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Effect of Extrusion Parameters on Nitrogen Solubility Index of Fonio-Locust Powder Extrudates

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ABSTRACT
This study evaluated the influence of extrusion processing variables on the nitrogen solubility index (NSI) and compositional quality of protein-enriched fonio–edible locust powder extrudates. A three-factor, five-level central composite rotatable design was employed to assess the effects of edible locust powder inclusion, screw speed and barrel temperature on extrudate functionality. Fonio flour and edible locust powder blends were conditioned to 20% moisture content and processed using a co-rotating twin-screw extruder at barrel temperatures of 96.36–163.64°C, screw speeds of 98.87–351.13 rpm and locust powder inclusion levels of approximately 3.6–36.7%. Proximate composition and NSI were determined using standard analytical procedures, with NSI evaluated at pH 3.5, 7.0 and 9.0. Protein solubility exhibited a distinct pH-dependent U-shaped pattern, with minimum NSI at pH 7.0, intermediate solubility at pH 3.5 and maximum solubility under alkaline conditions at pH 9.0. NSI ranged from 25.36% to 60.29% at pH 3.5, 14.51% to 42.34% at pH 7.0 and 40.30% to 79.05% at pH 9.0. Response trends showed that moderate extrusion severity preserved protein dispersibility, whereas excessive screw speed and high thermo mechanical input promoted protein aggregation and reduced its solubility. These findings demonstrate that extrusion parameters can be strategically controlled to balance protein enrichment, texturization and functional protein recovery in fonio–insect-based snack systems, while supporting the development of sustainable, high-protein extruded foods from underutilized cereals and edible insect ingredients.

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INTRODUCTION

Nitrogen solubility index (NSI) remains one of the most widely used indicators of protein denaturation and functional quality in protein-rich food ingredients, particularly in systems where intense thermal and mechanical processing were used. NSI is typically defined as the ratio of water-soluble nitrogen to total nitrogen, expressed as a percentage, and is closely associated with the extent of protein unfolding, aggregation, and network formation in proucts(Pismag *et al*., 2023).More recently, the relevance of NSI is increasingly applied to novel protein sources including under-utilised grains and edible insects. Higher NSI values generally indicate more extractable, less denatured protein fractions that may confer favorable techno-functional behaviors in foaming, emulsification, and gelation; conversely, low NSI suggests extensive denaturation, aggregation and reduced water dispersibility (Pismag *et al*., 2023). Edible insects, by contrast, provide dense, high quality protein and lipid, as they are increasingly recognized as a sustainable, low emission alternative protein source compatible with circular bio economy principles. Reviews of insect protein functionality indicate that insect proteins display distinct solubility profiles, with minima near the isoelectric point and higher solubility under alkaline conditions, similar to plant proteins (Mosibo *et al*., 2022). Despite the growing body of work on insect protein functionality, extrusion studies involving insect powders remain relatively nascent and are often focused on physical, nutritional, and sensory outcomes rather than detailed NSI characterization

Extrusion cooking is a high-temperature, short-time (HTST) process combining thermal energy, pressure, and intense shear within a screw–barrel system to transform doughs into expanded, texturised, or instantised products (Choton *et al.*, 2020) The major extrusion parameters: feed moisture content, barrel temperature profile, screw speed, screw configuration, and feed composition interact to determine specific mechanical energy input, residence time, and localised temperature and shear histories. Proteins exposed to extrusion conditions undergo conformational transitions including unfolding, exposure of hydrophobic groups, disulfide interchange, aggregation, and in some cases covalent cross-linking, which collectively govern solubility and thus NSI. These findings emphasize that NSI is highly sensitive to both formulation and process conditions, and that its optimal range depends on the intended end use. Fonio (*Digitaria exilis*) is an underutilized West African cereal that is gaining attention as a climate resilient "minor millet" with short growth cycle, low input requirements, and adaptation to marginal environments. . Recent agronomic and compositional studies on fonio consistently highlight its role in climate smart and culturally significant food systems. Extrusion of fonio and related small millets has been reported to improve digestibility, reduce anti nutritional factors, and generate shelf stable, ready to eat products, although detailed reporting of NSI for fonio extrudates is still limited compared to the three major cereals. This study is therefore aimed at evaluating the influence of extrusion processing variables on NSI and compositional quality of protein-enriched fonio–edible locust powder extrudates. The study sought to determine how locust powder inclusion, screw speed, and barrel temperature affect protein solubility and related functional responses in order to identify processing conditions that maximize nutritional enrichment while preserving desirable extrusion performance. Research shows that incorporating insect powder into cereal based extrudates can increase protein content and modify expansion, colour, texture, and lipid oxidation stability, but usually reports protein solubility in a general sense (e.g., percentage soluble protein at a given pH) while embodying important knowledge gaps regarding how specific extrusion parameters affect insect protein functionality.

MATERIALS AND METHODS

A three-factor, five-level CCRD was used for the extrusion parameters for protein-enriched fonio snacks with edible locust powder. The independent variables and their ranges are presented in Table 1 below. The CCRD comprised 20 experimental runs for k= 3; to yield 8 factorial points, 6 axial points, and 6 center points for error estimation using the factorial equation 2^k+2k+n. This experimental design was run on Design-Expert version 13 Software (Sopyan *et al*., 2022) to develop an empirical model that will establish the relationship between process and response variables.

Table 1: Central Composite Rotatable Design (Ccrd) for Response Surface Methodology

Variables	Symbols	Coded variable Levels(-1,-α, 0,+α,+1)	Range
Edible Locust powder (%)	X_1	3.6,10,20,30, 36.7	3-37
Screw speed (rpm)	X_2	98.87, 150,225.300,351	98-360
Barrel temperature (ºC)	X_3	96.36,110,130,150, 163.64	96-164

Extrusion

Five variable combinations Fonio flour and locust powder were weighed and mixed in ratios according to the CCRD matrix in Table 2 below to obtain the raw composite blends (R).. The fonio- edible locust blend were hydrated to the target moisture content of 20% by adjusting their moisture contents to this target- moisture content in a continuous mixing environment, covered, and equilibrated at room temperature in sealed containers for 12 hours to obtain uniform moisture distribution appropriate for extrusion(Khanna *et al.,* 2019). This equilibration will improve the thermal behaviour and flow of the blend in the extruder barrel (Khanna *et al*., 2019). The samples were summed up to 300g for each sample run. The preconditioned blends of the fonio- edible locust composite with a moisture content of 20% was fed continuously through the hopper into the barrel of Berker Perkins MPF twin-screw extruder with co-rotating and intermeshing screws (figure 1 below).The extruding conditions were; a variable barrel temperature of between 96.36ºC to 168ºC, a variable screw speed of between 150 to 350 rpm, a variable Locust powder composition ranging from 3.6-37% and a fixed die diameter of 3 mm. The extrusion temperatures were set at a uniform value for the three zones of the extruder. The rotating twin screw conveyed the material through zones of increasing temperature of (96.36ºC–168°C) and pressure for extrusion cooking.

Figure 1: Berker Perkins MPF twin-screw extruder with co-rotating and intermeshing screws

The screw speed was set according to the order of extrusion in table 2 below. Each run was stabilized and tested for residence time before extrudate samples‘collection. Extrudates were air cooled at room temperature for 10 minutes and further dried in a cabinet air dryer at 25oC to 10% moisture content, and packaged in moisture-proof bags before being stored at room temperature for onward analysis.

Proximate and Functional properties Analysis

Moisture Content

Dry aluminum dish with cover was preheated at 130 ± 3°C, the heated dish was cooled in desiccator and weighed accurately (tare weight), 2 g of the sample was weighed into the dish, the combined weight of dish plus sample was recorded, the uncovered dish with sample was placed in the ventilated oven (130 ± 3°C), preheated and maintained at this the target temperature for 1 hour to ensure temperature stability. The dish was covered while still in the oven to prevent moisture absorption, the dish was transferred to a desiccator and cooled to room temperature (for about 20 minutes), the cooled dish with dried sample was weighed, drying will be repeated for one hour each until a constant weight is achieved or until difference between two successive weighing exceeds 0.5 mg (AOAC, 2023). Moisture content was calculated as:

$$\text{Moisture}(\%) = \frac{\text{Weight loss on drying}}{\text{Original sample weight (g)}} \times 100$$

Crude Fibre

Sample was defatted although visible fat was not noticed to avoid interference. Sample was homogenized and milled to pass through a 1 mm sieve for uniformity, 1–2 g of prepared sample was accurately weighed into a digestion flask or beaker, acid digestion was carried out by adding 200 ml of 1.25% sulfuric acid solution into the ample. The mixture was boiled under reflux for 30 minutes, stirring occasionally, the hot mixture was filtered through a suitable filter and the residue obtained, was washed with hot distilled water until free of acid. Alkali Digestion was done by transferring the washed residue back to the flask and adding, 200 ml of 1.25% Sodium hydroxide solution to the flask, the resultant solution was boiled under reflux for 30 minutes, with occasional stirring. The final residue was filtered and washed with hot distilled water until neutral and then washed once with 95% ethanol and then with acetone to remove impurities. The residue was dried in an oven at 102 ± 2°C to constant weight, and then cooled in a desiccator before being weighed as dried residue. The dried residue was incinerated in a muffle furnace at 475–500°C for 3–4 hours until white ash was obtained, cooled and weighed (AOAC, 2023). The crude fiber was obtained using:

$$\text{Crude fibre} = \frac{\text{Weight of fibre}}{\text{Weight of sample (g)}} \times 100$$

Crude Fat

A glass thimbles was pre- cleaned and pre-weighed, and sample was milled, homogenized and passed through a 1mm sieve for uniformity. The homogenized sample was placed in the glass thimble which was inserted into the Soxtec extractor and the crude fat present in the sample was extracted with diethyl ether in the Soxtec apparatus for 30-60 minutes with continuous solvent reflux. The solvent was evaporated by gently heating it to boiling point, while the fat residue was dried to a constant weight in the oven at 100-105^{0}C (AOAC, 2023). A blank sample was run to correct for solvent and impurities. The flask was cooled, weighed and crude fat content of the extrudate was calculated as:

$$\text{Crude Fat}(\%) = \frac{\text{Weight of fat(g)}}{\text{Weight o sample(g)}} \times 100$$

Ash Content

Extrudate sample was homogenized and milled to pass through a 1 mm sieve for uniformity, and 1–2 g of sample was accurately weighed into a pre-weighed crucible. The sample in the crucible was dried at 105^0 C until a constant weight was achieved. The crucible with the dried sample was placed in a muffle furnace preheated to 550 ± 25°C and ignited for 3 hours continuously until a white ash was obtained, the crucible was removed from the oven and cooled in a desiccator, before being weighed to obtain the weight of the crucible and ash. The crude ash content of the extrudate was obtained as (AOAC.2023):

$$\text{Crude Ash }(\%) = \frac{\text{Weight of Ash}}{\textit{Weight of sample}} \times 100$$

Crude Protein Analysis

Sample was ground and homogenized thoroughly, 0.5–1.0 g of ground sample was accurately weighed into the digestion flask, catalyst mixture (of 0.3 g CuSO4•5H,O + 3 g K,SO4) was added, 15–20 ml concentrated H,SO4 was carefully added. The flask was gradually heated to boiling until solution clarifies and sample was fully digested. Excess NaOH was added to the digest to convert ammonium ions to ammonia gas and the gas was distilled into 50 ml of boric acid solution containing mixed indicator and collected or for a fixed time ensuring complete ammonia capture, the boric acid solution containing trapped ammonia was titrated with standardized acid (HCl or H,SO4) until endpoint indicated by color change (green to pink) is obtained. A reagent blank was run alongside to correct for background nitrogen (AOAC, 2023). Percentage Nitrogen was calculated as;

$$\text{N\%} = \frac{\text{vol of titrant for sample (ml)} - \text{vol of titrant for blank (ml)} \times \text{Norm of titrant} \times 14.01}{\text{weight of sample (g)}} \times 100\text{n}$$

$$Crude\ \text{Protein }(\%) = \text{Nitrogen}(\%) \times \text{F}$$

Where F = Nitrogen-to-Protein conversion factor (commonly 6.25 for general feeds).

Carbohydrate (by difference)

Carbohydrate content of the extrudate sample was calculated directly by subtracting the sum of moisture, protein, fat, ash, and fiber from 100% (AOAC, 2023) as follows;
Carbohydrate (%) = 100− (Moisture + Protein + Fat + Fibre+ Ash)

Nitrogen Solubility Index of Samples

Nitrogen solubility index (NSI) was measured following method used by Chan *et al.*, 2023. 5 grams of extrudate was weighed into a beaker, the weighed extrudate was ground into a uniform size of 250µm. The ground extrudate sample in the beaker was transferred to a centrifuge tube and 200ml of pre-warmed distilled water (300C) was added to it. The mixture was stirred at 120 rpm in a shaking water bath (SW22 model of Julabo, Seelbach brand, Germany) for 2 hours at 30^{0}C to extract soluble nitrogen. The sample was then re- suspended for 30 seconds in every 30 minutes to prevent the particles of the sample from settling. 10ml of the aliquot mixture was drawn into a centrifuge tube and centrifuged (Sorvall RC6 plus model, of Thermo Fischer Scientific, brand, USA) at 4180xg for 10 minutes and the supernatant was filtered using Whatman no.2 filter paper, with minor modifications. The quantity of water soluble nitrogen in the filtrate was determined according to AACCI method 46-16.01, while Nitrogen content in the original sample was determined using the Kjeldahl method to quantify soluble nitrogen content (Chan *et al.*, 2023) and the NSI, expressed as percentage (Ma *et al.*, 2018) was calculated as;

$$\text{NSI}(\%) = \frac{\text{Water soluble Nitrogen}}{\text{Total Nitrogen in blend}} \times 100$$

RESULTS AND DISCUSSION

Nutritional Composition of Blends and Extrudates

Across blends R1–R5, mean moisture content ranged from 10.7% to 12.8% (Table 2). Blends with higher locust powder inclusion exhibited slightly higher moisture, consistent with the high water-binding capacity of insect proteins and the reported water absorption of fonio and related small-grain cereals (Vanqa *et al.*, 2022). Higher moisture levels (R3, R4) may shorten shelf life by increasing water activity, but can also promote steam generation and cell expansion during extrusion, whereas lower moisture (R1, R5) improves storage stability and reduces microbial risk while generally limiting expansion. Ash content was moderate in all blends, with R3 and R4 showing the highest values, reflecting the mineral contribution of the locust fraction. This may affect the colour and taste of extrudates due to the darker hue and characteristic flavour of edible insect ingredients. Fibre content ranged from 0.9% to 3.0%, with the lowest value in R5, which contained the least edible locust powder. This is consistent with the contribution of chitin and chitin-associated polysaccharides in insect exoskeletons to total dietary fibre. Elevated fibre levels (R1, R3) can enhance nutritional quality but typically reduce starch gelatinisation and bubble growth during extrusion, yielding denser, less expanded products, whereas lower fibre (R4) favours smoother extrusion, higher expansion and crisper textures because fibre interferes with cell wall thinning and gas cell coalescence in expanded snacks(Kasparak *et al*. 2013).

Fat content varied between 4.2% (R4) and 7.9% (R3), largely driven by the oil-rich locust fraction. Higher fat levels (R3, R5) imparted a slightly clumpy feel to the blends and are expected to decrease expansion during extrusion by lubricating the melt, lowering specific mechanical energy and limiting starch gelatinisation, while also increasing susceptibility to oxidative rancidity Gao *et al.*, 2024).. Lower fat content (R4) generally supports better puffing and crispness but may require additional fat or flavouring for optimal sensory quality. Protein content ranged from 9.3% (R4) to 19.5% (R3), with R2, R3 and R5 classified as high-protein blends suitable for protein-fortified extrudates. Elevated protein levels improve nutritional density but can compete with starch for available water, limiting starch gelatinisation and expansion and thereby modifying cell structure and texture. In contrast, the lower-protein blend (R4) is more starch-driven and is expected to produce lighter, highly expanded extrudates. Protein–starch interactions formed during extrusion, including non-covalent complexes and Maillard-type linkages, are known to influence colour, texture and digestibility of starch-based products

Carbohydrate content was highest in R4 and lowest in R3, reflecting the reciprocal dilution of starch by protein and fat. High carbohydrate levels favour extensive starch gelatinisation, gas cell stabilisation and crisp texture in extruded snacks and breakfast cereals, while lower carbohydrate, as in high-protein, high-fat blends (R3), tends to reduce expansion but enhance nutritional density. Overall, the compositional gradients in moisture, fibre, fat, protein and carbohydrate across R1–R5 are expected to translate into distinct extrusion behaviours and product properties, underpinning the need to optimise formulation and process parameters jointly for targeted functional and nutritional outcomess (Sabowale *et al*., 2023). The mean proximate composition of the fonio–locust extrudates is summarized in Table 2 below.

Table 2: Proximate Compsition of the Raw Fonio-Locu St Powder Blends

Blends	Moisture (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	Carbohydrate (%)
R1	10.7	3.3	2.2	6.8	12.4	64.6
R2	11.6	2.6	1.4	6.5	18.5	59.3
R3	11.8	3.4	2.6	7.6	18.5	56.1
R4	10.9	2.2	0.9	4.2	9.3	72.5
R5	12.8	3.9	3.0	7.9	19.5	52.9

Carbohydrate content ranged from 60.72% (S8) to 75.88% (S1), compared with 53.8–72.5% in the corresponding raw blends, reflecting both concentration effects after moisture loss and extrusion induced structural changes in starch and non starch polysaccharides. Extrusion promotes water removal, disrupting native granular and cell wall matrix, making starch more accessible to analytical extraction and enzymatic hydrolysis(Zhang et al., 2023).Recent work shows that extrusion decreases relative crystallinity, gelatinization enthalpy, slowly digestible starch (SDS) and resistant starch (RS), while increasing the proportion of gelatinized, enzyme-accessible starch that is quantified as "available carbohydrate" in standard assays(Đermanović *et al*., 2021), as well as destroying the compact granular structure of starch converting it into a more open, porous matrix (Zhang *et al.*2023). The inclusion of edible locust powder, which contributes less carbohydrate and more protein and fat than fonio, initially lowers the carbohydrate fraction of the raw blends relative to pure cereal flour. During extrusion, insect proteins denature and may form new protein–protein and protein–starch associations, but the dominant carbohydrate still arises from fonio starch. High mechanical energy and shear in the barrel can partially disrupt protein matrices and surface protein layers around starch granules in both cereal and insect tissues, exposing encapsulated starch and facilitating its conversion from SDS and RS into more rapidly digestible fractions. Collectively, these effects: moisture removal, destruction of semi-crystalline regions, depolymerization of starch chains, and partial breakdown of fibre and cell-wall polysaccharides help explain why the extrudates exhibit higher measured carbohydrate percentages than the corresponding raw fonio–locust blends, and while their starch is expected to be more digestible.

The ash content of the extrudates ranged from 1.68% to 2.90%, representing a modest decrease compared with the raw fonio–locust blends (2.2–3.9%). This reduction is consistent with reports that extrusion can lower measured ash in some cereal systems, largely due to concentration of organic matter, matrix changes, and analytical variability rather than true mineral losses (Azzollini *et al*., 2018). Most bulk minerals (e.g. Ca, Fe, Zn, K, Na) are thermally stable at typical extrusion temperatures; however, recent reviews indicate that total mineral content is generally preserved, although mineral bioaccessibility can change as binding to phytic acid, phenolics, dietary fibre and proteins are altered. Therefore, the lower ash values observed in the fonio–locust extrudates likely reflect changes in mineral distribution and extractability, and a relative dilution effect by more extractable organic solids, rather than a meaningful loss of minerals during processing. Extrusion at high temperature and shear can hydrolyze phytate to lower inositol phosphates, releasing minerals and inorganic phosphate, while also changing their solubility and distribution (Diop *et al*., 2024). Heat-labile vitamins and cofactors (some B-vitamins present in fonio) also assist in mineral reduction/chelation and are markedly degraded during extrusion, especially at high barrel temperatures and low feed moisture. Also High screw speed and specific mechanical energy (SME) generate intense shear, which breaks cell walls and protein networks, releasing minerals from localized spots (insect tissues) into the melt; this occurs via rupture of hydrogen bonds, electrostatic interactions, and some ester linkages in the cell wall–phenolic complex. Sobota *et al*. 2010 reported that extrusion of cereal blends at higher screw speeds and lower moisture increased fines formation and changed ash content slightly, attributing changes to segregation and loss of mineral-rich bran particles. For insect-enriched cereals, Azzollini *et al* 2018 observed that process conditions (barrel temperature, screw speed) significantly affected composition as well as that macro and micro-nutrient levels in the final products which differed slightly from the blends, consistent with non-uniform distribution and possible fines loss. The dietary fibre content of the fonio–locust extrudates ranged from 0.17% (S16) to

2.20% (S17), lower than the 0.9–3.0% measured in the corresponding raw blends.

This reduction is consistent with researches that extrusion cooking decreases total dietary fibre (TDF), particularly the insoluble fraction (IDF), while increasing soluble dietary fibre (SDF) through thermomechanical depolymerization of cell-wall polysaccharides. In the raw fonio–locust matrix, fibre arises from cereal components (cellulose, arabinoxylans, β-glucans, hemicelluloses, lignin) while from insect, it is derived from the chitin–protein complexes (Boakaye *et al.,* 2023). Studies by Azzollini *et al* (2018) reports that extrusion disrupts glycosidic bonds in non-starch polysaccharides, converting some IDF into SDF and low-molecular-weight carbohydrates. These heat-labile‖ fibre components which no longer behave as gravimetrically recoverable fibre, decrease the measured fibre fraction, while the dietary fibre contents of the raw fonio-locust power blend could be lost due to the heat induced cleavage of feruloylated arabinoxylans in the cell walls of the fonio grains due to extrusion temperatures. Hydrolysis of ferulate esters breaks cross-links, loosens the insoluble network, while releaseing phenolic acids, thereby: decreasing the rigidity of the insoluble fibre matrix.while increasing solubility of the remaining polysaccharide fragments which contributes to loss of IDF and a reduction in measured fibre content.. Edible locusts provide chitin, a β(1→4) linked polymer of N acetyl D glucosamine associated with cuticular proteins, which contributes to the insoluble fibre fraction. High extrusion temperatures and shear can depolymerise chitin chains and promote partial deacetylation towards chitosan like structures, disrupting the ordered chitin–protein matrix and increasing solubility of degradation products. As a result, material that originally behaves as insoluble dietary fibre can be converted into more soluble carbohydrate or nitrogenous fragments that escape quantification in conventional fibre assays

Table 3: Mean Proximate Composition of the Extrudates

Sample	Moisture (%)	Ash (%)	Fibre (%)	Fat (%)	Protein (%)	Carbohydrate (%)
S1	6.00	2.24	1.22	2.87	16.27	71.44
S2	5.17	2.03	1.22	3.42	15.94	72.23
S3	5.65	2.34	1.21	2.95	18.70	69.17
S4	6.80	1.68	1.36	3.11	17.18	69.89
S5	5.51	1.94	1.93	4.79	23.23	62.61
S6	7.90	1.90	1.42	2.87	15.33	70.60
S7	7.09	2.12	1.21	2.86	20.22	66.51
S8	7.59	2.48	1.87	4.67	22.68	60.72
S9	7.65	1.70	0.44	1.15	13.27	75.79
S10	7.57	1.88	1.66	2.51	15.59	70.81
S11	5.30	1.93	1.19	2.63	16.52	72.44
S12	6.54	2.03	1.22	2.45	17.25	70.55
S13	9.76	1.91	1.06	1.96	15.86	69.45
S14	9.07	2.34	0.97	2.37	10.92	74.34
S15	8.26	1.89	1.11	1.85	15.22	71.68
S16	8.87	1.92	0.17	1.48	11.69	75.88
S17	7.40	2.79	2.20	4.86	20.79	61.97
S18	7.23	2.24	1.01	2.16	12.49	74.89
S19	5.84	2.90	2.06	6.31	20.47	62.47
S20	7.60	2.78	2.11	4.59	20.96	61.98

Thereby lowering the measured fibre content of the extrudates even though total non-digestible material may be redistributed rather than lost(Gadzama., 2025). Fat content also decreased markedly during extrusion, from 4.2–7.9% in the raw fonio–locust blends to 1.15–4.86% in the extrudates. Similar reductions have been observed in extruded cereal and oilseed products and can be attributed to a combination of thermal oxidation of unsaturated lipids, physical redistribution and loss of surface lipids under high shear, and stronger entrapment of lipids within the starch–protein matrix, which reduces recovery in solvent based fat extraction. Fonio and edible locusts both contain appreciable amounts of linoleic, α linolenic and oleic acids that are susceptible to oxidation at typical barrel temperatures (100–160 °C), especially under low moisture conditions that favour rapid heating and oxygen exposure at the die.

Extrusion has been shown to increase the susceptibility of lipid rich extrudates to oxidation during storage unless antioxidants or protective matrices are present, indicating that part of the apparent fat loss may reflect oxidative degradation to volatile products not captured in gravimetric fat measurements. The Protein content of the extrudates ranged from 13.27% (S9) to 23.23% (S5), demonstrating effective protein fortification by the edible locust powder. Sample S9 prepared from an R4 fonio-locust blend containing 3.18% edible locust powder (dry basis) and a blend protein level of 9.3% (Table 3) had an increase in protein content to 13% which illustrates how small additions of insect powder, which typically contain >50% crude protein on a dry basis, substantially increase the blend's protein concentration. The observed rise in extrudate protein is attributable to both compositional effect and to changes induced by extrusion cooking: high temperature, shear, and pressure remove moisture and some non-protein solids (concentrating protein), while disrupting non-covalent interactions and some disulfide bonds (causing protein unfolding and partial aggregation). The break in starch–protein and chitin–protein associations, together also increases protein solubility and apparent digestibility (Combryznski *et al*., 2023; Fu *et al*., 2024). Fonio, an underutilized cereal with 7 g protein/100 g raw grain, further contributes to the final protein content

Nitrogen Solubility Index

Protein solubility, assessed by the nitrogen solubility index (NSI), displayed a U-shaped pH profile with minimum solubility at pH 7.0, increased solubility at pH 3.5 (30–55%) and highest solubility at pH 9.0 (65–80%) from Table 4 below. The minimum near neutrality indicates the composite fonio–locust system's isoelectric region, where reduced net charge promotes protein–protein attraction, aggregation and precipitation. Increased NSI at pH 3.5 reflects partial protonation and aggregate disruption, while the high NSI at pH 9.0 reflects extensive deprotonation, increased net negative charge, unfolding and enhanced dispersibility of cereal and insect proteins (Chan *et al*., 2023). Extrusion reduced overall NSI relative to native material—consistent with denaturation and insoluble aggregate formation reported for thermomechanical processing—yet substantial solubility at alkaline pH shows many peptide chains remain small or charged enough to stay dispersed (Patel *et al*., 2015; Samard *et al*., 2021). Sample-level variability (e.g., higher NSI for S8, S9, S13, S18; low NSI for S6, S12, S20) correlates with formulation (fonio:locust ratio, protein content) and processing severity (SME, barrel temperature, screw speed, residence time), where milder energy input preserved greater solubility and harsher conditions promoted aggregation. Overall, the NSI profile indicates substantial but incomplete denaturation—compatible with retained functional properties and acceptable digestibility for snack applications, while permitting alkaline extraction for protein recovery if required (Samard *et al.,* 2021).

Effect of Extrusion Variables on the Nitrogen Solubility Index of the Extrudates

Extrusion markedly altered the nitrogen solubility index (NSI) of fonio–locust extrudates, producing a characteristic U-shaped solubility pH profile with a minimum near pH 7.0 (Table 5), higher NSI at pH 3.5 (30–55%) and maximal NSI at pH 9.0 (65–80%). These results indicate substantial thermo mechanical denaturation and partial aggregation while retaining significant alkaline solubility. the NSI variation correlated with process severity and formulation: higher barrel temperatures, greater specific mechanical energy (SME), increased screw speed and longer residence times promoted protein unfolding, intermolecular cross linking and insoluble aggregate formation, lowering NSI across pH (S6, S12, S20). Conversely, lower SME and shorter residence time (e S8, S9, S13, and S18) limited cross linking, preserving dispensability and yielding higher NSI. The pH dependence reflects isoelectric precipitation near neutrality and enhanced electrostatic repulsion under acidic or alkaline conditions that redisperse partially denatured peptides. Overall, extrusion produced partially texturized but not over cross linked protein networks suitable for snack matrices and amenable to alkaline extraction for protein recovery consistent with established effects of thermo mechanical processing on protein solubility (Patel *et al*., 2015; Samard *et al*., 2021; Chan *et al*., 2023). Table 5 below, shows that NSI is consistently highest at pH 9.0, intermediate at pH 3.5, and lowest at pH 7.0. This supports research works on the solubility of insect proteins under alkaline conditions, (Pismag *et al* 2024, Silvestre *et al*., 2020). However, edible locust powder protein molecules acquired greater net charge and higher electrostatic repulsion due to increase isoelectric points in alkaline pH conditions which increased hydration and subsequently solubility. This shows that protein solubility was greatly impacted by the barrel temperature in connection with screw speed. At low screw speed, increasing temperature from about 110°C to 150°C reduced predicted NSI. At high screw speed, however, increasing barrel temperature raised NSI. In food extrusion, the product experiences simultaneous heating, pressure, mixing and mechanical shear, so barrel temperature and screw speed jointly determine residence time, melt viscosity, protein unfolding, starch–protein interactions and aggregation. From table 1 above, a second-order quadratic response-surface model for each NSI response: pH 3.5, pH 7.0 and pH 9.0 can be fitted an can be given below

$$X1 = \frac{T-130}{20}$$

$$X2 = \frac{S-225}{75}$$

$$X3 = \frac{C-20}{10}$$

Table 4: Nitrogen Solubility Indices of Extrudates at Different Extrusion Parameters

Sample	Barrel Temp (0C)	Screw Speed (rpm)	Locust Powder Composition (%)	NSI at pH 3.5(%)	NSI at pH 7.0(%)	NSI at pH 9.0(%)
S1	110	150	10	43.71	28.57	72.75
S2	110	150	30	52.57	35.26	78.32
S3	150	150	10	38.92	27.52	65.11
S4	150	150	30	45.27	19.30	63.32
S5	110	300	10	29.66	20.35	70.48
S6	110	300	30	25.36	18.32	40.30
S7	150	300	10	41.65	21.78	71.81
S8	150	300	30	59.01	40.33	76.61
S9	130	225	3.18	60.29	38.66	78.48
S10	130	225	36.82	58.47	39.47	69.59
S11	96.36	225	10	52.31	25.35	76.05
S12	163.64	225	20	35.81	20.38	62.77
S13	130	98.87	20	54.38	35.27	79.05
S14	130	351.13	20	35.22	24.95	56.95
S15	130	225	20	49.16	20.30	70.39
S16	130	225	20	34.34	17.28	63.27
S17	130	225	20	39.31	20.38	67.87
S18	130	225	20	53.18	42.34	72.36
S19	130	225	20	38.36	24.68	69.51

Sample	Barrel Temp (0C)	Screw Speed (rpm)	Locust Powder Composition (%)	NSI at pH 3.5(%)	NSI at pH 7.0(%)	NSI at pH 9.0(%)
S20	130	225	20	28.87	14.51	52.76

At pH 3.5(figure 2), the surface suggests the most favourable NSI region at relatively low barrel temperature and low screw speed (130ºC, 225rpm).

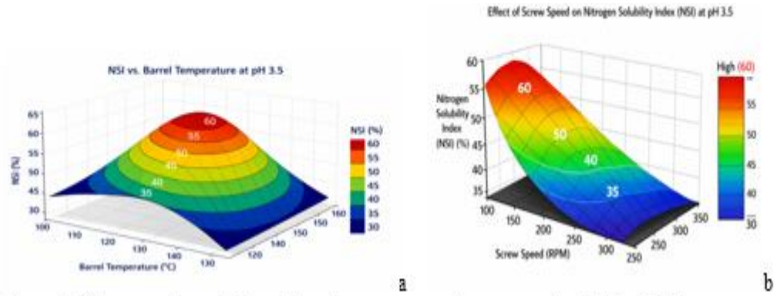

Figure 2: 3D contour plots of effect of barrel temperatures and screw speed on NSI at 3.5 pH

Under these gentler extrusion conditions, less thermal/shear denaturation and fewer insoluble aggregates are expected, so more nitrogenous material remains extractable. At this pH NSI values for locust powder extrudates span roughly 25–60%, indicating substantial but incomplete solubilization of insect proteins as many side chains are protonated, electrostatic repulsion is reduced, and chitin–protein interactions are strengthened, which makes the system sensitive the unfolding and aggregation of the edible locust proteins At pH 7.0(figure 3), the contour pattern is similar (130ºC, 225 rpm, 42. 34% NSI) but the NSI values are much lower. This implies that neutral extraction did not sufficiently disrupt the aggregated edible locust protein matrix formed during extrusion.

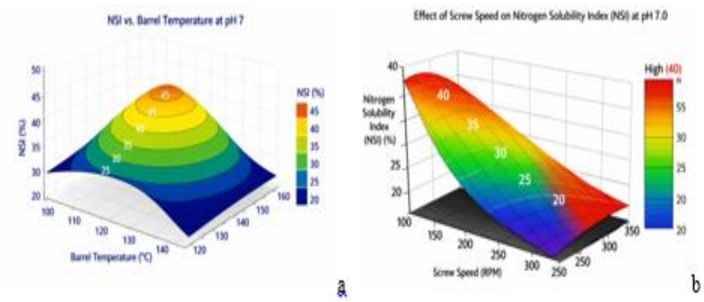

Figure 3: 3D contour plots of effect of barrel temperatures and screw speed on NSI at 7.0 pH

The model fit is also weakest at pH 7.0 as the aggregated structures formed during extrusion of insect protein–chitin matrices are not efficiently dispersed, even when some unfolding occurs At pH 9.0(figure 4), the contours show much higher NSI values and a clearer response surface. Alkaline pH promotes protein ionization and electrostatic repulsion, improving the release of soluble nitrogen, as alkaline extraction can recover a large proportion of insect protein despite extrusion

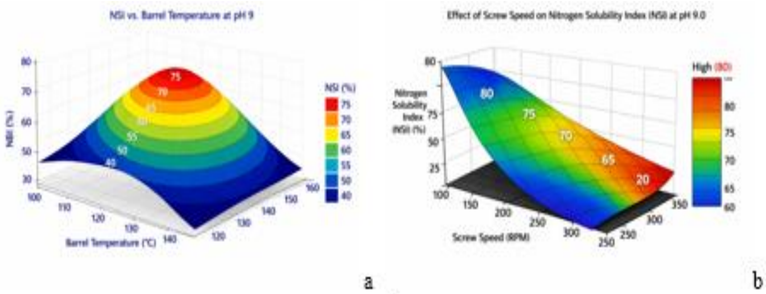

Figure 4: 3D contour plots of effect of barrel temperatures and screw speed on NSI at 9.0 pH

Research shows that alkaline conditions enhance solubility of insect proteins by increasing net negative charge, disrupting protein–protein and protein–chitin associations, and improving hydration of hydrophobic surfaces exposed during extrusion. However, the high NSI at pH 9 does not mean the proteins are undamaged; rather, alkaline extraction can solubilize fractions that remain poorly soluble at neutral pH (Pismag *et al* 2024). At pH 9.0 there is a broad high-NSI plateau at intermediate barrel temperatures (120–140 °C) and moderate screw speeds (150–225 rpm), where NSI nears or exceeds 70% (S9, S10, S13, S18). At very high screw speeds (351 rpm; S14), NSI drops despite alkaline conditions, indicating that excessive shear can create heavily aggregated or cross linked insect protein structures that remain only partially soluble even at pH 9.0, a behaviour observed when insect proteins are over-processed in high-shear rates. However, low-temperature, moderate-speed conditions. (96 °C, 225 rpm; S11) still yielded high NSI (76%), confirming that for insect protein the balance between moderate unfolding and limited aggregation is more critical than pushing temperature to the upper extremes.

CONCLUSION

This research shows that: there is an optimal extrusion severity at intermediate barrel temperature and screw speed that maximizes insect protein extractability, particularly evident at pH 9.0 but also reflected at pH 3.5. Neutral pH measurements underestimate the "recoverable" functionality of the insect protein system, while alkaline NSI better captures how process conditions modulate the underlying protein structure, Also, Insect-specific features such as the presence of chitin and higher natural hydrophobicity likely shift the NSI response surfaces making the high-shear/high-temperature more detrimental to the protein structure.

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