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RESEARCH ARTICLE

Protein fortification in oat flour gel using various dairy protein ingredients: An evaluation of textural and pasting properties

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The effects of dairy ingredient substitution including whey protein concentrate (WPC), whey lactalbumin concentrate (WLAC) and skim milk powder (SMP) on functional properties of oat flour were investigated. Pasting analysis revealed that adding dairy ingredients at low substitution concentrations (5% and 10%) weaken the gel strength. WPC and WLAC resulted in increased hot paste stability compared to SMP as indicated by results obtained from a rapid visco analyser (RVA). Textural analysis suggested that WPC and SMP resulted in more elastic gels in comparison with WLAC, whereas WLAC reduced the elasticity of gel owing to the high denaturation resistance of α-lactalbumin.

Keywords Whey protein concentrate, Texture, New product development, Milk proteins, Fortification, Microstructure.

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INTRODUCTION

Increased rates of metabolic diseases such as diabetes and obesity have increased the demand for a well-balanced, nutritionally rich diet. Oats (*Avena sativa*) are rich sources of dietary fibre, particularly β-glucan, which makes it a most valuable cereal among consumers (Weightman *et al*. 2004; Brennan and Cleary 2005; Brennan *et al*. 2012; Shah *et al*. 2016; Shoukat and Sorrentino 2021). Compared to other cereals, oat contains 9–15% protein and is a rich source of lysine. In addition, oats have a β-glucan content of 2–8% and a lipid content of 3–11%, which is higher than other cereals (Rasane *et al*. 2015). Although oats contain more lysine than many other cereal grains, its levels might still be suboptimal when the total protein requirements and the recommended ratios of essential amino acids are considered (Culetu *et al*. 2021). Although there has been an increase in the attention of using oat material in terms of oat milk products as well as oat fibre, from a phytochemical and nutritional basis, there is still a question regarding the protein quality of these items and consumer appreciation (Jiang *et al*. 2021; Riofrio and Baykara 2022). Thus, proteins from animal sources can be utilised to develop more nutritionally balanced foods with oats. Specifically, dairy proteins such as whey have antioxidant, immunostimulatory and anticarcinogenic properties (HewaNadungodage *et al*. 2022). In addition to providing high-quality protein for nutrition, they are also ideal for fortifying foods (Hoppe *et al*. 2008). More recently, there has been an interest in developing whey protein and plant protein-based combinations such as those derived from pea protein extraction (Kristensen *et al*. 2021). Oats are also used in baked foods, granola bars, dairy and vegan products. Hence, becoming a common food component of the food industry (Butt *et al*. 2008; Zhu 2017).

In oat-based fortified systems, oat flour can interact with the dairy ingredients in several ways. For instance, oat flour consists primarily of oat starch, which can interact with dairy proteins in fortified systems. However, the incompatible thermodynamic nature of β-glucan with milk proteins affects the food item's texture, viscoelastic nature and phase stability (Sharafbafi *et al*. 2014). Most studies have been conducted on isolated starches with milk proteins and considerably less on complex mixtures of native flours (Noisuwan *et al*. 2008; Kumar *et al*. 2018, 2022a, 2022b). In case of mixture of oat starch and dairy protein ingredients, some detailed studies were conducted by our group (Kumar *et al*. 2018, 2022a, 2022b), where milk protein ingredients such as, whey protein concentrate (WPC), α-lactalbumin enriched WPC (α-Lac-WPC), skim milk powder (SMP), whey protein isolate (WPI) and calcium caseinates were investigated. Kumar *et al*. (2018) reported that incorporating milk components (WPC, α-Lac-WPC, SMP) into oat starch supported its hot paste stability and restricted its peak viscosity. Following this, when WPI was added to oat starch, it resulted in honeycomb-like gel lattice, with a subsequent increase in the hot paste viscosity, stability, and an enhancement in the starch's ordered structures and thermal resilience (Kumar *et al*. 2022b). Similarly, the addition of calcium caseinate (CaCn) in oat starch led to a rise in breakdown, final, and setback viscosities, and an elevated gelatinisation temperature (Kumar *et al*. 2022a). However, there are limited studies on the interaction between oat flour and milk proteins, where flour is replaced with milk protein constituents, thus affecting the properties of the native flour (Zhou *et al*. 2021).

Hence, this study aimed to determine the effect of substituting oat flour with milk protein ingredients at 5% and 10% concentrations on hydration, pasting, viscoelastic and syneresis properties of the resultant oat-dairy blends subjected to thermal treatment. The impact of oat flour substitution with dairy protein ingredients was also studied in relation to textural properties in starch-dominated food systems. The effect of dairy protein ingredients on the texture and viscoelastic behaviour of oat flour/milk protein gels/paste was studied with back extrusion. Large deformation mechanical tests imitate the industrial processing of semi-solid foods and can also characterise rheology, which is correlated to sensory attributes (Bollaín *et al*. 2005; Roopa and Bhattacharya 2008). This research will improve the structural integrity of such systems when protein-rich fractions are substituted at low levels. This information will assist in producing new oat-dairy-based products and possibly using these systems as texture modifiers or stabilisers. The research findings will be helpful in the manufacturing of bakery foods, ready-to-eat snacks, dairy and vegan food products.

MATERIAL AND METHODS

Materials and sample preparation

The organic oat flour sample (Harraway's & Sons Ltd, Dunedin, New Zealand) was bought from an Organic wholefood supermarket (Piko, Christchurch, New Zealand). The oat flour sample comprises 54.3% carbohydrates, 14% proteins, 11.6% moisture, 6.8% fat, 11.2% dietary fibre and 1.65% ash. Whey protein concentrate (WPC) (protein 77.5%, fat 7.2%, ash 3.1%, moisture 3.1%) and whey lactalbumin enriched WPC (WLAC) (protein 79.2%, fat 10.5%, ash 2.9%, moisture 5%) and skim milk powder (SMP) (protein 32.6%, fat 0.8%, ash 8.5%, moisture 4.3%) provided by Oceania Dairy Limited, New Zealand. WLAC contained 30.3% of α-lactalbumin and 18.1% of β-lactoglobulin of total protein. The oat flour and dairy ingredients (WPC, WLAC and SMP) were prepared as 100:0, 95:5 and 90:10 (w/w) for each dairy ingredient. The sample suspensions obtained by dispersing oat flour and oat-dairy blends in water were used for pasting and penetration tests with a content of 10.71% (w/w); and, 10% (w/v) and 5% (w/w) were used for hydration, syneresis and back extrusion test.

Pasting properties

Rapid Visco Analyser (Newport Scientific Inc., Jessup, MD, USA) was used to measure the oat flour pasting behaviour based on the method used by Kumar *et al*. (2018). Using standard profile 1, the samples underwent a systematic heating and cooling cycle. Starting at an initial temperature of 50°C, followed by an elevation to 95°C at a consistent rate of 6°C/min. Upon reaching 95°C, the temperature was held steady for a duration of 1.5 min. The samples were then cooled to 50°C at a similar rate and subsequently stabilised at this temperature for 2 min. The stirring speed during the whole heating and cooling cycle was maintained at 160 rpm.

Hydration properties (water absorption index and water solubility index)

The water absorption index (WAI) and water solubility index (WSI) were analysed according to Choi *et al*. (2012) with slight changes. First, aforementioned sample suspensions (10% w/v) were prepared and heated in a water bath at 75°C for 30 min. Then, samples were centrifuged at 3000 *g* for 15 min. Finally, the supernatant was collected and dried overnight at 105°C for WSI measurement.

The WAI was calculated as

$$\mathrm{WAI} = \frac{m'}{M}$$

m' = the mass of the wet pellet after centrifugation; M= the dry mass of the pellet.

The WSI was calculated as

$$\mathrm{WSI} = \frac{m''}{m} \times 100\%$$

m'' = dry mass dissolved in the supernatant; m = mass of flour sample.

Syneresis

Oat flour paste (5%, w/w dry basis) was prepared using a rapid visco analyser, as described previously in Pasting properties section. The resulting paste was kept at 4°C for 21 h and was warmed up at 30°C for 3 h in a water bath. The samples were centrifuged at 1000 *g* for 20 min, and the supernatant was weighed. Syneresis was expressed as

$$\text{Syneresis} = \frac{w}{p} \times 100\%$$

w = weight of water expelled from the pellet; p = mass of the initial paste.

Large deformation textural properties

Penetration test

A penetration test was conducted using TA-XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK) with 5 Kg load cell at a constant crosshead speed of 1 mm/s to measure the texture of the samples. The method was based on Kumar *et al*. (2018). The samples were prepared in RVA and stored at 4°C for 24 h prior to analysis. The samples were subjected to a 10 mm distance of single compression with a 5 mm cylindrical plunger (p/5) at room temperature. Hardness (the maximum force) and brittleness (the distance of penetration at peak force) were detected during the compression cycle. Adhesiveness was the opposing force observed while removing the probe. The initial linear region gradient of the curve is reported as 'Elasticity'.

Back extrusion test

The back extrusion textural analysis was done using a back-extrusion cell (A/BE Back Extrusion Rig) on TA-XT2i Texture Analyzer (Stable Micro Systems Ltd, Surrey, UK) at room temperature (Angioloni and Collar 2009). The samples were prepared in RVA, cooled down to room temperature and analysed using a flat disc, following a compression cycle at a constant speed of 1 mm/s to a distance of 25 mm and then returning to initial position with same speed. Three parameters were recorded during the cycle and analysed by inbuilt software; cohesiveness (negative peak force during return of disc), index of viscosity (area under back extrusion) and consistency (positive area during compression).

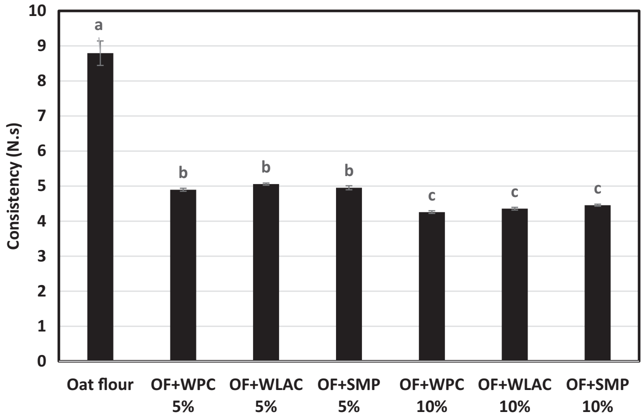

Figure 1 Consistency of oat flour paste containing milk components. Values expressed are means ± SD (n = 3). Means in the same column followed by different superscript differ significantly at $P < 0.05$. OF, Oat Flour; SMP, Skim Milk Powder; WLAC, Whey Lactalbumin; WPC, Whey Protein Concentrate.

Statistical analysis

All results were statistically analysed by using Minitab (version 19) from three replications. The analysis of variance (ANOVA) and Tukey's post-hoc test was used to differentiate among samples at $P < 0.05$ level of confidence. BioRender software was used to create Figure 3 to describe possible effects of substitution of oat flour with dairy ingredients on textural dynamics.

RESULTS AND DISCUSSION

Pasting properties

The pasting behaviours of the oat flour-dairy samples are shown in Table 1.

The composition and concentration of milk components significantly affect oat flour's peak, trough, setback and final viscosities. The peak viscosity, was attained prior to a sharp decline in the RVA profile, showed a significant reduction ($P < 0.05$) upon substituting oat flour with all types of the tested dairy ingredients. For example, at 5% WPC substitution, the peak viscosity reduced by 23.9% and it further reduced to 33.2% at 10% substitution level as compared to the control sample. Similar trends were observed for WLAC and SMP substituted samples. The results in Table 1 are consistent with those observed in previous work (Kumar *et al*. 2018), where a similar effect was observed on peak viscosity when oat starch was substituted with milk components. In summary, the higher the milk solid content, the lower the peak, final and setback viscosities. However, it is interesting to note that reduction extent in peak viscosity was more drastic for the first 5% of substitution and the further substitution of 5% had relatively lower effect in reducing peak viscosity.

These observations suggest that protein at low concentrations might act as filler, which gets adsorbed at the periphery of starch granules, restricts the hydration of starch molecules, and reduces the swelling of the starch granules. Consequently, reduced starch swelling results into reduced peak viscosity, as suggested by other works (Sopade *et al*. 2006; Considine *et al*. 2011; Kumar *et al*. 2017). Furthermore, the current result confirms that a starch-dominating network prevails at low concentrations of milk components. For SMP samples, the reduction in peak viscosity can be due to high lactose, casein, minerals and salt,

Table 1 Pasting characteristics of oat flour with milk components.

Samples	Peak Viscosity (cP)	Trough Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Stability ratio	Setback ratio	Relative breakdown	Pasting Temperature (°C)
Oat flour (OF)	1466.5 ± 14.50^{a}	1262 ± 12.00^{a}	204.5 ± 2.50^{a}	2547 ± 24.00^{a}	1285 ± 12.00^{a}	0.86 ± 0.00^{d}	2.01 ± 0.00^{de}	0.15 ± 0.00^{a}	90.55 ± 0.00^{b}
OF + WPC[1] 5%	1116 ± 58.00^{bc}	983 ± 88.00^{b}	133 ± 30.00^{bc}	2038.5 ± 132.50^{b}	1055.5 ± 44.50^{bc}	0.87 ± 0.00^{cd}	2.07 ± 0.05^{cd}	0.12 ± 0.00^{ab}	90.47 ± 0.02^{b}
OF + WLAC[2] 5%	1088.5 ± 8.50^{c}	986.5 ± 4.50^{b}	102 ± 13.00^{c}	2133.5 ± 10.50^{b}	1147 ± 6.00^{b}	0.90 ± 0.01^{bc}	2.16 ± 0.00^{b}	0.08 ± 0.01^{bc}	90.10 ± 0.40^{b}
OF + SMP[3] 5%	1158.5 ± 2.50^{b}	1009.5 ± 18.50^{b}	149 ± 21.00^{b}	2125.5 ± 26.50^{b}	1116 ± 8.00^{b}	0.87 ± 0.01^{cd}	2.10 ± 0.01^{bc}	0.13 ± 0.01^{ab}	91.70 ± 0.35^{a}
OF + WPC 10%	979.5 ± 7.50^{d}	934.5 ± 4.50^{b}	45 ± 12.00^{d}	1878 ± 19.00^{c}	943.5 ± 14.50^{d}	0.95 ± 0.01^{a}	2.00 ± 0.01^{e}	0.04 ± 0.01^{c}	90.50 ± 0.00^{b}
OF + WLAC 10%	784.5 ± 22.50^{e}	735.5 ± 21.50^{c}	49 ± 1.00^{d}	1719.5 ± 38.50^{d}	984 ± 17.00^{d}	0.93 ± 0.00^{ab}	2.33 ± 0.01^{a}	0.04 ± 0.00^{c}	90.50 ± 0.40^{b}
OF + SMP 10%	923.5 ± 4.50^{d}	828.5 ± 11.50^{c}	95 ± 7.00^{c}	1785 ± 22.00^{cd}	956.5 ± 10.50^{d}	0.89 ± 0.00^{bcd}	2.15 ± 0.00^{b}	0.09 ± 0.00^{b}	92.05 ± 0.00^{a}

Values expressed are means ± standard deviation ($n = 3$). Means in the same column followed by different superscript differ significantly at $P < 0.05$.

[1] WPC, Whey protein Concentrate.

[2] WLAC, Whey lactalbumin enriched WPC.

[3] SMP, Skim Milk Powder.

which results in particle rigidity of starch and other constituents. However, the mechanism of this effect is not fully understood yet, similar results were reported by Kumar *et al*. (2018). No significant difference was observed among the milk components between the peak viscosity of WPC and SMP. Whereas the WLAC resulted in significantly lower peak viscosity at both substitution levels (5% and 10%) compared with other dairy ingredients. This trend may be due to the composition of WLAC, which has a higher concentration of α-lactalbumin (30.3%) over β-lactoglobulin (18.1%) compared to a standard WPC. Since α-lactalbumin has a low number of thiol groups and high denaturation resistance compared to β-lactoglobulin (Wijayanti *et al*. 2014), it results in fewer protein–protein interactions. Therefore, a lower peak viscosity for the WLAC samples (Wijayanti *et al*. 2014; Kumar *et al*. 2017).

The stability ratio expresses hot paste stability, which explains high shear and temperature resistance (Shafie *et al*. 2016). The stability ratio was increased significantly when oat flour was substituted with milk protein components at 5% WLAC and 10% of WPC or WLAC in comparison with the control. At the same time, SMP did not show a significant effect on paste stability at 5% or 10%. In a previous study (Kumar *et al*. 2018), the authors showed that the substitution of SMP induced a significant increase in stability ratio in the oat starch system, and the effect was more than WPC and WLAC. However, in this study, SMP had no such effect observed on paste stability, possibly due to the complex nature of oat flour which includes fat, β-glucan and proteins besides oat starch.

Final viscosity, Setback viscosity and Setback Ratio elucidate the oat flour paste retrogradation tendency. A higher Setback value indicates high retrogradation and *vice versa*. An increase in the concentration of milk components caused a reduction in the setback viscosity compared to the control. However, there was no significant difference among the Setback viscosity of WPC, WLAC and SMP at their respective substitution rates of 5% and 10%. WPC substituted samples had the least Setback ratio at 5% and 10% compared to WPC and WLAC. At 10% substitution, all three milk components had significantly different Setback ratios, refer to Table 1. Relative breakdown (RB) is the ratio of breakdown to setback viscosity, indicating the degree of starch disruption during the pasting process. Increasing the concentration of milk components reduced the relative breakdown of the oat flour, especially in the case of WPC and WLAC. At 5% substitution, the WLAC exhibited significantly lower relative breakdown than the control. While at 10% substitution, WPC and WLAC had the highest effect on relative breakdown.

Pasting temperature is when oat flour starts to absorb water and starch starts to swell, followed by an increase in viscosity. A high pasting temperature indicates higher resistance of the flour components to swell and a high cooking temperature is required to functionalise food ingredients. Table 1 illustrates that at both 5% and 10% substitution levels, WPC and WLAC had no significant effect on the pasting temperature of the samples. However, for SMP-substituted samples, a significant increase in Pasting temperature was evident. Noisuwan *et al*. (2007, 2008) also reported the same behaviour of SMP on gelatinisation temperature. SMP contains more lactose, minerals and casein proteins than WLAC and WPC for the same quantity of substituted solids. The findings are in agreement with results reported by Kumar *et al*. (2018), where SMP was substituted for oat starch. Zhang *et al*. (2013) also reported that not the type but the sugar concentration affects tapioca starch's thermal and rheological properties. Perry and Donald (2002) hypothesised that adding low molecular weight sugar can reduce the plasticisation of starch granules and increase the gelatinisation temperature. As a result, the gelatinisation temperature increased significantly at the minerals' low concentration (<1 M).

Hydration properties

The WAI and WSI values are reported in Table 2, WAI ranged from 4.82 in oat flour to 3.97 in oat flour substituted with 10% SMP. Replacing oat flour with 5% and 10% milk components results in a significant reduction ($P < 0.05$) in WAI capacity. However, no significant difference was observed between each dairy ingredient at their respective concentrations of 5% and 10%. Lupano (2000) observed a similar trend in starch-whey mixtures where an increase in whey protein concentrate resulted in a decrease in the water absorption of starch. WSI was increased significantly when oat flour was substituted with dairy protein ingredients. According to Osundahunsi *et al*. (2003), WSI measures the solutes leached from the starch granule, such as amylose and amylopectin, during starch gelatinisation under high temperature and shear. Due to the soluble nature of whey proteins and lactose (Joshi *et al*. 2014), dairy ingredients have significantly higher WSI than oat flour. However, SMP showed lower WAI and WSI values compared to WPC and WLAC at both substitution levels, which was also earlier reported in another study by Kumar *et al*. (2018).

Syneresis analysis

Syneresis in thermally treated oat flour is due to expulsion of water from the gel structure. Syneresis in these type of systems follows the typical retrogradation behaviour of starch molecules present in oat flour. As cooling of heated paste/gel progress, the leached starch molecules lead to a more firm structure based on leached molecules ratio (Muadklay and Charoenrein 2008). Syneresis analysis can be very helpful to understand the strength of starch gels to endure physical changes during cooling and thawing (Karim 2000). Substituting oat flour with milk components affects syneresis (Table 2). Increasing the concentration of

Table 2 Hydration and syneresis properties of oat flour containing dairy ingredients.

Samples	Water absorption index	Water solubility index (%)	Syneresis (%)
Oat Flour	4.82 ± 0.12^{a}	5.55 ± 0.38^{f}	15.59 ± 0.34^{c}
Oat flour + WPC 5%	4.33 ± 0.09^{b}	10 ± 0.88^{cd}	17.36 ± 0.06^{b}
Oat flour + WLAC 5%	4.31 ± 0.02^{b}	9.22 ± 0.50^{de}	17.44 ± 0.11^{b}
Oat flour + SMP 5%	4.28 ± 0.13^{bc}	7.44 ± 1.01^{e}	17.092 ± 0.58^{b}
Oat flour + WPC 10%	4.07 ± 0.03^{cd}	12.44 ± 0.69^{ab}	18.74 ± 0.44^{a}
Oat flour + WLAC 10%	4.01 ± 0.02^{d}	13.66 ± 0.66^{a}	18.73 ± 0.48^{a}
Oat flour + SMP 10%	3.97 ± 0.02^{d}	11.33 ± 0.00^{bc}	18.73 ± 0.29^{a}

Values expressed are means ± SD ($n = 3$). Means in the same column followed by different superscript differ significantly at $P < 0.05$. SMP, Skim Milk Powder; WLAC, Whey Lactalbumin; WPC, Whey protein Concentrate.

milk fractions significantly increased ($P < 0.05$) the syneresis. However, no significant difference in syneresis values was observed among each dairy ingredient at both levels of substitutions. This observation suggest that in the substituted samples, structural strength is more dependent upon flour fraction instead of dairy ingredient type. Kumar *et al*. (2018) also reported similar findings when oat starch gels were substituted with milk components; however, they reported a significant effect of different milk constituents on oat starch. As oat flour has a more complex structure than oat starch, thus, compositional differences can be the reason for these contrasting results.

Large deformation textural properties

Large deformation mechanical tests were performed using a penetration and back extrusion analysis, which provides information about the sample's textural integrity. As shown in Table 3, all penetration textural parameters significantly decreased upon substitution of oat flour with milk protein ingredients ($P < 0.05$). The hardness of the gels is based on amount of amylose and other constituents present within the continuous network. Hardness of the samples containing dairy ingredients was significantly lower than oat flour alone. WPC and SMP-enriched samples showed more hardness at 5% and 10% substitution levels than WLAC-enriched samples. A similar trend was observed in elasticity and brittleness. WLAC, which is comprised of higher α-lactalbumin to β-lactoglobulin ratio than WPC, is unable to form α-lactalbumin-mediated S = S-bridged protein–protein interactions *via* the heat-induced S = S group and thiol group inter-exchange reactions (Yang *et al*. 2015). This leads to a weaker paste texture of WLAC-enriched samples than WPC-enriched samples (hardness and elasticity), as shown in Table 3. Similar results were observed (Kumar *et al*. 2018) in oat starch and milk component gels. WPC tends to form whey protein aggregates due to heat treatment. These aggregates may act as pore fillers and strengthen oat flour gel in comparison with WLAC. Furthermore, SMP addition resulted in stiff gels similar to WPC, the hardness was attributed to presence of soluble lactose, colloidal protein aggregates (casein and whey) and minerals which led to more junction zones between amylose chains (Onwulata *et al*. 2003; Gunaratne *et al*. 2007). Berski *et al*. (2016) reported that the oat flour gels were stiffer when sugars were included in the samples. Moreover, brittleness results indicated that the samples containing WLAC (5% and 10%) had resulted in significantly more brittle gels in comparison with WPC and SMP. WPC and SMP at both

Table 3 Textural properties of oat flour/dairy ingredient mixtures.

Samples	Hardness (N)	Brittleness (mm)	Elasticity (N.s)	Adhesiveness (N)
Oat flour	0.141 ± 0.014^{a}	5.46 ± 1.90^{a}	0.029 ± 0.001^{a}	-0.068 ± 0.001^{d}
Oat flour + WPC 5%	0.122 ± 0.013^{b}	4.49 ± 0.51^{ab}	0.023 ± 0.001^{b}	-0.062 ± 0.002^{c}
Oat flour + WLAC 5%	0.095 ± 0.006^{c}	4.09 ± 0.13^{b}	0.019 ± 0.001^{cd}	-0.060 ± 0.003^{c}
Oat flour + SMP 5%	0.127 ± 0.010^{ab}	5.20 ± 0.39^{ab}	0.020 ± 0.000^{c}	-0.056 ± 0.002^{b}
Oat flour + WPC 10%	0.093 ± 0.004^{cd}	4.36 ± 0.18^{ab}	0.016 ± 0.000^{ef}	-0.050 ± 0.000^{a}
Oat flour + WLAC 10%	0.077 ± 0.006^{d}	4.02 ± 0.42^{b}	0.014 ± 0.000^{f}	-0.053 ± 0.003^{ab}
Oat flour + SMP 10%	0.096 ± 0.007^{c}	4.29 ± 0.33^{ab}	0.018 ± 0.001^{de}	-0.053 ± 0.001^{ab}

Values expressed are means ± SD ($n = 3$). Means in the same column followed by different superscript differ significantly at $P < 0.05$. SMP, Skim Milk Powder; WLAC, Whey Lactalbumin; WPC, Whey protein Concentrate.

concentrations showed no significant difference in brittleness of gels in comparison with oat flour only. Adhesiveness was decreased in all samples containing milk protein ingredients. At 5%, adhesiveness of gels containing WPC and WLAC was more than SMP. However, at 10%, no significant difference was observed between milk protein ingredients. Therefore, at the same solid concentration level, WPC and SMP are more functional dairy ingredient for fortifying protein content in oat flour/oat starch containing semi-solid food products. This will result into less compromised textural strength in comparison with WLAC.

Table 3 demonstrates that the elasticity of oat flour gel decreased significantly ($P < 0.05$) when oat flour was substituted with dairy ingredients. An increase in the concentration of the milk components caused a decrease in gel elasticity. For instance, the elasticity of oat flour gel was 0.029 N.s; upon substituting the oat flour with 5% WPC, gel elasticity reduced to 0.023 N.s and further reduced to 0.016 N.s for 10% WPC substitution. All other components had comparable trends. Similar results were reported by Min *et al*. (2022), who observed that adding flaxseed proteins in mung bean starch, resulted in a decrease in gel elasticity due to the instable packed structure.

Back extrusion analysis of oat flour paste revealed several parameters such as consistency, index of viscosity and cohesiveness (Figures 1 and 2). Oat flour paste consistency was reported as a representation of the extrusion force profiles in relation to time. Consistency (area under the curve of extrusion work) is used to determine textural properties in dairy products such as yoghurt (Angioloni and Collar 2009). Figures 1 and 2 showed that adding milk constituents to the oat flour results in a significant drop in back extrusion-based textural properties. A significant decline in the consistency of oat flour was noted upon substituting 5% and 10% of the oat flour fraction with dairy ingredients. Nonetheless, the reduction in consistency was comparatively smaller with a 10% substitution as opposed to the 5% substitution. No significant variation was observed in the consistency values among the individual components of milk at each substitution rate.

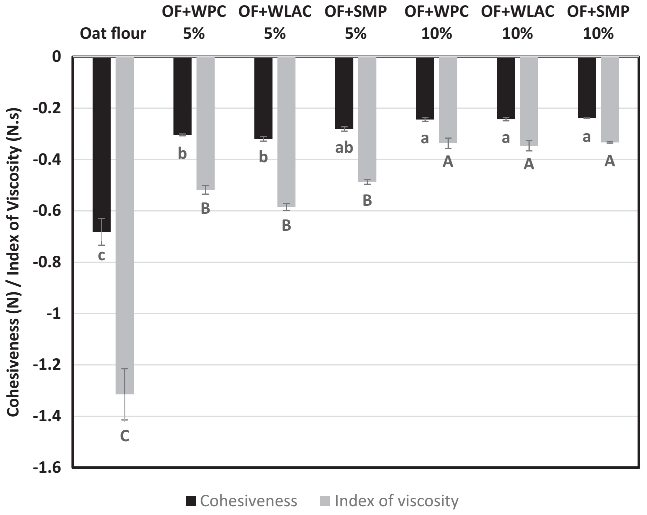

Figure 2 Cohesiveness and index of viscosity of oat flour paste containing dairy ingredients. Values expressed are means ± SD ($n = 3$). Means in the same column followed by different superscript differ significantly at $P < 0.05$. OF, Oat Flour; SMP, Skim Milk Powder; WLAC, Whey Lactalbumin; WPC, Whey Protein Concentrate.

Cohesiveness is the maximum negative force measured during back extrusion, it is a measure of cohesive force between particles and can be associated with the stickiness of a product. In addition, it indicates the gel's internal mechanical strength. Substitution of the oat flour with milk components significantly reduced ($P < 0.05$) the cohesiveness of the oat flour gel. Since cohesiveness appears to be related to the breakdown of starch granules and the network of starch molecules and other components present in the system during heating and stirring, this result can give insight into developing a food product with tailored functionality and quality attributes. The lowest cohesiveness value among all the samples was reported at 10% SMP substitution. The viscosity index gives an indirect measurement of the flow behaviour of oat flour paste, which is used in determining the process conditions such as flowability. Figure 2 depicted that at 5% and 10% substitution levels, all the dairy ingredients resulted in a significant decrease in the viscosity index, with a greater reduction for the first 5% substitution. Notably, the concentration of milk components plays a vital role in determining the textural properties of oat flour gel. For consistency and cohesiveness, the dropping rate was significantly higher for the first 5% of substitution compared to 10%. Interestingly, no difference was observed among dairy ingredients considering their significant differences in composition.

Possible effects of oat flour substitution with dairy protein ingredients in terms of textural properties

The possible effects of the relative substitution of flour with dairy protein ingredients on the structural integrity of oat flour gels are summarised in Figure 3. Oat flour primarily consists of starch, followed by dietary fibres, lipids, sugar and other micronutrients. The formation of oat flour gel can be primarily attributed to the development of junction zones, where the leached starch molecules (amylose) interact to form the 3D structure of gels. The physical and thermal properties of the gel are dictated by the orientation and geometries of the junction zones (Saha and Bhattacharya 2010). As depicted in Figure 3, the gel without any substitution exhibits the highest structural strength due to close packing, indicating a higher degree of cross-linking between polymers, specifically amylose and amylopectin. However, when oat flour is substituted with dairy protein ingredients, the gel network fails to maintain its structural

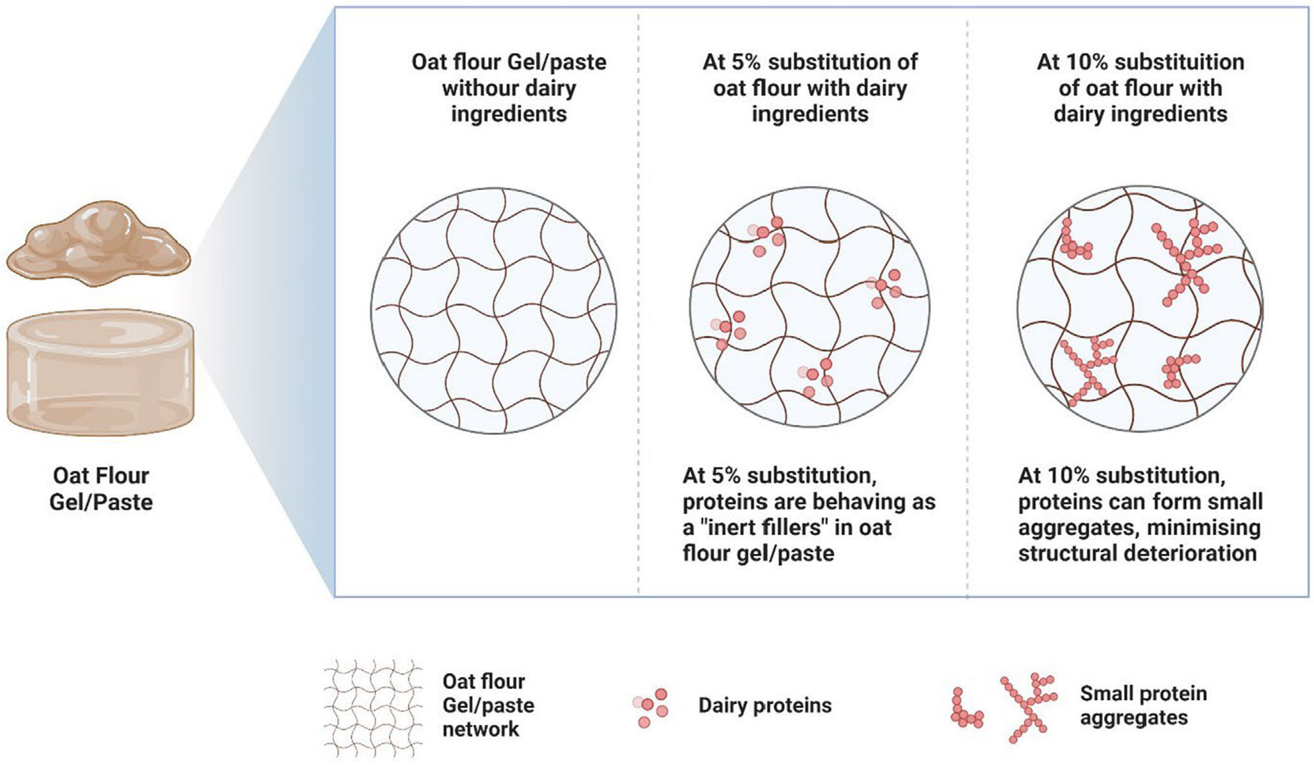

Figure 3 Deduced mechanisms of interaction between oat flour and dairy ingredients at varied concentrations.

integrity due to the hindering or disrupting effects of milk proteins and/or sugars. The addition of proteins may hinder the development of junction zones in starch-dominated structures and the rearrangement of starch molecules during retrogradation due to competitive hydration between starch and protein molecules, as well as the space-filling effect of protein assemblies formed during heating. Starch molecules readily absorb water compared to proteins and begin to swell. During the cooling period, denatured proteins form localised aggregates, inhibiting the formation of a closely packed gel network and weakening the gel structure (Saha and Bhattacharya 2010; Joshi *et al*. 2014; Cao and Mezzenga 2020). At low substitution levels of 5%, a significantly higher loss in textural and pasting profiles is observed, indicating that at lower concentrations, the proteins might act as inert fillers with little contribution to the mechanical strength of the gel network. The loss of hardness, cohesiveness and consistency affirms this phenomenon and is majorly related to substituting the starch fraction. Likewise, high water absorption and peak viscosity for the oat flour gel and its subsequent reduction after WPC, WLAC and SMP substitution suggest that these milk components restrict starch swelling and leaching of amylose and amylopectin, which form the backbone of the continuous structural matrix in starch-dominated gels.

However, as mentioned earlier, the decrease in structural integrity is not as pronounced at higher concentrations (e.g. 10% substitution level). At higher concentrations, the proteins begin to interact with themselves and other components present in the flour, especially lipids, sugar, protein and starch may form complexes, which to some extent reinforce the 3D networking of the flour gel (Kumar *et al.* 2017, 2018). The current results align with Joshi *et al.* (2014), who investigated the properties of gels made of lentil starch and lentil protein and found that protein negatively influences the structural integrity of the gel networks. The starch gel structure dominates the composite gel structure as the protein gel structure is weak and formed relatively slowly. The enriched proteins aggregate and formed colloidal protein assemblies causing spacing between starch networks, eventually weakening the composite gel structure compared to the starch gel networks (Joshi *et al*. 2014).

CONCLUSION

The substitution of oat flour with dairy ingredients led to a decrease in oat flour peak, breakdown and final viscosities. Pasting properties revealed that whey protein concentrate (WPC) and whey lactalbumin enriched WPC (WLAC) increased hot paste stability when used at a 10% substitution level. Among the samples, those containing skim milk powder (SMP) exhibited the highest pasting temperature due to the presence of lactose and minerals, which hindered starch swelling. The pasting properties indicated that WPC and WLAC (dairy ingredients rich in whey protein concentrate), had a significant impact on oat flour viscosity compared to SMP. Furthermore, the substitution of oat flour with dairy ingredients, increased the syneresis and reduced the water adsorption index, regardless of the type of dairy ingredient used. Textural properties, as assessed by penetration test, indicated that hardness, brittleness, elasticity and adhesiveness decreased when oat flour was replaced with milk components. Large-deformation back extrusion studies demonstrated that SMP, WLAC and WPC reduced the consistency and cohesiveness of the oat flour gel. Notably, a sudden loss in textural integrity was observed in oat flour gel properties with the first 5% substitution of dairy ingredients, as compared to the control. However, from 5% to 10% substitution, minimal differences were observed, suggesting that proteins acted as fillers at low concentrations and effectively modified the texture of oat flour gel. However, at higher substitution levels, the textural effect did not increase significantly, indicating an optimum substitution level of 5%. The experimental data and knowledge acquired from this study provide valuable insights into the textural behaviour of model oat flour gels. The outcome of this study will also aid in optimising the use of dairy ingredients in conjunction with oat flour to develop novel food products with superior textures and overall acceptability.

AUTHOR CONTRIBUTIONS

Lokesh Kumar: Conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing – original draft; writing – review and editing. **Margaret Brennan:** Project administration; supervision; writing – review and editing. **Haotian Zheng:** Funding acquisition; resources; supervision; writing – review and editing. **Gaurav Kumar:** Writing – review and editing. **Charles Brennan:** Conceptualization; funding acquisition; project administration; resources; supervision; writing – review and editing.

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding authors.

REFERENCES

Angioloni A and Collar C (2009) Small and large deformation viscoelastic behaviour of selected fibre blends with gelling properties. *Food Hydrocolloids* **23** 742–748.

Berski W, Krystyjan M, Zięć G, Litwinek D and Gambuś H (2016) The influence of starch hydrolyzate on physicochemical properties of pastes and gels prepared from oat flour and starch. *Journal of Cereal Science* **70** 16–22.

Bollaín C, Angioloni A and Collar C (2005) Bread staling assessment of enzyme-supplemented pan breads by dynamic and static deformation measurements. *European Food Research and Technology* **220** 83–89.

Brennan C S and Cleary L J (2005) The potential use of cereal (1→3, 1→4)-β-d-glucans as functional food ingredients. *Journal of Cereal Science* **42** 1–13.

Brennan M A, Derbyshire E J, Brennan C S and Tiwari B K (2012) Impact of dietary fibre-enriched ready-to-eat extruded snacks on the postprandial glycaemic response of non-diabetic patients. *Molecular Nutrition and Food Research* **56** 834–837.

Butt M S, Tahir-Nadeem M, Khan M K I, Shabir R and Butt M S (2008) Oat: Unique among the cereals. *European Journal of Nutrition* **47** 68–79.

Cao Y and Mezzenga R (2020) Design principles of food gels. *Nature Food* **1** 106–118.

Choi I, Han O-K, Chun J, Kang C-S, Kim K-H, Kim Y-K, Cheong Y-K, Park T-I, Choi J-S and Kim K-J (2012) Hydration and pasting properties of oat (*Avena sativa*) flour. *Preventive Nutrition and Food Science* **17** 87–91.

Considine T, Noisuwan A, Hemar Y, Wilkinson B, Bronlund J and Kasapis S (2011) Rheological investigations of the interactions between starch and milk proteins in model dairy systems: A review. *Food Hydrocolloids* **25** 2008–2017.

Culetu A, Stoica-Guzun A and Duta D E (2021) Impact of fat types on the rheological and textural properties of gluten-free oat dough and cookie. *International Journal of Food Science and Technology* **56** 126–137.

Gunaratne A, Ranaweera S and Corke H (2007) Thermal, pasting, and gelling properties of wheat and potato starches in the presence of sucrose, glucose, glycerol, and hydroxypropyl β-cyclodextrin. *Carbohydrate Polymers* **70** 112–122.

HewaNadungodage N D, Torrico D, Brennan M A and Brennan C S (2022) Nutritional, physicochemical, and textural properties of gluten-free extruded snacks containing cowpea and whey protein concentrate. *International Journal of Food Science and Technology* **57** 3903–3913.

Hoppe C, Andersen G S, Jacobsen S, Mølgaard C, Friis H, Sangild P T and Michaelsen K F (2008) The use of whey or skimmed milk powder in fortified blended foods for vulnerable groups. *The Journal of Nutrition* **138** 145S–161S.

Jiang Y, Shi Y, Li R, Hang F, Zhao J, Zhang H and Chen W (2021) The peptides in oat and malt extracts that are preferentially absorbed by lactobacillus plantarum and stimulates its proliferation in milk. *International Journal of Food Science and Technology* **56** 4690–4699.

Joshi M, Aldred P, Panozzo J F, Kasapis S and Adhikari B (2014) Rheological and microstructural characteristics of lentil starch-lentil protein composite pastes and gels. *Food Hydrocolloids* **35** 226–237.

Karim A (2000) Methods for the study of starch retrogradation. *Food Chemistry* **71** 9–36.

Kristensen H T, Christensen M, Hansen M S, Hammershøj M and Dalsgaard T K (2021) Protein–protein interactions of a whey–pea protein co-precipitate. *International Journal of Food Science and Technology* **56** 5777–5790.

Kumar L, Brennan M A, Mason S L and Zheng Hand Brennan C S (2017) Rheological, pasting and microstructural studies of dairy protein-starch interactions and their application in extrusion-based products: A review. *Starch/Staerke* **69** 1–2.

Kumar L, Brennan M, Zheng H and Brennan C (2018) The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch. *Food Chemistry* **245** 518–524.

Kumar L, Brennan M, Brennan C and Zheng H (2022a) Thermal, pasting and structural studies of oat starch-caseinate interactions. *Food Chemistry* **373** 131433.

Kumar L, Brennan M, Brennan C and Zheng H (2022b) Influence of whey protein isolate on pasting, thermal, and structural characteristics of oat starch. *Journal of Dairy Science* **105** 56–71.

Lupano C E (2000) Gelation of mixed systems whey protein concentrate-gluten in acidic conditions. *Food Research International* **33** 691–696.

Min C, Ma W, Kuang J, Huang J and Xiong Y L (2022) Textural properties, microstructure and digestibility of mungbean starch–flaxseed protein composite gels. *Food Hydrocolloids* **126** 107482.

Muadklay J and Charoenrein S (2008) Effects of hydrocolloids and freezing rates on freeze–thaw stability of tapioca starch gels. *Food Hydrocolloids* **22** 1268–1272.

Noisuwan A, Hemar Y, Bronlund J E, Wilkinson B and Williams M A K (2007) Viscosity, swelling and starch leaching during the early stages of pasting of normal and waxy rice starch suspensions containing different milk protein ingredients. *Starch/Staerke* **59** 379–387.

Noisuwan A, Bronlund J, Wilkinson B and Hemar Y (2008) Effect of milk protein products on the rheological and thermal (DSC) properties of normal rice starch and waxy rice starch. *Food Hydrocolloids* **22** 174–183.

Onwulata C I, Konstance R P, Cooke P H and Farrell H M (2003) Functionality of extrusion—Texturized whey proteins. *Journal of Dairy Science* **86** 3775–3782.

Osundahunsi O F, Fagbemi T N, Kesselman E and Shimoni E (2003) Comparison of the physicochemical properties and pasting characteristics of flour and starch from red and white sweet potato cultivars. *Journal of Agricultural and Food Chemistry* **51** 2232–2236.

Perry P and Donald A (2002) The effect of sugars on the gelatinisation of starch. *Carbohydrate Polymers* **49** 155–165.

Rasane P, Jha A, Sabikhi L, Kumar A and Unnikrishnan V S (2015) Nutritional advantages of oats and opportunities for its processing as value added foods – a review. *Journal of Food Science and Technology* **52** 662–675.

Riofrio A and Baykara H (2022) Techno-environmental and life cycle assessment of 'oat-milk' production in Ecuador: A cradle-to-retail life cycle assessment. *International Journal of Food Science and Technology* **57** 4879–4886.

Roopa B S and Bhattacharya S (2008) Alginate gels: I. Characterization of textural attributes. *Journal of Food Engineering* **85** 123–131.

Saha D and Bhattacharya S (2010) Hydrocolloids as thickening and gelling agents in food: A critical review. *Journal of Food Science and Technology* **47** 587–597.

Shafie B, Cheng S C, Lee H and Yiu P (2016) Characterization and classification of whole-grain rice based on rapid visco analyzer (RVA) pasting profile. *International Food Research Journal* **23** 2138–2143.

Shah A, Masoodi F A, Gani A and Ashwar B A (2016) Newly released oat varieties of himalayan region -techno-functional, rheological, and nutraceutical properties of flour. *LWT – Food Science and Technology* **70** 111–118.

Sharafbafi N, Tosh S M, Alexander M and Corredig M (2014) Phase behaviour, rheological properties, and microstructure of oat β-glucan-milk mixtures. *Food Hydrocolloids* **41** 274–280.

Shoukat M and Sorrentino A (2021) Cereal β-glucan: A promising prebiotic polysaccharide and its impact on the gut health. *International Journal of Food Science and Technology* **56** 2088–2097.

Sopade P A, Hardin M, Fitzpatrick P, Desmee H and Halley P (2006) Macromolecular interactions during gelatinisation and retrogradation in starch-whey systems as studied by rapid visco-analyser. *International Journal of Food Engineering* **2** 1–18.

Weightman R M, Heywood C, Wade A and South J B (2004) Relationship between grain (1→3, 1→4)-β-ᴅ-glucan concentration and the response of winter-sown oats to contrasting forms of applied nitrogen. *Journal of Cereal Science* **40** 81–86.

Wijayanti H, Bansal B and Nand Deeth H C (2014) Stability of whey proteins during thermal processing: A review. *Comprehensive Reviews in Food Science and Food Safety* **13** 1235–1251.

Yang N, Ashton J and Kasapis S (2015) The influence of chitosan on the structural properties of whey protein and wheat starch composite systems. *Food Chemistry* **179** 60–67.

Zhang X, Tong Q, Zhu W and Ren F (2013) Pasting, rheological properties and gelatinization kinetics of tapioca starch with sucrose or glucose. *Journal of Food Engineering* **114** 255–261.

Zhou R, Mitra P, Melnychenko A and Rizvi S S H (2021) Quality attributes and rheological properties of novel high milk protein-based extrudates made by supercritical fluid extrusion. *International Journal of Food Science and Technology* **56** 3866–3875.

Zhu F (2017) Structures, properties, modifications, and uses of oat starch. *Food Chemistry* **229** 329–340.