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Understanding the Mechanism of How Pulsed Electric Fields Treatment Affects the Digestibility and Characteristics of Starch in Oat Flour

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Abstract: The objective of this study was to evaluate the effect of pulsed electric fields (PEF) on the *in vitro* starch digestibility properties of oat flour. A wide range of PEF process intensity was investigated (electric field strength between 2.1 and 4.5 kV/cm and specific energy inputs between 52 and 438 kJ/kg using 20 µs square wave bipolar pulse at 100 Hz). The results revealed that PEF applied at a high electric field strength and energy <216 kJ/kg was favourable in slowing down the rate of starch digestibility (by 48%) during *in vitro* gastrointestinal digestion. This is accompanied by a significant decrease (from 15% to 7–10%) in the proportion of rapidly digestible starch (RDS) and a significant increase (from 77% to 84–85%) in resistant starch (RS) fraction. The application of PEF at energy level >421 kJ/kg at any field strength intensities raised the RDS (from 15% to 19–20%), but the rate of starch digestion was not affected (maintained at 3.3–3.7 $\times$ 10^{-2} min^{-1} vs. untreated at 3.8 $\times$ 10^{-2} min^{-1}). Further analysis of the structure, particle size, and thermal stability of PEF-treated oat flour through fractionation into three distinct flour segments revealed that PEF could cause major modifications in the particle size, damage and aggregation of starch granules, and destruction of the long- and short-range ordered structures of starch. Data gathered in this study indicate that PEF treatment can be a reliable strategy to modulate the *in vitro* starch digestibility of oat flour, either by reasonably slowing down the digestion rate or enabling a slightly higher amount of starch to be readily accessible by digestive enzymes without affecting the digestion rate.

Keywords: pulsed electric fields; oat; starch digestibility; gastrointestinal digestion; particle size; starch structure; starch crystallinity

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1. Introduction

Oat is one of the world's important food crops, which ranks seventh in terms of global cereal production volume [1]. It is recognized as an excellent source of nutrition and a multipurpose ingredient that has attracted attention in the recent years. Oat is a complex food system that is made up of ~60% starch, ~9–15% protein, ~3–11% lipid, and ~2–8% β-glucan [2]. Oats, when milled, produce a heterogeneous flour [3] that can be used directly as an ingredient for oat-based food products (e.g., snack bars, breakfast porridge) without requiring further heating. Because of the presence of β-glucan, a soluble dietary fibre, oat-based products are generally classified as low or medium glycaemic index food (GI between 44–69), which elicits a slow increase in blood glucose levels when consumed [4,5]. This is because β-glucan has been observed to form a network encapsulating protein and starch, thus limiting enzyme accessibility, and slows down the digestion of oat starch [6]. However, heat treatment application (in the presence of moisture) on oat flour will cause starch to

gelatinise and increase the rate of starch digestion, where GI was estimated to rise beyond 78 [5]. In conjunction, a significant increase in the proportion of rapidly digestible starch (RDS) and reduction in resistant starch fraction (RS) were observed [7]. Acknowledging the fact that oat starch is sensitive to structural and physical changes during processing, novel food processing technologies that can maintain the low GI response of oat without triggering a significant increase in starch digestion rate is worth exploring.

Pulsed electric fields (PEF) is an emerging processing technique that has been recently on the spotlight to be considered on starch-rich food products such as rice, wheat, potato, and pea starches [8,9], as well as oat and sorghum flours [10,11]. This is because PEF may offer a novel opportunity to alter the physicochemical and structural characteristics of starches [12], which may lead to changes in product functionality including the thermal, pasting, and starch digestibility properties [13,14]. The extent of PEF treatment influence is dictated by the different process parameters applied, including the electric field strength, pulse shape, pulse width, pulse number, specific pulse energy, and frequency [15,16]. Equally, the inherent properties of the food material (e.g., Type A, B, or C starch polymorph; fresh or pre-treated) prior to processing were also found to substantially determine the magnitude of PEF treatment effect and the modifications brought about on product functionality [9,10]. In this context, if PEF can influence oat structure and properties, the impact of PEF on the release of glucose during gastrointestinal process becomes of particular interest. Previous study by Li et al. [9] showed that the effect of PEF treatment on the starch digestibility was found to be more pronounced in wheat (Type A, i.e., considerable increase in %RDS and decrease in % slowly digestible starches (SDS)) and potato (Type B, i.e., increase in %RDS, %RS and decrease in %RS) starches compared to that of pea starch (Type C, i.e., no changes in any starch fraction).

This research aimed to understand the mechanism of how PEF treatment affects the starch digestibility of oat flour. In this study, oat flour was prepared using raw oats and it was then subjected to 2 h long simulated gastrointestinal process to estimate the rate of starch digestibility and evaluate the proportion of different starch fractions (RDS, SDS, and RS). Fractionating oat flour based on gravity was conducted after PEF treatment. Afterwards, systematic and thorough structural and physicochemical analyses were carried out for each segment of PEF-treated oat flour alongside a control (no PEF treatment). Modifications in particle size, volume distribution, morphological characteristics, long- and short-range ordered structures of starch, and thermal properties under PEF influence were analytically assessed. At the end of this study, a possible mechanism based on the physicochemical properties of each oat segment was postulated.

2. Materials and Methods

2.1. Oat Sample Preparation and Pulsed Electric fields (PEF) Treatment

Dehulled intact oat groats (*Avena sativa* cv. Armstrong) donated by Harraway and Sons, Ltd. (Green Island, Dunedin, New Zealand) were milled to flour and sieved (850 µm aperture, BS410/1986, Endecotts Ltd., London, England). Only the fine portions (containing 77% total starch, 11% crude protein, 5% crude fat, and 1% β-glucan content (dry weight basis)) were collected, vacuum-packed (Audion Elektro, Weesp, TheNetherlands) and stored at −20 °C until use.

PEF treatment was conducted using a batch setting of ELCRACK® HVP 5 PEF system (German Institute of Food Technologies, Quakenbrück, Germany) with a treatment chamber made up of two parallel stainless steel (4 cm distance) electrodes. For each PEF treatment, thirty-five milliliters of flour suspension (8% oat flour dissolved in cold distilled water) was subjected to PEF treatment at electric field strength levels of 2.1–2.2 kV/cm (referred as "2") and 4.2–4.5 kV/cm (referred as "4"), with pulse energy from 1.2 to 9.7 J. This is combined with a range of specific energy levels of 42–53 kJ/kg (referred as "A"), 216–218 kJ/kg (referred as "B"), and 421–438 kJ/kg (referred as "C"). The different combinations of PEF treatment parameters reported in this study were coded as: electric field strength-specific energy input (i.e., "2-A" is an oat flour that was PEF-treated at 2.2 kV/cm and 53 kJ/kg).

The PEF processing conditions were achieved by applying 20 µs square wave bipolar pulse at 100 Hz with pulse number ranging from 307 to 7778. The pulse characteristics (shape: square wave, width: 20 µs, rise and fall times: <1 µs) during PEF treatment was monitored using a digital oscilloscope (model UTD2042C, UNI-T, Dongguan City, Guangdong, China). The flour suspension was freshly prepared exactly 3 min before PEF treatment. The flour and cold water were appropriately mixed to break flour clumps and ensure no bubble formation. The temperature and conductivity of the flour suspension were monitored before (kept below 9 °C) and after PEF processing using CyberScan CON 11 conductivity meter (Eutech Instruments, Singapore).

2.2. Evaluation of the Starch Digestibility of PEF-Treated Oat Flour Using an In Vitro Static Digestion Method

The extent of starch digestion in both PEF-treated and untreated oat flour (0.5 g per sample), within a 3-stage static *in vitro* digestion method [17] with modifications [18], was determined. At oral phase (stage 1), oat flour was firstly diluted with saliva juice (consisted of 2 mM NaCl, 2 mM KCl, and 25 mM $NaHCO_3$) before α-amylase solution (0.0125 g/mL) was added. Then, 1 M HCl (Ajax Finechem, Seven Hills, New South Wales, Australia) was added until pH 3.0 ± 0.2 was reached to deactivate the enzyme prior to the commencement of gastric phase. The acidic mixture was added with 2 mL gastric juice, which consisted of pepsin (4% *w*/*v*) in 151 mM NaCl and 28 mM KCl in 1 L 1 mM HCl at pH 3, and incubated for 120 min at 37 °C. Then, 1 M NaOH was added until pH 7.0 ± 0.2 was reached. The gastric-chyme sample mixture was then added with 4 mL intestinal juice consisted of porcine pancreas pancreatin (1% *w*/*v*) and porcine bile extract (0.8452% (*w*/*v*)) in 0.1 M $NaHCO_3$ at pH 7. Small intestinal phase digestion was allowed to proceed for a total of 120 min. One mL aliquot digest from each flour sample was removed at 0, 20, 60, 90, and 120 min along the 2 h long intestinal digestion. The aliquots were immediately added with 80% (*v*/*v*) ethanol (Anchor Ethanol, Auckland, New Zealand) to deactivate the digestive enzymes, mixed thoroughly, centrifuged (1090× *g*, 20 min, 4 °C), and the supernatant was removed and analysed immediately. All incubation during digestion was conducted at 37 °C (Jeio Tech IB-15G, Seoul, Korea) at 55 strokes per min using rocking shaker (DLAB SK-R1807-S, Dr. Lab Technology Company, New Territories, Hong Kong).

D-glucose (due to starch hydrolysis during digestion) content in the oat digest was determined using glucose oxidase/peroxidase (GOPOD) enzyme (K-GLUC, Megazyme, Bray, Ireland). Firstly, the collected supernatant from the digest was added with 0.05 mL amyloglucosidase (3300 U/mL, Megazyme, Wicklow, Ireland), vortexed for 3 sec, and maintained at 50 °C (Grant Instruments water bath, Cambridgeshire, United Kingdom) for 60 min. Then, samples were diluted with distilled water to appropriate volume and centrifuged (1090× *g*, 10 min, 4 °C). Supernatant (0.05 mL) was then reacted with 1.5 mL GOPOD, and kept at 50 °C for 20 min. Absorbance of the coloured mixture was determined using Ultrospec 3300 Pro UV/visible spectrophotometer (Amersham Biosciences, Uppsala, Sweden) at 510 nm. D-glucose (1 mg/mL) provided by Megazyme K-GLUC kit was used as standard. The measured glucose in the digest is expressed as mg glucose released per mL of digest.

Three different starch fractions during *in vitro* digestibility were estimated [19]: (1) readily digestible starch (RDS), defined as the amount of D-glucose (mg/mL digest) released after 20 min of *in vitro* intestinal digestion ($Glucose_{20min}$), (2) slowly digestible starch (SDS), defined as amount of glucose (mg/mL digest) released between 20 and 120 min of *in vitro* intestinal digestion ($\Delta Glucose = Glucose_{120min} - Glucose_{20min}$), and resistant starch (RS) were calculated using Equations (1)–(3), respectively.

$$\text{RDS } (\%) = \frac{Glucose_{20min} \times 0.9}{\text{Total starch}} \times 100\% \quad (1)$$

$$\text{SDS } (\%) = \frac{\Delta Glucose \times 0.9}{\text{Total starch}} \times 100\% \quad (2)$$

$$\text{RS}\ (\%) = \text{Total starch} - (\text{RDS} + \text{SDS}) \quad (3)$$

To convert the measured D-glucose in the digest based on the molecular mass ratio of starch to glucose (162/180), 0.9 factor value was used. Total starch content of the undigested samples was determined using the Megazyme kit (K-TSTA, Bray, Ireland) based on GOPOD format with modifications [20].

To estimate the extent (G_f, mg/mL digest) and the rate of starch hydrolysis (k, min^{-1}) of oat flour during the 2 h *in vitro* small intestinal digestion, the experimental data was fitted into a first-order fractional conversion kinetic model (Equation (4)).

$$G\ (t) = G_f + (G_0 - G_f)\ \times\ \exp^{(-k\ t)} \quad (4)$$

where $G(t)$ is the glucose released (mg/mL digest) at digestion time t and G_0 is the amount of glucose (mg/mL digest) at the start of small intestinal phase (0 min). Three relevant starch digestion parameters k, G_0 and G_f were estimated using R function "nls: Nonlinear Least Squares" (version 4.0.4 2021). Approaches to evaluate the goodness of fit of the fractional conversion model (Equation (4)) to the experimental data obtained in this study include calculation of adjusted R^2, and visual assessment of residual and parity plots. R Studio (version 1.4.1103 2021) was used to execute the R programming language.

2.3. Systematic Investigation to Understand the Mechanism How PEF Affects the Physicochemical Properties of Oat Flour

The overview of the experimental design to understand the impact of PEF treatment application on oat flour properties is presented in Figure 1. Due to the heterogenous nature of oat flour, different oat flour segments were collected after PEF treatment and further analysed.

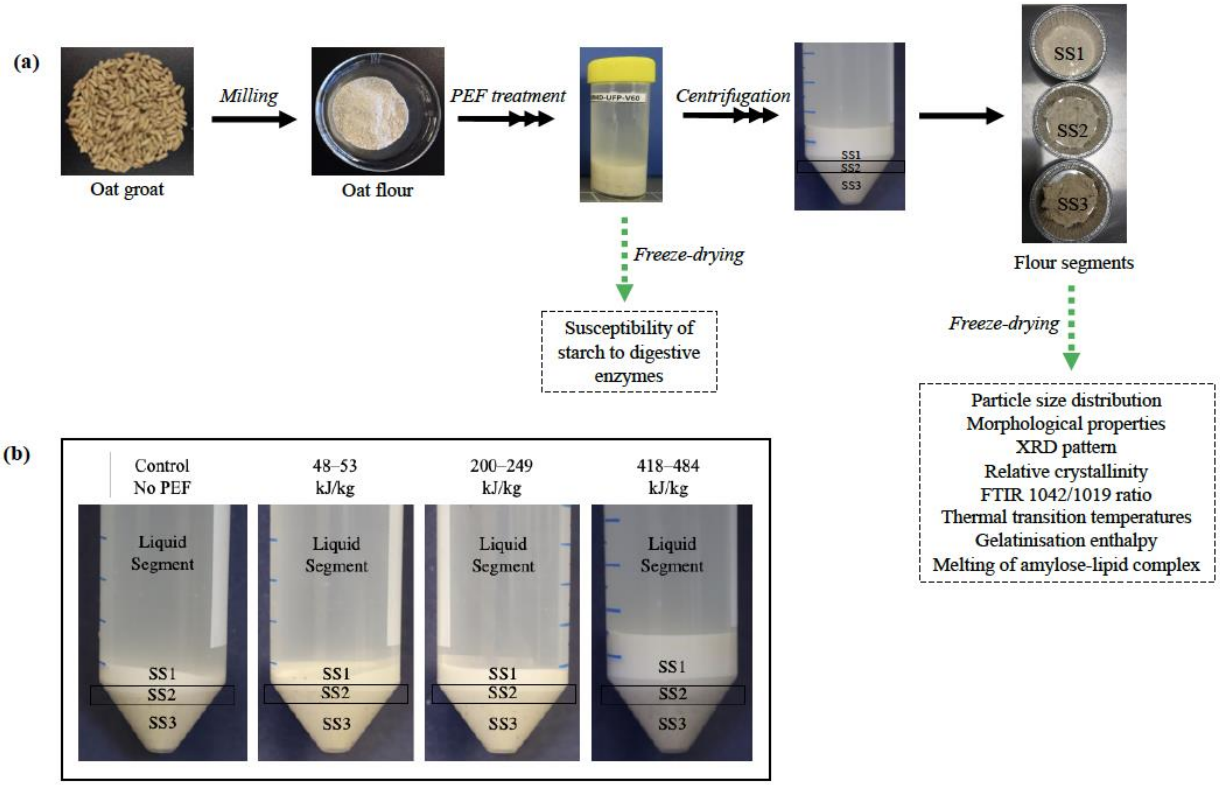

Figure 1. (**a**) Experimental design used in understanding the impact of PEF treatment application on oat flour properties through a systematic evaluation of its flour segments. (**b**) Different flour segments observed after PEF treatment of oat flour. "SS" refers to Solid Segment.

2.3.1. Fractionation of Oat Flour Segments

Non-PEF-treated (Control) and PEF-treated oat flour suspensions were centrifuged at 453× *g* for 10 min at 20 °C (Eppendorf, Hamburg, Germany). The individual solid segments were scraped out carefully. Samples obtained from the topmost solid layer were referred to as "Solid Segment 1 or SS1". Samples obtained from the succeeding layers were referred to as "Solid Segment 2 or SS2" and "Solid Segment 3 or SS3", respectively. SS2 is made up of residual SS1 and portions of SS3. SS1 exhibited a "cloudy" feature, while SS3 displayed a typical "floury" appearance. From the 24–34 independent oat flour PEF treatments, each flour segment was collected and pooled. Pooled segments from each treatment were then freeze-dried and stored at −20 °C until use. The physicochemical profile of the different flour segments collected was determined based on the distribution of its particle size, morphological characteristics, short- and long-range structures of starch, and thermal properties.

2.3.2. Investigation of the Physicochemical Properties of Oat Flour

To elucidate the mechanism of how PEF treatment affects the digestibility properties of starch in oat, the collected solid segments (Section 2.3.1) were evaluated for its particle size distribution, morphological properties, long- and short-range starch ordered structure characteristics, and thermal properties according to Duque et al. [10] and Duque et al. [21]. For particle size distribution, d (0.1), d (0.5), d (0.9) measurements were taken and peak shoulder observations in the volume distribution were noted. For the study of morphological properties, micrographs at different magnifications (1000×, 2000×) were captured and assessed. For the evaluation of the long-range ordered structure of starch, X-ray diffraction patterns were processed and visualised. For the analysis of short-range ordered structure of starch, height of the peaks found around 1042 and 1019 cm^{-1} was used to evaluate the properties of the crystalline and amorphous structures of starch, respectively. Lastly, thermal properties were evaluated based on the onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), gelatinisation enthalpy, and amylose-lipid complex melting.

2.4. Statistical Analyses

All analyses were carried out in triplicates (n = 3) unless otherwise stated. The significant difference between mean values was evaluated using analysis of variance (ANOVA) followed by a post hoc Tukey's Honestly Significant Difference (HSD) test at 95% level of confidence. All the statistical tests were executed using Minitab®® Version 17.2.1 statistical software (Minitab, LLC, State College, PA, USA).

3. Results

3.1. Susceptibility of PEF-Treated Oat Flour to Digestive Enzyme Action

The susceptibility of starch in PEF-treated oat flour (as a whole) towards the action of digestive enzymes was studied using a static *in vitro* simulated human digestion assay. Data presented in Figure 2 shows the amount of glucose released per volume of digest (mg/mL) during the small intestinal phase of oat flour digestion at different sampling times (0, 20, 60, 90, 120 min).

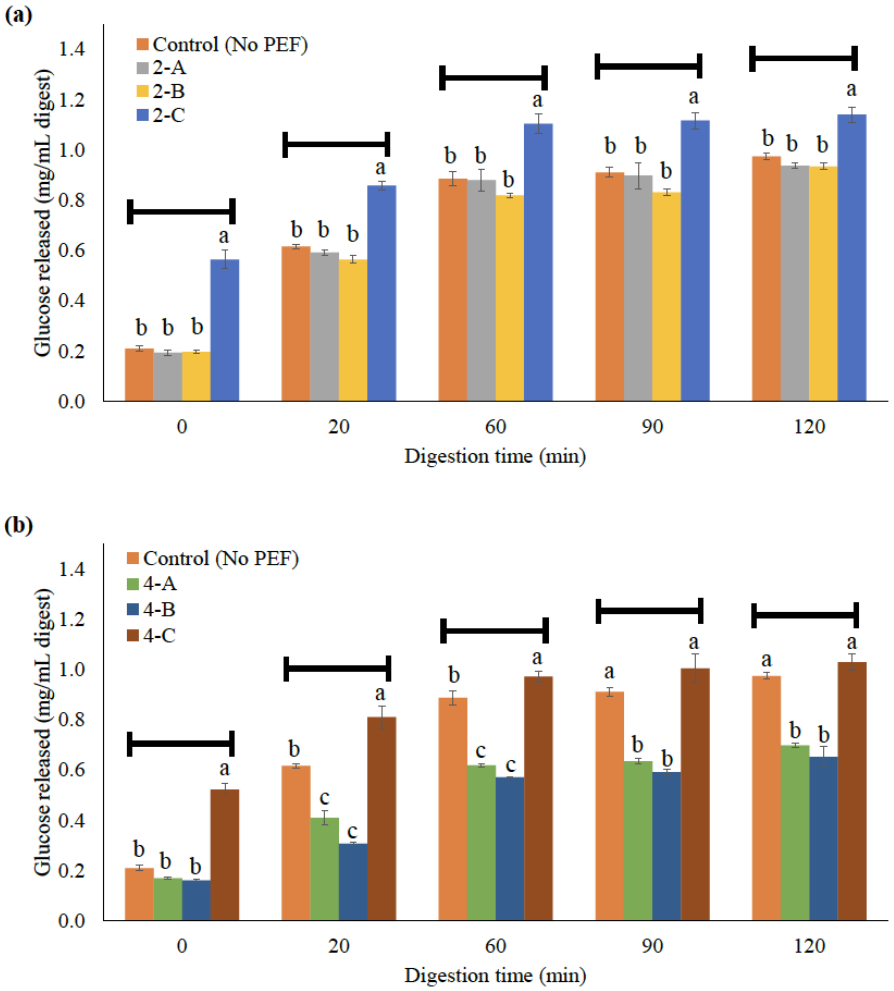

Figure 2. Glucose released (mg/mL digest) during the *in vitro* small intestinal digestion phase of PEF-treated oat flours (n = 3). PEF treatment was applied at electric field strength levels of (**a**) 2.1–2.2 kV/cm and (**b**) 4.2–4.5 kV/cm. Specific energy input ranges from 52 to 53 kJ/kg (A), 216 to 218 kJ/kg (B), and 421 to 438 kJ/kg (C). Bars showing different letters indicate significant difference ($p < 0.05$) between mean values of samples collected from the same time interval (e.g., Control vs. 2-A/B/C or Control vs. 4-A/B/C at time 0). Errors bars were generated using the calculated standard error of mean as positive and negative error values.

Findings from this study showed the impact of varying levels of PEF treatment on the susceptibility of starch to digestive enzymes. Oat flour processed at 421 kJ/kg and 2.2 kV/cm displayed significantly higher overall starch susceptibility (2-C, Figure 2) than Control, as indicated by higher levels of glucose released. Aligned with this finding, the resulting proportion of rapidly digestible starch (RDS) for 2-C sample was the highest compared to Control and other PEF-treated oat flours (Table 1). However, it is worth noting that the estimated rate of *in vitro* starch digestibility (k) for 2-C sample fell within the same range (3.68–3.83 $\times$ 10^{-2} min^{-1}) as the Control. At lower specific energy input application of 52–218 kJ/kg at similar electric field strength level (2.2 kV/cm), PEF treatment of oat flour did not result in any significant modification in its starch susceptibility properties (2-A and 2-B, Figure 2). These samples shared similar proportion of starch fractions with the Control in terms of RDS, slowly digestible starch (SDS) and resistant (RS). However, the %SDS and %RS of these samples were significantly lower and higher than the high-energy treated 2-C sample, respectively (Table 1).

Table 1. Calculated proportions of readily digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) and the estimated kinetic parameters of *in vitro* starch digestibility for PEF-treated oat flours.

PEF Process Parameters		Proportion of Starch Fractions [#]			Estimated Kinetic Parameters of *In Vitro* Starch Digestibility [##]		
		RDS (%)	SDS (%)	RS (%)	G_0 (mg/mL Digest)	G_f (mg/mL Digest)	k ($\times 10^{-2}$ min^{-1})
Control	No PEF	$14.83 \pm 0.41_{\ b}$	$8.50 \pm 1.05_{\ a}$	$77.17 \pm 0.41_{\ bc}$	0.21 ± 0.01	0.96 ± 0.01	3.83 ± 0.25
2.2 kV/cm, 53 kJ/kg	2-A	$14.17 \pm 0.75_{\ b}$	$8.17 \pm 0.75_{\ ab}$	$78.00 \pm 0.63_{\ b}$	0.19 ± 0.01	0.92 ± 0.01	4.01 ± 0.27
2.2 kV/cm, 218 kJ/kg	2-B	$13.33 \pm 1.03_{\ b}$	$8.67 \pm 1.86_{\ a}$	$78.00 \pm 1.10_{\ b}$	0.20 ± 0.02	0.91 ± 0.02	3.46 ± 0.31
2.1 kV/cm, 421 kJ/kg	2-C	$20.00 \pm 0.63_{\ a}$	$6.50 \pm 1.05_{\ ab}$	$73.17 \pm 1.17_{\ d}$	0.56 ± 0.02	1.15 ± 0.02	3.68 ± 0.47
4.5 kV/cm, 52 kJ/kg	4-A	$9.67 \pm 1.03_{\ c}$	$7.00 \pm 0.89_{\ ab}$	$83.50 \pm 0.55_{\ a}$	0.17 ± 0.01	0.70 ± 0.01	3.02 ± 0.27
4.3 kV/cm, 216 kJ/kg	4-B	$7.17 \pm 0.41_{\ d}$	$8.17 \pm 2.04_{\ ab}$	$84.67 \pm 1.51_{\ a}$	0.15 ± 0.02	0.70 ± 0.03	1.99 ± 0.33
4.2 kV/cm, 438 kJ/kg	4-C	$18.83 \pm 2.56_{\ a}$	$5.33 \pm 2.50_{\ b}$	$76.00 \pm 1.55_{\ c}$	0.52 ± 0.02	1.04 ± 0.02	3.25 ± 0.57

[#] Data is presented as mean ± standard deviation ($n = 6$). Means sharing same lowercase letters in subscript within the same column are not significant different ($p > 0.05$) based on post hoc Tukey's HSD test. [##] Data is presented as estimated kinetic parameter ± standard error of the estimated value based on the fractional conversion model (Equation (4)).

Likewise, the application of elevated electric field strength (4.2–4.5 kV/cm) at high levels of specific energy input (421–438 kJ/kg) on oat flour led to increased starch digestibility. Compared to Control, PEF-treated oat flour at 4.2 kV/cm, 438 kJ/kg released significantly higher levels of glucose up to the 60th min during intestinal digestion (4-C, Figure 2). Moreover, the %RDS for 4-C sample was significantly higher than Control while the %SDS was significantly lower than Control (Table 1). It is interesting to note that PEF treatment at lower specific energy input of 48–249 kJ/kg, at similar electric field strength levels (4.3–4.5 kV/cm), resulted in oat flours that are less susceptible to digestive enzymes compared to Control (4-A and 4-B, Figure 2). Lower levels of glucose released were noted for 4-A and 4-B samples from 20 min onwards during simulated human digestion. Moreover, 4-A and 4-B samples displayed significantly lower proportion of RDS and higher proportion of RS compared to Control and other PEF-treated oat flours (Table 1). In particular, the estimated rate of *in vitro* starch digestibility (k) of 4-B sample was the lowest (1.99×10^{-2} min^{-1}) amongst PEF-treated oat flours, in which its starch digestion was 48% slower than the Control. Overall, PEF treatment of oat flour at elevated specific energy input increases the susceptibility of starch to the action of digestive enzymes. On the other hand, increased electric field strength application leads to greater proportion of RS and effectively slowed down the starch digestion rate and thus, lowering the amount of RDS fraction.

3.2. Modifications in the Structural and Thermal Properties of Flour Segments Due to PEF Treatment

3.2.1. Particle Size and Volume Distribution

Table 2 revealed that segments from Control and PEF-treated oat flour exhibited a range of particle sizes, which were dictated by the processing parameters involved during PEF treatment and the location from which samples were collected (i.e., Solid Segment 1/SS1, Solid Segment 2/SS2 or Solid Segment 3/SS3). These segment samples also exhibited either a unimodal or multimodal size distribution with well-defined peaks and shoulders (Figure 3).

Table 2. Particle size distribution of different flour segments collected after PEF treatment of oat flour.

Particle Size (µm)	Control No PEF	2-A 2.2 kV/cm 53 kJ/kg	2-B 2.2 kV/cm 218 kJ/kg	2-C 2.1 kV/cm 421 kJ/kg	4-A 4.5 kV/cm 52 kJ/kg	4-B 4.3 kV/cm 216 kJ/kg	4-C 4.2 kV/cm 438 kJ/kg
Solid Segment 1 (SS1)							
d (0.1)	5–6 $^{c}{}_{B}$	4–5 $^{c}{}_{B}$	5 $^{c}{}_{C}$	14–20 $^{b}{}_{A}$	5–6 $^{c}{}_{B}$	6 $^{c}{}_{B}$	44–45 $^{a}{}_{A}$
d (0.5)	15–18 $^{c}{}_{B}$	14–16 $^{c}{}_{B}$	15–16 $^{c}{}_{B}$	85–102 $^{b}{}_{A}$	18 $^{c}{}_{B}$	15–16 $^{c}{}_{B}$	134–143 $^{a}{}_{A}$
d (0.9)	51–56 $^{c}{}_{B}$	44–58 $^{c}{}_{B}$	54–62 $^{c}{}_{B}$	188–216 $^{b}{}_{A}$	54–66 $^{c}{}_{B}$	54–57 $^{c}{}_{B}$	338–552 $^{a}{}_{A}$
Solid Segment 2 (SS2)							
d (0.1)	5–6 $^{c}{}_{B}$	6–7 $^{bc}{}_{B}$	6–7 $^{c}{}_{B}$	7–8 $^{b}{}_{B}$	5–6 $^{c}{}_{B}$	5 $^{c}{}_{C}$	11–12 $^{a}{}_{B}$
d (0.5)	16–19 $^{b}{}_{B}$	16–21 $^{b}{}_{B}$	17–21 $^{b}{}_{B}$	20–22 $^{b}{}_{B}$	20–21 $^{b}{}_{B}$	18–19 $^{b}{}_{B}$	44–49 $^{a}{}_{B}$
d (0.9)	44–62 $^{b}{}_{B}$	40–66 $^{b}{}_{B}$	52–88 $^{b}{}_{B}$	66–98 $^{b}{}_{B}$	69–126 $^{b}{}_{B}$	121–167 $^{a}{}_{B}$	164–185 $^{a}{}_{B}$
Solid Segment 3 (SS3)							
d (0.1)	7–8 $^{a}{}_{A}$	8–9 $^{a}{}_{A}$	8 $^{a}{}_{A}$	8 $^{a}{}_{B}$	8 $^{a}{}_{A}$	8 $^{a}{}_{A}$	8–9 $^{a}{}_{C}$
d (0.5)	31–36 $^{a}{}_{A}$	31–33 $^{a}{}_{A}$	32–36 $^{a}{}_{A}$	26–32 $^{a}{}_{B}$	33–35 $^{a}{}_{A}$	33–43 $^{a}{}_{A}$	32–34 $^{a}{}_{C}$
d (0.9)	247–336 $^{ab}{}_{A}$	262–284 $^{ab}{}_{A}$	351–426 $^{a}{}_{A}$	154–249 $^{b}{}_{A}$	343–378 $^{a}{}_{A}$	327–468 $^{a}{}_{A}$	297–337 $^{ab}{}_{AB}$

Particle size data are presented as a range of values from test replicates (n = 3, collected and pooled from 24–34 independent samples). Means sharing the same lowercase letters in superscript, within the same row, indicate no significant difference ($p > 0.05$) between treatments (Control vs. 2-A vs. 2-B vs. 2-C vs. 4-A vs. 4-B vs. 4-C) based on post hoc Tukey's HSD test. Means sharing the same uppercase letters in subscript, within the same column, indicate no significant difference ($p > 0.05$) between mean values of different flour segments within the same test parameter based on post hoc Tukey's HSD test (e.g., d (0.1)–SS1 vs. SS2 vs. SS3).

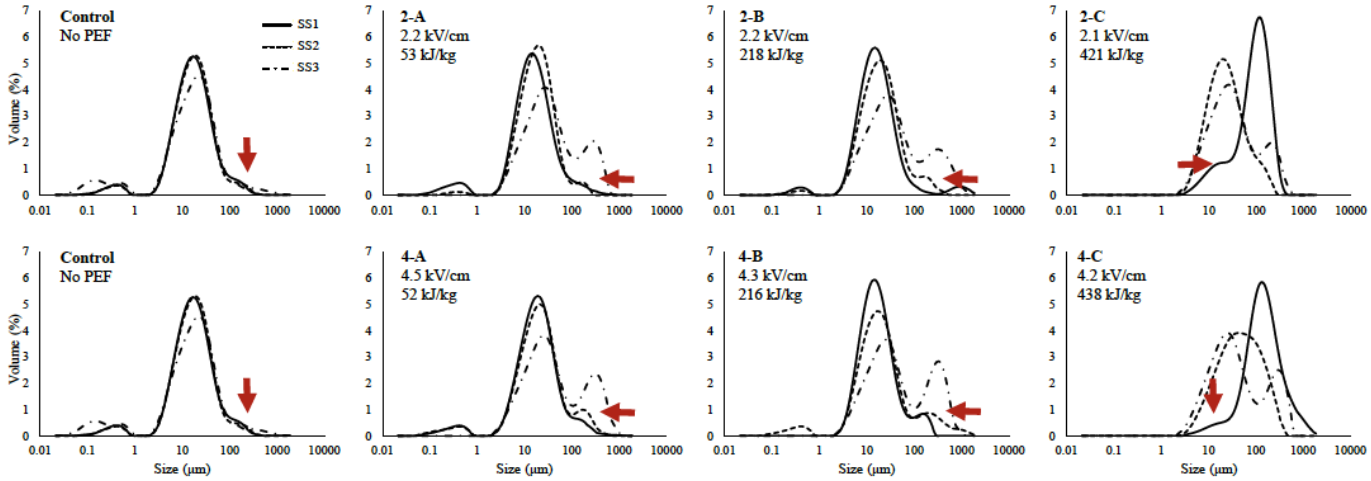

Figure 3. Volume distribution of particles in selected representative flour segments (Solid Segment 1/SS1, Solid Segment 2/SS2, and Solid Segment 3/SS3) obtained from PEF-treated oat flour. Red arrows on the figure points out to the shoulders (indicate particle aggregation) observed on the distribution curve of the samples.

Evaluation of flour segment data showed that SS1 from PEF-treated oat flour at 421–438 kJ/kg exhibited the largest particle size (2-C and 4-C, Table 2). SS1 also displayed the greatest extent of modification in terms of overall particle size. More specifically, SS1 samples demonstrated up to an eight-fold increase in particle size after oat flour PEF treatment at 421–438 kJ/kg (2-C and 4-C, Table 2). A further significant increase in size was also detected when the electric field strength was increased from 2.1 to 4.2 kV/cm (2-C and 4-C, Table 2). On the other hand, only a two- to three-fold increase in size was displayed by its corresponding SS2 samples (4-C, Table 2). In contrast, the overall impact of oat flour PEF treatment to SS3 samples was not evident based on the comparable size profiles of the samples (Table 2).

When examining the three separate oat flour segments within a treatment, it was observed that samples from SS3 generally displayed the biggest particle sizes compared to SS1 and SS2. However, SS3 samples from 2-C and 4-C (421–438 kJ/kg, Table 2) are of exception. It is interesting that SS3 from 2-C and 4-C were the smallest, while SS1 samples were the biggest within the treatment (Table 2). The largest mean particle size of 2-C and 4-C SS1 samples were found to be 85–102 µm and 134–143 µm, respectively ($d_{0.5}$, 2-C and 4-C, Table 2). On the other hand, the corresponding SS3 samples from 2-C and 4-C only recorded 26–32 µm and 32–34 µm, respectively ($d_{0.5}$ for 2-C and 4-C, Table 2).

Apparent differences in the volume distribution of particles in segments obtained from PEF-treated oat flour were also observed (Figure 3). A high volume of big particles (>100 µm) was exhibited by SS1 collected from PEF-treated oat flour at 421–438 kJ/kg compared to Control (solid line, 2-C and 4-C, Figure 3). Similarly, greater volumes of big particles (>100 µm) were apparent for SS3 samples obtained from PEF-treated oat flour (2-A/B/C and 4-A/B/C) compared to its corresponding Control (dash dotted line, Figure 3).

3.2.2. Morphological Characteristics

SEM micrographs were taken to observe the starch granule morphology of SS1 (Figure 4) and SS3 (Figure 5) samples. The size, shape, and starch granule integrity of the different segment samples were examined. SS1 from Control and PEF-treated oat flour at 52–218 kJ/kg displayed polygonal and intact starch granules (yellow arrows, Figure 4). In contrast, SS1 from PEF-treated oat flour at 421–438 kJ/kg exhibited starch granule damage. This is evident based on the observed deformation on the shape of starch granules and the loss of granule integrity (red arrows, Figure 4). Interestingly, SS3 from the same PEF-treated oat flour (421–438 kJ/kg) did not manifest severe granule damage but rather showed a seemingly unaffected granules of starches (yellow arrows, 2-C and 4-C, Figure 5).

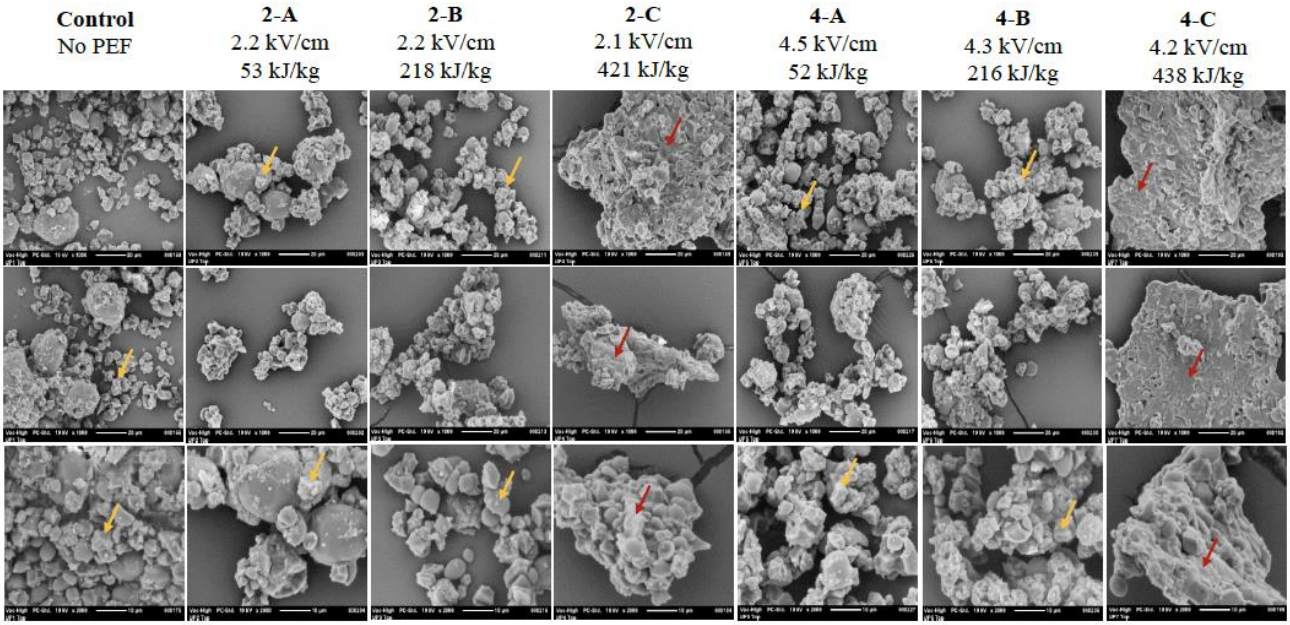

Figure 4. Scanning electron micrographs of Solid Segment (SS1) from PEF-treated oat flour. SEM images are presented at 1000× (rows 1 and 2) and 2000× (row 3) magnifications. Yellow and red arrows point to intact and damaged starch granules, respectively.

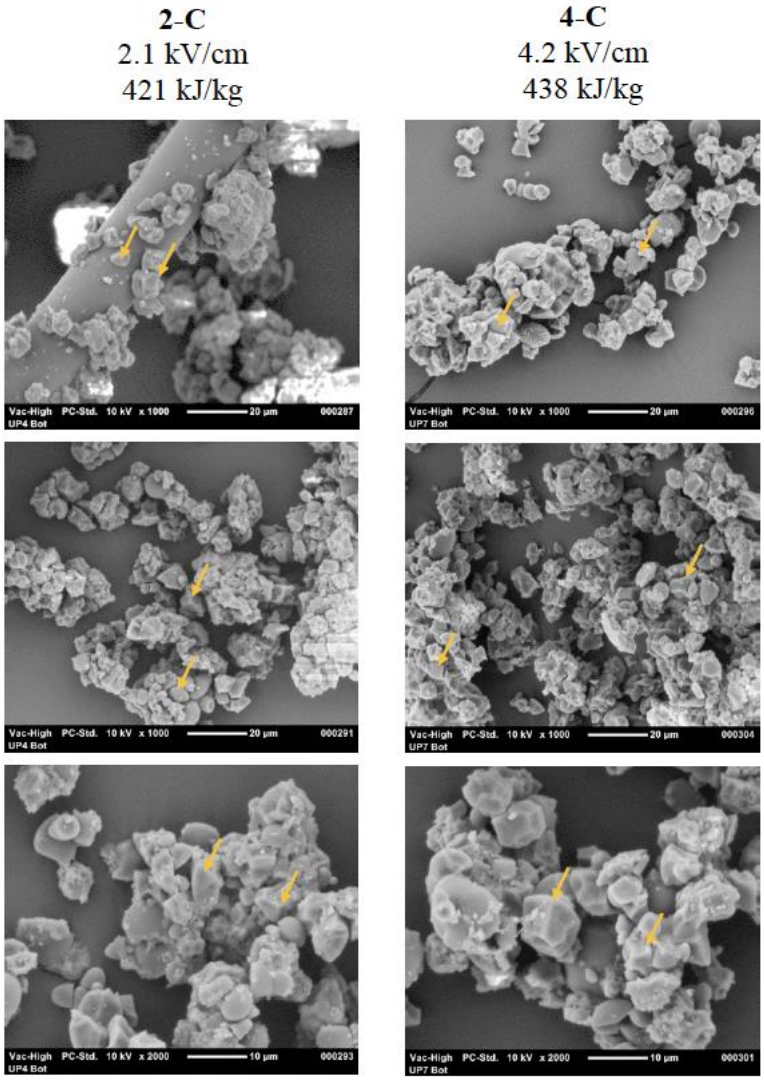

Figure 5. Scanning electron micrographs of selected Solid Segment 3 (SS3) samples from PEF-treated oat flour. SEM images are presented at 1000× (rows 1 and 2) and 2000× (row 3) magnifications. Yellow arrows point to the intact/undeformed granules of starch indicating that PEF treatment of oat flour at 2.1 kV/cm, 421 kJ/kg (2-C) and 4.2 kV/cm, 438 kJ/kg (4-C) did not cause significant impact on starch granules comprising SS3 samples.

Extensive aggregation was evident in SS1 segments obtained from PEF-treated oat flour at 421–438 kJ/kg (2-C and 4-C, Figure 4). On the contrary, the SS3 segments collected from the same PEF-treated oat flour displayed no indication of severe aggregation (2-C and 4-C, Figure 5). These data also support the recorded larger particle size of samples that was presented in the previous section (Section 3.2.1).

3.2.3. Starch Long-Range Ordered Structure of Flour Segments

Modifications in the long-range ordered structure of flour segments were studied based on the alterations observed in X-ray diffractograms (XRD). XRD data of each flour segment sample obtained from Control and selected PEF-treated oat flour are presented in Figure 6. SS1, SS2, and SS3 from Control displayed the typical A-type pattern expected of cereal starches. More specifically, peaks at 15.1°, 23°, doublet peak at 17.1° and 18.2°, and a small peak at 20° were recorded. Similarly, SS1, SS2, and SS3 obtained from PEF-treated oat flour processed at 52–218 kJ/kg displayed comparable XRD patterns with Control (data not shown). On the contrary, XRD patterns of SS1 collected from PEF-treated oat flour at 421–438 kJ/kg (2-C and 4-C) displayed weakened or loss of some peaks in comparison

to Control. In particular, peaks at 15.1°, 17.1°, 18.2°, and 23° (2θ) appeared to have been altered after PEF treatment of oat flour (Green dots, Figure 6). Moreover, a new peak at 13° (2θ) became apparent for these same samples after PEF treatment of oat flour at 421–438 kJ/kg (Red dots, Figure 6).

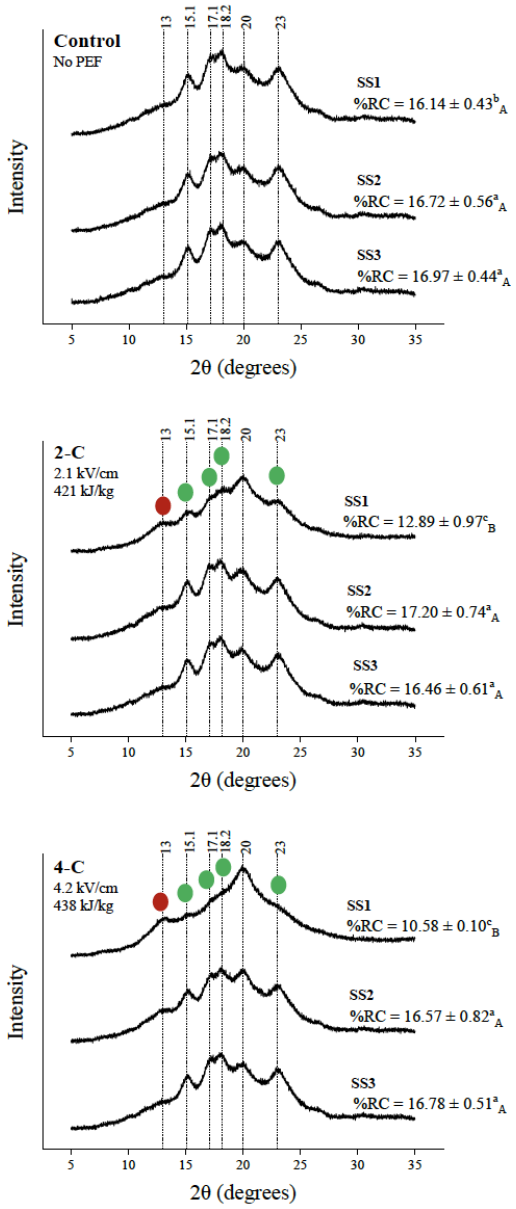

Figure 6. X-ray diffractograms and percent relative crystallinity (%RC) of flour segments collected from PEF-treated oat flour at 418–484 kJ/kg. The %RC values are displayed as mean ± standard error of mean (n = 3, collected and pooled from 24–34 independent samples). Means sharing the same lowercase letters in superscript indicate no significant difference ($p < 0.05$) between treatments obtained from the same segment (e.g., 2-C, SS1 vs. SS2 vs. SS3). Means sharing the same uppercase letters in subscript indicate no significant difference ($p < 0.05$) between flour segments obtained from different treatments (e.g., SS1, Control vs. 2-C vs. 4-C). Green dots direct to the modification of specific peaks due to PEF treatment. Red dots highlight the new peaks observed in flour segments collected from PEF-treated oat flour.

Based on the XRD pattern, the percent relative crystallinity of each segment was determined to evaluate any alterations in its crystallinity properties as a consequence of oat flour PEF treatment. Considering all the segments evaluated in this study, the percent

relative crystallinity of samples ranged from 10.58–19.17%. SS1 collected from PEF-treated oat flour at 52–218 kJ/kg did not display significant change in percent relative crystallinity in comparison to Control (data not shown). However, SS1 from PEF-treated oat flour at 421–438 kJ/kg revealed a 20–34% reduction in percent relative crystallinity in comparison to SS1 from Control (%RC, Control, 2-C, and 4-C, Figure 6).

3.2.4. Starch Short-Range Ordered Structure

FTIR spectroscopy was used to evaluate the short-range molecular order of starch in segment samples, which were collected from PEF-treated oat flour. The data gathered was useful in estimating the properties of the double helices structure in starch [22]. Spectral deconvolution and peak analyses around 1019 and 1042 cm^{-1} were found to be valuable in obtaining characteristic data on the amorphous and crystalline structures of starch, respectively.

All flour segment samples displayed corresponding crystalline and amorphous peaks on the regions around 1039–1050 cm^{-1} and 1016–1027 cm^{-1}, respectively, when oat flour was treated at low (~2 kV/cm) and high (~4 kV/cm) intensity electric field strengths (Table 3). Relevant FTIR peaks for most flour segments were found to be situated around 1042 and 1019 cm^{-1}. However, SS1 obtained from PEF-treated oat flour at 4.2 kV/cm, 438 kJ/kg revealed a distinct shift in the positions of its relevant peaks (4-C, Table 3). FTIR peaks associated with its crystalline and amorphous structures were identified to be positioned at 1050 cm^{-1} and 1027 cm^{-1}, respectively. The same sample (4-C, SS1) also displayed the lowest recorded wavelength ratio in terms of peak height values amongst the samples tested (Table 3). On the other hand, the rest of the segment samples did not manifest significant difference in its FTIR ratio compared to Control.

Table 3. Ratio of the crystalline to amorphous regions of starch in three different flour segments that were collected after PEF treatment of oat flour.

Treatment	Flour Segment	Peak Positions (cm^{-1})	Peak Height Ratio (1042/1019 *)
Control No PEF	SS1	1042, 1019	$1.08 \pm 0.10^{ab}{}_{A}$
	SS2	1040, 1019	$1.06 \pm 0.22^{a}{}_{A}$
	SS3	1042, 1019	$1.15 \pm 0.32^{a}{}_{A}$
2-A 2.2 kV/cm 53 kJ/kg	SS1	1042, 1020	$1.42 \pm 0.43^{ab}{}_{A}$
	SS2	1042, 1020	$1.20 \pm 0.18^{a}{}_{A}$
	SS3	1040, 1019	$1.58 \pm 0.15^{a}{}_{A}$
2-B 2.2 kV/cm 218 kJ/kg	SS1	1043, 1020	$1.06 \pm 0.13^{ab}{}_{A}$
	SS2	1042, 1020	$0.95 \pm 0.07^{a}{}_{A}$
	SS3	1042, 1022	$1.04 \pm 0.17^{a}{}_{A}$
2-C 2.1 kV/cm 421 kJ/kg	SS1	1041, 1016	$0.57 \pm 0.03^{ab}{}_{B}$
	SS2	1041, 1019	$1.40 \pm 0.13^{a}{}_{A}$
	SS3	1042, 1020	$0.93 \pm 0.15^{a}{}_{AB}$
4-A 4.5 kV/cm 52 kJ/kg	SS1	1041, 1019	$2.00 \pm 0.52^{a}{}_{A}$
	SS2	1041, 1018	$1.20 \pm 0.30^{a}{}_{A}$
	SS3	1041, 1019	$1.36 \pm 0.12^{a}{}_{A}$
4-B 4.3 kV/cm 216 kJ/kg	SS1	1039, 1019	$1.96 \pm 0.40^{a}{}_{A}$
	SS2	1042, 1020	$1.24 \pm 0.07^{a}{}_{A}$
	SS3	1042, 1020	$1.14 \pm 0.10^{a}{}_{A}$
4-C 4.2 kV/cm 438 kJ/kg	SS1	1050, 1027	$0.03 \pm 0.02^{b}{}_{B}$
	SS2	1039, 1019	$1.80 \pm 0.43^{a}{}_{A}$
	SS3	1044, 1019	$1.00 \pm 0.08^{a}{}_{AB}$

Peaks around 1039–1050 and 1016–1027 represent the crystalline region and amorphous region of starch, respectively. * Wavenumbers vary depending on the exact peak position in the FTIR spectra. Means sharing the same lowercase letters in superscript indicate no significant difference ($p > 0.05$) between treatments from the same flour segment based on post hoc Tukey's HSD test (SS1–Control vs. 2-A vs. 2-B vs. 2-C vs. 4-A vs. 4-B vs. 4-C). Means sharing the same uppercase letters in subscript indicate no significant difference ($p > 0.05$) between mean values of different flour segments within a treatment based on post hoc Tukey's HSD test (Control–SS1 vs. SS2 vs. SS3).

Evaluation of three separate segment samples within a treatment showed variation in peak height ratio, particularly after PEF treatment of oat flour at high specific energy input level of 421–438 kJ/kg. Looking closely within the treatment, SS1 from PEF-treated oat flour at 421–438 kJ/kg exhibited the lowest peak height ratio compared to SS2 and SS3 (2-C and 4-C, Table 3). On the other hand, segment samples (SS1, SS2, and SS3) within Control and other PEF-treated oat flour did not manifest noteworthy variation of its 1042/1019 peak height ratio.

3.2.5. Thermal Properties

DSC analysis of each oat flour segment allowed the evaluation of its thermal properties. Data gathered revealed the impact of oat flour PEF treatment on transition temperatures and enthalpies of the different flour segments. Table 4 shows that the thermal properties of sample segments collected from PEF-treated oat flour varied according to the intensity of the processing applied. Modifications in transition temperatures (onset temperature or T_o, peak temperature or T_p, and conclusion temperature or T_c) and enthalpy (ΔH) were observed (Table 4). These specific parameters provided insight towards properties related to starch crystallite melting and starch gelatinisation, as well as the melting of amylose-lipid complex, which are further discussed in Sections 3.2.5.1 and 3.2.5.2, respectively.

Table 4. Thermal properties of three separate flour segments that were collected after PEF treatment of oat flour.

DSC Parameters	Control No PEF	2-A 2.2 kV/cm 53 kJ/kg	2-B 2.2 kV/cm 218 kJ/kg	2-C 2.1 kV/cm 421 kJ/kg	4-A 4.5 kV/cm 52 kJ/kg	4-B 4.3 kV/cm 216 kJ/kg	4-C 4.2 kV/cm 438 kJ/kg
Solid Segment 1 (SS1)							
T_o^1 (°C)	$51.64 \pm 0.06^{e}{}_{B}$	$52.21 \pm 0.06^{de}{}_{A}$	$52.95 \pm 0.19^{cde}{}_{A}$	$56.10 \pm 0.16^{ab}{}_{A}$	$54.22 \pm 0.05^{bcd}{}_{A}$	$54.64 \pm 0.06^{bc}{}_{B}$	$56.81 \pm 1.12^{a}{}_{A}$
T_p^1 (°C)	$57.23 \pm 0.04^{d}{}_{B}$	$57.71 \pm 0.04^{d}{}_{A}$	$58.04 \pm 0.15^{cd}{}_{A}$	$61.21 \pm 0.38^{b}{}_{A}$	$58.78 \pm 0.07^{c}{}_{A}$	$59.03 \pm 0.06^{c}{}_{B}$	$62.57 \pm 0.40^{a}{}_{A}$
T_c^1 (°C)	$63.10 \pm 0.09^{c}{}_{B}$	$63.95 \pm 0.20^{c}{}_{A}$	$63.35 \pm 0.31^{c}{}_{A}$	$70.23 \pm 1.57^{b}{}_{A}$	$63.89 \pm 0.12^{c}{}_{A}$	$64.28 \pm 0.07^{c}{}_{A}$	$74.40 \pm 1.19^{a}{}_{A}$
ΔH^1 (J/g)	$6.63 \pm 0.17^{b}{}_{A}$	$6.80 \pm 0.09^{b}{}_{B}$	$7.55 \pm 0.17^{a}{}_{A}$	$3.03 \pm 0.16^{c}{}_{B}$	$7.81 \pm 0.06^{a}{}_{A}$	$7.71 \pm 0.16^{a}{}_{AB}$	$0.59 \pm 0.01^{d}{}_{C}$
T_o^2 (°C)	$84.74 \pm 0.76^{b}{}_{A}$	$85.40 \pm 0.27^{b}{}_{A}$	$84.82 \pm 0.24^{b}{}_{A}$	$86.81 \pm 0.29^{b}{}_{A}$	$84.70 \pm 0.27^{b}{}_{A}$	$85.01 \pm 0.71^{b}{}_{A}$	$97.45 \pm 0.61^{a}{}_{A}$
T_p^2 (°C)	$94.64 \pm 0.28^{b}{}_{A}$	$94.44 \pm 0.36^{b}{}_{A}$	$93.98 \pm 0.31^{b}{}_{A}$	$95.43 \pm 0.31^{b}{}_{A}$	$94.57 \pm 0.69^{b}{}_{A}$	$94.96 \pm 0.16^{b}{}_{A}$	$99.68 \pm 0.33^{a}{}_{A}$
T_c^2 (°C)	$103.36 \pm 0.26^{ab}{}_{A}$	$103.82 \pm 0.22^{ab}{}_{A}$	$103.06 \pm 0.08^{b}{}_{A}$	$105.20 \pm 0.29^{a}{}_{A}$	$103.35 \pm 0.81^{ab}{}_{A}$	$103.83 \pm 0.04^{ab}{}_{A}$	$102.58 \pm 0.48^{b}{}_{A}$
ΔH^2 (J/g)	$1.73 \pm 0.05^{b}{}_{A}$	$1.76 \pm 0.08^{b}{}_{A}$	$1.77 \pm 0.08^{b}{}_{A}$	$1.92 \pm 0.02^{b}{}_{A}$	$1.75 \pm 0.04^{b}{}_{A}$	$1.72 \pm 0.09^{b}{}_{A}$	$3.38 \pm 0.07^{a}{}_{A}$
Solid Segment 2 (SS2)							
T_o^1 (°C)	$51.82 \pm 0.15^{c}{}_{AB}$	$52.12 \pm 0.44^{bc}{}_{A}$	$52.32 \pm 0.57^{abc}{}_{A}$	$53.48 \pm 0.81^{abc}{}_{B}$	$55.01 \pm 0.31^{a}{}_{A}$	$54.68 \pm 0.08^{ab}{}_{B}$	$54.22 \pm 0.98^{abc}{}_{A}$
T_p^1 (°C)	$57.05 \pm 0.23^{b}{}_{B}$	$57.71 \pm 0.34^{ab}{}_{A}$	$57.59 \pm 0.26^{ab}{}_{A}$	$58.65 \pm 0.58^{ab}{}_{B}$	$59.47 \pm 0.28^{a}{}_{A}$	$58.96 \pm 0.09^{ab}{}_{B}$	$58.85 \pm 0.75^{ab}{}_{B}$
T_c^1 (°C)	$63.36 \pm 0.23^{ab}{}_{AB}$	$62.94 \pm 0.26^{b}{}_{B}$	$63.00 \pm 0.16^{b}{}_{A}$	$63.94 \pm 0.36^{ab}{}_{B}$	$64.40 \pm 0.49^{ab}{}_{A}$	$64.04 \pm 0.26^{ab}{}_{A}$	$64.76 \pm 0.45^{a}{}_{B}$
ΔH^1 (J/g)	$6.66 \pm 0.48^{b}{}_{A}$	$7.42 \pm 0.14^{ab}{}_{AB}$	$7.62 \pm 0.22^{ab}{}_{A}$	$6.62 \pm 0.22^{b}{}_{A}$	$7.58 \pm 0.34^{ab}{}_{A}$	$8.05 \pm 0.11^{a}{}_{A}$	$5.13 \pm 0.20^{c}{}_{B}$
T_o^2 (°C)	$86.44 \pm 1.24^{a}{}_{A}$	$84.38 \pm 0.17^{a}{}_{A}$	$84.71 \pm 0.37^{a}{}_{A}$	$85.78 \pm 0.05^{a}{}_{AB}$	$85.00 \pm 0.55^{a}{}_{A}$	$84.64 \pm 0.25^{a}{}_{A}$	$85.28 \pm 0.51^{a}{}_{B}$
T_p^2 (°C)	$95.37 \pm 0.12^{a}{}_{A}$	$93.91 \pm 0.50^{a}{}_{A}$	$94.63 \pm 0.44^{a}{}_{A}$	$95.12 \pm 0.20^{a}{}_{A}$	$95.22 \pm 0.65^{a}{}_{A}$	$95.34 \pm 0.37^{a}{}_{A}$	$95.20 \pm 0.33^{a}{}_{B}$
T_c^2 (°C)	$103.06 \pm 0.47^{a}{}_{A}$	$102.41 \pm 0.77^{a}{}_{A}$	$103.14 \pm 0.34^{a}{}_{A}$	$102.91 \pm 0.78^{a}{}_{B}$	$102.98 \pm 0.66^{a}{}_{A}$	$104.65 \pm 0.21^{a}{}_{A}$	$104.00 \pm 0.23^{a}{}_{A}$
ΔH^2 (J/g)	$1.52 \pm 0.02^{bc}{}_{B}$	$1.48 \pm 0.03^{bc}{}_{B}$	$1.91 \pm 0.10^{a}{}_{A}$	$1.92 \pm 0.06^{a}{}_{A}$	$1.40 \pm 0.04^{c}{}_{B}$	$1.71 \pm 0.00^{ab}{}_{A}$	$1.95 \pm 0.07^{a}{}_{B}$
Solid Segment 3 (SS3)							
T_o^1 (°C)	$52.20 \pm 0.13^{b}{}_{A}$	$52.98 \pm 0.01^{b}{}_{A}$	$52.96 \pm 0.54^{b}{}_{A}$	$54.67 \pm 0.10^{a}{}_{AB}$	$55.06 \pm 0.25^{a}{}_{A}$	$55.11 \pm 0.03^{a}{}_{A}$	$55.74 \pm 0.05^{a}{}_{A}$
T_p^1 (°C)	$57.86 \pm 0.10^{c}{}_{A}$	$58.26 \pm 0.08^{bc}{}_{A}$	$58.24 \pm 0.50^{c}{}_{A}$	$59.35 \pm 0.16^{ab}{}_{B}$	$59.62 \pm 0.24^{a}{}_{A}$	$59.47 \pm 0.09^{a}{}_{A}$	$60.04 \pm 0.12^{a}{}_{B}$
T_c^1 (°C)	$63.90 \pm 0.17^{b}{}_{A}$	$63.75 \pm 0.05^{bc}{}_{AB}$	$64.19 \pm 0.34^{ab}{}_{A}$	$64.16 \pm 0.19^{ab}{}_{B}$	$64.97 \pm 0.26^{a}{}_{A}$	$64.22 \pm 0.21^{ab}{}_{A}$	$65.01 \pm 0.16^{a}{}_{B}$
ΔH^1 (J/g)	$6.75 \pm 0.11^{b}{}_{A}$	$7.46 \pm 0.20^{ab}{}_{A}$	$7.15 \pm 0.20^{ab}{}_{A}$	$6.97 \pm 0.15^{ab}{}_{A}$	$7.63 \pm 0.32^{a}{}_{A}$	$7.36 \pm 0.06^{ab}{}_{B}$	$7.43 \pm 0.12^{ab}{}_{A}$
T_o^2 (°C)	$84.98 \pm 0.77^{a}{}_{A}$	$84.58 \pm 0.41^{a}{}_{A}$	$84.49 \pm 0.35^{a}{}_{A}$	$85.26 \pm 0.32^{a}{}_{B}$	$86.18 \pm 0.75^{a}{}_{A}$	$86.25 \pm 0.29^{a}{}_{A}$	$84.88 \pm 0.21^{a}{}_{B}$
T_p^2 (°C)	$95.38 \pm 0.22^{a}{}_{A}$	$94.64 \pm 0.07^{a}{}_{A}$	$95.07 \pm 0.20^{a}{}_{A}$	$95.62 \pm 0.24^{a}{}_{A}$	$95.67 \pm 0.37^{a}{}_{A}$	$95.52 \pm 0.49^{a}{}_{A}$	$94.93 \pm 0.25^{a}{}_{B}$
T_c^2 (°C)	$103.05 \pm 0.07^{a}{}_{A}$	$103.39 \pm 0.26^{a}{}_{A}$	$102.73 \pm 0.16^{a}{}_{A}$	$103.35 \pm 0.18^{a}{}_{AB}$	$103.77 \pm 0.32^{a}{}_{A}$	$103.65 \pm 0.45^{a}{}_{A}$	$103.95 \pm 0.35^{a}{}_{A}$
ΔH^2 (J/g)	$1.43 \pm 0.05^{a}{}_{B}$	$1.50 \pm 0.06^{a}{}_{AB}$	$1.34 \pm 0.01^{a}{}_{B}$	$1.29 \pm 0.05^{a}{}_{B}$	$1.41 \pm 0.07^{a}{}_{B}$	$1.53 \pm 0.05^{a}{}_{A}$	$1.53 \pm 0.06^{a}{}_{C}$

DSC data are presented as mean ± standard error of mean (n = 3, collected and pooled from 24–34 independent samples). T_o–onset temperature; T_p–peak temperature; T_c–conclusion temperature; ΔH–enthalpy. [1] Low-temperature endotherm: describes the starch crystallite melting and starch gelatinisation. [2] High-temperature endotherm: describes the amylose-lipid complex melting. Means sharing the same lowercase letters in superscript, within the same row, indicates no significant difference ($p > 0.05$) between treatments (Control vs. 2-A vs. 2-B vs. 2-C vs. 4-A vs. 4-B vs. 4-C) based on post hoc Tukey's HSD test. Means sharing the same uppercase letters in subscript, within the same column, indicates no significant difference ($p > 0.05$) between mean values of different flour segments under the same test parameter based on post hoc Tukey's HSD test (e.g., T_c^1—SS1 vs. SS2 vs. SS3).

3.2.5.1. Melting of Starch Crystallites and Starch Gelatinisation Behaviour

- Transition temperatures

Data evaluation on the impact of PEF processing intensities applied to oat flour showed that with increased specific energy input and electric field strength, there was a significant shift towards higher transition temperatures. SS1 from Control exhibited a transition temperature range of 51.64–63.10 °C (T_o^1, T_p^1, T_c^1 of Control, Table 4). With PEF treatment of oat flour at 52–216 kJ/kg and 4.3–4.5 kV/cm, there was an observed notable increase in onset (54.22–54.64 °C) and peak temperatures (58.78–59.03 °C) for the SS1 flour segment (T_o^1, T_p^1 of 4-A and 4-B, Table 4). Moreover, further increase in the specific energy input applied (421–438 kJ/kg) demonstrated a shift to higher overall transition temperature range of 56.10–74.40 °C for SS1 (T_o^1, T_p^1, T_c^1 of 2-C and 4-C, Table 4).

SS2 sample properties were also found to be affected by the intensity of PEF treatment applied on oat flour. More specifically, higher onset (55.01 vs. 51.82 °C) and peak temperatures (59.47 vs. 57.05 °C) were exhibited by SS2 from PEF-treated oat flour at 4.5 kV/cm, 52 kJ/kg compared to Control (refer to T_o^1, T_p^1, 4-A for Table 4). Similar observation on higher onset temperature (54.68 vs. 51.82 °C) was also manifested by SS2 from PEF-treated oat flour at 4.3 kV/cm, 216 kJ/kg compared to Control (refer to T_o^1 for 4-B, Table 4).

Contrary to what was observed in SS1 and SS2 samples, SS3 collected from PEF-treated oat flour at 2.2 kV/cm, 53–218 kJ/kg did not manifest significant changes in its transition temperatures compared to Control (T_o^1, T_p^1, T_c^1, ΔH^1 of Control, 2-A and 2-B, Table 4). However, PEF treatment of oat flour at higher field strength (4.2–4.5 kV/cm, 4-A, 4-B, 4-C) and specific energy input (421–438 kJ/kg, 2-C and 4-C) led to increased transition temperatures of its SS3 segment, compared to Control (Table 4).

- Gelatinisation enthalpy

SS1 collected from PEF-treated oat flour at specific energy input of 421–438 kJ/kg demonstrated lower gelatinisation enthalpy than the Control (ΔH^1 of Control, 2-C and 4-C, Table 4). A 91% decrease in the gelatinisation enthalpy was exhibited by SS1 from 2-C and 4-C in comparison to Control. It was also evident that with increased electric field strength (2.1 vs. 4.2 kV/cm) application, at the same level of specific energy input applied, (421–438 kJ/kg) a more drastic change in the gelatinisation enthalpy was noted. With respect to the Control, a 54% and 91% reduction was recorded for SS1 obtained from PEF-treated oat flour at 2.1 kV/cm and 4.2 kV/cm, respectively (ΔH^1 of 2-C and 4-C, Table 4). On the other hand, it is interesting to note that oat flour PEF treatment at a lower specific energy input levels of 52–218 kJ/kg produced SS1 samples with higher gelatinisation enthalpy compared to Control (refer to ΔH^1 for 2-B, 4-A, 4-B, Table 4). A 14–18% increase in the recorded gelatinisation enthalpy was observed for SS1 from PEF-treated oat flour at 52–218 kJ/kg.

SS2 from PEF-treated oat flour at 2.1–2.2 kV/cm, 53–421 kJ/kg (2-A, 2-B, and 2-C) did not display significant changes in its gelatinisation enthalpy compared to Control (ΔH^1, Table 4). However, oat flour PEF treatment at 4.3 kV/cm, 216 kJ/kg produced an SS2 sample with substantially higher gelatinisation enthalpy than Control (8.05 vs. 6.66 J/g) (ΔH^1 of 4-B, Table 4). On the contrary, oat flour PEF treatment at 4.2 kV/cm, 438 kJ/kg produced an SS2 sample with a significantly lower gelatinisation enthalpy than Control (5.13 vs. 6.66 J/g, ΔH^1 of 4-C, Table 4).

SS3 from Control and PEF-treated oat flour did not show significant difference in gelatinisation enthalpy except for SS3 from PEF-treated oat flour at 4.5 kV/cm, 52 kJ/kg (ΔH^1 of 4-A, Table 4). A 9% increase in the required energy needed to melt starch crystallites was exhibited by SS3 from 4-A compared to Control.

Overall comparison of the different flour segments showed that SS1 from PEF-treated oat flour at 421–438 kJ/kg (2-C and 4-C) generally displayed the highest transition temperatures compared to SS2 and SS3. Conversely, SS1 also exhibited the lowest gelatinisation enthalpy amongst samples within a treatment.

3.2.5.2. Melting of the Amylose-Lipid Complex

The specific thermal property parameters associated with amylose-lipid complex melting are represented by T_o^2 (onset temperature), T_p^2 (peak temperature), T_c^2 (conclusion temperature), and ΔH^2 (enthalpy) in Table 4. In comparison to Control, all three segments from PEF-treated oat flour did not manifest any significant change in its onset, peak, and conclusion temperatures, except for SS1 from PEF-treated oat flour at 4.2 kV/cm, 438 kJ/kg (T_o^2, T_p^2 of 4-C, Table 4). SS1 from 4-C also displayed a substantial 13 °C increase in onset temperature (97.45 vs. 84.74 °C) and a significant 5 °C increase in peak temperature (99.68 vs. 94.64 °C) compared to Control.

In terms of enthalpy that is related to amylose-lipid complex melting, SS1 from PEF-treated oat flour at 4.2 kV/cm, 438 kJ/kg was the only SS1 sample that exhibited a noteworthy 95% increase in enthalpy compared to Control (3.38 vs. 1.73 J/g, ΔH^2 of 4-C, Table 4). Such increase in enthalpy was also exhibited by SS2 from PEF-treated oat flour at 218–438 kJ/kg (ΔH^2 of 2-B, 2-C and 4-C, Table 4), with a recorded 26–28% improvement in enthalpy compared to Control. SS3 from PEF-treated oat flour, on the other hand, did not manifest any significant modification in enthalpy properties compared to Control.

4. Discussion

Oat flour that underwent PEF treatment (different electric field strength and specific energy input levels) demonstrated variation in its starch digestibility properties. The amount of glucose released from hydrolysed starches in the sample correlates with the susceptibility of starch to digestive enzymes. Compared to Control, the increased levels of glucose released from PEF-treated oat flour at 421–438 kJ/kg during digestion suggests an increased susceptibility of starch to the action of enzymes (Figure 2). It is presumed that the modifications in the structural properties of starch in oat (e.g., deformation of starch granules, damage in the crystalline regions of starch), as a result of PEF treatment at elevated specific energy input, have improved its starch digestibility properties as demonstrated by a higher proportion of starch that is rapidly digestible (i.e., significant increase in RDS fraction) during *in vitro* digestion (Table 1). On the other hand, lower levels of glucose released from digested samples (particularly those processed at increased electric field strength levels of 52–218 kJ/kg) indicate increased resistance of starch towards enzyme action. This finding was supported by both increment in the %RS and a considerable reduction in the rate of *in vitro* starch digestibility, particularly for sample 4-B (Table 1). Castro et al. [23] have outlined consistent reports that PEF treatment can improve starch digestibility with increasing electric field strength application. For example, Zeng et al. [14] reported that the application of higher electric field strength (30, 40, and 50 kV/cm) on waxy rice starch led to increased starch digestibility, which was expressed as increased levels of rapidly digestible starch. Similar observations were also reported by Simonis et al. [24] in studying the impact of increasing electric field strength application (2.86, 4.29, 5.71, 7.14, and 8.57 kV/cm) to wheat, potato, and pea starches.

In this study, the potential to manipulate the susceptibility of starch to digestion was determined by varying the levels of PEF treatment intensity applied. Increased susceptibility of starch to digestion was associated with elevated specific energy input (421–438 kJ/kg) application while increased resistance of starch to digestion may be achieved with increased electric field strength application at 52–218 kJ/kg PEF treatment. Production of starch-rich foods with low GI has been a recent priority in the food industry in the context of health concerns against the risk of obesity and related diseases among population [25]. Findings from the present study clearly demonstrated the feasibility of PEF as a reliable technology, if applied at appropriate intensity, to maintain or even slow down the rate of starch digestion of starch-rich foods such as oats.

By fractionating PEF-treated oat flour into three distinct flour segments, it was possible to identify that PEF treatment elicited varying levels of impact on a heterogenous material, that is oat flour. The uneven PEF treatment effect was evident in the changes observed in terms of particle size, damage and aggregation of starch granules, and destruction of

the long- and short-range ordered structures of starch between the flour segments within a treatment (e.g., 2-A–SS1 vs. SS2 vs. SS3). Firstly, the greatest extent of modification (in terms of particle size, starch ordered structure and thermal properties) exhibited by SS1 samples from PEF-treated oat flour indicates that flour components that are most susceptible to property alteration, with PEF treatment application, are most likely to be found on SS1 or the topmost segment. On the other hand, the overall insignificant change in the morphology, gelatinisation enthalpy, and percent relative crystallinity of SS3 samples, even after PEF treatment at high specific energy input level of 421–438 kJ/kg, indicates that components of oat flour that exhibited resistance to the impact of PEF treatment are most likely to be found in SS3 or the bottom segment.

Secondly, the wide range of particle sizes (5–552 μm) exhibited by different segments investigated demonstrate the range of particle size heterogeneity, particularly within a treatment (e.g., 4-C–SS1 vs. SS2 vs. SS3, Table 2). The varying extent of change in the particle size (two- to eight- fold increase) of flour components that was observed in various flour segments also stresses the uneven PEF treatment effect on oat flour components. In addition, the unimodal and multimodal size distribution displayed by each flour segment sample (Figure 3), which indicates clusters of varying particle sizes, is a demonstration of the asymmetrical impact of PEF treatment on a heterogeneous material.

Lastly, although the aforementioned cases highlight the variation of properties within a treatment, it cannot be denied that alterations exhibited by certain flour segments would impart a major impact on the overall property of the entire sample (e.g., starch susceptibility to digestive enzymes). This is most substantially observed in PEF-treated oat flour samples at 421–438 kJ/kg (2-C and 4-C), which was found to be the most susceptible to digestive enzymes amongst the samples analysed (Figure 2). The SS3 samples from 2-C and 4-C did not manifest significant difference from the corresponding SS3 segment of the Control in terms of granule morphology, particle size, XRD pattern, relative crystallinity, FTIR ratio, and thermal enthalpies. However, the SS1 segments from the same samples (2-C and 4-C) displayed the greatest degree of modification in the overall properties evaluated.

Findings from this study have consistently showed that varying degrees of modification in the particle size and morphological characteristics observed in flour segments obtained from PEF-treated oat flour is indicative of PEF influence. Firstly, the bigger particle size exhibited by segment samples obtained from PEF treatment of oat flour at 421–438 kJ/kg (Table 2) can be attributed to particle aggregation based on the small shoulders observed on the side of well-defined peaks (Figure 3). This finding was validated by the observed agglomeration of starch granules that was captured in SEM micrographs (Figure 4). Herrera-Gómez et al. [26] previously reported that starch granules tend to aggregate in the presence of gelatinised starch, which acted as an "adhesive" to cluster starch granules and hold the aggregates together. Secondly, the observed loss of starch granule integrity and starch deformation for samples that was processed at 421–438 kJ/kg implies that starch granules in oats can be very sensitive to structural modification under the influence of high specific energy input application. It is also likely that the collective impact of PEF treatment and the concomitant temperature increase (up to 33 °C temperature change) during processing rendered starch granules to be more vulnerable to alteration. Lastly, data on the particle size distribution of segment samples revealed that the size of the granule cannot be used to generalise denseness of oat flour components (e.g., big particles or starch aggregates tend to settle at the bottom). This is particularly applicable for segment samples collected after oat flour PEF treatment at 421–438 kJ/kg (2-C and 4-C). With the biggest component particle sizes manifested by samples collected from the top layer (SS1) of 2-C and 4-C, PEF treatment of oat flour at 421–438 kJ/kg would have caused alteration in the structural packing within the starch granule that resulted in larger but less dense segments.

Investigation of various flour segments, through the collective XRD, FTIR, and DSC analyses, permitted the characterisation of ordered structure for starch. The data gathered provided a comprehensive overview on how starch would interact with other molecules, such as water. XRD is the only analytical technique that may be used to evaluate and

quantify the long-range order in starch [27] based on the loss of intensity in crystalline peaks after atoms deviate from their ideal positions [28]. Data on the long-range molecular order of starch allowed the evaluation of the degree of starch crystallinity. On the other hand, FTIR study of the short-range ordered structure of starch, which reflects the local organization of helices into crystalline arrays, allowed the investigation of modifications in its helical properties. The 1042/1019 ratio was a beneficial tool in assessing changes in the starch structure [29] and reflects the short-range crystallinity that is related to the double-helix packing within the inner granule microstructure [30]. Lastly, DSC analysis enabled the evaluation of the thermal properties of oat flour to reveal the influence of PEF processing on the (1) transition temperatures and (2) enthalpies related to either starch gelatinisation or amylose-lipid complex melting.

Kaur and Singh [31], Ren et al. [32], Shah et al. [33], and Tang et al. [34] reported an A-type XRD pattern for oat starch, which displayed solid diffraction peaks around 15° and 23°, a doublet peak at 17° and 18°, and a small peak at 20° (2θ). The comparable XRD pattern exhibited by most segment samples from Control and PEF-treated oat flour (Figure 6) indicates closely packed monoclinic structure of starch that is stable and can inhibit chemical reactions [35]. On the other hand, flour segments from PEF-treated oat flour at 421–438 kJ/kg (2-C and 4-C) that displayed weakened XRD peaks indicates some degree of transformation in the crystalline region of starch in oat. Derived from the XRD pattern, the percent relative crystallinity was evaluated to determine how PEF treatment affected the crystalline regions of starch in flour segments. The observed decreased on the percent relative crystallinity of segment samples due to PEF treatment of oat flour (i.e., SS1 from 2-C and 4-C, Figure 6) is likely an indication of crystallite disruption or reorientation [36]. On the other hand, higher percent relative crystallinity of starch implies a more stable and crystalline structure. Findings from previous studies on potato and pea starches reported a similar decrease in percent relative crystallinity after PEF-treatment application [9,37]. It is likely that the compact starch chains in the granules were affected by PEF treatment and led to the alteration of the inner structures of starch, resulting in altered relative crystallinity. In addition, Dias et al. [38] and Wang et al. [39] attributed changes in relative crystallinity of starch, as a result of processing, to several factors including the amount of crystalline regions in the sample, double helices orientation in the crystalline area, and the extent of double helices interaction. The observation of additional peak at 13° (2θ) in the XRD pattern of SS1 samples has not been reported in literature to be one of the effects of PEF treatment of starch or starchy materials. Nevertheless, previous study on the impact of dual autoclaving-retrogradation processing of oat starch reported a similar 13° (2θ) XRD peak observed post processing [40]. New peaks observed in the XRD (e.g., 13° at 2θ) patterns typically indicate new crystalline regions were formed.

Compared to Control, the significant change in the FTIR ratio (1042/1019) of flour segment samples from PEF-treated oat flour (Table 3) signifies disturbance in double helix packing of the inner starch granule. More specifically, the observed lower 1042/1019 ratio compared to the Control indicates decreased molecular order of the double helix short-range molecules in the granules owing to PEF treatment application (Table 3). This finding verifies previous report on the impact of PEF treatment on the ordered structure of starch [9]. Moreover, the observed prominent shift of major FTIR peak positions (from 1042 to 1050 cm^{-1} and 1019 to 1027 cm^{-1}) also demonstrates the complexity of the impact of PEF treatment on oat flour.

Modifications in the observed thermal properties of segment samples reflect the influence of oat flour PEF treatment on the transition temperature and enthalpy, which are related to gelatinisation and amylose-lipid complex melting in starch. The notable increase in transition temperatures that was displayed by flour segments collected from PEF-treated oat flour, at high specific energy input, (Table 4) substantiates higher temperature and longer time requirements for the facilitation of (1) starch swelling, (2) starch gelatinisation, and (3) amylose-lipid complex melting. Depending on the strength of PEF intensity applied, earlier studies reported either a higher or lower transition temperature with PEF-treated

starches and PEF-assisted chemically modified starches [8,41,42]. In this study, the observed increase in transition temperature of segment samples was likely an effect of granule aggregation, which induced physical reorganisation in the samples and enhanced its structural stability. Alterations on the gelatinisation enthalpy exhibited by flour segments after PEF treatment of oat flour indicates some degree of change in the water-starch molecules association/interaction in the sample [43]. Lower gelatinisation enthalpy suggests that water molecules were able to react with starch molecules more easily in the crystalline region. In other words, the packing within the inner granule structure have been relaxed after PEF treatment and this can affect the accessibility of starch molecules. On the other hand, higher gelatinisation enthalpy of flour segments from PEF-treated oat indicates a more crystalline sample and therefore, a more compact starch granule packing that would require larger amount of energy to cause melting of starch crystals. Increased enthalpy associated with amylose-lipid complex melting, due to PEF treatment, was not previously reported in the literature. Flour segments from PEF-treated oat that exhibited higher enthalpy would demand increased energy requirements to melt the amylose-lipid complex in the system. It is likely that the hydrophobic tail buried in the inner part of the amylose-lipid complex helical structure increased the degree of entanglements among starch molecules [40,44] and caused the observed increase in enthalpy. Moreover, the amylose-lipid complex forms the V-amylose crystalline units, which was reported to be the fifth type of resistant starch. This affirms the additional peak (13° at 2θ) that was observed in the XRD pattern of flour segments from PEF-treated oat flour, which was previously reported to be also related to formation of amylose-lipid complex [45].

4.1. Possible Mechanism of PEF Treatment Impact on Starch Digestibility of Oat Flour

This study clearly shows that the extent of PEF treatment effect on oat flour was dependent on PEF intensity applied (levels of electric field strength and specific energy input). It is noteworthy to mention that with increasing specific energy input application, there is also an inevitable increase in sample temperature during PEF processing, which may be attributed to the electric current that was applied and the dissipation of electric energy to thermal energy within the system. Although the observed increase in temperature during PEF processing is intermittent and short, its influence on the different physicochemical properties of PEF-treated oat flour should not be dismissed. In this context, the synergistic effect of PEF treatment and intermittent temperature increase during oat flour processing is recognised. Based on the findings presented in this study, the potential mechanism of PEF treatment effect on starch digestibility of oat flour is proposed. Figure 7 shows the overview of the impact of PEF treatment, which encompasses both the physical and molecular structures of starch in oat flour. Firstly, PEF treatment of oat flour leads to the observed modification in the physical characteristics of flour components. At certain PEF treatment levels, the surface properties of oat starch are affected. Starch granules with deformed or irregular shapes clump together and produce large clusters of aggregates that resulted in bigger particle size measurement. Secondly, PEF treatment of oat flour affects both the crystalline and amorphous domains of starch in several ways: (1) damage in the starch molecular structure that caused instability in the crystalline region and prompting the conversion of starch crystals to its amorphous form, or (2) the enhanced stability of the crystalline structure, making it denser and more compact. These modifications in the crystalline region further affect the accessibility of starch molecules for possible interactions with other molecules. Any observed change in the molecular structure of starch will impact how it interacts with water, which affects the thermal properties of the sample.

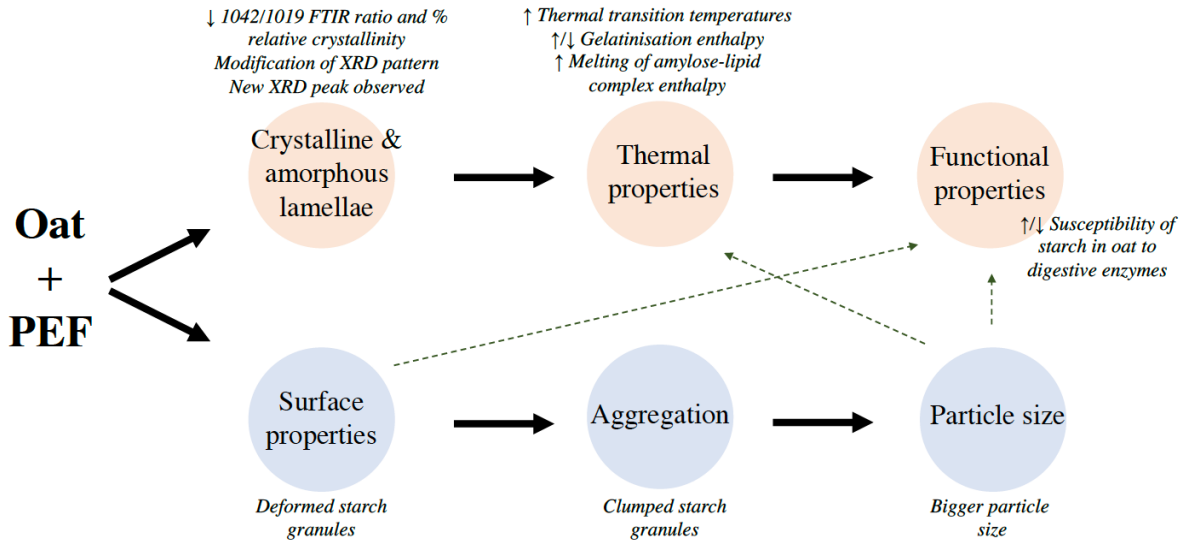

Figure 7. Possible mechanism of PEF treatment impact on oat flour properties. The symbols "↑" and "↓" denote increase and decrease of specific properties, respectively.

Starch, being the major component of oat, is the main driver of oat functionality. With the observed changes in the physical and molecular structures of starch, it is expected that oat functionality, such as its susceptibility to digestive enzymes, will also be altered. PEF treatment of oat at 418–484 kJ/kg caused instability in the crystalline structure, which allowed easier access of water to starch molecules and permitted improved facilitation of enzymatic hydrolysis of starch in oat flour. Hence, an increased starch digestibility was observed. On the other hand, the enhanced stability of the crystalline structure in starch appeared to be prohibitive in terms of water access to starch. That is, with PEF treatment, swollen granules of starch will command higher energy requirements to cause the melting of amylopectin crystalline structures and amylose double helices, which leads to increased starch resistance to digestion.

In addition to starch, the presence of protein (11%), lipids (5%), and β-glucan (1%) in lower amounts and the interaction effects of these macromolecules with starch in oat flour may pose minimal impact on starch digestibility and other related functional characteristics under the influence of PEF. Oat protein may act as a physical barrier to starch digestion [46,47]. On the other hand, lipids have been reported to limit enzyme hydrolysis by blocking the absorption sites, trapping the starch granules, and promoting the formation of lipid-amylose complex [48,49]). Lastly, starch hydrolysis may be negatively affected with the presence of β-glucan due to increased viscosity and reduced amylolytic activity [5,50]. Tosh et al. [51] also reported that β-glucan may inhibit enzyme action by encapsulating protein and starch.

4.2. Potential of PEF Treatment to Tailor the Starch Digestibility of Oat Flour

The susceptibility of a food product (e.g., oat starch) to the action of digestive enzymes is correlated with how slow or how fast it affects the blood glucose level after food ingestion. This rate of change in the blood glucose level is significant because it was widely reported that foods causing rapid increase in blood glucose levels (high GI food) are associated with obesity, cardiovascular disease, and Type 2 diabetes [52,53]. Data gathered from this study clearly showed that PEF treatment prompted significant alterations in different levels of oat starch structure, which led to changes in its starch digestibility properties. While most techniques (steaming, steaming + toasting, autoclaving, extrusion) used to process oat tend to increase starch susceptibility to digestive enzymes [7,54], the use of appropriate levels of PEF intensities (e.g., higher electric field strength) was found to maintain or slow down the starch digestibility of oat. Hence, with this technology, it is possible to produce oat products with a much lower GI. In a different perspective, the reported increase in the

starch digestibility of oat with PEF treatment (at elevated specific energy input) may be particularly beneficial to consumers (e.g., athletes) who desires high GI product for energy source, and at the same time enjoy the other nutritional benefits of oat.

These important findings present the potential of PEF treatment as an attractive processing strategy for the production of new lines of oat products and ingredients with targeted functional properties (e.g., increased or decreased starch digestibility) suited for the varying needs of different consumers. These findings also offer an interesting new area of research that can broaden the application of PEF treatment in the food industry.

5. Conclusions

Results from the present study clearly demonstrated that the *in vitro* starch digestibility properties of oat flour can be preserved and even slowed down after PEF treatment is applied. Non-conventional approach was adopted to understand the underlying mechanism on how PEF can influence the *in vitro* starch digestibility properties of oat flour. Specifically, flour segments exhibiting deformation of starch granules, increased particle size, modification of XRD pattern, decreased percent relative crystallinity, decreased 1042/1019 FTIR ratio, and lower gelatinisation enthalpy were associated with oat flour treated with PEF at elevated specific energy input levels, which influenced its thermal stability and starch digestibility properties. Overall, this study implies that PEF treatment can be utilized as a processing tool to tailor the *in vitro* starch digestibility properties of oat flour.

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