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Enhancing Pea Flour Characteristics Through Radio Frequency Heating: Effects on Composition, Flavour, Anti-Nutrients and Functionality

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ABSTRACT

Peas and other legume crops are sources of sustainable plant-based proteins. Dry pea seeds can be processed into whole seed flour or dehulled milled flour and used as food ingredients. However, the widespread consumption of plant-based ingredients from peas is limited by their poor sensory properties, limitations in their functionality, nutritional properties and presence of anti-nutritional factors. Many of these negative attributes could potentially be overcome by using novel processing technologies. In this study, radio frequency (RF) pre-treatment of whole dry yellow peas was carried out at two temperatures (85°C and 115°C) and their effects on the macromolecular composition, amino acid profiles, anti-nutritional content, techno-functional properties and flavour chemistry were analysed. Significant reduction in the amounts of 12 out of 18 amino acids were observed in RF-treated samples compared to untreated control, while the other six amino acids showed a slight increase after RF treatment. Upon RF treatment, total phenolic content increased, while saponin content, trypsin inhibitor activity and lipoxygenase enzyme activity showed a reduction. Volatolomics studies revealed the generation of a number of new pyrazine class of volatile flavour compounds *in situ*, only in the RF treatment at the higher temperature of 115°C. Techno-functional analysis of dehulled milled flours prepared from RF-treated seeds showed an increase in water holding capacity, while the oil absorption capacity, foaming and emulsion properties decreased compared to untreated control. The significant findings of this investigation include the identification of a set of pyrazine compounds that enhance the flavour profile of RF-treated seeds and an improvement in water holding capacity of the flour that can increase its utilization in meat-analogue applications.

Praiya Asavajaru and Pabitra Chandra Das made equal contributions.

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1 | Introduction

Pea (*Pisum sativum* L.) is a legume crop utilized globally as both a fresh vegetable and dry seeds. They are gaining popularity as an alternative protein source to animal proteins due to their high availability, affordability and sustainability of production (Meinlschmidt et al. 2016). Pea seeds contain protein, starch, minerals, vitamins and fibres, while having low fat content (Boye, Zare, and Pletch 2010; Tulbek et al. 2017). Consuming peas might help lower the risk of certain diseases, such as cardiovascular diseases, diabetes and cancer (Kumar et al. 2022; Patel et al. 2017). Dry pea seeds are commercially processed into flour or air-classified protein-rich concentrate, as well as wet-extracted protein isolate (Schutyser et al. 2015). Pea flours serve as ingredients in various food applications to enhance protein content and add interesting techno-functional properties. Incorporating pea flours into cereal and bakery products can improve their nutritional and functional qualities. Additionally, pea flours can be utilized in pasta, noodles, snacks, beverages and meat analogue applications (Banaszek et al. 2019; Lu et al. 2020; Philipp et al. 2018 2017; Tulbek et al. 2017). Pea proteins are a good source of some essential amino acids such as leucine, lysine, phenylalanine and threonine, but are limited in tryptophan and sulphur-containing amino acids like methionine and cysteine (Boye, Zare, and Pletch 2010). Further, pea protein ingredients contain certain anti-nutritional factors, such as saponins, phenolics, protease inhibitors, lectins, oligosaccharides and phytic acid, which can affect protein digestibility, bioavailability or have some adverse physiological effects (Deshpande 2000; Sandberg 2002). Peas also have suboptimal sensory properties with off-flavour attributes such as beany and grassy flavour notes that can reduce consumer acceptance (Bott and Chambers 2006; Trindler, Annika Kopf-Bolanz, and Denkel 2022; Vara-Ubol, Chambers, and Chambers 2004). Lastly, there is also a need to modify the functional properties of pea ingredients to better suit the food applications that they are used for.

Although plant breeding can address some of these challenges, it takes a long time for the development and release of new crop varieties. Post-harvest processing technologies have the potential to modify and improve the quality attributes of pea seeds and ingredients derived from them. Thermal treatment technologies have been explored for the processing of various pulses and they have been effective in modifying the flavour, anti-nutritional content and functionality of treated seeds and ingredients (Bühler et al. 2020; Ma et al. 2011; Trindler, Annika Kopf-Bolanz, and Denkel 2022; Zhou et al. 2024). Another important application of thermal treatments is in the production of ingredients such as flours and protein-enriched concentrates using dry processing steps. Dry processing involves dehulling and milling, followed by air classification and is considered more sustainable than wet extraction of proteins, as it requires less energy, chemical and water inputs and does not require wastewater recycling. However, unlike wet extraction that incorporates a thermal treatment step such as pasteurization, dry processing leads to sub-optimal deactivation of components such as microbes, protease inhibitors, amylase inhibitors, lipoxygenase (LOX), lectins etc., leading to potential food safety and quality issues (Schutyser et al. 2015). Among thermal treatments, RF is becoming increasingly popular as a cost-effective method for treating pulses (Hassan, Pawelzik, and Von Hoersten 2016; Jiao et al. 2018; Piyasena et al. 2003; Zhang et al. 2022). RF utilizes non-ionizing electromagnetic waves in the range of 10–300 MHz to achieve uniform dielectric heating, induced by molecular friction within food matrices (Piyasena et al. 2003; Wang et al. 2006). RF has greater penetration depth compared to infrared or microwave, due to their long wavelength (Das et al. 2025), making them suitable for the processing of liquids, semi-solids, powders and even intact seeds. Further, they cause uniform volumetric heating of materials and are effective and energy efficient, as they do not rely on heat transfer through conduction or convection like traditional dry and steam heating techniques (Costa and Marra 2024). RF treatment has been effectively applied in food processing for drying, defrosting, pasteurization, pest control and inactivation of enzymes and microbes (Costa and Marra 2024; Li et al. 2017). While RF heating offers several advantages, studies have also highlighted its potential negative effects on grain quality due to denaturation of proteins and carbohydrates (Ling, Cheng, and Wang 2020). For example, RF heating above 100°C reduced the yield, purity, solubility and foaming properties of rice bran protein isolate, but improved the emulsifying and absorption capacities (Ling, Ouyang, and Wang 2019a). Similarly, in wheat germ, RF heating enhanced colour and water/oil absorption but decreased protein solubility and foaming stability (Ling, Ouyang, and Wang 2019b). Despite some negative effects on protein functionality, RF heating technology has gained significant scientific and industrial interest due to its superior product safety and suitability for scaling up applications. The benefits, particularly in microbial inactivation and preservation of key quality attributes, outweigh the limitations, making it an increasingly promising technology for the food industry.

RF heating remains relatively unexplored for processing of dry peas, despite its potential as an innovative processing technology. A notable exception is a study demonstrating that RF treatment effectively blanched fresh high moisture containing peas by inactivating LOX and peroxidase enzymes, thereby extending shelf-life of frozen green peas (Zhang et al. 2022, 2021). To our knowledge, no studies have systematically investigated RF heating as a pre-treatment for dry pea seeds prior to their conversion into flours and functional ingredients, nor have the impacts on critical quality parameters such as amino acid composition, flavour characteristics and functional properties been investigated. Thus, the objectives of the present study were to explore the effectiveness of RF based pre-treatment of dry whole yellow pea seeds and generating a detailed understanding of the effects of RF treatment on the composition, anti-nutrients, flavour chemistry and techno-functional properties of the pea flour ingredient.

2 | Materials and Methods

2.1 | Materials

Whole yellow pea (*P. sativum* var. CDC Lewochko) seeds cultivated in the year 2021 were obtained from Ardell Seeds Ltd. (Vanscoy, SK, Canada). The seeds were estimated to have a moisture content of 13.98%. Although RF treatment could be performed on dehulled yellow pea seeds, whole yellow pea seeds

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were used for this study. Dehulling of seeds tend to reduce their moisture content by approximately 2%–4% (Saldanha do Carmo et al. 2020). In preliminary trials using dehulled split yellow peas that had a moisture content of 9.94%, it was noted that the heat generated in the material was low, due to inefficient RF coupling. Hence, the whole seeds with a higher moisture content of 13.98% were used in this study.

2.2 | Methods

2.2.1 | RF Processing

The RF treatments were done using the pilot-scale GP-15 RF equipment (QMTI, Prince Albert, SK, Canada). The RF equipment works at a frequency of 27.12 MHz and is capable of a maximum power output of 15 kW. This is a continuous-flow processing equipment in which 40 kg of seeds were loaded on a hopper bin that sits on top of a conveyor belt (Figure 1). The feeder gap at the bottom of the bin was adjusted to create a seed bed height of 30 mm on the moving conveyor belt. In the first set of process conditions named as 'RF-treated – Low', the conveyor belt speed was set at 0.2 m/min and the electrode gap was set at 86 mm, resulting in an average temperature of 85°C on the seeds. In the second set of process conditions named as 'RF-treated – High', the conveyor belt speed was set at 0.08 m/min and the electrode gap was set at 85 mm, resulting in an average temperature of 115°C on the seeds. The residence times for RF treatment were calculated to be 7.6 min for the RF-treated – Low and 19 min for the RF-treated – High, based on the electrode length and belt speed. The temperature of the seeds was measured by IR sensors at the outfeed of the equipment and also using temperature-sensitive indicator strips embedded within the seed bed on the conveyor belt. The RF-treated seeds were cooled down to room temperature (RT, 20°C–23°C).

2.2.2 | Dehulling and Milling

Fifteen kilograms each of three yellow pea samples (untreated control, RF-treated – Low and RF-treated – High) were dehulled using a laboratory sheller from SK Engineering. To generate flour, the dehulled yellow pea seeds were passed through a hammer mill (Jacobson Model 120-B, Minneapolis, MN) with a screen size of 3.175 mm, followed by fine milling using a Buhler Laboratory Mill (model# MLU-202). Mass balances of cotyledon and flour production were recorded.

2.2.3 | Proximate and Amino Acid Composition Analysis of pea Flours

Seed samples collected from control and RF treatments were ground into flour using a bench top lab mill (Wonder Mill). Near-infrared (NIR) spectrum-based analysis of whole yellow pea flours was performed using the DA7250 analyser (Perten Instruments AB, Hagersten, Sweden). Analysis of flour sample was performed using a round sample cup (43.47 mL).

2.2.4 | Functional Property Measurements

Water Holding Capacity (WHC). The WHC of flour samples were determined by following the method of AACCI (2017). Briefly, 1 ± 0.02 g (wb) of flour sample was placed in a culture test tube. Deionized water was added and mixed gently with a glass rod to form a saturated (thoroughly wetted) mixture. Then, a folded filter cloth was placed at the end of the test tube. The test tube was then placed in a syringe barrel by positioning it upside down, and the syringe barrel was placed into a 50 mL centrifuge tube. After that, the mixture was centrifuged (Eppendorf AG 22331 Hamburg, Germany) at RT at a speed of 300 ×*g* for 10 min to remove the extra water from the saturated mixture. The WHC capacity was calculated and expressed as the amount of water (g) absorbed by 1 g of flour.

Oil Absorption Capacity (OAC). For OAC, a previously developed method was followed (Wang et al. 2020). Briefly, 0.5 ± 0.05 g (wb) of flour sample was mixed with 1.5 ± 0.1 g of canola oil in a culture test tube using a glass rod. Then, the mixture was vortexed for 5 s at 10 min intervals, for a total period of 20 min. A folded filter cloth was placed at the opening of the test tube and it was gently inserted into a syringe barrel by positing it upside down. The syringe barrel was placed in a 50 mL centrifuge tube and was centrifuged (Eppendorf AG 22331 Hamburg, Germany) at 600 ×*g* for 25 min. The OAC was calculated using the formula below:

$$\textit{Oil absorption capacity}, \text{OAC}\ (g/g, db) = \frac{\textit{Amount of oil asbsorbed by the sample}\ (g)}{\textit{Sample used for estimation}\ (g)} \times \frac{100}{100 - M.C.}$$

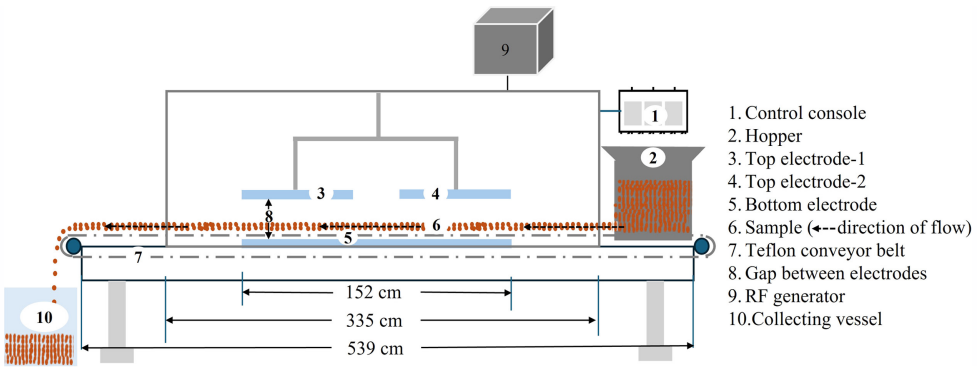

FIGURE 1 | Schematic layout of pilot-scale capacity RF processing equipment.

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where, M. C. = moisture content (%) of the sample.

Foaming Capacity (FC) and Foaming Stability (FS). The FC and FS of flour samples were measured according to a previously developed method (Guldiken et al. 2022; Sathe, Deshpande, and Salunkhe 1982). A 2% (w/w) flour solution was prepared by dissolving 2 g (dry weight) of flour into 98 g of distilled water. The solution was then stirred overnight at 400 rpm for 5 min to create a homogenous mixture. Then, the solution was further homogenized using a polytron (POLYTRON PT 10-35 GT-D, Switzerland) with tooth diameter of 20 mm for 1 min at 13,500 rpm to create foam. The volume of generated foam was measured at 0 min and 60 min. The FC and FS were calculated using the equations below:

$$\text{Foaming capacity FC}(\%) = \frac{\text{Volume of foam at 0 min}}{\text{Volume of flour solution (100 ml)}} \times 100$$

$$\text{Foaming stability FS}(\%) = \frac{\text{Volume of foam at 60 min}}{\text{Volume of foam at 0 min}} \times 100$$

Emulsion Capacity (EC). The EC of flour samples was determined using a modification of a previous method (Stone et al. 2015). The method follows protein inversion point theory. One hundred millilitres of 1% (w/v) flour solution was prepared by dissolving 1 g of flour into 99 mL of distilled water using magnetic stirrer at refrigerated condition (4°C) overnight. Then, a series of emulsions were prepared by adding various amounts (by weight) of canola oil to 3.00 mL of 1% flour solution in 50 mL centrifuge tubes. Each sample was homogenized (POLYTRON PT 10-35 GT-D, Switzerland) with a tooth diameter of 12 mm for 1 min at speed 17,500 positioning saw tooth probe at the oil–water interface. Immediately following homogenization, sample conductivity was measured using a conductivity meter (fisher brand accumet XL200, Fisher Scientific, Singapore). The EC was determined to be the point in which an oil-in-water emulsion changed to a water-in-oil emulsion (inversion point) as indicated by a large drop in conductivity. The EC was defined as the mL (average) of oil emulsified before and after the inversion point per g of flour.

Emulsion Stability (ES). The ES of flour samples was determined according to a previous method, with slight modifications (Stone et al. 2015). A 0.5% (w/w) flour solution was prepared by dissolving 0.5 g into 99.5 g of 10 mM sodium phosphate buffer (pH 7.00). The solution was stirred in a refrigerator at 4°C overnight. A 50:50 oil-in-water emulsion was prepared in a 50 mL centrifuge tube and homogenized for 2 min at 8000 rpm. The developed emulsion was immediately transferred to a graduated cylinder and allowed to separate for 1 h. Volume of aqueous layer (bottom) and turbid layer (upper) were recorded and ES was calculated using following formula:

$$\text{Emulsion stability, ES}(\%) = \frac{V_0 - V_1}{V_0} \times 100$$

where V_0 and V_1 are the volume of aqueous phase before homogenization and after separation time (1 h), respectively.

Least Gelation Concentration (LGC). The LGC determines the minimum amount of flour needed to form a gel and was determined by slightly modifying previously established methods (O'Kane et al. 2005; Sun and Arntfield 2010). A number of 5 mL flour solutions were prepared at concentration of 15%–25% (w/w) of flour with required amount of distilled water, followed by mixing for 1 min to make a homogenous mixture. Then the solutions were heated in a hot water bath at 95°C for 30 min, followed by cooling for 5 min in cold water and placed at RT for 1 h. The samples were then allowed to stand in a refrigerator (4°C) overnight. On the following day, the sample containing tubes were placed inverted on a tissue paper and the liquid dripping out from the set gel was observed. The minimum concentration at which no dripping of liquid was noticed was recorded as the LGC.

2.2.5 | Volatolomics

The volatile compounds from flour samples were analysed using a HS-SPME GC/MS method modified from Azarnia et al. (2011). An Agilent 6890 GC/5973 MSD equipped with a PAL RSI 85 autosampler (Agilent Technologies, Santa Clara, CA, USA) was used to analyse the volatile profiles. Two grams of each sample was weighed into clear glass vials (20 mL; Chromatographic Specialties, Brockville, ON, Canada). A blank was prepared with 2 g of NaCl. The vials were stored at 4°C and were brought to RT (21°C–23°C) 1 h before volatile analysis. Volatile compounds in the free head space above the samples were extracted by placing them in an agitator set at 50°C for 45 min and were adsorbed on a SPME fibre. A carboxen/polydimethylsiloxane (CAR/PDMS) (Supelco, Bellefonte, PA, USA) fibre was conditioned at 285°C for 3 min before the volatile compound adsorption. GC front inlet was set as pulsed splitless mode. The SPME fibre desorption was performed at 285°C for 0.3 min, followed by elution at 35°C using pure helium (1.5 mL/min) on a DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent Technologies). The gradient was held at 35°C for 3 min, increased to 80°C at a rate of 6°C/min, then up to 280°C at a rate of 20°C/min and held at 280°C for 2 min. The MS was acquired by scan from 35 to 200 m/z with the source set at 230°C, quad set at 150°C and 70.3 eV of fixed electron energy. Compound identification was done using library search (NIST 20.L database) using the Agilent Mass Hunter software.

2.2.6 | Anti-Nutritional and Enzyme Activity Analyses

Total Phenolics. The total phenolic compounds (TPC) of flour samples taken from control and RF-treated materials were measured using the method of Attard (2013) with some modifications. Phenolic compounds were extracted from 50 mg of flour samples by adding 500 μL of 80% (v/v) aqueous methanol at RT (21°C–23°C) in capped 1.5 mL centrifuge tubes. The tubes were placed on a shaker for 60 min and then centrifuged (Eppendorf Centrifuge 5417R) at 17,900 × *g* for 10 min to collect the supernatant. A 1:10 (v/v) Folin–Ciocalteu working solution was prepared using the Folin–Ciocalteu reagent (cat# 47641, Sigma-Aldrich Co., St Louis, MO, USA) and distilled water. Supernatant (20 μL) was mixed with 100 μL of the diluted Folin–Ciocalteu reagent and 80 μL of 1 M sodium bicarbonate solution. A reagent blank was prepared using 20 μL of 80% (v/v) aqueous methanol instead

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of the sample supernatant. After a 15-min incubation at RT, the absorbance of the sample mixture was measured at 765 nm (BioTeK Synergy H1 Hybrid Reader, Agilent Technologies, Santa Clara, CA, USA). To prepare the calibration curve, serial dilutions (250, 125, 62.5, 31.25 and 15.63 μg/mL) of gallic acid (Sigma, Cat#398225) were prepared from a 1-mg/mL stock of gallic acid in 80% (v/v) aqueous methanol. The TPC quantity was calculated as mg of gallic acid equivalent (GAE)/g of dry sample weight.

Saponins. The saponin content from flour samples was determined using a modified procedure as described by Le et al. (2018). Briefly, 500 μL of 80% (v/v) aqueous methanol was added to 50 mg of sample in a 1.5 mL tube placed on a shaker for 60 min and incubated at RT. The tubes were centrifuged (Eppendorf Centrifuge 5417R) at 17,900 ×*g* for 10 min to collect the supernatant for saponin analysis. The reaction was carried out in 96-well plate (VWR, Cat #10062-900). In each well, 30 μL of the above supernatant saponin extract was mixed with 15 μL of 8% vanillin solution in ethanol (w/v) and 170 μL of 50% sulphuric acid. After incubation of the plate for 30 min at 55°C, the absorbance of the mixture was measured at 540 nm using a plate reader (BioTeK Synergy H1 Hybrid Reader, Agilent Technologies, Santa Clara, CA, USA). The total saponin content was calculated as mg aescin equivalents (AE) per g of dry sample weight. A standard curve consisting five concentrations of aescin (15, 7.5, 3.75, 1.87 and 0.94 mg/mL) was generated for the quantification.

Trypsin Inhibitor Activity (TIA) Assay. The TIA was determined based on the AOCS method (Method Ba 12a-2020) (Liu 2021), with some modifications. Fifty milligrams of sample was mixed with 2.5 mL of 10 mM sodium hydroxide extraction buffer in a 15-mL tube and mixed by rotation for 3 h at RT. The extracts were centrifuged at 20,000 ×*g* for 10 min before use. The assay reaction was carried out on a microplate in the reading chamber of a plate reader (BioTeK Synergy H1 Hybrid Multi-Mode Reader). For the sample assays, reaction buffer (135 μL, containing 99 μL of 50 mM Tris buffer pH 8.2 and 36 μL of 1 mM hydrochloric acid solution), diluted sample extract (40 μL), BAPA (Na-Benzoyl-D, l-arginine 4-nitroanilide hydrochloride) solution (1 μL, 40 mg/mL), trypsin (4 μL, 0.2 mg/mL) were added sequentially. The dynamic absorbance changes at 410 nm were recorded every 15 s for 10 min after the addition of trypsin. Acetic acid (20 μL, 30% solution) was added to stop the reaction 10 min after the addition of trypsin and the final absorbance at 410 nm was recorded for TIA calculation. A separate control reaction with same amounts of reagents was added, but acetic acid was added before the addition of BAPA to prevent trypsin activity. One trypsin unit is defined as an increase of 0.114 absorbance unit at 410 nm in a 200-μL volume reaction mixture. TIA is reported as the amount of trypsin units inhibited per mg of sample dry weight.

LOX Assay. The LOX activity was determined based on modifications to published methods (McCurdy et al. 1983 ; Pathiratne et al. 2015). To 50-mg of each sample, 1 mL of extraction buffer (50 mM sodium phosphate buffer at pH 6, with 2% PVPP) was added and was incubated for 60 min at 4°C in a rotatory shaker. Extracts were centrifuged for 20 min at 16,000 ×*g* at 4°C, and aliquots of the supernatant were taken for protein concentration measurement by using the Bio-Rad protein assay kit according to the manual. The extract was diluted to 0.025 mg/mL and used for the LOX assay. The LOX assay was performed at RT using 10 mM linoleic acid as the substrate. One unit of LOX activity is equivalent to an increase in absorbance of 0.001 units per minute at 234 nm.

2.3 | Statistical Analysis

The data obtained from experimental analyses were analysed statistically to assess the impact of RF heating on the studied quality parameters. A one-way analysis of variance (ANOVA) was conducted to evaluate any significant differences among the groups using IBM SPSS Statistics software (version 25). Following this, post-hoc testing using Tukey's Honest Significant Difference (HSD) test was performed at a 5% significance level to distinguish specific differences between the groups.

3 | Results

3.1 | Changes in Proximate and Amino Acid Composition Associated With RF Pre-Treatments

Flours of samples collected from untreated control and RF pre-treatments were used for compositional analysis using NIR spectroscopy. Both RF treatments showed significant reduction of moisture content compared to untreated control, as well as to each other. The initial moisture content of the untreated control was 13.98 ± 0.52%. This was reduced on a temperature-dependant manner to 10.94 ± 0.48% and 8.14 ± 0.23% in the RF-treated – Low and RF-treated – High seeds, respectively (Table 1). There were small but significant changes to the total protein content between the three sample sets after adjustments for moisture content were made and the data was calculated on dry sample weight basis. However, the changes in protein content did not follow a temperature-dependent pattern, with the RF-treated – Low sample having the lowest protein and the RF-treated – High having slightly higher protein content than the untreated sample. No significant changes were observed in the

TABLE 1 | Proximate composition of radio-frequency (RF)-treated and untreated peas.

Sample	Moisture (%)	Protein (% db)	Fat (% db)	Starch (% db)	Ash (% db)
Untreated	13.98 ± 0.52 [a]	29.06 ± 0.44 [b]	1.89 ± 0.39 [a]	53.16 ± 0.30 [b]	1.93 ± 0.08 [a]
RF-treated – Low	10.94 ± 0.48 [b]	27.05 ± 0.22 [c]	1.73 ± 0.36 [a]	54.69 ± 0.54 [a]	1.10 ± 0.11 [b]
RF-treated – High	8.14 ± 0.23 [c]	30.08 ± 0.27 [a]	1.29 ± 0.14 [a]	53.78 ± 0.24 [ab]	0.89 ± 0.05 [c]

Note: % db – percentage dry basis. Values are expressed as mean ± standard deviation (triplicate). Mean with superscripts a–c in the same column indicate significant difference ($p < 0.05$).

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fat content between the RF treatments and control. The starch content increased from 53.16± 0.30% in untreated control to 54.69± 0.54% in the RF-treated – Low and 53.78± 0.24% in RF-treated – High samples. Finally, there was a small but significant decrease in ash content in both RF treatments compared to control, showing a clear temperature-dependant reduction trend.

The NIR spectroscopic analysis of the amino acid composition (Table 2) of the untreated control revealed a profile that was typical for pea proteins, with a low content of Trp (0.229± 0.012), as well as the sulphur-containing amino acids Met (0.314± 0.010) and Cys (0.380± 0.005). The amino acids Glx (Glu + Gln) (5.909±0.058) followed by Asx (Asp + Asn) (3.964 ±0.003) were the highest in the untreated control sample. The RF treatments showed a consistent temperature-dependent reduction pattern of several amino acids, such as His, Ser, Arg, Gly, Asx, Glx, Pro, Lys, Val, Ile, Leu and Phe, compared to control. Among these, major reductions were observed for the amino acids Arg, Asx and Glx in the RF-treated samples compared to control. There was a small but significant increase in the content of Ala, Cys, Tyr, Met, Trp and Thr in the RF treatments compared to control. While six out of nine essential amino acids showed a reduction upon RF treatment, it should be noted that the amounts of the two lowest essential amino acids in the control (Trp and Met) increased slightly after RF treatments.

3.2 | Changes in Anti-Nutritional Factor Content and Enzyme Activity Due to RF Pre-Treatments

The presence of compounds such as phenolics and saponins affects the taste, colour and mouth-feel of food ingredients, as well as have other bioactivities. The levels of total phenolics and saponins were estimated using colorimetric spectroscopic assays from flour samples obtained from untreated and RF-treated seeds (Table 3). Total phenolic content of untreated seed flour was 0.289± 0.005 mg GAE/g dry weight. However, there was a significant increase in the level of phenolic compounds to 0.436± 0.003 and 0.447± 0.004 mg GAE/g dry weight in RF-treated – Low and RF-treated – High samples, respectively. The saponin content of the untreated sample was 42.04± 1.33 mg AE/g dry weight. This decreased significantly to 32.35± 2.07 and 34.47± 2.61 mg AE/g dry weight in the RF-treated – Low and RF-treated – High samples, respectively. Trypsin inhibitors (TI) prevent the proper digestion of proteins and decrease the availability of nutrients. The TIA in the untreated and RF-treated seeds were estimated using trypsin enzyme inhibition assay. The untreated control sample showed a TIA of 2.76± 0.03 TIU/mg of sample dry weight. There was no significant change in TIA in the RF-treated – Low sample that was subjected to a lower temperature of 85°C. However, the TIA reduced significantly to 0.85± 0.17 TIU/mg of sample dry weight in the RF-treated – High sample that was exposed to a higher temperature of 115°C.

LOX is an enzyme involved in the lipid oxidation pathway that could lead to the generation of several volatile flavour compounds that are associated with off-flavour aroma characteristics. The activity of LOX enzyme before and after RF thermal treatments was determined. The untreated control showed a high LOX activity of 92,960.72± 2506.81 units/mg of extracted protein. This activity was significantly reduced in a temperature-dependent

TABLE 2 | Amino acid composition of radio-frequency (RF)-treated and untreated peas.

Sample	His	Ser	Arg	Gly	Asx	Glx	Ala	Pro	Cys
Untreated	0.667±0.003^{a}	1.442±0.059^{a}	2.868±0.073^{a}	1.360±0.004^{a}	3.964±0.003^{a}	5.909±0.058^{a}	1.159± 0.040^{b}	1.368± 0.039^{a}	0.380±0.005^{b}
RF-treated – Low	0.629±0.015^{b}	1.392± 0.037^{a}	2.489±0.020^{b}	1.310±0.013^{b}	3.672± 0.031^{b}	5.379±0.040^{b}	1.291 ±0.007^{a}	1.265±0.005^{b}	0.438±0.002^{a}
RF-treated – High	0.592±0.014^{c}	1.263±0.031^{b}	2.384±0.042^{b}	1.281±0.009^{c}	3.567±0.040^{c}	5.117±0.093^{c}	1.281 ±0.003^{a}	1.234±0.017^{b}	0.443±0.005^{a}
Sample	**Lys**	**Tyr**	**Met**	**Val**	**Ile**	**Leu**	**Phe**	**Trp**	**Thr**
Untreated	2.325±0.009^{a}	0.922±0.035^{b}	0.314±0.010^{b}	1.217±0.025^{a}	1.155±0.010^{a}	2.236± 0.023^{a}	1.457±0.008^{a}	0.229±0.012^{b}	1.054±0.008^{b}
RF-treated – Low	2.294±0.018^{a}	0.981± 0.018^{a}	0.356±0.005^{a}	1.175±0.004^{b}	1.097±0.004^{b}	2.197±0.008^{ab}	1.441 ±0.020^{a}	0.273± 0.008^{a}	1.149±0.023^{a}
RF-treated – High	2.235±0.026^{b}	0.962±0.006^{ab}	0.359± 0.001^{a}	1.168±0.006^{b}	1.078± 0.012^{b}	2.166±0.013^{b}	1.397± 0.006^{b}	0.261±0.001^{a}	1.125± 0.006^{a}

Note: Values are expressed as mean± standard deviation (triplicate). Mean with superscripts a–c in the same column indicate significant difference ($p < 0.05$).
Abbreviations: Ala – alanine, Arg – arginine, Asx – aspartic acid (aspartate) + asparagine, Cys – cysteine, Glx – glutamic acid (glutamate) + glutamine, Gly – glycine, His – histidine, Ile – isoleucine, Leu – leucine, Lys – lysine, Met – methionine, Phe – phenylalanine, Pro – proline, Ser – serine, Thr – threonine, Trp – tryptophan, Tyr – tyrosine, Val – valine.

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manner after the RF treatments to 74,750.24± 2444.15 and 26932.57± 712.19 units in the RF-treated – Low and RF-treated – High samples, respectively, showing effective enzyme deactivation.

3.3 | Analysis of Volatolomes of Untreated and RF-Treated Pea Samples

The subset of metabolites that are emitted from untreated and RF-treated samples as volatile organic compounds, comprising their respective volatolomes, were analysed using HS-SPME GC/MS (Figure 2). Drastic changes were observed between the chromatograms of the untreated and RF-treated samples. The untreated control showed a prominent peak at retention time 4.8 min identified as the aromatic hydrocarbon compound toluene (Figure 2A). In the RF-treated – Low sample, a new peak emerged at 5.6 min identified as the saturated aldehyde compound hexanal (Figure 2B). This hexanal peak in the RF-treated – Low sample was much higher compared to the toluene peak at 4.8 min. While the prominent hexanal peak at 5.6 min was still present in the RF-treated – High sample, there were several additional peaks in the chromatogram that belonged to various alkyl pyrazine compounds (Figure 2C). The relative peak abundance (RPA %) of each compound or group of compounds identified above were determined based on their individual peak area with respect to the total peak area of all compounds (Table 4). Toluene was

TABLE 3 | Anti-nutritional composition of radio-frequency (RF)-treated and untreated peas.

Sample	TPC (mg GAE/g DW)	Saponin (mg AE/g DW)	TIA (TIU/mg DW)	LOX (units/mg of extracted protein)
Untreated	0.289±0.005 [c]	42.04±1.33 [a]	2.76±0.03 [a]	92960.72±2506.81 [a]
RF-treated – Low	0.436±0.003 [b]	32.35±2.07 [b]	2.83±0.10 [a]	74750.24±2444.15 [b]
RF-treated – High	0.447±0.004 [a]	34.47±2.61 [b]	0.85±0.17 [b]	26932.57± 712.19[c]

Note: Values are expressed as mean ± standard deviation (triplicate). Mean with superscripts a- c in the same column indicate significant difference ($p < 0.05$).
Abbreviations: AE – aescin equivalent, DW – dry weight of sample, GAE – gallic acid equivalent, LOX – lipoxygenase enzyme activity, TIA – trypsin inhibitor activity, TIU – trypsin inhibitor units, TPC – total phenolic compounds.

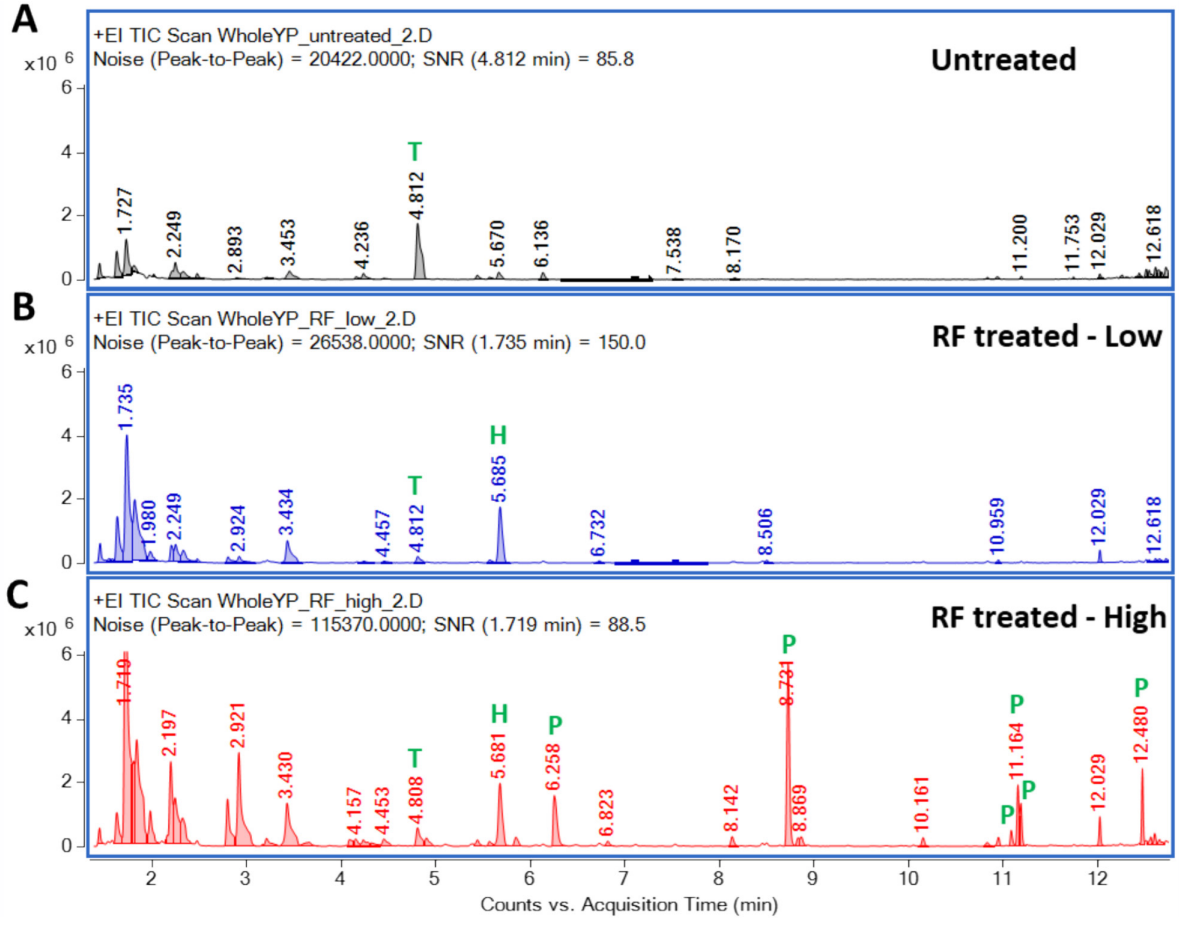

FIGURE 2 | Volatile chemistry analysis by HS-SPME GC/MS. Total ion chromatograms of untreated (A), RF-treated – Low (B) and RF-treated – High (C) yellow peas. Abbreviations: H – hexanal, P – pyrazine-class of compounds, T – toluene.

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the most dominant compound in terms of both its peak height and peak area in the untreated control sample. The RPA of toluene in the untreated control was 16.18% and it reduced to less than 1.4% in the RF-treated samples. Hexanal was not detected in the untreated control sample. However, with the RF treatments its relative abundance increased to 8.91% and 4.17% in the RF-treated – Low and RF-treated – High samples, respectively. Ten compounds belonging to the pyrazine class of molecules appeared specifically in the RF-treated – High sample, but were not detected from the untreated control and the RF-treated – Low samples. The combined RPA of all the new pyrazine-class compounds was 20.86%, revealing the high abundance and diversity of these molecules in samples exposed to the higher temperature of 115°C.

TABLE 4 | Volatile compound identification and relative abundance.

Sample name	CAS #	Compound name	Retention time (min)	Toluene RPA (%)	Hexanal RPA (%)	Pyrazine RPA (%)
Untreated	108-88-3	Toluene	4.811	16.18	n.d.	n.d.
RF-treated – Low	108-88-3	Toluene	4.814	1.12	8.91	n.d.
	66-25-1	Hexanal	5.685			
RF-treated – High	290-37-9	Pyrazine	4.092	1.36	4.17	20.86
	108-88-3	Toluene	4.809			
	66-25-1	Hexanal	5.683			
	109-08-0	Pyrazine, methyl—	6.258			
	123-32-0	Pyrazine, 2,5-dimethyl—	8.733			
	13925-00-3	Pyrazine, ethyl—	8.837			
	5910-89-4	Pyrazine, 2,3-dimethyl—	8.876			
	13925-03-6	Pyrazine, 2-ethyl-6-methyl—	11.093			
	14667-55-1	Pyrazine, trimethyl—	11.164			
	13925-03-6	Pyrazine, 2-ethyl-6-methyl—	11.195			
	13360-65-1	Pyrazine, 3-ethyl-2,5-dimethyl—	12.481			
	15707-34-3	2,3-Dimethyl-5-ethylpyrazine	12.61			
	18138-05-1	Pyrazine, 3,5-diethyl-2-methyl—	13.46			

Note: RPA – Relative peak abundance represents the relative percentage of a particular compound peak in relation to the total peak area of all the compounds in the chromatogram. RPA (%) = Peak area of a target compound/Total peak area * 100.

TABLE 5 | Techno-functional properties of radio-frequency (RF)-treated and untreated peas.

Sample	WHC (g/g DW)	OAC (g/g DW)	FC (%)	FS at 60 min (%)	EC (mL oil/g of sample)	ES at 60 min (%)	LGC (%)
Untreated	0.73 ± 0.02^{b}	0.30 ± 0.02^{a}	29.8 ± 1.9^{a}	83.9 ± 3.4^{a}	489	48.0 ± 2.0^{a}	22
RF-treated – Low	1.12 ± 0.05^{a}	0.26 ± 0.03^{a}	25.3 ± 3.2^{ab}	87.3 ± 2.4^{a}	383	44.4 ± 2.1^{a}	22
RF-treated – High	1.16 ± 0.05^{a}	0.13 ± 0.02^{b}	23.0 ± 1.0^{b}	72.5 ± 2.7^{b}	350	20.0 ± 2.0^{b}	25

Note: Values are expressed as mean ± standard deviation (triplicate). Mean with superscripts a- c in the same column indicate significant difference ($p < 0.05$). Abbreviations: DW – dry weight of sample, EC – emulsion capacity, ES – emulsion stability, FC – foaming capacity, FS – foaming stability, LGC – least gelation concentration; OAC – oil absorption capacity, WHC – water holding capacity.

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3.4 | Changes in the Techno-Functional Properties of the Pea Flour After RF Treatments

The effects on functional properties of the flour ingredient produced after RF treatments were assessed. Table 5 depicts the different functional properties WHC, OAC, FC, FS, EC, ES and LGC of the flours produced from untreated and RF-treated pea seeds. RF treatment resulted in a significant improvement of WHC of the pea flour. The untreated flour had a WHC of 0.73 ± 0.02 g/g of sample dry weight, which increased to 1.12 ± 0.05 and 1.16 ± 0.05 g/g of sample dry weight for RF-treated – Low and RF-treated – High flours, respectively. However, it should be noted that the difference between the WHC of the lower and higher temperature RF-treated flours was not statistically significant. In terms of OAC, RF heating showed the opposite behaviour of WHC. The untreated sample had an OAC of 0.30 ± 0.02 g/g of sample dry weight, while the RF-treated – Low sample did not show a significant change in OAC, though the RF-treated – High sample showed a significant reduction in OAC to 0.13 ± 0.02 g/g of sample dry weight. RF treatment negatively affected the foaming properties of pea flour. The FC values of the samples were 29.8 ± 1.9, 25.3 ± 3.2 and 23.0 ± 1.0% for the untreated, RF-treated – Low and RF-treated – High samples, respectively. The FS of untreated flour was 83.9 ± 3.4%. Heating to a higher temperature during the RF-treated – High processing significantly reduced FS to 72.5 ± 2.7% in comparison to the untreated pea flour. The RF-treated – Low processing did not show a significant change in FS when compared to the untreated control. RF treatments significantly impacted both EC and ES as the values reduced due to the treatments. Untreated flour had an EC of 489 mL oil/g of sample and an ES of 48.0 ± 2.0% at 60 min. RF-treated – Low processing resulted in slight reduction of EC and ES than untreated pea flour. However, the RF-treated – High processing resulted in greater reduction of emulsion properties, with a significant drop in ES to 20.0 ± 2.0% compared to the control. The LGC analysis showed that both untreated and RF-treated – Low samples required 22% of flour to form a firm gel, whereas the RF-treated – High sample required 25% of flour for formation of a stable gel. Overall, temperature-dependant trends on functional properties were observed, with the RF-treated – Low processing condition showing no significant changes or an intermediate change in functional properties compared to control and the RF-treated – High processing showing more extreme and significant changes in functionality due to the higher temperatures attained in this treatment.

4 | Discussion

4.1 | Changes in Macromolecular Composition and Antinutrients of Peas Due to RF Treatments

RF and alternative volumetric heating methodologies, including microwave irradiation, can affect the nutritional and anti-nutritional attributes of food constituents. As illustrated in Table 1, RF heating exerted an influence on the proximate components, including protein, fat, starch and ash. In the case of protein, this study identified a marginal increase attributable to RF-treated – High in comparison to the untreated control sample. Conversely, RF-treated – Low resulted in a slight decrease in protein content. No significant alterations were observed in fat content; however, a noteworthy reduction was evident in ash content. Our observations regarding proximate composition are consistent with prior investigations concerning analogous pulse crops. For example, Zhong, Wang, and Zhao (2015) noted more or less similar results for protein, fat, starch and ash content when black soybeans underwent RF heating at 27 MHz and 6 kW for a duration of 30 min. The modifications noted in these characteristics are primarily ascribed to structural transformations, thermal degradation phenomena and the production of other compounds (Das et al. 2025). These findings across diverse legume species imply that RF treatment may elicit analogous compositional alterations in various pulse crops, likely via comparable thermal modification mechanisms.

Amino acids, the building blocks of proteins, were influenced by RF treatment (Table 2). A prior investigation on the dielectric heating of black gram seeds utilizing RF, reported significant modifications of various amino acids (Tiwari et al. 2021). RF heating can cause protein denaturation, resulting in conformational changes that may hinder amino acid accessibility. This denaturation process disrupts the native conformations of proteins, potentially lowering their digestibility and modifying the bioavailability of amino acids (Han et al. 2018; Yu et al. 2017). The magnitude of these effects is contingent upon variables such as RF exposure duration, intensity and the thermal stability of the specific protein.

Anti-nutrients in pulses include phytic acid, protease inhibitors (e.g., trypsin and chymotrypsin inhibitors), saponins, lectins and polyphenols. These compounds can bind minerals, inhibit enzyme activity and reduce protein digestibility, thereby lowering the nutritional value of pulses (Faizal et al. 2023; Zhou et al. 2024). As shown in Table 3, RF preheating at 85°C and 115°C of peas significantly increased the total phenolic content but decreased the saponins and LOX activity. The TIA was also reduced significantly for RF-treated – High (115°C) peas, but did not change significantly for RF-treated – Low (85°C) sample. The rise in phenolic compounds may be attributed to enhanced extraction efficiency caused by RF heating, which creates a more porous structure in the peas. Phenolic compounds are often bound to cellular structures, such as lignin and hemicellulose, which can limit their extraction. RF heating, through its ability to disrupt these structures, releases these bound phenolics, making them more accessible for extraction (Markhali and Teixeira 2024; Sánchez et al. 2023). Saponins are non-volatile, surface-active compounds, and previously reported to be reduced around 22% from black soybean due to 30 min of RF heating (Zhong, Wang, and Zhao 2015). In this study, around 18% and 23% reduction of saponins were noticed by RF-treated – High and RF-treated – Low pea samples, respectively, which is similar to the report of Zhong, Wang, and Zhao (2015). TI and LOX are among the most significant anti-nutrients in peas. The activity of TI and LOX was reduced around 70% due to RF-treated – High (115°C), which demonstrates the effectiveness of RF heating on diminishing of these anti-nutrients. Both these components are proteins and the temperature-dependent reduction in their activities observed here is likely caused by the denaturation of their molecular

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structure leading to their inactivation. Oke (2021) similarly reported around 80% reduction of TI due to RF-heating of chickpea at 115°C. Based on the observed results, this study demonstrates that RF heating serves as a viable alternative processing method for peas, effectively reducing anti-nutritional factors while maintaining minimal impact on their essential nutrient profile. The technique strikes a balance between anti-nutrient degradation and preservation of most macronutrients, suggesting its potential as a sustainable thermal treatment for legume processing.

4.2 | Molecular Changes Due to RF Treatments Lead to the Modification of Flavour Profile of Pea Flour

Flavour is an important quality attribute of a food ingredient that dictates consumer acceptance of new products. The volatile compounds that emanate from a material are perceived as favourable or unfavourable aromas. Ingredients from peas and other pulses tend to be associated with unfavourable aroma profiles that are often described as beany, green and grassy notes (Karolkowski et al. 2021; Trindler, Annika Kopf-Bolanz, and Denkel 2022). Previous studies have tried to identify the predominant volatile compounds present in peas, describe their flavour properties and characterize the variations in these compounds between varieties, growth in different environments and year of harvest (Azarnia et al. 2011; Malcolmson et al. 2014). Although hundreds of volatile compounds have been identified by GC–MS analysis, only a small subset of these compounds were recognized as odour active in human sensory evaluations (Liu, Cadwallader, and Drake 2023; Zhogoleva, Alas, and Rosenvald 2023). This low number of odour active compounds could be because many of the volatile compounds could be at concentrations below the odour perception thresholds of human olfactory sensation (Czerny et al. 2008). In the current study, the primary volatile compound identified from unprocessed pea sample was the aromatic hydrocarbon compound toluene. Although toluene is produced industrially for applications as a solvent, it is also known to be biochemically synthesized by plants (Heiden et al. 1999). Heiden et al. (1999) used ^{13}C labelled carbon dioxide to show that sunflower and pine plants were able to incorporate the labelled carbon into the toluene emitted. Further, they observed that the production of toluene increased with stress due to tissue damage. A study on the volatile chemistry of pea cultivars also identified toluene as a volatile component of pea seeds (Azarnia et al. 2011). In both the RF-treated samples, the toluene content had reduced compared to the untreated control, while the aldehyde compound hexanal increased in relative abundance. While there are no previous studies that have reported the changes in volatile chemistry due to RF heating of peas, a relevant study on microwave-treated rapeseed demonstrated that hexanal levels increased with prolonged processing time (Zhang et al. 2023). Hexanal is a well characterized odour active compound from pea and other pulses that is known to be produced during processing and storage of various ingredients (Murat et al. 2013; Zhogoleva, Alas, and Rosenvald 2023). A study investigating the odour active compounds commonly associated with foods defined the aroma profile of hexanal as green and grassy. Further, they had determined that this compound had a low odour threshold of 2.4 μg/L of water (Czerny et al. 2008). Although many volatile compounds have their own unique flavour attributes, they can either augment or suppress the perception of other compounds to influence the overall aroma profile of the ingredient. It has been reported that although the primary odour description of hexanal is green and grassy, when combined with other compounds, such as 1-octen-3-one, 3-methyl-1-butanol, (*E*)-2-nonenal, (*E,E*)-2,4-nonadienal and (*E*)-2-hexenal, it can intensify the perception of beany flavour (Bott and Chambers 2006; Trindler, Annika Kopf-Bolanz, and Denkel 2022). Hexanal and other off-flavour compounds are generated by breakdown of fatty acids such as linoleic acid and their production is mediated by endogenous enzymes, such as lipase, LOX and hydroperoxide lyase (Trindler, Annika Kopf-Bolanz, and Denkel 2022; Zhao et al. 2025). Interestingly, while we observed that the relative abundance of hexanal increased in the RF treatments, the LOX enzyme activity decreased in a temperature-dependent manner, probably due to thermal-induced protein denaturation. This contradiction exists because there is an alternative autoxidative pathway of production of hexanal that induced by heat, light or the presence of metal ions. In a study of the development of volatile compounds through various stages of pea protein isolate production, it was observed that the amount of hexanal increased from 0.624 μg/g in dry flour to 83 μg/g in protein isolate, while LOX activity was only detected in the flour and not in the isolate (Murat et al. 2013). Thus, it can be concluded that the increase in hexanal in the RF-treated pea flours is mostly due to the heat-induced autoxidation of fatty acids.

The other major change in the volatolome was observed specifically in the RF-treated – High sample that was processed at a higher temperature of 115°C, where the relative peak area of the pyrazine class of compounds increased to 20%. These compounds were not detected in the untreated control as well as the RF-treated – Low sample that was subjected to a lower processing temperature of 85°C. Pyrazines are important odour active aroma compounds present in various foods such as coffee, cocoa, chocolate, roasted nuts and roasted meat products that impart a characteristic nutty or roasted flavour to foods. Chemically, their core structure is made of a monocyclic aromatic ring with two nitrogen atoms. Incorporation of various functional groups to the carbons around the ring can lead to a large number of pyrazine molecules (García-Lomillo and González-SanJosé 2019). Although these compounds are formed in certain processed foods, they are also produced industrially for use as high-value flavouring additives in several food formulation applications (Mortzfeld et al. 2020). Under certain thermal processing condition such as dry roasting, baking or deep frying, Maillard reactions between amino acids and reducing sugars lead to the formation of these compounds through Strecker degradation chemistry (Amrani-Hemaimi, Cerny, and Fay 1995; Ren et al. 2024). Studies using various Maillard model systems have shown that the amino acids such as Gln, Asn, Glu and Asp are major contributors to the production of pyrazine compounds (Chun and Ho 1997; Martin and Ames 2001). Interestingly, the amino acid composition analysis of the RF-treated – High samples showed that it had significant reduction in the amounts of both Glx and Asx compared to the untreated control and RF-treated – Low samples. This finding in an actual thermal

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processing system in the current study is in good agreement with these previous studies that used simulated model systems.

A previous study that analysed the flavour profiles and sensory properties of dry roasted peas reported an increase in both the variety and relative abundance of pyrazines, as well as a modification to the sensory properties which were reported as nutty and popcorn-like (Bi et al. 2020). Although the current study does not extend to human sensory analysis, it can be reported anecdotally that the RF-treated – High peas had a roasted aroma compared to the other samples. This previous study had placed a batch of pea seeds in a forced-air oven that was maintained at 160°C for a duration of 30 min (Bi et al. 2020). Pyrazine compound generation during thermal processing has been usually observed at higher treatment temperatures (> 120°C) and longer processing durations (longer than 30 min) (Liao et al. 2019). Whereas, the current study has employed a pilot-scale continuous flow RF processor that treated the seeds at a lower temperature of 115°C, as well as a lower residence time of 19 min. This ability of RF waves to penetrate deeply and produce volumetric heating in the material leading to the generation of pyrazine compounds at lower temperatures could potentially be useful in the production of flavour-modified functional pulse ingredients.

4.3 | RF-Induced Changes in Functional Attributes of the Flour Ingredient Could Influence Its Food Applications

Functional characteristics of the flour can provide insight into how the food ingredient will influence the texture, structure, appearance and other physical attributes of a food product. As seen from Table 5, RF heating had profound influence on the functionality of the pea flours. WHC defines the ability of the flour to physically hold water against gravity (Ma et al. 2011). The WHC increased significantly due to RF treatment. Similar results were also obtained by other authors for processing of different seeds/proteins using RF, such as maize grain (Hassan et al. 2019), grass carp myofibrillar protein (Wang et al. 2021), rice bran protein (Ling et al. 2019a), etc. The increase of WHC might be the result of formation of more porous structure, which helped in entrapment of more water inside the samples (Ma et al. 2011). Pulse or legume flours are rich in polysaccharides and proteins that show varying affinities to water molecules (Kaur and Singh 2005). Protein denaturation, starch gelatinization and the swelling of crude fibre during heating or cooking can contribute to increased WHC (Aguilera et al. 2009). Improving the WHC is important in food processing for applications such as cake batters and sausages, since they are required to provide the right texture, mouth feel and product density (Hassan et al. 2019). Thermal treatments have been employed as a tool for increasing the water holding capacity of plant proteins such as soy and faba bean, to increase their utilization in meat analogue applications (Bühler et al. 2020; Peng et al. 2021). Thus, increasing of WHC may be desirable for development of different plant-based meat products. Though the WHC positively increased due to RF heating, OAC reduced significantly. This is contrary to previous reports that noted that RF treatment might cause an increase in OAC (Hassan et al. 2019) by exposing the buried hydrophobic groups in proteins, which could be responsible for increased lipid–protein interactions (Kaur and Singh 2005; Prakash and Ramanatham 1995). It is possible that the effects of RF treatment on OAC might be influenced by multiple factors such as thermal stability of proteins, influence of the matrix composition and temperature and duration of treatments. Proteins and carbohydrates play a significant role in foam formation and its stability. Proteins provide an interfacial layer that helps to keep air bubbles afloat and inhibit their coalescence (Ma et al. 2011). The assessment of foaming properties of pea flours in this study revealed that both FC and FS decreased due to RF treatment at the higher temperature. A study on microwave and hot air-assisted RF heating of foxtail millet flour also found a decrease of both FC and FS (Rao et al. 2023). High temperature (80°C) roasting of different pulse flours such as lentil, chickpea and yellow pea (with or without hull) resulted in significant decreasing of FC (Ma et al. 2011). Similarly, isolated proteins from rice bran (Ling et al. 2019a) and maize grain (Hassan et al. 2019) showed either similar or less FC and stability. As like WHC and OAC, foaming properties also depend on molecular interactions of proteins with other components mediated through hydrogen bonding, as well as electrostatic and hydrophobic interactions (Ma et al. 2011). Typically, the dispersion of proteins at the water–air interface is the main factor influencing FC. In contrast, the creation of a cohesive, dense layer surrounding the air bubble regulates the FS (Khan et al. 2011). The current study found that the disruption of FC and FS was temperature-dependent and the lower RF temperature treatment that did not affect these properties could be considered if foaming attributes are important for downstream ingredient applications. As with foaming, emulsifying properties were also negatively influenced due to RF processing. Although few studies have reported that RF heating improves the emulsifying ability and stability (Hassan et al. 2019; Rao et al. 2023), its effect on different food matrices might not be the same. Gelling capacity is important for food systems that need thickening and gelling. This process involves denatured molecules linking together to create aggregates, which are stabilized by various types of bonds, such as electrostatic interactions, hydrogen bonds, hydrophobic interactions and/or disulphide bonds (Sathe, Deshpande, and Salunkhe 1982). This gelation capacity was assessed employing the LGC as an indicator. A flour with lower LGC value is more suitable indicating better gelation capacity. As seen in Table 5, high temperature RF heating resulted in higher LGC (25%) whereas RF-treated – Low and untreated pea flours had lower LGC values (22%). Differences in gelling capabilities have been scientifically linked to variations in the proportions of proteins, lipids and carbohydrates in flours. The gelation mechanism and appearance are influenced by the balance between repulsive electrostatic interactions and attractive hydrophobic contacts (Aguilera et al. 2009).

5 | Conclusion

With increasing focus on sustainable use of every component of available bioresources and efforts made towards the reduction of by-product and co-product streams from manufacturing, flours or protein-enriched concentrates are gaining prominence as ingredients in comparison to purer protein isolates. Products such as plant-based meat analogues are being formulated using flour ingredients from various crops (Kyriakopoulou, Keppler, and Van Der Goot 2021). Thermal

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treatment is a widely used method for processing pulses and enhancing their quality. This study investigated the effects of RF preheating on pea seeds, focusing on composition, anti-nutrient content, flavour profile and functional properties. While few adverse effects on macro nutrients and functionalities were noted, RF pre-treatment significantly improved overall pea quality by markedly reducing anti-nutrients, generating new flavour compounds such as pyrazines, which impart a desirable roasted flavour. Moreover, it has enhanced water-holding capacity, which not only may improve texture but also can make peas more suitable for meat analogue applications, as it contributes to a juicier mouthfeel. Overall, this study demonstrates RF heating as a novel processing technique for modifying properties of pea flours for use in plant-based meat alternatives.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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