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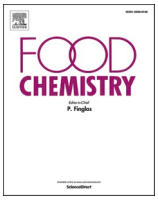

Novel palm shortening substitute using a combination of rapeseed oil, linseed meal and beta-glucan

Shirley L. Sampaio [a,1], Timothy Chisnall [a,2], Stephen R. Euston [b], Catriona Liddle [a], Julien Lonchamp [a,*]

[a] School of Health Sciences, Queen Margaret University, Queen Margaret University Drive, Edinburgh EH21 6UU, United Kingdom
[b] Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, Edinburgh EH14 4AS, United Kingdom

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ABSTRACT

This study investigated the potential of a novel sustainable ingredient composed of rapeseed oil, linseed meal and beta-glucan (PALM-ALT) to mimic palm shortening functionality in cake. The combined functional properties of linseed meal and beta-glucan led to stable semi-solid emulsion-gels (20–31 μm oil droplet size, 105–115 Pa.s viscosity and 60–65 Pa yield stress). PALM-ALT contained 25 and 88% less total and saturated fat than palm shortening, whilst PALM-ALT cakes contained 26 and 75% less total and saturated fat than the palm-based control. PALM-ALT cakes matched the flavour profile of the palm-based control, while rapeseed oil cakes tasted more sour and less sweet than the control ($p < 0.05$). PALM-ALT cakes proved less hard and more cohesive than the control ($p < 0.05$), with 100% of the consumer panel preferring PALM-ALT formulations. This study demonstrated the unique potential of PALM-ALT as healthier, sustainable and competitive alternative to palm shortening.

1. Introduction

Due to its unique composition and functionality, high yield and low production costs, palm oil has become the main functional fat ingredient across the food sector (Nor Aini & Miskandar, 2007), with the industry dependent on the expansion of palm cultivation to meet its increasing supply requirements. This expansion and associated global transportation of palm oil have led to devastating environmental impacts, including destruction of tropical rainforest at a rate of 117,000 ha/yr between 1995 and 2015 (Austin et al., 2017), destruction of animal habitat with a 25% decline in Bornean orangutan populations between 2007 and 2017 (Santika et al., 2017), loss of biodiversity with 68 to 77% lower densities of bird species in palm plantations in comparison with natural habitats in Thailand (Jaroenkietkajorn, Gheewala, & Scherer, 2021) and greenhouse gas emissions of 371 Mt. CO_2 eq per year (Chiriacò, Galli, Santini, & Rulli, 2024). As a result, the food industry has developed a number of strategies to reduce this impact, including the use of palm oil alternatives. The high saturated fat content (49%) of palm oil is also being questioned due to the association between high saturated fat consumption and increased risk of cardiovascular diseases (Briggs, Petersen, & Kris-Etherton, 2017).

Due to its unique fatty acid composition (with an almost 50–50 split between saturated and unsaturated ones) and distribution on triglycerides (with significant amounts of trisaturated and asymmetrical monounsaturated triglycerides), palm oil crystallises in β' form (Smith, 2001), which makes it semi-solid at room temperature and an ideal plastic shortening (Nor Aini & Miskandar, 2007). Following fractionation, the stearin fraction of palm oil (containing the highly saturated triglycerides) is used as high-melting hard stock in shortening blends (Wassell, 2006). In bakery, palm fat is used for its shortening, foam stabilising, texturising, mouthfeel and preservation (due to its high oxidative stability) properties (Pande, Akoh, & Lai, 2012). In cakes, the small β' crystals of palm shortening provide batter aeration for adequate cake texture as they can locate at the air–oil interface and stabilise air bubbles (Nor Aini & Miskandar, 2007).

Alongside efforts to develop more sustainable cultivation practises

Abbreviations: FA, Full Acidity; H-FA, Heated Full Acidity; SA, Semi Acidity; H-SA, Heated Semi Acidity; NA, No Acidity.

* Corresponding author at: School of Health Sciences, Queen Margaret University, Queen Margaret University Drive, Edinburgh EH21 6UU, United Kingdom.
E-mail address: JLonchamp@qmu.ac.uk (J. Lonchamp).
[1] Present address: Kenvue, São José dos Campos, São Paulo, Brasil
[2] Present address: NEC Software Solutions, iMex Centre, Hemel Hempstead, UK

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including RSPO (Roundtable on Sustainable Palm Oil) standards, palm fat replacement strategies have led to a number of alternatives including coconut oil, other tropical oils, hydrogenated oils and oleogels, however their impact and expansion have been limited so far due to health, cost, availability, legislation and/or technological issues, and there is currently no palm shortening alternative that is at the same time functional, sustainable, nutritionally-balanced and competitive. Due to their lower saturated fat levels (7–15%) and higher unsaturated fat levels (82–89%) including polyunsaturated fat (29–68%), liquid oils including rapeseed, sunflower and soybean are healthier than palm oil but exhibit low oxidative stability and are liquid at room temperature, so are inadequate for hard or aerated products including bakery. The use of hydrogenated oils as bakery shortenings has significantly reduced in the industry (Hinrichsen, 2016) due to the increased risk of coronary heart disease associated with the generation of trans fatty acids during partial hydrogenation (Bendsen, Christensen, Bartels, & Astrup, 2011). The high saturated fat content (90%) of coconut fat makes it solid at room temperature but is limiting its expansion due to health concerns, while other tropical oils including shea, sal and illipé have limited availability and high costs (Hinrichsen, 2016). A number of oleogel systems (in which oils are converted into semi-solid gels) have been shown to replicate palm shortening functionality in products including cakes via the use of phytosterols (Willett & Akoh, 2019), waxes (Oh, Amoah, Lim, Jeong, & Lee, 2017), methyl cellulose and xanthan gum (Patel, Cludts, Sintang, Lesafferb, & Dewettincka, 2014) or shellac (Patel et al., 2014), however their application has been limited so far due to complex technology, legislation issues, cost or availability.

There is a need to explore novel synergistic blends of sustainable ingredients capable of producing semi-solid emulsions, including combinations of amphiphilic proteins, which can adsorb at the oil-water interface, and polysaccharides, which can increase the viscosity by forming networks. The design of emulsion-gels, in which emulsified oil droplets are immobilised in a gelled aqueous phase, offers an opportunity as these systems can be prepared with proteins and polysaccharides commonly used in the food industry (including whey proteins, soy proteins, carrageenans, alginates, gums, pectins and starches) (Abdullah, Javed, & Xiao, 2022).

A preliminary study carried out in our laboratory screened a range of plant proteins, seed meals, fibre extracts, starches and their combinations for their potential to produce emulsion-gels with rapeseed oil. The results highlighted that combinations of defatted linseed meal and beta-glucan extract were able to create semi-solid emulsions (data not shown). This novel ingredient mix, composed of defatted linseed meal, beta-glucan, rapeseed oil, water and acidifying agent, was named PALM-ALT. Leahu, Ropciuc, Ghinea, and Damian (2023) previously reported the potential of other oilseed meals (sesame and walnut) to form low-fat mayonnaise-like emulsion-gels using vegetable oil, whilst an oat bran extract containing 22% beta-glucan was used to create semi-solid emulsion-gels with olive oil (Pintado, Herrero, Jiménez-Colmenero, and Ruiz-Capillas (2016).

Rapeseed, linseed and oat are grown on all continents (except Antarctica), so PALM-ALT has the potential to be produced locally on the global scale. A life-cycle analysis of the novel ingredient is ongoing, with early results indicating that PALM-ALT produced and used in the EU and UK could generate up to 3.57 times less CO_2 emissions than non-RSPO palm oil produced in South-East Asia and subsequently imported, due to the impact of deforestation and transport (data not shown).

The first component of this system, defatted linseed (flaxseed) meal is the solid co-product remaining following pressing of the seeds to extract the oil and subsequent defatting. Despite high concentrations of proteins, fibre and polyunsaturated fatty acids, this co-product is still predominantly used as animal feed or fertiliser, with food industry applications limited to nutritional fortification. Linseed meal contains a number of components exhibiting functional properties, including proteins with emulsifying, foaming and rheological properties, soluble mucilage fibre compounds with gelling, emulsion-stabilising and foam-stabilising properties (including gums, arabinoxylans and rhamnogalacturonans) and insoluble fibre compounds with rheological properties (including cellulose, hemicellulose and lignin). A protein concentrate from defatted linseed meal was shown to stabilise soybean oil-in-water emulsions, with high-viscosity high-oil phase ones proving the most stable (Wang, Li, Wang, & Özkan, 2010), while a linseed protein concentrate showed good emulsifying activity and emulsion- and foam-stabilising capacities (Martínez-Flores et al., 2006). A demucilaged linseed protein isolate exhibited high emulsifying properties (Kaushik et al., 2016), while protein isolates from defatted linseed meal displayed gelling properties (Krause, Schultz, & Dudek, 2002). Linseed mucilage gum solutions showed high water-holding capacity, viscosity and cold-set gelling properties (Chen, Xu, & Wang, 2006), while linseed mucilage extracts exhibited high foaming properties (Kaur, Kaur, & Punia, 2018). Dev and Quensel (1988) initially reported that the co-precipitation of mucilage with linseed or linseed meal protein concentrates led to higher foaming capacity and emulsifying properties as the negative charge of mucilage resulted in an increase in droplet repulsion and decrease of creaming rate. Nasrabadi et al. (2019) further showed that nano-assemblies of linseed proteins and mucilage adsorbed onto the surface of emulsion droplets, forming a protecting layer preventing flocculation and coalescence, thus improving emulsion stability via a Pickering-type mechanism.

Oat beta-glucan, the second component of the PALM-ALT ingredient, is widely used as functional ingredient in the food industry due to its water-binding, thickening, gelling and emulsion- and foam-stabilising properties, which result from the formation of junction zones and intermolecular hydrogen bonding between its polysaccharide chains (Burkus & Temelli, 2000; Laitinen, Mäkelä-Salmi, & Maina, 2023). Karp, Wyrwisz, and Kurek (2020) applied oat beta-glucan as gluten replacer in cake formulations and reported structure-forming properties. Oat beta-glucan concentrate exhibited emulsion-stabilising properties in emulsion-gel systems prepared with unsaturated oils via the formation of a network around the oil droplets, with potential application as saturated fat mimetics (Karp, Wyrwisz, & Kurek, 2019; Sereti, Kotsiou, Biliaderis, Moschakis, & Lazaridou, 2023).

A number of studies highlighted mechanisms of molecular interaction and conjugation between beta-glucan and proteins, leading to aggregation or gelation. Zielke, Lu, Poinsot, and Nilsson (2018) reported that oat beta-glucan and whey protein or gliadin aggregated at low pH due to electrostatic interactions. Zhao, Wu, Li, and Liu (2014) found that beta-glucan facilitated the aggregation and gelation of soy protein isolate through hydrophobic interactions, Shen, Liu, Dong, Si, & Li, 2015) studied the gelation patterns of mixtures of oat β-glucan and soy protein isolate and reported their conjugation via hydrogen bonds.

The PALM-ALT ingredient offers a number of benefits in comparison with current palm shortening alternatives, including its lower saturated fat content, clean label status, availability (with all its components currently in use by the industry) and cost-competitiveness. The purpose of this paper is to ascertain if the combination of rapeseed oil, defatted linseed meal and beta-glucan of the PALM-ALT ingredient can effectively replace palm shortening in a model cake formulation. We hypothesised that the combined emulsifying and rheological properties of defatted linseed meal and beta-glucan would create a stable emulsion-gel system able to mimic palm shortening functionality during baking, resulting in the maintenance of the sensory profile of the cake product.

2. Material and methods

2.1. Materials

The defatted linseed meal and beta-glucan extract used were Food-Solute Linsol Flour Organic (Th. Geyer, Germany) and PromOat Beta Glucan Gluten-Free (Lantmännen Oats AB, Sweden). The defatted linseed meal contained 10% of oil, 34% protein and 47% fibre (product specification sheet), while the beta-glucan extract contained 43.5%

carbohydrates, 5% protein and 36% fibre including 29% beta-glucan (product specification sheet) (Supplementary table 1).

The shortening Trex (UK), composed of 57% RSPO-certified palm oil and 43% rapeseed oil, was used to formulate the control cake product. Albex Crystal rapeseed oil (Kent Foods), which contains 7 g saturated fat, 63 g monounsaturated fat and 29 g polyunsaturated fat (product specification sheet), was used to formulate the PALM-ALT ingredients and rapeseed oil cakes. A 6% acidity cider vinegar (Aspall, UK) was used as acidifying agent and purchased from Sainsbury's (UK).

2.2. PALM-ALT preparation

Five prototypes of the palm shortening substitute were formulated to study the impact of temperature and pH on its structure and functionality: FA (Full Acidity), H-FA (Heated Full Acidity), SA (Semi Acidity), H-SA (Heated Semi Acidity) and NA (No Acidity) (Table 1). A high internal phase emulsion system close to that of a mayonnaise was chosen, with 75% of rapeseed oil and 20 to 23% of water.

The dry mix components (defatted linseed meal and beta-glucan extract powders) were combined using a handled whisk. The rapeseed oil was added and the mixture was gently whisked until uniform. The aqueous phase (water and cider vinegar) was then added and the mixture was gently whisked until uniform. The H-FA (Heated Full Acidity) and H-SA (Heated Semi Acidity) mixtures were heated at 70 °C for 1 min. All mixtures were let to rest for 20 min before homogenisation using a L5M mixer (Silverson, UK) fitted with a standard emulsor screen at 2000 rpm for 60 s then 5800 rpm for 60 s. An additional set of FA, H-FA, SA and H-SA samples were heated at 80 °C for 30 min in a water bath, producing the P-FA (Pasteurised Full Acidity), P-H-FA (Pasteurised Heated Full Acidity), P-SA (Pasteurised Semi Acidity) and P-H-SA (Pasteurised Heated Semi Acidity) ingredients. All the samples were stored at 4 °C for 8 days.

Table 1
Composition of the PALM-ALT prototypes and of the cake formulations prepared with palm shortening, rapeseed oil and the PALM-ALT prototypes.

PALM-ALT ingredient prototypes			
Ingredient (weight in g)	Full Acidity (FA)	Semi Acidity (SA)	No Acidity (SA)
Dry mix			
Defatted linseed meal	2.26	2.26	2.26
Beta-glucan extract	0.38	0.38	0.38
Water phase			
Water	20.40	21.53	22.66
Cider vinegar (6% acidity)	2.26	1.13	0.00
Oil phase			
Rapeseed oil	74.70	74.70	74.70
Total	100.00	100.00	100.00

Cake formulations						
Ingredient	Palm shortening		Rapeseed oil		PALM-ALT	
	Weight (g)	%	Weight (g)	%	Weight (g)	%
Self-raising flour	187.5	28.7	187.5	28.7	187.5	28.7
Baking powder	5.0	0.8	5.0	0.8	5.0	0.8
Salt	1.0	0.1	1.0	0.1	1.0	0.1
Caster sugar	172.5	26.4	172.5	26.4	172.5	26.4
Whole eggs	150.0	23.0	150.0	23.0	150.0	23.0
Full-fat milk	22.0	3.3	22.0	3.3	22.0	3.3
Vanilla extract	3.0	0.5	3.0	0.5	3.0	0.5
Palm shortening	112.5	17.2	0.0	0.0	0.0	0.0
Rapeseed oil	0.0	0.0	112.5	17.2	0.0	0.0
PALM-ALT	0.0	0.0	0.0	0.0	112.5	17.2
Total	653.5	100	653.5	100	653.5	100

2.3. PALM-ALT characterisation

2.3.1. Methodology

The protein composition of the defatted linseed meal was determined by proteomic analysis and gel electrophoresis. The PALM-ALT ingredient prototypes (FA, H-FA, SA, H-SA and NA) were characterised in terms of pH, oil droplet size distribution, rheology and nutritional profile, whilst their structure was observed by confocal microscopy. The nutritional profiles of the PALM-ALT prototypes were calculated based on the composition specification sheets of the different components used.

2.3.2. Proteomic composition of the defatted linseed meal

Ten mg of defatted linseed meal were mixed with 200 µl of Laemmli 2× concentrate sample buffer (Sigma Aldrich Ltd., UK) and heated at 70 °C for 5 min in a DB-3 thermomixer (Techne, US). The samples were prepared for proteome analysis via Filter-Aided Sample Preparation (FASP) following the method of Le Bihan et al. (2011). Thirty µl of the samples were mixed with 200 µl of UA solution, which contained 8 M urea (U5128, Sigma Aldrich Ltd., UK) in 0.1 M Tris/HCL pH 8.5), in filter units and centrifuged at 14,000 x*g* for 15 min, following which the flow-through from the collection tubes was discarded. One hundred µl of IAA solution, which contains 0.05 M iodoacetamide (Sigma Aldrich Ltd., UK) in UA, were added and the mixtures were mixed at 600 rpm in the thermomixer for 1 min and incubated without mixing for 20 min. The filter units were centrifuged at 14,000 x*g* for 10 min. One hundred µl of UA solution were added to the filter units and the mixtures were centrifuged for 10 min at 14,000 g, with this step repeated twice. One hundred µl of a 50 mM ammonium bicarbonate (Sigma Aldrich Ltd., UK) solution were added to the filter units and the mixtures were centrifuged for 10 min at 14,000 g, with this step repeated twice. One hundred and twenty µl of ABC solution, which contained 0.05 M NH_4HCO_3 (Sigma Aldrich Ltd., UK) in water with 20 µg/ml trypsin (Sigma Aldrich Ltd., UK) (at a 1:100 enzyme to protein ratio) were added and the mixtures were mixed at 600 rpm for 1 min. The filter units were incubated at 37 °C overnight, transferred to new tubes and centrifuged at 14,000 x*g* for 10 min. Fifty µl of a 10% acetonitrile solution (HPLC quality, Fisher, UK) were added and the filter units were centrifuged at 14,000 x*g* for 10 min. The samples were acidified with trifluoroacetic acid (99% purity sequencing grade, Sigma Aldrich Ltd., UK) and dried in a vacuum centrifuge.

The resulting peptide residues were analysed using a nanoflow HPLC Electrospray Tandem Mass Spectrometry (nLC-ESI-MS/MS) (Thermo Scientific, US). The samples were solubilised in 20 µl of 5% acetonitrile (HPLC quality, Fisher, UK) with 0.5% formic acid (Suprapure 98–100%, Merck, Germany) using the auto-sampler of the RSLCnano uHPLC system (Thermo Scientific, US). The peptide ions were detected by electrospray ionisation mass spectrometry MS/MS with an Orbitrap Elite MS (Thermo Scientific, US). Ionisation of LC eluent was performed by interfacing the LC coupling device to an NanoMate Triversa (Advion Bioscience) with an electrospray voltage of 1.7 kV. Injection volumes of 5 µl of the reconstituted protein digest were desalted and concentrated for 10 min on trap column (0.3 × 5 mm) using a 25 µl/min flow rate of 1% acetonitrile with 0.1% formic acid. Peptide separation was performed on a Pepmap C18 reversed phase column (50 cm × 75 µm, particle size 3 µm, pore size 100 Å) (Thermo Scientific, US) using a solvent gradient at a fixed flow rate of 0.3 µl/min. The solvent composition was 0.1% formic acid in water (A) (HPLC quality, Fisher, UK) and 0.08% formic acid in 80% acetonitrile 20% water (B). The solvent gradient was 4% B for 12 min, 4 to 60% B for 90 min, 60 to 99% B for 14 min and held at 99% B for 5 min.

The Orbitrap Elite MS acquired a full-scan MS in the range of 300 to 2000 *m/z* for a high-resolution precursor scan at 60,000 resolution (at 400 m/z), while simultaneously acquiring up to the top 15 precursors which were isolated at 0.7 m/z width and subjected to CID fragmentation (35% normalised collision energy) in the linear ion trap using rapid

scan mode. Singly charged ions were excluded from selection, while selected precursors were added to a dynamic exclusion list for 30 s.

Protein identification was performed using the Mascot version 2.6.2 search engine (Matrix Science Ltd., UK) against the NCBIprot database using both the family *Linaceae* (including the *Linum* genus or linseed) and order *Malpighiales* (including *Linaceae*). A mass tolerance of 10 ppm was allowed for the precursor and 0.3 Da for MS/MS matching. The exponentially modified Protein Abundance Index (emPAI) was calculated for each protein based on the method defined by Ishihama et al. (2005). Four replicates were analysed.

2.3.3. Gel electrophoresis of the PALM-ALT components

Three types of PAGE (polyacrylamide gel electrophoresis) were carried out on the defatted linseed meal (DLM), beta-glucan (BG) and 6:1 DLM:BG mixture (replicating the 6:1 DLM:BG ratio in the PALM-ALT ingredient) using a Mini-Protean Tetra Cell System with TGX 4–20% Tris-glycine gels (Bio-Rad Laboratories Ltd.,UK): Native PAGE, non-reducing SDS–PAGE (Sodium Dodecyl Sulfate) and reducing SDS-PAGE (with addition of β-mercaptoethanol). The method described by Havea, Watkinson, and Kuhn-Sherlock (2009) was followed with minor modifications. Three sets of 500 μl solutions (2% *w*/w solid) of the samples were prepared in deionised water and stirred for one hour. The first set (Native PAGE) was mixed with 500 μl of Novex native TrisGly 2× sample buffer (Life Technologies, UK), the second set (non-reducing SDS-PAGE) with 500 μl of Laemmli 2× concentrate sample buffer (Sigma Aldrich Ltd., UK) and the third set (reducing SDS-PAGE) with 475 μl of Laemmli 2× concentrate sample buffer and 25 μl β-mercaptoethanol (Sigma Aldrich Ltd., UK). The mixtures were heated at 70 °C for 10 min in a DB-3 thermomixer (Techne, US). Twenty μl of sample or 30 μl of the Precision Plus protein standard (Bio-Rad Laboratories Ltd., UK) were loaded onto the wells. The gels were run for 1 h at 100 V in Tris/Glycine/SDS buffer for the SDS-PAGE gels (Bio-Rad Laboratories Ltd., UK) and in native buffer for the Native PAGE gels (Bio-Rad Laboratories Ltd., UK). The gels were stained in a Coomassie brilliant blue solution (VWR Ltd., UK) and destained in glacial acetic acid:methanol: deionised water at a 7:5:88 ratio (HPLC grade, Fisher, UK). The gels were scanned using a Biorad Gel Doc EZ imaging system (Bio-Rad Laboratories Ltd., UK) and the analysis was carried out using the associated Image Lab software.

2.3.4. Oil droplet size distribution

The average oil droplet size distribution of the PALM-ALT ingredients or D[3,2] (surface weighted mean) was measured using a Mastersizer 2000 (Malvern Instruments Ltd., UK) set at the refractive index of rapeseed oil (1.474) and at 10% laser obscuration adjustment. The experiment was repeated thrice, with three replicates per sample for each experiment.

2.3.5. Rheology

The rheological profile of the PALM-ALT ingredients was determined using a Kinexus Pro+ rheometer (Netzsch, Germany) equipped with a 4°/40 mm cone (gap 144 μm). An amplitude sweep was carried out, with the shear viscosity (Pa.s) measured through a complex shear strain increase from 0 to 100%. The impact of a three-step shear on the shear viscosity was then measured, with an initial shear rate of 0.1 s^{-1} for 10 min followed by 100 s^{-1} for the next 10 min and finally 0.1 s^{-1} for the last 10 min. Finally, the yield stress of the samples was determined by measuring the shear viscosity through a shear stress increase from 0 to 100 Pa. Measurements were carried out at 25 °C and a frequency of 1 Hz. The experiment was repeated twice, with three replicates per sample for each experiment.

2.3.6. Confocal microscopy

The PALM-ALT ingredients were imaged using a Leica TCS2 confocal laser scanning microscope (Leica Microsystems, Germany). The micrographs were recorded at a 512 × 512 pixel resolution and analysed using the DM SDK software version 4.2.1 (Leica Microsystems, Germany). The fluorescent dye Rhodamine B (Sigma Aldrich Ltd., UK) was added to stain a number of linseed meal and oat components including proteins and carbohydrate molecules. The dye was excited at 514 nm, the collection range was set at 600–700 nm and a 10×/0.25 dry lens was used.

2.4. Cake preparation

A standard Madeira-type pound cake formulation was chosen to assess the potential of PALM-ALT prototypes as palm shortening substitutes. Five products were formulated with the same amount of functional fat (17.2% *w*/w): positive control (palm shortening), negative control (rapeseed oil) and three PALM-ALT formulations (FA, H-FA and SA prototypes) (Table 1). Two additional products were prepared using the FA prototype to test the functionality of PALM-ALT at reduced concentrations: FA-20P (prepared with a 20% lower FA ingredient level) and FA-20 T (prepared with a 20% lower total ingredient weight). Preliminary tests showed that H-SA and FA-20P cakes had less potential to match the sensory profile of the palm shortening control (data not shown), so these formulations were not assessed by the panel as six cakes was the maximum number of products that the panellists could test before generating sensory fatigue.

The other ingredients were used in the same proportions in all products: self-raising flour, baking powder, salt, caster sugar, whole eggs, full-fat milk and vanilla extract (Sainsbury's, UK). The cakes were baked in a pre-heated oven at 170 °C for 40 min. The nutritional profiles of the cakes were calculated based on the specification sheets of the ingredients used.

2.5. Cake characterisation

2.5.1. Methodology

The cakes were characterised 24 h after baking in terms of height, sensory profile, water activity and instrumental texture and colour profiles, whilst their structure was observed by SEM (Scanning Electron Microscopy). The nutritional profiles of the different cakes were calculated based on the composition specification sheets of the different ingredients used.

2.5.2. Sensory analysis

The sensory profile of the cake products was analysed by both trained and consumer panels. The sensory analysis procedures were submitted to and approved by Queen Margaret University's research ethics committee. Written consent was obtained from each panellist. All procedures were carried out in compliance with Queen Margaret University's health and safety guidelines and risk assessments.

Sensory tests were carried out under controlled lighting and temperature conditions in sensory booths using the Compusense Cloud software (Compusense Inc., Canada). The cakes were served at room temperature. A 12-member panel (seven females and five males aged 39–65 years old) was selected and trained to objectively rate the intensity of the sensory attributes based on a QDA (Quantitative Descriptive Analysis) procedure. The training included basic odour, taste and texture recognition, sensory attribute generation and selection, exposure to commercial products, intensity rating and familiarisation with the software. Thirteen sensory attributes were selected: crust colour, crumb colour, crumb density, overall odour, overall flavour, fatty flavour, sourness, sweetness, firmness, crumbliness, moistness, fat mouthfeel and overall sensory quality. The attributes were scored on an intensity of perception scale of 1 to 9 with 1 (very low), 3 (low), 5 (medium), 7 (high) and 9 (very high). The test was replicated twice.

A consumer panel of 42 people (27 females and 15 males aged 28–70 years old) indicated their hedonic preference for one of the six samples.

2.5.3. Physico-chemical characterisation

The maximum and minimum heights of the different cake products were measured using a ruler. The texture of the cakes was analysed by TPA (Texture Profile Analysis) using a TA.XT Plus texture analyser (Stable Micro Systems, UK) equipped with a P/25 25 mm diameter cylindrical probe (Stable Micro Systems, UK). Squares of 4 × 4 cm of cake were prepared, and a double compression test was performed at a 1 mm/s crosshead speed and 75% compression rate. The following parameters were recorded: hardness (g), springiness, cohesiveness, gumminess, chewiness and resilience. The results were analysed using the associated Exponent software (Stable Micro Systems, UK). The experiment was repeated thrice, with six replicates per cake analysed for each experiment.

The colour profiles of the crust and crumb of the different cakes were separately assessed using a PCE-CSM 7 colorimeter (PCE Instruments Ltd., UK). Twelve areas were measured for crust and crumb of each cake. The colourimeter was calibrated using a white reference tile and a light trap. The illuminant chosen was D65 and the observer used was 10°. Five colour readings were measured: L* (lightness index scale ranging from 0 for black to 100 for white), a* (degree of redness if positive and greenness if negative), and b* (degree of yellowness if positive and blueness if negative), C* (chroma) and h* (hue angle).

The water activity (a_w) of the products was measured using a Novasina LabTouch water activity meter (Novatron Scientific Ltd., UK).

2.5.4. Scanning-electron microscopy

The microstructure of the cake formulations was analysed using a Quanta FEG 650 scanning electron microscope (FEI, US). The samples were examined in low vacuum mode using a backscattered detector. The SEM was operated at 20 kV accelerated voltage, 0.825 Torr pressure and x100 magnification. Three replicates of each product were imaged.

2.6. Statistical analysis

Statistical analysis of the rheological, sensory, texturometry and colourimetry data was carried out using the SPSS Statistics 23.0 software (IBM, USA). One-way ANOVA tests were performed on the rheological, texturometry and colourimetry data, while two-way ANOVA tests were carried out on the sensory data. The ANOVA tests were followed by post hoc Tukey's HSD (Honestly Significant Difference) tests for multiple pair-wise comparison. A p value of 0.05 was used as cut-off for significance.

3. Results and discussion

3.1. Proteomic composition of the defatted linseed meal

The major proteins detected in the defatted linseed meal are presented in Table 2. Three low-molecular weight oleosins (between 15 and 16 kDa) and one high-molecular weight oleosin (19 kDa) were identified among the six most concentrated proteins reported. Plankensteiner, Hennebelle, Vincken, and Nikiforidis (2024) reported high oil-in-water emulsifying and emulsion-stabilising properties for oleosin extracts, which adsorbed onto oil droplets as individual proteins or micelles and exhibited steric and electrostatic repulsion. A conlinin (albumin) was also identified at 19 kDa as the ninth most abundant protein based on the *Linaceae* database search. Conlinin was previously reported as the main protein in linseed gum and as a key contributor to its high emulsifying and stabilising properties due to its surfactant activity at the oil-water interface (Liu, Shim, Poth, & Reaney, 2016).

Four legumins or legumin-like proteins and one vicilin-like protein were detected based on the *Malpighiales* database search. Legumins and vicilins are the main proteins found in legumes and showed high emulsifying, foaming and gelling properties (Chang et al., 2022; Mession, Chihi, Sok, & Saurel, 2015). Three amaranthin-like lectins were detected. Lectins are carbohydrate-binding proteins, and a number of plant lectins have been reported to bind with beta-glucan (De Coninck & Van Damme, 2022). A 93 kDa SMP-LTD domain-containing protein was identified. Plant synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domains have been shown to bind to lipids (Benitez-Fuente & Botella, 2023).

3.2. Gel electrophoresis of the PALM-ALT components

The Native-PAGE, non-reduced SDS-PAGE and reduced SDS-PAGE profiles of the defatted linseed meal (DLM), beta-glucan (BG) and DLM-BG mixture are presented in Fig. 1. Several concentrated low-molecular weight bands (A to F) were reported in the reduced SDS-PAGE gel for DLM and DLM-GB (Fig. 1c). Band B matched the molecular weights of the three low molecular weight oleosin isoforms (15 to 17 kDa) identified by proteomics (Table 2), while band C corresponded to the high molecular weight oleosin isoform and to conlinin (18 to 19 kDa). Band F (circa 45 kDa) matched the molecular weight of one of the legumins (legumin B precursor). Conlinin was previously predicted at 19 kDa (Liu et al., 2016).

Bands C, D and E significantly weakened on the non-reduced SDS-PAGE gel for both DLM and DLM-GB (Fig. 1b), whilst a large concentrated band (circa 35 to 60 kDa) appeared (band H), suggesting that some of the proteins of bands C, D and E were present as dimers or trimers in non-reducing SDS conditions and were fragmented into their monomeric form in reducing SDS conditions. Band H also matched the molecular weights of the legumin A, vicilin-like protein and one of the amaranthin-like lectins (55 to 57 kDa), suggesting that these proteins could have fragmented into smaller monomeric forms corresponding to bands B, C, D or E in reducing SDS conditions (Fig. 1c).

Chung, Lei, and Li-Chan (2005) obtained similar gel electrophoresis profiles for non-defatted linseed, with three major bands reported at 20, 23 and 31 kDa in reducing SDS-PAGE conditions (corresponding to bands C, D and E) and two major bands identified in non-reducing conditions at 40 and 48 kDa (corresponding to the large extended band H).

The Native PAGE profiles of the DLM and DLM-BG samples (Fig. 2a) displayed two major bands at circa 170 and 270 kDa (I and J), corresponding to large oligomers or polymers which fragmented into oligomeric and monomeric forms in non-reducing and reducing SDS conditions (Fig. 2b and c). Band J matched the molecular weight of linin (11-12S globular protein), which was previously reported by Chung et al. (2005) at 252–298 kDa as the major linseed protein. Krause et al. (2002) identified four linin subunits (36, 46, 50 and 55 kDa), which could be present in the enlarged band H and separated from each other in non-reducing SDS conditions.

A fraction of the DLM and DLM-BG samples did not migrate on the Native PAGE and non-reduced SDS-PAGE gels, leading to the concentrated bands K in the wells. These bands were composed of very large proteins or aggregates, which were broken down into oligomers or monomers in reducing SDS conditions. Chung et al. (2005) previously identified a 365 kDa oligomeric protein in defatted linseed.

The electrophoretic profiles of the beta-glucan sample exhibited two major bands in reducing SDS conditions (L and M) and one predominant band (N) in non-reducing SDS conditions. The band N matches the molecular weight (54 kDa) of the 12S oligomeric globulin fraction of oats in non-reducing SDS conditions (Ercili-Cura et al., 2015). The 12S globulin dissociates into two subunits of 22 and 32 kDa in reducing SDS conditions (Ercili-Cura et al., 2015), corresponding to the bands L and M. Fat-binding, gelling and foaming properties have been reported for the oat globulin fraction (Kumar, Sehrawat, & Kong, 2021).

3.3. Oil droplet size distribution of the PALM-ALT ingredient prototypes

The oil droplet size distributions measured at days 1 and 8 for the PALM-ALT prototypes are presented in Fig. 2 alongside confocal images of the emulsion structures. The FA (Full Acidity) and SA (Semi Acidity)

Table 2
Proteomic composition of the defatted linseed meal.

Code	Identification	Database	Reported functionality	Molecular mass (kDa)	SDS-PAGE band		emPAI	
					Without β-ME	With β-ME	*Linaceae* database	*Malpighiales* database
ADD54611.1	Amino acid transporter, partial	*Linum usitatissimum*		10,759	**A**	**A**	0.46 ± 0.06 (7)	n/a
KDP22870.1	hypothetical protein JCGZ_00457	*Malpighiales*		12,197	**A**	**A**	n/a	0.40 ± 0.06 (6)
XP_002298046.1	nuclear transcription factor Y subunit C-3	*Malpighiales*		12,941	**A**	**A**	n/a	0.37 ± 0.05 (7)
KAB5564697.1	hypothetical protein DKX38_004751	*Malpighiales*		13,061	**A**	**A**	n/a	0.37 ± 0.03 (8)
ABB01620.1	**Oleosin low molecular weight isoform, partial**	*Linum usitatissimum*	**Emulsion, foam**	14,982	B-H	B	**1.29 ± 0.09 (2)**	**0.74 ± 0.06 (3)**
ABB01619.1	**Oleosin low molecular weight isoform, partial**	*Linum usitatissimum*	**Emulsion, foam**	15,013	B-H	B	**0.74 ± 0.07 (5)**	**0.74 ± 0.07 (4)**
ABB01618.1	**Oleosin low molecular weight isoform**	*Linum usitatissimum*	**Emulsion, foam**	16,029	B-H	B	**0.68 ± 0.05 (6)**	**0.68 ± 0.05 (5)**
AFN53709.1	LEA group 1-embryo development protein	*Linum usitatissimum*		16,795	B-H	B	0.84 ± 0.10 (3)	0.28 ± 0.04 (9)
AFU96921.1	30S ribosomal protein S7, partial	*Linum usitatissimum*		18,345	C-G-**H**	**C**	0.25 ± 0.03 (8)	n/a
ABB01616.1	**Oleosin high molecular weight isoform**	*Linum usitatissimum*	**Emulsion, foam**	18,689	C-G-**H**	**C**	**4.95 ± 0.29 (1)**	**1.44 ± 0.09 (2)**
CAC94011.1	**Conlinin**	*Linum usitatissimum*	**Emulsion**	19,456	C-G-**H**	**C**	**0.24 ± 0.02 (9)**	**0.24 ± 0.02 (10)**
KAF2298697.1	hypothetical protein GH714_025292	*Malpighiales*		21,131	C-D-**H**	**C-D**	n/a	0.22 ± 0.03 (11)
AMY26645.1	Chromosome maintenance element 4	*Linum usitatissimum*		21,405	C-D-**H**	**C-D**	0.21 ± 0.03 (10)	n/a
TKR74437.1	hypothetical protein D5086_0000294700	*Malpighiales*		24,483	D-**H**	**D**	n/a	0.19 ± 0.03 (12)
AMY26651.1	Late Embryogenesis Abundant group 1 protein	*Linum usitatissimum*		24,891	D-**H**	**D**	0.18 ± (11)	0.18 ± 0.02 (13)
TKS08074.1	hypothetical protein D5086_0000107240	*Malpighiales*		25,705	D-**H**	**D**	n/a	0.18 ± 0.03 (14)
PNT53255.1	hypothetical protein POPTR_001G076400	*Malpighiales*		34,416	**H**	**E**	n/a	0.15 ± 0.01 (19)
AEG76894.1	Glycosyl transferase family 1 protein	*Linum usitatissimum*		35,459	**H**	**E**	0.13 ± 0.02 (14)	n/a
PNT48064.1	hypothetical protein POPTR_002G059100	*Malpighiales*		36,757	**H**	**E**	n/a	0.12 ± 0.02 (20)
AFN53654.1	Transcription factor HBP-1b	*Linum usitatissimum*		38,830	**H**		0.11 ± 0.02 (15)	n/a
AMY26626.1	Unidentified	*Linum usitatissimum*		40,561	**H**		0.11 ± 0.01 (16)	n/a
AND01208.1	Unidentified	*Linum usitatissimum*		42,784	**H**		0.10 ± 0.02 (17)	n/a
EEF28917.1	**legumin B precursor**	*Malpighiales*	**Emulsion, gel, foam**	45,652	**H**	F	n/a	0.10 ± 0.01 (21)
KDP40078.1	hypothetical protein JCGZ_02076	*Malpighiales*		47,026	**H**	F	n/a	0.09 ± 0.01 (22)
AMY26641.1	Cation efflux protein	*Linum usitatissimum*		50,322	**H**	F	0.18 ± 0.02 (12)	n/a
AFJ52983.1	UDP-glycosyltransferase 1	*Linum usitatissimum*		52,617	**H**		0.08 ± 0.01 (19)	n/a
KDP26529.1	hypothetical protein JCGZ_17687	*Malpighiales*		52,924	**H**		n/a	0.08 ± 0.01 (23)
AFJ53024.1	UDP-glycosyltransferase 1	*Linum usitatissimum*		53,001	**H**		0.08 ± 0.01 (20)	n/a
XP_002300775.1	**legumin A**	*Malpighiales*	**Emulsion, gel, foam**	54,751	**H**		n/a	0.17 ± 0.02 (15)
XP_012064865.1	**vicilin-like seed storage protein At2g28490**	*Malpighiales*	**Emulsion, gel, foam**	56,827	**H**		n/a	0.16 ± 0.02 (18)
AIU47284.1	**Amaranthin-like lectin**	*Linum usitatissimum*	**Binding to β-glucan**	57,232	**H**		0.08 ± 0.01 (18)	n/a
AIU47276.1	**Amaranthin-like lectin**	*Linum usitatissimum*	**Binding to β-glucan**	66.887			0.13 ± 0.02 (13)	n/a
AND01162.1	**SMP-LTD domain-containing protein**	*Linum usitatissimum*	**Binding to lipids**	93,394			0.05 ± 0.01 (23)	n/a
AIU47275.1	**Amaranthin-like lectin**	*Linum usitatissimum*	**Binding to β-glucan**	118,971			0.04 ± 0.01 (24)	n/a
XP_021631311.1	**legumin A-like**	*Malpighiales*	**Emulsion, gel, foam**	n/a			n/a	0.17 ± 0.02 (16)
XP_021640278.1	**legumin A-like**	*Malpighiales*	**Emulsion, gel, foam**	n/a			n/a	0.17 ± 0.02 (16)

emPAI: exponentially modified Protein Abundance Index, β-ME: β-mercaptoethanol.

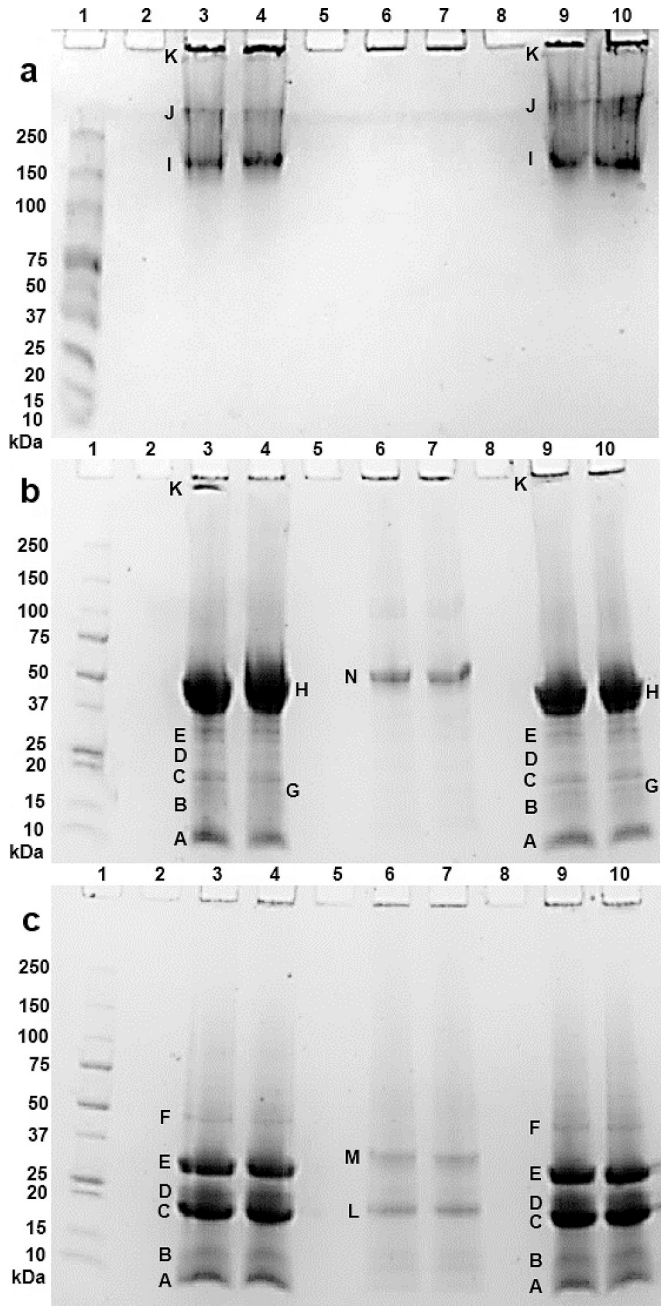

Fig. 1. Native-PAGE (a), non-reduced SDS-PAGE (b) and reduced SDS-PAGE (c) profiles of defatted linseed meal (DLM), beta-glucan (BG) and DLM-BG mixture (2% w/w solid solutions).
Precision Plus reference marker [lane 1], blank [2], defatted linseed meal [3–4], blank [5], beta-glucan [6–7], blank [8], DLM-BG mixture [9–10].

samples displayed similar mean oil droplet sizes of 29 μm (D[3,2] surface weighted means) at day 1, while the NA (No Acidity) sample showed a higher value (41 μm) (Fig. 2a). The addition of a heating step during the preparation of the FA and SA prototypes did not significantly affect the mean oil droplet sizes, with 22 and 31 μm reported for H-FA (Heated Full Acidity) and H-SA (Heated Semi Acidity) at day 1. Similarly, the subsequent pasteurisation of the ingredients did not significantly increase the mean oil droplet sizes, with D[3,2] values of 28, 20, 29 and 33 μm measured for P-FA (Pasteurised Full Acidity) P-H-FA (Pasteurised Heated Full Acidity), P-SA (Pasteurised Semi Acidity) and P-H-SA (Pasteurised Heated Semi Acidity) at day 1 (Fig. 2c, P-FA and P-H-SA not shown). All the acidified samples showed a relatively stable

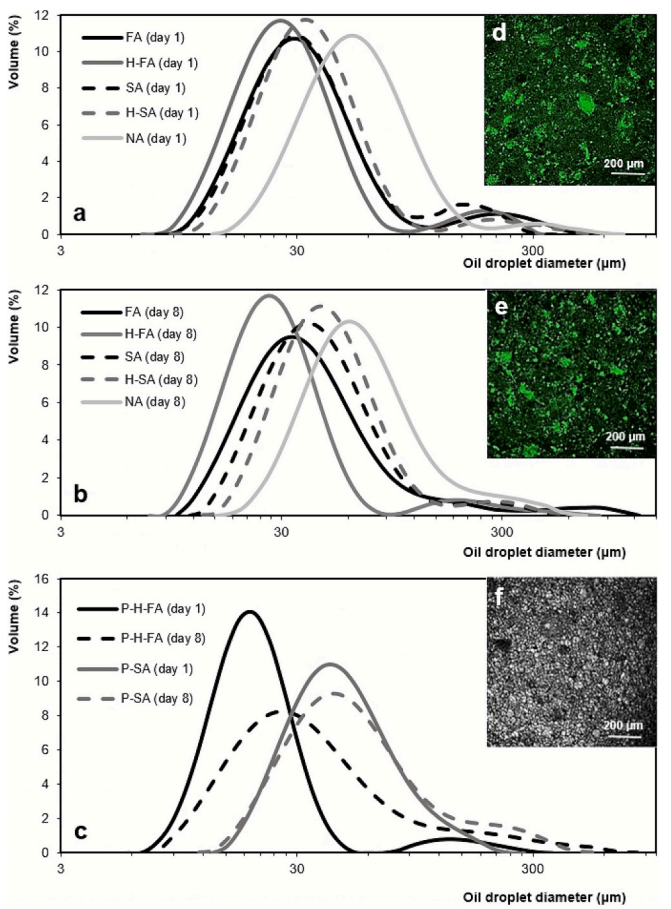

Fig. 2. Oil droplet size distribution of the PALM-ALT ingredient prototypes at day 1 (a), day 8 (b), following pasteurisation (c) and confocal micrographs of the H-FA prototype at day 1 (d) and FA prototype at day 8 (e and f).
FA (Full Acidity), H-FA (Heated Full Acidity), SA (Semi Acidity), H-SA (Heated Semi Acidity), NA (No Acidity), P-H-FA (Pasteurised Heated Full Acidity) and P-SA (Pasteurised Semi Acidity).

mean oil droplet sizes after 7 days of storage: 31 μm (FA), 36 μm (SA), 24 μm (H-FA), 41 μm (H-SA), 32 μm (P-FA), 25 μm (P-H-FA), 38 μm (P-SA) and 43 μm (P-H-SA) (Fig. 2b and c, P-FA and P-H-SA not shown). The NA ingredient proved less stable with a D[3,2] of 56 μm on day 8. These results highlighted that acidification resulted in smaller mean oil droplet size and higher emulsion stability, while the influence of the heating step on the PALM-ALT emulsifying properties was not significant.

The pH values of the NA, SA and FA samples were 6.11, 5.08 and 4.62. A concentration of large, aggregated structures was observed on the confocal micrographs of the acidified PALM-ALT emulsions (Fig. 2d and e) in comparison with the NA one, which contributed to their higher stability by limiting droplet coalescence (Fig. 2f) and the resulting emulsion destabilisation. These structures could be aggregates of linseed proteins, which previously exhibited low solubility between pH 4.9 and 5.8 (Krause et al., 2002) and/or aggregates of beta-glucan and linseed proteins formed at low pH via electrostatic interactions, as found by Zielke et al. (2018) for combinations of oat beta-glucan and whey protein or gliadin. Gao et al. (2022) reported the presence of similar aggregates in the continuous phase of emulsions stabilised by combinations of whey proteins and pectin.

The formation and stabilisation of the PALM-ALT emulsions can be explained by a potential synergy of action between a number of components. Due to their interfacial properties, a number of identified linseed proteins (Table 2) had the ability to form and initially stabilise oil droplets, including oleosins (Plankensteiner et al., 2024), conlinin (Liu et al., 2016), legumins and vicilins (Chang et al., 2022; Mession

et al., 2015). Kaushik et al. (2016) previously reported a higher stability at low pH for emulsions stabilised by a linseed protein isolate, whilst Nwachukwu and Aluko (2018) attributed the high emulsifying properties of linseed proteins in acidic conditions to the contribution of conlinin. Linseed mucilage potentially co-adsorbed with linseed proteins onto the surface of the oil droplets, further stabilising them via repulsion and/or Pickering mechanisms, as reported by Dev and Quensel (1988) and Nasrabadi et al. (2019). Finally, linseed mucilage carbohydrates (including gums, arabinoxylans and rhamno-galacturonans) and/or oat beta-glucan potentially contributed as emulsion stabilisers with the formation of stabilising networks around the emulsified droplets, as previously observed by Li et al. (2023) and Karp et al. (2019).

3.4. Rheological profile of the PALM-ALT ingredient prototypes

The yield stress, amplitude sweep and three-step shear test profiles of the FA, H-FA, SA, H-SA and NA emulsions are presented in Fig. 3. The consistency observed for the PALM-ALT prototypes was similar to that of a semi-solid emulsion-gel system (Fig. 3d).

The NA emulsion exhibited a lower yield stress (40 Pa) than the acidified samples (between 60 and 65 Pa) (Fig. 3a). The NA prototype also showed a lower viscosity (75 Pa.s) than the acidified ones (between 105 and 115 Pa.s) in the linear region of the amplitude sweep test (Fig. 3b), while the FA, H-FA, SA and H-SA emulsions displayed similar viscosity profiles. Finally, the NA sample exhibited a small level of hysteresis during the three-step flow test (Fig. 3c), while the acidified samples instantly recovered their initial viscosity values.

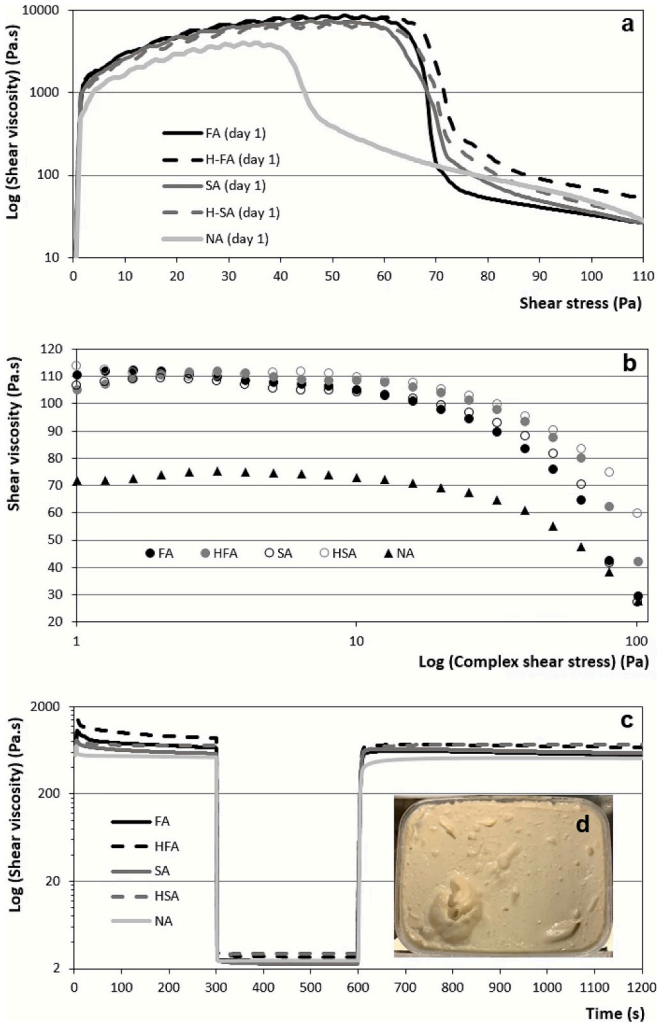

Fig. 3. Rheological profiles of the PALM-ALT ingredient prototypes at day 1 (a to c) and digital photography of the H-FA prototype (d).
Yield stress test (a), Amplitude sweep test (b), Three-step shear test (c).
FA (Full Acidity), H-FA (Heated Full Acidity), SA (Semi Acidity), H-SA (Heated Semi Acidity) and NA (No Acidity).

Similarly to the oil droplet size distribution profiles, these profiles indicated that acidified conditions led to emulsions which were more resistant to deformation and shear, while the heating step of the PALM-ALT process did not significantly influence the rheological properties. The concentration of aggregated structures in acidified PALM-ALT emulsions (Fig. 2d and e) contributed to their higher viscosity and resistance to shear in comparison with the NA emulsion.

A number of PALM-ALT components contributed to the viscosity and gel-like properties of the emulsions, including linseed proteins (gelling properties as reported by Krause et al., 2002), linseed mucilage (water-holding capacity, viscosity and cold-set gelling properties as reported by Chen et al., 2006) and oat beta-glucan (water-binding, thickening, gelling and emulsion-stabilising properties as reported by Laitinen et al., 2023). As found by Zielke et al. (2018) for combinations of oat beta-glucan and whey protein or gliadin, a potential aggregation of linseed proteins and oat beta-glucan via electrostatic interactions in acidified conditions could have also contributed to the higher rheological profiles obtained in comparison with the NA sample.

The gel-like texture observed for the acidified emulsions suggested the presence of a gel-like network in the aqueous phase surrounding the emulsified droplets, further increasing the emulsion stability. This structure could be formed by linseed mucilage carbohydrates and/or oat beta-glucan, as previously reported by Li et al. (2023) and Karp et al. (2019), or result from the conjugation and co-gelation of linseed proteins and oat beta-glucan, as identified for mixtures of soy protein isolates and oat beta-glucan (Shen et al., 2015; Zhao et al., 2014).

3.5. Nutritional profiles

The nutritional profiles of palm shortening, rapeseed oil, the PALM-ALT prototypes (FA, H-FA, SA and FA-20 T) and cakes prepared with these ingredients are presented in Table 3. The saturated fat contents of the PALM-ALT ingredient prototypes were >30% lower (88%) than that of palm shortening so they qualified for a reduced saturated fat claim. The PALM-ALT ingredients also showed a 25% lower total fat level and a 76% higher polyunsaturated fat level than palm shortening.

The saturated fat contents of cakes prepared with the PALM-ALT ingredient prototypes were >30% lower (75 to 78%) than that of the palm shortening control cake, so they qualified for a reduced saturated fat claim. In addition, the total fat content of the FA-20 T cake (prepared using a 20% lower total ingredient weight) was >30% lower (36%) than that of the palm shortening cake and amounted to 1.5 g/100 g so the formulation also qualified for both reduced saturated fat and low saturated fat claims. The other PALM-ALT cake formulations (prepared with the FA, H-FA and SA ingredients) showed a 26% lower total fat content and a 25% higher polyunsaturated fat content in comparison with the palm shortening product.

3.6. Sensory profile of the palm-free cake formulation

The sensory and instrumental analysis profiles of cakes prepared with palm shortening, rapeseed oil or the PALM-ALT ingredient prototypes are presented in Table 4. Scanning-electron micrographs and digital photos of the cakes are shown in Fig. 4.

The heights of the FA, H-FA, SA and FA-20P (20% PALM-ALT reduced) formulations were 21 to 25% higher than those of the palm shortening and rapeseed oil cakes (Table 4 and Fig. 4), while the 20% total weight reduced PALM-ALT formulation (FA-20 T) showed a comparable height to the palm shortening control. The presence of the acidifying agent in the acidified PALM-ALT prototypes potentially enhanced the acid-base reaction involving the self-raising flour and

Table 3
Nutritional profile of the PALM-ALT prototypes and cake formulations prepared with palm shortening, rapeseed oil and the PALM-ALT prototypes.

Nutrient (per 100 g)	Palm shortening (PS)	Rapeseed oil (RO)	PALM-ALT FA and H-FA	PALM-ALT SA	PALM-ALT FA-20 T	% difference FA, H-FA, SA (versus PS)	% difference FA, H-FA, SA (versus RO)	% difference FA-20 T (versus PS)	% difference FA-20 T (versus RO)
Ingredient									
Energy (kJ)	3765	3696	2843	2843	2843	- 24.5%	- 23.1%	- 24.5%	- 23.1%
Energy (kcalories)	900	899	679	679	679	- 24.6%	- 24.5%	- 24.6%	- 24.5%
Fat (g)	**100.0**	**100.0**	**74.9**	**74.9**	**74.9**	**- 25.1%**	**- 25.1%**	**- 25.1%**	**- 25.1%**
Saturated fat (g)	**42.2**	**6.6**	**5.0**	**5.0**	**5.0**	**- 88.3%**	**- 24.9%**	**- 88.3%**	**- 24.9%**
Monounsaturated fat (g)	44.1	57.0	42.6	42.6	42.6	- 3.5%	- 25.3%	- 3.5%	- 25.3%
Polyunsaturated fat (g)	**13.6**	**32.0**	**23.9**	**23.9**	**23.9**	**+ 75.8%**	**- 25.3%**	**+ 75.8%**	**- 25.3%**
Carbohydrate (g)	0.0	0.0	0.2	0.2	0.2	n/a	n/a	n/a	n/a
Sugar (g)	0.0	0.0	0.1	0.1	0.1	n/a	n/a	n/a	n/a
Fibre (g)	0.0	0.0	0.9	0.9	0.9	n/a	n/a	n/a	n/a
Protein (g)	0.0	0.0	0.8	0.8	0.8	n/a	n/a	n/a	n/a
Sodium as salt (g)	0.0	0.0	0.0	0.0	0.0	n/a	n/a	n/a	n/a
Cake formulation (pre-bake stage)									
Energy (kJ)	1653	1653	1454	1460	1417	- 11.7%	- 11.7%	- 14.3%	- 14.3%
Energy (kcalories)	395	395	348	349	339	- 11.7%	- 11.7%	- 14.3%	- 14.3%
Fat (g)	**19.9**	**19.9**	**14.5**	**14.6**	**12.7**	**- 26.6%**	**- 26.6%**	**- 36.2%**	**- 36.2%**
Saturated fat (g)	**6.8**	**1.9**	**1.7**	**1.7**	**1.5**	**- 75.0%**	**- 10.5%**	**- 77.9%**	**- 21.1%**
Monounsaturated fat (g)	8.9	10.6	8.1	8.2	6.9	- 7.9%	- 23.6%	- 22.5%	- 34.9%
Polyunsaturated fat (g)	**3.2**	**5.8**	**3.9**	**4.0**	**3.3**	**+ 25.0%**	**- 32.8%**	**+ 3.1%**	**- 43.1%**
Carbohydrate (g)	48.0	48.0	48.0	48.0	49.7	n/a	n/a	n/a	n/a
Sugar (g)	26.8	26.8	26.8	26.8	27.7	n/a	n/a	n/a	n/a
Fibre (g)	0.9	0.9	1.0	1.0	1.0	n/a	n/a	n/a	n/a
Protein (g)	5.8	5.8	6.0	6.0	6.2	n/a	n/a	n/a	n/a
Sodium as salt (g)	1.9	1.9	1.9	1.9	1.9	n/a	n/a	n/a	n/a

baking powder, thus increasing the release of carbon dioxide bubbles and the resulting rise of the cakes.

A number of PALM-ALT components contributed to the formation and stabilisation of the carbon dioxide bubbles produced, with high foaming properties reported for legumins and vicilins (Chang et al., 2022; Mession et al., 2015) and mucilage (Kaur et al., 2018) and foam-stabilising properties observed for oat beta-glucan (Burkus & Temelli, 2000). The crumb density values of the PALM-ALT products (assessed visually) were significantly higher than those of the palm shortening and rapeseed oil formulations ($p < 0.05$), except for the FA-20 T ingredient, which showed similar values to the two controls (Table 3). The digital photographs of cross-sections of the acidified PALM-ALT cakes showed a higher number and density of small bubbles and fewer large bubbles than the palm shortening and rapeseed oil formulations (Fig. 4), which was in agreement with the higher crumb densities perceived by the trained panel for these products.

The rapeseed oil product proved statistically more sour and less sweet than the palm shortening one ($p < 0.05$), while the PALM-ALT formulations scored similar values to those of the palm shortening cake. The higher sourness and lower sweetness of the rapeseed oil control cake could be due to the generation of acidic, bitter or rancid compounds from non-emulsified rapeseed oil during baking, which would have partially masked the sweetness. Majchrzak et al. (2017) previously reported the development of rancid and acidic off-flavours for rapeseed oil heated at 100 °C and 180 °C, respectively.

The trained panel scored the semi-acidified PALM-ALT cake (SA) with a significantly higher overall sensory quality than the rapeseed oil control product ($p < 0.05$). The other parameters were similar between the products. A hundred percent of the consumer panel indicated their preference for one of the PALM-ALT cakes, including a 60% preference for the SA cake.

3.7. Instrumental characterisation of the palm-free cake formulations

The results indicated a number of statistical differences in instrumental colour and texture profiles between the products ($p < 0.05$) (Table 4). The hardness values of the FA, H-FA, SA and FA-20P (20% PALM-ALT reduced) formulations were significantly lower than those of the palm shortening and rapeseed oil cakes ($p < 0.05$), while the FA-20 T (20% total weight reduced) product showed similar hardness to the two controls. The palm shortening cake was statistically less cohesive than the other formulations ($p < 0.05$), while the FA-20 T cake proved significantly gummier than the other products ($p < 0.05$). The chewiness values reported for the FA, H-FA, SA formulations were comparable to that of the palm shortening cake and statistically higher than those of the rapeseed oil, FA-20P and FA-20 T products ($p < 0.05$). The lower cohesiveness of the palm shortening control (Table 4) was potentially related to the lower crumb density reported by the trained panel (Table 3), as previously suggested by Renzetti and van der Sman (2022) in sugar-reduced cakes.

The scanning-electron micrographs of the palm shortening cake showed the coating of gluten, starch and other microstructures by relatively large pools of fat (Fig. 4a), while the PALM-ALT formulation micrographs (FA, H-FA, SA and FA-20 T) displayed an even coating of these structures by small oil droplets (Figs. 4b to 4e). The FA-20P cake micrograph showed a similar microstructure to the other PALM-ALT products, while the rapeseed oil cake micrograph displayed a similar oil distribution to the palm shortening formulation (micrographs not shown). These observations suggested that the oil fraction of the PALM-ALT ingredient remained emulsified during the baking process. The lower hardness and higher cohesiveness measured for PALM-ALT cakes in comparison with the palm shortening product could be due to a lower overall coverage of the gluten structures, which would have allowed the gluten network to develop more extensively, leading to a softer but more cohesive texture as reported by the trained panel. Karp et al. (2020) previously reported the ability of oat beta-glucan to create gluten-like structures in gluten-free cake formulation, which could have reinforced the viscoelastic network and the resulting higher cohesiveness reported by the trained panel for PALM-ALT cakes.

Differences in hue angle were reported for both crust and crumb. The crust hue angle of the palm shortening cake was significantly different to those of all products ($p < 0.05$) except the FA-20P formulation, while the crumb hue angle values of the FA, FA-20P and FA-20 T cakes statistically differed from those of the other formulations ($p < 0.05$). However, none

Table 4
Sensory and instrumental analysis profiles of the cake formulations prepared with palm shortening, rapeseed oil and PALM-ALT prototypes.

Properties	Palm shortening control	Rapeseed oil control	PALM-ALT FA (Full Acidity)	PALM-ALT H-FA (Heated Full Acidity)	PALM-ALT SA (Semi Acidity)	PALM-ALT FA-20P (20% PALM-ALT reduced)	PALM-ALT FA-20 T (20% Total reduced)
1. Quantitative Descriptive Analysis (QDA)							
Crust colour intensity	6.0 ± 1.2	5.7 ± 0.7	6.2 ± 0.8	5.8 ± 0.2	5.7 ± 0.9	n/a	5.6 ± 0.9
Crumb colour intensity	5.3 ± 0.3	5.1 ± 0.9	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.3	n/a	4.9 ± 0.4
Crumb density (visual)	**4.3 ± 0.9 c**	**4.5 ± 1.2 c**	**5.8 ± 1.1 ab**	**5.5 ± 0.4 ab**	**6.2 ± 0.9 a**	n/a	**4.7 ± 0.8 bc**
Overall odour intensity	4.8 ± 0.6	4.7 ± 0.7	4.7 ± 1.1	4.9 ± 0.6	4.2 ± 1.2	n/a	5.1 ± 1.1
Overall flavour intensity	5.5 ± 1.0	4.6 ± 1.0	5.4 ± 0.4	4.6 ± 0.6	5.3 ± 0.4	n/a	4.5 ± 1.2
Fatty flavour	5.3 ± 1.0	5.0 ± 0.5	4.4 ± 0.8	4.6 ± 0.6	4.3 ± 0.9	n/a	4.9 ± 0.9
Sourness	**3.6 ± 0.8 b**	**5.2 ± 1.1 a**	**4.4 ± 0.9 ab**	**4.6 ± 0.9 ab**	**4.0 ± 1.2 b**	n/a	**4.9 ± 0.8 ab**
Sweetness	**6.2 ± 0.6 a**	**5.1 ± 1.1 b**	**5.5 ± 0.5 ab**	**5.7 ± 0.6 ab**	**5.3 ± 0.7 ab**	n/a	**5.4 ± 1.1 ab**
Firmness	5.7 ± 0.3	5.1 ± 0.6	5.1 ± 0.4	5.6 ± 0.7	5.3 ± 0.4	n/a	5.7 ± 0.7
Crumbliness	5.5 ± 0.7	4.8 ± 1.0	5.5 ± 0.8	4.5 ± 0.7	4.7 ± 0.6	n/a	4.7 ± 0.7
Moistness	4.1 ± 1.2	4.6 ± 0.5	4.9 ± 0.7	5.0 ± 0.4	5.1 ± 1.2	n/a	4.2 ± 1.1
Fat mouthfeel	5.6 ± 0.7	5.9 ± 0.9	5.2 ± 0.5	5.2 ± 0.6	4.9 ± 0.5	n/a	4.8 ± 1.0
Overall sensory quality	**5.8 ± 1.0 ab**	**5.0 ± 0.9 b**	**5.7 ± 1.0 ab**	**5.4 ± 0.5 ab**	**6.7 ± 1.1 a**	n/a	**5.3 ± 1.3 ab**
2. Consumer analysis							
Preference	0%	0%	10%	10%	60%	n/a	20%
3. Height							
Height - maximum (cm)	4.0 ± 0.1	4.1 ± 0.1	5.4 ± 0.1	5.2 ± 0.1	6.2 ± 0.2	5.5 ± 0.1	4.4 ± 0.1
Height - minimum (cm)	2.8 ± 0.1	2.9 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	2.6 ± 0.1
4. Texture Profile Analysis (TPA)							
Hardness (g)	**7362 ± 452 a**	**6157 ± 772 b**	**5901 ± 655 b**	**5678 ± 457 b**	**5450 ± 776 b**	**6101 ± 295 b**	**6760 ± 327 ab**
Springiness	0.63 ± 0.08	0.72 ± 0.03	0.68 ± 0.01	0.70 ± 0.07	0.69 ± 0.06	0.70 ± 0.06	0.74 ± 0.02
Cohesiveness	**0.35 ± 0.01 b**	**0.47 ± 0.04 a**	**0.45 ± 0.03 a**	**0.46 ± 0.01 a**	**0.48 ± 0.01 a**	**0.49 ± 0.02 a**	**0.46 ± 0.01 a**
Gumminess	**2548 ± 149 b**	**2872 ± 462 b**	**2640 ± 267 b**	**2647 ± 272 b**	**2611 ± 363 b**	**2988 ± 182 b**	**3120 ± 141 a**
Chewiness	**1607 ± 191 b**	**2060 ± 378 a**	**1782 ± 194 b**	**1851 ± 54 b**	**1824 ± 371 b**	**2088 ± 237 a**	**2314 ± 122 a**
Resilience	0.10 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.01
5. Colourimetry							
Lightness - crust (L*)	33.12 ± 2.23	30.10 ± 1.54	29.93 ± 0.07	29.76 ± 0.60	30.04 ± 0.56	32.80 ± 0.61	29.75 ± 0.92
Redness - crust (a*)	7.06 ± 0.12	6.58 ± 0.09	6.54 ± 0.04	6.43 ± 0.09	6.62 ± 0.11	7.03 ± 0.17	6.84 ± 0.06
Yellowness - crust (b*)	8.65 ± 1.56	7.55 ± 0.62	7.27 ± 0.12	7.24 ± 0.47	7.49 ± 0.45	9.61 ± 0.51	7.51 ± 0.55
Chroma - crust (C*)	11.66 ± 0.91	10.12 ± 0.59	9.90 ± 0.06	9.86 ± 0.28	10.05 ± 0.40	11.94 ± 0.50	10.27 ± 0.37
Hue angle - crust (h*)	**58.67 ± 0.71 a**	**47.27 ± 0.97 b**	**44.57 ± 0.37 b**	**44.79 ± 2.24 b**	**47.51 ± 1.39 b**	**52.46 ± 1.42 ab**	**45.73 ± 1.12 b**
Lightness - crumb (L*)	34.84 ± 0.01	35.09 ± 0.44	34.44 ± 0.36	33.81 ± 0.74	34.98 ± 0.88	36.60 ± 0.22	30.78 ± 0.95
Redness - crumb (a*)	2.16 ± 0.56	1.81 ± 0.23	1.83 ± 0.08	1.85 ± 0.09	1.79 ± 0.14	1.57 ± 0.06	2.55 ± 0.29
Yellowness - crumb (b*)	6.20 ± 0.07	5.98 ± 0.15	5.50 ± 0.29	5.78 ± 0.34	6.10 ± 0.01	6.29 ± 0.08	4.78 ± 0.11
Chroma - crumb (C*)	6.85 ± 0.14	6.69 ± 0.19	5.99 ± 0.25	6.16 ± 0.24	6.55 ± 0.11	6.57 ± 0.05	5.89 ± 0.13
Hue angle - crumb (h*)	**68.57 ± 2.42 b**	**68.51 ± 1.91 b**	**77.66 ± 2.09 a**	**70.46 ± 2.87 b**	**68.46 ± 3.37 b**	**74.40 ± 1.00 a**	**78.38 ± 1.78 a**

Different letters indicate statistically significant differences ($p < 0.05$) between samples.

of the other colourimetry parameters proved statistically different between the samples, which was in agreement with the absence of overall colour difference reported by the trained panel for both crust and crumb (Table 3).

The water activity values of the PALM-ALT cakes were slightly higher (0.86 to 0.89) than the palm shortening and rapeseed oil products (0.82 and 0.83), while the H-FA formulation proved comparable (0.83).

4. Conclusions

This paper demonstrated the potential of an innovative ingredient composed of defatted linseed meal, beta-glucan, rapeseed oil, water and cider vinegar (PALM-ALT) as novel palm shortening substitute with a unique combination of environmental and health benefits. The results confirmed the hypothesis of the study, with the combined emulsifying and rheological properties of defatted linseed meal and beta-glucan creating a stable semi-solid emulsion-gel able to mimic palm shortening functionality during baking and maintain the sensory profile of the cake product. The acidification of the PALM-ALT ingredient enhanced its emulsion stability and rheological properties via the formation of aggregates of linseed proteins and/or beta-glucan in the aqueous phase.

The emulsification of rapeseed oil in the PALM-ALT ingredients prevented the loss of flavour quality observed for the rapeseed oil cake in comparison with the palm shortening formulation. The FA-20 T formulation (prepared with a 20% lower total ingredient weight)

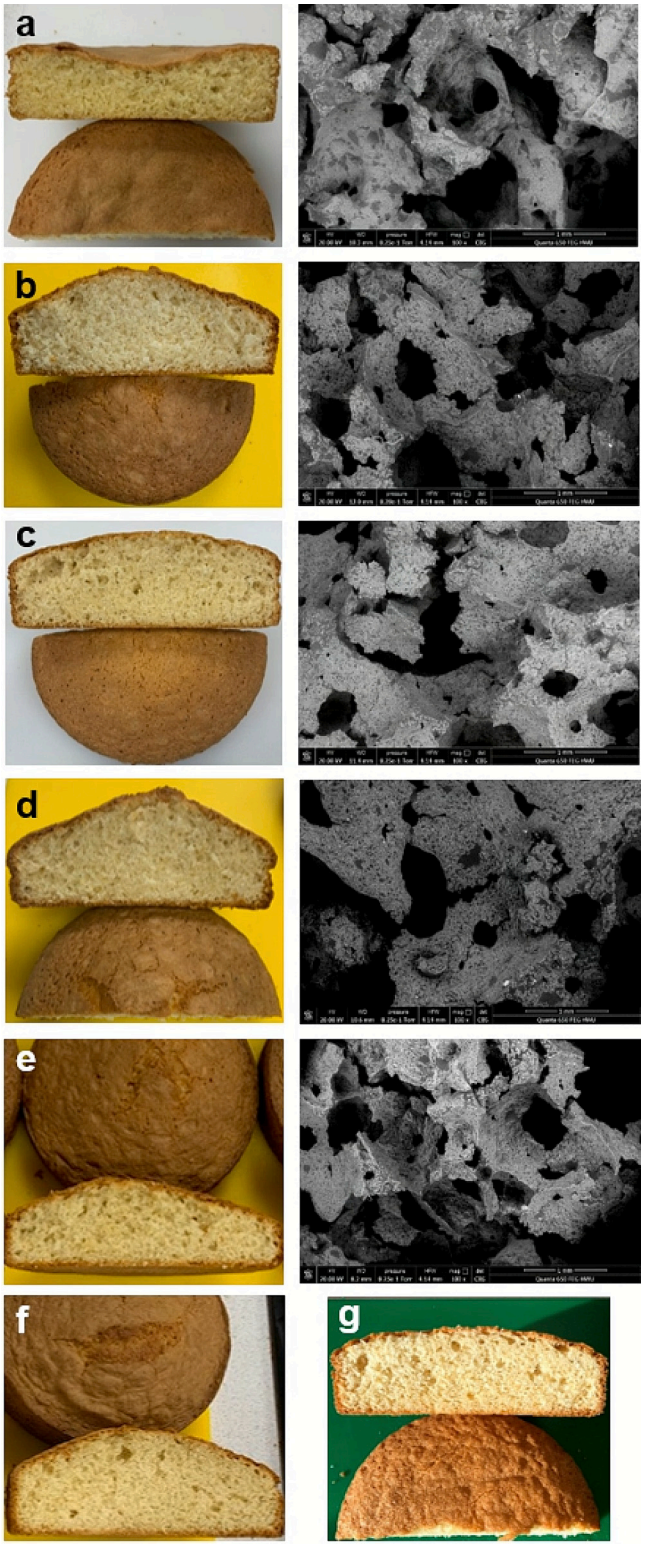

Fig. 4. Digital photography and Scanning Electron Microscopy (SEM) of the cake formulations prepared with palm shortening, rapeseed oil and PALM-ALT ingredient prototypes.
Palm shortening (a), FA (b), H-FA (c), SA (d), FA-20 T (e), FA-20P (f), Rapeseed oil (g).
FA (Full Acidity), H-FA (Heated Full Acidity), SA (Semi Acidity), FA-20 T (20% lower total ingredient weight) and FA-20P (20% lower FA ingredient level).

exhibited a similar sensory profile to the palm shortening cake, which enhances the cost-competitiveness of PALM-ALT in comparison with other palm shortening substitutes.

This work highlighted a novel high value-added application for linseed meal. Linseed meal proteins contributed to the formation and stabilisation of oil droplets in the PALM-ALT emulsion and of carbon dioxide bubbles in the cake formulations, whilst linseed mucilage and oat beta-glucan further stabilised the emulsion and the cake foam, in addition to the large aggregates of linseed proteins and/or beta-glucan observed in the aqueous phase.

Similar results were obtained with different batches, types or brands of defatted linseed meal, beta-glucan extract, rapeseed oil and cider vinegar than the ones used in this paper, which highlighted the repeatability and reproducibility of the PALM-ALT process and functionality. All PALM-ALT components are currently used in the food industry, so the ingredient does not require a novel food authorisation.

Additional studies are needed to further understand the mechanisms underpinning the functional contribution of the different PALM-ALT components (linseed proteins, linseed mucilage and oat beta-glucan) and their synergy in the ingredient and formulations. Future work will investigate the optimisation of the PALM-ALT ingredient, its upscaling at pilot and industry scales and its application in other bakery formulations and other products.

CRediT authorship contribution statement

Shirley L. Sampaio: Validation, Methodology, Investigation, Formal analysis, Data curation. **Timothy Chisnall:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Stephen R. Euston:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Catriona Liddle:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julien Lonchamp:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140134.

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