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Research Article

Comparison of the Resistant Starch Content and Physical Properties of Extruded Pea Flour-Based Snacks Enriched with Protein and Dietary Fibre

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Abstract

Increasing public health concerns necessitate the production of healthier snacks, preferably with a lower glycaemic index. Resistant starch (RS) is a functional bioactive ingredient with many health benefits, including the modulation of blood glucose levels. By selecting a desirable combination of raw materials, extruded food products with lower starch digestibility can be developed. This study compares the RS content and physical properties of extruded pea-flour-based snacks enriched with protein and dietary fibre. Four recipes were formulated using pea flour (77%), two types of protein concentrate (20%, pea and faba bean protein concentrate) and two types of insoluble fibre (3%, yellow pea and maize fibre). All recipes qualified for high protein and high fibre nutrition claims. The recipe mixes were extruded using a co-rotating, twin-screw extrusion cooker and the resulting pellets were expanded in a hot-air oven. RS content, expansion ratio (ER), bulk density (BD), textural attributes (hardness, crunchiness and crispiness), moisture content and water activity values were determined. The highest ER and low BD and hardness values were obtained with the faba bean protein/maize fibre recipe. This recipe also registered the highest RS content (3.2%, $p < .05$). Therefore, this sample was the most preferred snack with desirable quality attributes and high nutritional value. This preliminary study demonstrated that it was possible to increase the RS content of the snacks by ingredient substitution without adversely impacting their physical properties.

Keywords: Extrusion; Snack; Resistant Starch; Expansion Ratio; Protein; Fibre

Background

Extrusion cooking is an efficient continuous process, which uniquely combines several unit operations, namely mixing, shearing, heating, pumping, forming and sizing [1]. Most extruded snacks are made from refined flour (primarily cereals) and starch as the main ingredient. They, therefore, are not a good source of dietary fibre and other health-promoting compounds [2,3].

During extrusion, flour is subjected to high temperature (100 - 180°C) and high shear at relatively low levels of moisture content (10 - 40% on a wet weight basis), which can effectively modify the functionality of composite food matrices by dextrinisation of starch, denaturation of protein, modification of lipids as well as inactivation of enzymes and antinutritional factors [4,5]. The ingredients used in extrusion cooking are transformed into a fluid melt by the shearing action at the high temperature and pressure in the extruder. Different types and levels of starch, protein and other ingredients (i.e., dietary fibre, oil or salt) produce different viscosities and, therefore, extrudates with distinct quality characteristics (i.e., expansion ratio, bulk density, hardness, crispiness etc. [6]. Starch is a structure-forming material in extrusion and plays a critical role in creating well-expanded cellular structures, which is a highly desirable attribute [7,8].

The design of extruded products with high-protein and high-fibre content as healthier options is still challenging due to the interaction of these components under thermomechanical treatment and their impact on extrudate structure and texture [3,9]. High protein and fibre content dilutes the starch concentration and interferes with its gelatinisation during extrusion. This subsequently hinders the expansion of the product as it exits the extruder die [10-14].

As a commonly utilised legume, pea-based ingredients, mainly pea flour, have been used as an ingredient to improve the nutritional properties of extruded products [10,13,15-18]. Legumes offer great potential as ingredients in extruded snacks since they are a low-cost and sustainable source of plant protein, dietary fibre and bioactive ingredients [3,19,20]. They are also a good source of slowly digested starch (SDS) and resistant starch (RS) and, therefore, can help reduce post-prandial glucose responses [21,22]. RS is a functional bioactive ingredient with many health benefits, including modulation of blood glucose and cholesterol levels, homeostasis of gut microbiota, prevention of colon cancer and metabolic diseases, improvement of the immune system and management of obesity and body weight [23].

During extrusion, the gelatinisation of starch and the reduction in the molecular weight of amylose and amylopectin due to shearing and heating increase the digestibility of starch [6] and decrease the RS content [24-27]. The type of RS formed during food processing is retrograded starch (known as type 3 resistant starch, RS3), and it involves changes in the starch crystalline structure [28]. The increase in starch digestibility post extrusion often results in products with a high glycaemic index, which is undesirable. Thus, producing extruded snacks with increased RS content can be beneficial to improve public health.

By selecting a combination of raw materials and extrusion processing conditions (feed moisture, extrusion temperature and screw speed), extruded food products with lower starch digestibility can be developed [28]. This study uses raw materials (protein and fibre) to increase the RS content of pea-flour-based extruded snacks. Several studies investigated the effect of either protein or fibre on the extrudate characteristics, but only a few looked at the combined effect of both (using rice and corn-based extrudates) [10,11,13,19]. Moreover, no information is available concerning the combined effect of the two ingredients on the RS content of the extrudates. Therefore, using an extrudate with pea ingredients as a control (i.e., pea flour, pea protein and yellow pea fibre), this preliminary study looks at the impact of substituting the protein (with faba bean protein) and fibre (with maize fibre) on the RS content and physical properties (expansion ratio, bulk density and textural properties) of the extruded snacks.

Materials and Methods

Materials

Clean flavour pea flour (HOMECRAFT ® Pulse CT 1203), faba bean protein concentrate (Vitessence Pulse CT 3602) and pea protein concentrate (Vitessence Pulse CT 1552) were supplied by Ingredion Incorporated (Westchester, USA). Yellow pea fibre (Emfibre EF 60) and maize fibre (Sofabran 184-80) were obtained from Emsland Group (Germany) and Limagrain Ingredients (France), respectively. The protein and fibre content of the ingredients are shown in table 1.

Table 1. Protein and dietary fibre content of the ingredients as provided by suppliers.

	Ingredients	Protein (%)	Dietary fibre (%)
Main ingredient	Pea flour	11.8	4.6
Sources of protein	Pea protein concentrate	51.0	13.2
	Faba bean protein concentrate	56.0	14.4
Sources of fibre	Yellow pea fibre	10.8	59.3
	Maize fibre	4.7	70.0

Four extruded snack recipes were formulated using pea flour (77%), two types of protein concentrate (20%, pea and faba bean protein concentrate) and two types of insoluble fibre (3%, yellow pea and maize fibre). Four samples were produced containing pea flour as the base ingredient: PP-YPF: Pea protein concentrate and yellow pea fibre, PP-MF: Pea protein concentrate and maize fibre, FP-YPF: Faba bean concentrate and yellow pea fibre and FP-MF: Faba bean concentrate and maize fibre. Sample PP-YPF was used as a control. The samples' theoretical protein, dietary fibre and energy content were calculated using NutriCalc® nutritional software. The levels of protein and fibre supplementation were chosen for the snacks to qualify for the nutrition claims "high protein" (where the protein provides at least 20% of the energy content of the food) and "high fibre" (where the food provides at least 6 g dietary fibre per 100g) [30].

Extrusion processing

Extrusion processing was conducted using a co-rotating, twin-screw extruder cooker (Baker Perkins MP19c) with a screw diameter of 19 mm and a length-to-diameter ratio (L/D) of 26. The extruder barrel consisted of five independently temperature-controlled sections. From feed to die plate, the temperatures of these sections were 50, 105, 105, 105 and 95°C, respectively. The extrudate temperature at the die face was 98°C. Water at ambient temperature was injected through a port to achieve total moisture contents of 32 to 35% in the barrel. A feed rate of 3.5 to 4.5 kg/h was used, with screw speeds ranging between 150 to 260 rpm. Extrusion processing variables were recorded, such as motor torque, specific mechanical energy, barrel temperatures, die temperature and pressure, screw speed, and material and water feed rates.

The extrudate strips were produced using a slotted die having a width of 20.0 mm and a slit depth of 0.5 mm. Emerging strips of extrudate were manually placed under tension to provide an extrudate thickness that was reduced from 1 mm to a range of 0.65 to 0.85 mm. Strips of pellets were cut into pieces approximately 1.5 cm in length and dried in a hot-air oven (70°C) to reach a final moisture content of c.10 to 13%. Dried pellets were cooled to room temperature, placed in sealed bags and stored at 18°C until expanded in a Torbed Compact Bed Reactor (Torftech Technologies) at 270-280°C for 10-13 s. Expanded pellets were stored in sealed bags at 18°C until measurements were taken.

Moisture content (MC)

The moisture content of the expanded pellets was measured according to BS 4317-2:1968 (Methods of test for cereals and pulses: Determination of moisture content of cereals and cereal products). Samples were kept in an oven at 130°C until they reached a constant weight.

Water activity (a_w)

The water activity of each sample was determined in triplicate using a calibrated AquaLab Water Activity Meter (Decagon Devices, Inc., Pullman, WA) at 21.2°C.

Expansion ratio (ER)

The expansion ratio (%) was calculated as the thickness of the expanded product divided by the thickness of the extrudate. Ten pellets were collected randomly, and the thickness of extruded and expanded pellets was measured with a Vernier calliper. The following formula was used to calculate ER:

ER (%) = [Thickness of an extruded pellet (mm)/Thickness of an expanded pellet (mm)] *100

Bulk density (BD)

Bulk density is defined as the mass of extrudate particles divided by the total volume occupied, including particle volume, internal pore volume and interparticle void volume [14]. BD was calculated after measuring the weight of a standard volume of expanded pellets. The volume of the pellets was measured by the solid displacement method [31], using poppy seeds as the solid material. The following formula obtained BD

BD = Weight of the extruded product (g)/volume of the container (dm^3)

Texture measurements

The texture characteristics of the snacks were measured using a Stable Micro System TA-TX2 Texture Analyser (Surrey, UK) fitted with a Mini Ottawa-Kramer Shear Cell (HDP/MK05). Five randomly collected expanded pellets (15g) for each sample were measured. A force-time curve was recorded and analysed for the hardness (positive area), crunchiness (linear distance) and crispiness (peak count) of the snacks. The test speed was 1 mm/s, and the distance was 10 mm.

Resistant starch (RS) analysis

RS content of the snacks was determined by the AOAC method 2002.02 using Megazyme Resistant Starch Assay Kit. The samples were incubated with pancreatic α-amylase and amyloglucosidase (AMG) in a shaking water bath for 16h at 37°C. The addition of ethanol terminated the reaction, and RS was recovered as pellets after centrifugation. Following this, RS was dissolved in KOH solution (2 M), neutralised with acetate buffer (1.2 M, pH 3.8) and hydrolysed to glucose by AMG. The amount of D-glucose was measured spectrophotometrically (at 510 nm) using a glucose oxidase peroxidase (GOPOD) reagent.

Statistical analysis

Statistical analyses were conducted on IBM SPSS Statistics (Version 28). The impacts of individual ingredient substitutions (and their interactions) were tested using a two-way analysis of variance (ANOVA) test, applied once to each measure. To compare the samples and as a post hoc analysis for those ANOVAs with significant interactions, pairwise Tukey comparisons were also performed between the four samples. The significance level for all statistical tests was set at $p < .05$.

Results

Figure 1 and Table 2 present the means for each measure applied to each recipe. Figure 1 shows that maize fibre and faba bean protein were associated with the highest RS content, expansion ratio (ER) and crispiness. Table 3 presents the two-way ANOVAs, showing a significant main effect (i.e., protein and fibre supplementation) in all three cases, with no significant interaction. The faba bean protein and maize fibre recipe (FP-MF) had the most RS, the highest ER and the crispiness value.

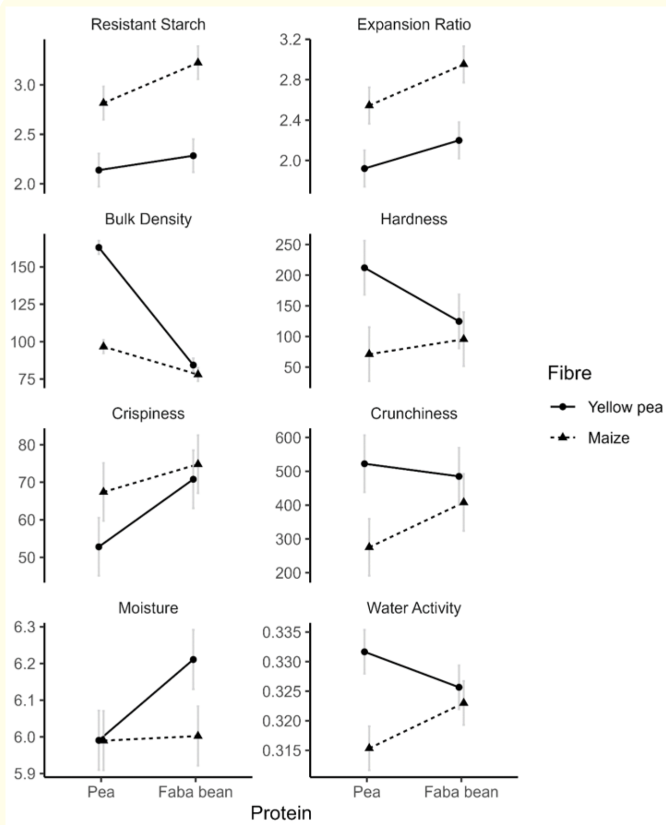

Figure 1. Estimated marginal means plots for all measures. Error bars show 95% confidence intervals.

Maize fibre had the lowest values for bulk density (BD), hardness and crunchiness (Figure 1). While the introduction of faba bean protein was associated with decreased BD, its impact on hardness and crunchiness appeared more complex. The interactions for these three measurements were significant (confirming a more complex result) (Table 3).

The following can be inferred from the Tukey pairwise comparisons in Table 2. The two faba bean protein recipes had lower BD than either of the two pea protein recipes ($ps < .008$), but the faba bean protein recipes could not be distinguished between ($p = .169$). The combination of pea protein and pea fibre had high hardness (PP-YPF), differing significantly from all other three recipes ($.001 < ps < .040$). None of the other three recipes could decisively be differentiated from the others ($ps > .296$). Regarding crunchi-

Table 2. Measurements obtained with the extrudates (mean ± SD).

	PP-YPF	PP-MF	FP-YPF	FP-MF
Resistant starch (g/100g)	2.1 ± 0.2[a]	2.8 ± 0.2[b]	2.3 ± 0.2[a]	3.2 ± 0.3[c]
Expansion ratio	1.9 ± 0.3[a]	2.5 ± 0.2[b]	2.2 ± 0.3[a]	3.0 ± 0.2[c]
Bulk density	163 ± 6[c]	97 ± 0[b]	84 ± 2[a]	78 ± 2[a]
Hardness (N)	212 ± 80[b]	71 ± 14[a]	125 ± 33[a]	96 ± 29[a]
Crispiness	53 ± 8[a]	67 ± 6[ab]	71 ± 13[b]	75 ± 4[b]
Crunchiness	522 ± 129[b]	275 ± 57[a]	485 ± 88[b]	408 ± 61[ab]
Moisture (%)	5.99 ± 0.05[a]	5.99 ± 0.06[a]	6.21 ± 0.05[b]	6.00 ± 0.08[a]
Water activity	0.332 ± 0.002[c]	0.315 ± 0.003[a]	0.326 ± 0.004[bc]	0.323 ± 0.002[b]
Protein content (%) *	19.6	19.4	20.6	20.4
Dietary fibre content (%)*	8.0	8.3	8.2	8.5
Energy from protein (%)*	21.3	21.1	22.3	22.1

*Theoretical values obtained using NutriCalc® nutritional software. PP-YPF: Pea protein concentrate and yellow pea fibre, PP-MF: Pea protein concentrate and maize fibre, FP-YPF: Faba bean concentrate and yellow pea fibre and FP-MF: Faba bean concentrate and maize fibre.

Values in a given row that share a superscript did not differ significantly from one another; alphabetical order of subscripts represents increasing values of the measure (superscript [a] denotes the lowest values, etc).

Table 3: Two-way analysis of variance (ANOVA) and effect estimates for main effects.

	F	p	Mean difference	95% CI
Resistant starch				
Protein	11.42	.002	0.277	[0.110, 0.443]
Fibre	97.58	<.001	0.809	[0.642, 0.976]
Interaction	2.56	.119		
Expansion ratio				
Protein	15.14	<.001	0.344	[0.164, 0.523]
Fibre	60.81	<.001	0.688	[0.509, 0.868]
Interaction	0.53	.470		
Bulk density				
Protein	643.5	<.001	-48.67	[-53.09, -44.24]
Fibre	358.67	<.001	-36.33	[-40.76, -31.91]
Interaction	244.53	<.001		
Hardness				
Protein	16.87	.148	-31.50	[-75.43, 12.43]
Fibre	2.31	.001	-85.12	[-129.04, -41.19]
Interaction	7.28	.016		
Crispiness				
Protein	12.22	.003	12.70	[5.00, 20.40]
Fibre	6.56	.021	9.30	[1.60, 17.00]
Interaction	2.13	.164		
Crunchiness				
Protein	16.78	.246	47.70	[-36.19, 131.59]
Fibre	1.45	.001	-162.08	[-245.97, -78.19]
Interaction	4.61	.047		
Moisture content				
Protein	11.05	.010	0.116	[0.036, 0.197]
Fibre	8.95	.017	-0.105	[-0.186, -0.024]
Interaction	8.8	.018		
Water activity				
Protein	0.27	.618	0.001	[-0.003, 0.005]
Fibre	34.94	<.001	-0.009	[-0.013, -0.006]
Interaction	18.08	.003		

Note: The reference categories for mean differences were pea protein and yellow pea fibre. A positive difference indicates that the other ingredient measured higher than the reference category.

ness, the pea protein/maize fibre (PP-MF) combination differed significantly from the yellow pea fibre recipes (ps < .009), while the best-performing recipe (FP-MF) did not (ps > .214). It was, however, not decisive that pea protein/maize fibre was lower than the faba bean/maize recipe (p = .123). Maize fibre had the lowest crunchiness, but unclear within this which type of protein was the least crunchy.

The faba bean protein/yellow pea fibre (FP-YPF) combination showed significantly higher moisture content than any of the other three recipes (*ps* < .013). The other three recipes could not be distinguished between (*ps* > .994). The two maize fibre recipes had the lowest water activity, although the combination with pea protein, this time, was lower and significantly lower than all other recipes (*ps* < .039). The two faba bean recipes came out very close to each other in the middle (*p* = .659), and the two pea fibre recipes showed the highest water activity, and they could not statistically be distinguished between (*p* = .111).

Discussion

Extrudates with low moisture content (often < 6%) tend to yield products with high crispiness [1,2,32]. Low a_w values (0.1 to 0.33) are desirable to ensure the stability of the extrudates against microbial growth during storage [1]. The samples' moisture content and a_w values were within the values stated in the literature.

The RS content of untreated pea flour was reported to range between 13.2 and 14.8% [33]. Mild extrusion conditions (high moisture content, low residence time, low temperature) tend to result in higher starch digestibility (i.e., lower RS values) due to the gelatinisation of starch and the inactivation of antinutritional factors interfering with enzyme activity [1]. A reduction in the RS content was reported when pea flour (20.1% dry weight basis, dwb) was extruded at a temperature of 90°C (16.8% in the native flour vs 4.7% in the extrudate) [34]. The samples in the current study had similar protein contents (c. 21% dwb) but registered lower RS contents (2.1 to 3.2%, see Table 2). The difference in the RS content could be caused by the raw materials used (pea flour vs the combination of pea flour with protein and fibre sources in the current study). Extrusion temperatures were lower in the study above, so less severe conditions could have prevented the melting of crystalline regions of retrograded starch (and hence registering a higher RS value). The use of different methods (Englyst's method vs the AOAC method) for RS analysis may be another factor for the discrepancy in the results. Other studies reported similar findings, i.e., lower RS content after extrusion with pea, rice and carob flour blends [35], cereal and legume-based snacks fortified with by-products from herbs and vegetables [36] and defatted chickpea flour [37]. Considering such possible losses in the RS content post-extrusion, it was promising to obtain increased RS contents with faba bean protein and maize fibre (Sample FP-MF as compared to PP-YPF), and the former still qualifying for nutrition claims, "high protein" and "high fibre"(Table 2).

How much an extruded product expands is a fundamental property that describes its structural and textural quality [12,38]. As the extruded melt exits the die, the difference between the water's vapour pressure at the melt's temperature and atmospheric pressure causes the formation of bubbles and subsequent expansion of the product [7,39]. ER is strongly correlated to the relative composition and interaction of starch, protein and fibre, with higher starch, lower protein and fibre contents resulting in higher values [40,41]. The protein and fibre contents of the snacks in the current study were similar. Still, sample FP-MF registered the highest ER and lowest BD value (Table 2 and Figure 1), perhaps due to a possible interaction between faba bean protein and maize fibre. ER and BD are interdependent physical attributes, i.e., the increase in ER corresponds to a decrease in BD and vice versa [42]. This was consistent in the study.

In a study, extrudates made from whole faba bean flour were observed to have higher ER values (p < .05) than those of lima, pinto and red kidney bean whole flours. However, the protein and crude fibre contents of the faba bean extrudates were significantly higher (29.8 and 5.3%, respectively) [43]. The authors attributed this to the unique functionality of faba bean protein (containing a high amount of globulins with lower amounts of albumins); but added that further research was necessary to explore this functionality. In the current study, extrudates containing faba bean protein resulted in significantly higher ER values despite the comparable protein and fibre content of the samples. In addition to the proposed functionality of the faba bean protein, its possible interaction with other ingredients could affect the expansion of the extrudates [7,28]. Further studies are required to investigate how faba bean protein interacts with maize fibre and the starch in pea flour, eventually giving rise to higher ER and RS values.

[44] investigated the effect of pea-protein fortification (using pea protein isolate - PPI) on expanded rice snacks' physical and microstructural properties. Extrudates with a PPI content of up to 10% (extrudate protein content c. 8%) were reported to have the highest expansion (and the lowest BD and hardness) and increasing the PPI content (to an extrudate protein content of c.38%), affected those properties negatively. The level of pea protein addition (20%) did not vary in the current study, resulting in all extrudates having similar protein contents (c.19-20%). The use of pea protein concentrate provided either comparable or lower ER values compared to the faba bean protein-containing counterparts (Table 2 and Figure 1).

Another study reported higher sectional expansion index values at pea protein fortification levels of 10 and 25% and pea fibre addition at 8 and 16% [13]. The combined use of 25% protein and 16% fibre was still able to result in an increase in expansion compared to pure rice starch (control). In the current study, the inclusion of 20.4% protein and 8.5% dietary fibre resulted in the highest ER value in sample FP-MF, which was significantly higher than the control (PP-YPF). Interestingly, the addition levels of protein and fibre for the highest expansion were similar in those studies, although the raw materials, blend formulations and extrusion conditions varied.

The texture of extruded food products is one of the key factors influencing consumer acceptance, and expanded snacks usually have a crisp and light (aerated) texture [3,45]. In a study with pea-protein fortified extruded rice snacks [45], described highly expanded snacks (with 13% PPI, corresponding to an extrudate protein content of c.20%) as crisp with low crunchiness and hardness values, while poorly expanded snacks (containing 45% PPI, extrudate protein content c.47%) were reported to be harder and crunchier with low crispiness. Likewise, sample FP-MF (protein content 20.4%) was the most expanded snack in the current study and registered the highest crispiness value and low hardness and crunchiness (Table 2).

High ER, low BD and hardness are generally regarded as outstanding characteristics of an extruded snack [40,46]. Indeed, sample FP-MF registered the highest ER and low BD and hardness values. As discussed earlier, it also showed the highest RS content (as well as having similar protein and fibre content as PP-YPF (control) and therefore qualifying for the nutrition claims). For those reasons, sample FP-MF could be proposed as the preferred snack with the highest nutritional value and the most desirable quality attributes.

Conclusions

The combination of faba bean protein and maize fibre showed the highest resistant starch, expansion ratio and crispiness and was among the lowest in bulk density, hardness and crunchiness. The differences were not statistically significant in those cases where the measured value was not the lowest for the last three parameters listed above. This preliminary study demonstrated that it was possible to increase the RS content by substituting the protein and fibre sources and without adversely impacting snacks` physical properties. The results are promising; however, further work needs to be carried out to establish optimum processing parameters and blend formulations to produce snacks with increased resistant starch content and within a reasonable cost range for the ingredients. Secondly, other nutritionally important starch fractions (i.e., rapidly and slowly digestible starch) could be measured to acquire further information on the rate of starch digestibility, which relates to the glycaemic index of the snacks.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting this study's findings are not openly available due to work carried out as part of a funded project involving third parties and are available from the corresponding author upon reasonable request.

Competing Interests

None.

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Authors` Contributions

Ayten Tas: Conceptualisation, methodology, formal analysis, writing original draft, project administration, review and editing.

Keith Brewood: Conceptualisation, methodology, investigation, review and editing.

Bukola Onarinde: Conceptualization, methodology, resources, review and editing, funding acquisition.

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