO2 e)**
Rye Flour	0.853	0.153	0.153	0.136
Whole Wheat Flour	0.855	0.111	0.111	0.102
Unfermented Pea Flour	0.337	0.101	0	0
SSF Yellow Pea Flour	6.56	0	1.968	0
SmF Yellow Pea Flour	6.41	0	0	1.923
Water (added to dough)	0.01	0.003	0.003	0.003
Rapeseed Oil	2.2851	0.114	0.159	0.159
Baking Powder	0.7421	0.007	0.007	0.007
Salt	0.315	0.003	0.003	0.003
Total Carbon Foodprint		0.660	2.573	2.502

Table 14: For Stage 1: The carbon footprint obtained for the ingredients for burger patty from SLU SAFAD, along with the carbon footprint adjusted according to the recipe for 1000g are listed in the table below.

Parameter	**Carbon Footprint (kg CO2 e)**	**CONTROL (kg CO2 e)**	**SSF patty (kg CO2 e)**	**SmF patty (kg CO2 e)**
Beef	30.76	10.150	10.150	10.150
Unfermented Pea Flour	0.3378,	0.111	0	0

Yellow Pea SmF	6.41	0	0	2.115
Yellow Pea SSF	6.56	0	2.164	0
Water (tap)	0.01	0.002	0.002	0.002
Sunflower oil	2.64	0.147	0.147	0.147
Salt	0.315	0.003	0.003	0.003
Methylcellulose	3.69	0.036	0.036	0.036
Total		10.452	12.506	12.456

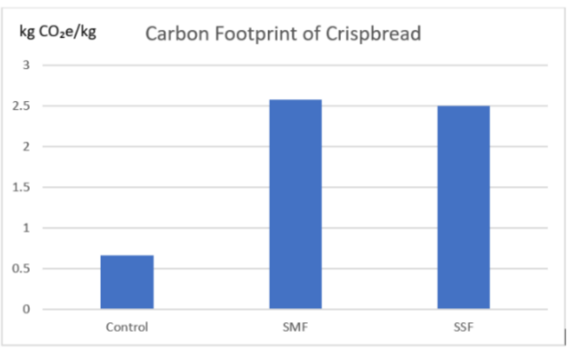
4.6.2. Process

Table 15: The carbon footprint obtained for the process followed in stage 2 for crisp bread.

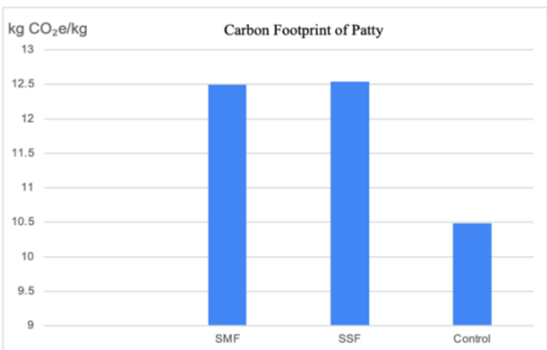
Parameter	**Power (kW)**	**Duration (h)**	**Emission (*Grid Factor) (g CO_2e)**	**(Kg CO_2 e/kg) of patty**
Baking	14.3	30	50.05	0.050
Total				0.050

Table 16: The carbon footprint obtained for the process followed in stage 2 for patty.

Parameter	**Power (kW)**	**Duration (h)**	**Emission (*Grid Factor) (g CO_2e)**	**(Kg CO_2 e/kg) of patty**
Grinding	0.8	1.333	7.466	0.007
Pan Frying	3.5	1.11	27.195	0.027
Total				0.034



(a)



(b)

Figure 8: Depicts Carbon Footprint (kg CO_2e) a) Crisp bread and b) Hybrid burger patty.

Note: SSF: Solid State Fermented flour, SmF: Submerged Fermented flour and Control: unfermented yellow pea flour, which is substituted in each of the product formulations across the beef patty and rye crispbread.

In cradle-to-gate, LCA for 1kg FU of the crispbread and hybrid yellow pea-beef patty, there are two different stages, firstly in preparation, which is considered from raw material acquisition and secondly, processing. In the LCA for crispbread, it was depicted that the total greenhouse gas emissions of 0.66 kg CO_2e for the unfermented Control, 2.57 kg CO_2e for the Solid-State Fermented, and 2.50kg CO_2e for the Submerged Fermented. The data demonstrates that the commercial rye crispbread generates a cradle to gate carbon footprint of around 1.02kgCO2e/kg (Carbon Cloud, 2023). While industrial facilities optimize emissions through massive economies of scale, continuous flow production and efficient heat recovery systems. Consequently, scaling these fermentation processes to a commercial level would significantly lower their carbon intensity below current market baselines.

On the other hand, the total greenhouse gases of 28.88 kg CO_2e for the commercial full beef burger patty, 10.45 kg CO_2e for the unfermented raw yellow pea flour-beef control patty, 12.50 kg CO_2e for the SSF-beef patty, and 12.45 kg CO_2e for the SmF-beef patty were observed. Commercial beef has a massive carbon footprint, which is due to enteric fermentation, like methane from cows. By replacing 50% of the beef with yellow pea, there is an effective way to cut the beef-related emissions into half. Legumes require minimal fertiliser and have incredibly low carbon footprints, leading to a drastic overall reduction in emissions from hybrid patties (SAFAD). For crispbread, analysis identified stage 1 as the dominant driver of the overall footprint, heavily dictated by the high upstream emission factors of the fermented SSF and SmF yellow pea flours (Fernando, 2025) . Additionally, in the case of patty the dominant driver of the overall footprint, heavily dictated by the upstream emissions of the beef cuts and the preliminary fermentation of the pea flour. Within stage 2, the manufacturing phases thermal processing steps (baking) emerged as the primary energy due to its high-power draw (14.3 kW) and in the case of patty, in the processes, the thermal processing step pan-frying emerged as the manufacturing energy hotspot due to its high-power draw (3.5 kW), which significantly outweighed the mechanical energy required for extrusion and grinding.

5. Conclusion

This research demonstrates that yellow pea flours can successfully serve as highly functional, sustainable ingredients to improve the nutritional profile and physical attributes of modern foods, showing that through fermentation configurations, the physical, chemical and organoleptic properties of yellow peas can be systematically altered to meet the structural and sensory objectives of diverse food matrices. In fulfilment of the first objectives, Submerged Fermentation (SmF) enhanced the flavour refinement and organic acidification, driving the liquid medium's pH down to 4.19 over 48 hours to eliminate bitter leguminous notes and mask "beany" off-flavours while recording highest crude protein concentration at 23.5% alongside high heat resistance and a delayed starch activation profile during pasting analysis.

Conversely, Solid State Fermentation (SSF) excelled at structural texturisation and moisture retention by inducing an intermediate acidification to pH 5.14, driven by alkaline buffering byproducts. The SSF flour's hydration properties yield a water holding capacity of 3.52g water/g dry matter and lower peak, trough, and setback viscosities to reduce the tendency of the starch to retrograde. Integrating the control, SmF and SSF fermented flours into a traditional rye crispbread format at a 30% substitution level achieved major nutritional strengths. Additionally, results showed that crude protein levels of over 12.4% & 12.9% for SSF & SmF respectively, were recorded compared to an unfermented control baseline of 8.33%. For the 50:50 beef-to-pea hybrid burger prototypes, which were developed in consultation with Solina A.B, the functional modifications induced during fungal SSF increased exposure of non-polar amino acid residues, enhanced structural water affinity, resulting in the lowest dimensional shrinkage of 3.82% and the lowest cooking loss of 5.68 % during pan-frying without structural breakdown, and while SmF patties experienced a higher cooking loss of 12.15%. The reduced pasting parameters observed in the SSF flour make it highly suitable for hybrid patty formulation, whereas the profile of the SmF flour proves ideal for crispbread applications.

Sensory testing on a 7-point hedonic scale drastically improved the palatability of pulse-based foods, showing that for the crispbread, the SmF formulation earned the highest panel marks for taste (5.90), aftertaste (5.70), and overall acceptability (6.40) due to its superior flavour masking, while the SSF formulation was rated highest in crispness and visual appeal driven by extensive Maillard browning, whereas the unseasoned hybrid burger prototypes successfully mitigated the intense bean-like notes. There was not much of a significant difference among the three samples. The panelist suggested adding spices to improve flavour and overall juiciness for better acceptability. However, the SSF patties had higher acceptance comparatively, indicating higher scopes of improvement in the future.

Ultimately, the comparative cradle-to-gate Life Cycle Assessment (LCA) verified the environmental viability of these formulations, identifying raw material acquisition as the primary global warming potential driver across both product categories and proving that the patty formulations reduced the massive upstream carbon footprint of a commercial all-beef patty by slashing more than half of emissions from 28.88 $kgCO_2e/kg$ down to a range of 10.45 to 12.50 $kgCO_2e/kg$ per kilogram of product, which, when combined with the minimal total emissions of the crispbread trials (2.57 $kgCO_2e/kg$), confirms that using targeted bacterial and fungal fermentations successfully bridges the gap between raw crops and competitive consumer options to create functional, nutrient-dense, and eco-friendly products that preserve natural resources.

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