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Published in:
Food Hydrocolloids

DOI:
10.1016/j.foodhyd.2023.109402

Published: 01/02/2024

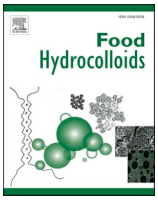
Document Version
Publisher's final version

Food Hydrocolloids 147 (2024) 109402

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd



Influence of oat flour characteristics on the physicochemical properties of oat-based milk substitutes

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ARTICLE INFO

Keywords:
Oats
Dairy alternative
Oat milk quality
Physical properties

ABSTRACT

This study aimed at investigating the effect of oat composition on the quality characteristics of oat-based milk substitute (OBMS). Raw materials for OBMS consisted of thirty pure cultivar oat flours produced by industrial milling process. The main steps in OBMS preparation included mixing of 10% oat flour with water, starch hydrolysis, solid-liquid separation and formulation of the final product. OBMS was characterised in terms of mass and protein yields, protein content, reducing sugars, particle size, viscosity, dispersion stability and colour. The different composition of oat flours affected the characteristics of the OBMS in terms of mass yield (81.3–88.1%), dry matter yield (56.7–73.8%), protein content (0.63–1.10%), protein yield (31.4–61.6%), content of free reducing sugars (13.1–19.1%), and physical properties including particle size, dispersion stability and viscosity. OBMS mass and dry matter yields correlated positively with both total and damaged starch contents of the oat flour as well as the share of damaged starch of the total starch of the sample, while flour starch content correlated negatively with protein content of OBMS. Protein content of the flour correlated negatively with the mass, dry matter and protein yields and positively with protein content of OBMS. All colour values (L*, a*, b*) of the flour correlated with colour value b* of the OBMS. Protein content and viscosity of OBMS correlated positively with dispersion stability of OBSM. In conclusion, the clear relation between oat composition and the final properties of OBMS suggest relevance of selecting specific oat raw materials to produce good quality OBMS.

1. Introduction

Consumer demand for plant-based liquid food products, especially dairy substitutes is constantly growing due to health, environmental and sociocultural reasons. As reviewed by Sethi, Tyagi, and Anurag (2016) most of the research regarding liquid food systems such as plant-based dairy alternatives, has concentrated on soy but recently more attention has also been given to cereal-, oilseed- and nut-based products. Oats offer a mild-tasting and nutritious raw material for various alternatives for traditional animal-origin foods (Yang et al., 2023). Recently, the popularity of oat-based milk substitutes (OBMS) has substantially increased and therefore, identifying the factors that influence the quality of the OBMS is essential and has also a considerable economic impact.

Processing of oats for a dairy substitute most commonly includes the following steps: milling the oat raw material and mixing it with water, homogenisation, enzymatic hydrolysis of starch followed by removal of insoluble particles, addition of other ingredients and additives, second homogenisation and heat treatment (Deswal, Deora, & Mishra, 2014; Mäkinen, Wanhalinna, Zannini, & Arendt, 2016). In the production of an OBMS, starch, being one of the major components in oats, plays a crucial role during processing due to gelatinization during heating/pasteurization. Therefore, starch hydrolysis by enzymes is usually a necessary step to avoid extensive viscosity formation (Deswal et al., 2014). In addition, starch hydrolysis affects the sensory quality of the final OBMS due to hydrolysis of the long carbohydrate chains into shorter chains and free sugars exhibiting sweetness. Presence of insoluble particles deriving from both dietary fibre and insoluble proteins affect negatively the properties of the OBMS since they may bring about a gritty and chalky mouthfeel and sedimentation during storage, and therefore solid-liquid separation step and homogenisation are typical processes during the manufacturing (McClements, Newman, & McClements, 2019; Sethi et al., 2016).

Abbreviations: OBMS, oat-based milk substitute; TSI, Turbiscan Stability Index.

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https://doi.org/10.1016/j.foodhyd.2023.109402
Received 8 June 2023; Received in revised form 18 September 2023; Accepted 8 October 2023
Available online 14 October 2023

At present, there are already several commercial OBMS products, some of which have also been studied in the scientific literature. The research has mainly focused on compositional and functional characterisation of OBMS (Jeske, Zannini, & Arendt, 2017; Mäkinen, Uniacke-Lowe, O'Mahony, & Arendt, 2015; Martínez-Padilla et al., 2020). Optimisation of the processing conditions such as alpha-amylase enzyme dosage and treatment time as well as dry matter content of initial dispersion (Deswal et al., 2014), understanding the impact of enzymatic and heat treatment on the properties of the OBMS (Bonke, Sieuwerts, & Petersen, 2020) and optimising the heat treatment of the drink prior to yoghurt manufacturing from oats (Demir, Simsek, & Yıldırım, 2021) have also been reported. However, relatively little is known about the impact of compositional and physical oat raw material properties on the quality of the final OBMS. For example, the sweetness and colour of the plant-based milk substitutes are crucial attributes for customers who want the plant-based milk substitute to resemble cow's milk. Furthermore, yields of different components from the raw material to the final product have a significant industrial relevance in terms of resource efficiency and side-stream generation. Since protein content of plant-based dairy substitutes is often considerably lower than that of cow's milk, identifying the most potential raw materials and processing parameters that result in high protein content would enhance the nutritional quality of OBMS.

Currently, the literature lacks systematic studies showing the impact of composition and physical properties of the oat raw material used in the preparation of the OBMS on the quality characteristics of the final product. Recently, Zhou et al. (2023) examined how chemical composition of oat groats affect the physical stability of OBMS. However, they only studied four oat varieties which limits drawing reliable conclusions. To build systematic understanding on the effect of raw material characteristics, the aim of this study was to investigate the impact of chemical composition (starch, damaged starch, protein, total dietary fibre, β-glucan, ash, lipids) and particle size of 30 pure cultivar Finnish oat flour samples on the properties of OBMS. More specifically, the objective was to understand how flour composition and particle size affect yields (mass, dry matter and protein yield), composition (dry matter, protein as well as free and total reducing sugars) and physical properties (dispersion stability, colour, particle size, viscosity) of the OBMS. The hypothesis was that the variation in the composition of oat samples, especially in terms of protein and starch, are likely to affect the final properties of the OBMS, but it is possible to find also other important correlations between the flour and OBMS.

2. Materials and methods

2.1. Materials and composition

Oat-based milk substitutes (OBMS) were prepared from thirty Finnish oat flour samples that were produced from heated oat groats after flaking in an industrial milling process at Vääksyn Mylly Ltd. (Asikkala, Finland) as described in Jokinen et al. (2021). Ten first samples (1–10) were milled from flakes using a stone mill with a used stone pair, while the samples 11–30 were milled with a newer stone pair and a hammer mill (0.5 mm sieve, Hamermolln mono 6, Werkhuizen Schepens NV, Dendermonde, Belgium) in order to unify the particle size of all 30 flours. Starch, protein, total dietary fibre, β-glucan and ash contents as well as colour values (L*, a* and b*) of the flours were analysed in Jokinen et al. (2021), while particle size was analysed in Jokinen et al. (2022) and damaged starch content in Jokinen, Silventoinen-Veijalainen, Lille, Nordlund, and Holopainen-Mantila (2023). This composition, colour and particle size data of the flours was used in this manuscript to determine correlations between the flour and OBMS properties.

2.2. Preparation of oat-based milk substitute

The process for preparing the OBMS is shown in Fig. 1. The oat flour was first mixed with water at 10% dry matter content and homogenised using an Ultra-Turrax Heidolph SilentCrusher M (Schwabach, Germany) equipped with Typ 18G generator for 3 min at 20 000 rpm. The homogenised sample was treated with an α-amylase BAN 480 L (Novozymes A/S, Bagsvaerd, Denmark) at 70 °C water bath for 15 min at a dosage of 0.5% (w/w dry matter) after which the enzyme was inactivated by heating the sample in a water bath to 90 °C (13 min) and holding the sample at 90 °C for further 10 min. During the inactivation, samples were shaken manually every 2 min. After the heat-treatment, the samples were cooled to 22 °C, salt (0.001%) was added to the sample and the pH was adjusted to 7.2 with 1 M NaOH. Centrifugation to remove the insoluble particles was carried out at 500×*g* at 20 °C for 10 min. The supernatant was separated from the solid residue by pouring the liquid through a sieve having openings of 0.8 mm * 0.8 mm. An aliquot of the supernatant was taken for DNS-analysis (section 2.3.4) at this point. Then, calcium phosphate ($Ca_5HO_{13}P_3$, Sigma-Aldrich, lot 95H0644) and rapeseed oil (Pirkka, Avena Nordic Grain Oy, Helsinki, Finland) were added to the liquid phase to reach concentrations of 1.2% (w/w) and 0.5% (w/w), respectively. The dispersion was again homogenised for 3 min at 20 000 rpm using Ultra-Turrax as described earlier. Final heat treatment was performed at 90 °C for 10 min with manual stirring every 2 min. Finally, the samples were cooled down to 22 °C. OBMS samples were prepared in duplicates for each of the thirty oat samples and within each duplicate the samples were prepared and treated as two separate dispersions until the sieving after solid-liquid separation was carried out. Aliquots of samples for compositional and colour analyses were frozen while dispersion stability, particle size and viscosity were measured from the samples directly after preparation.

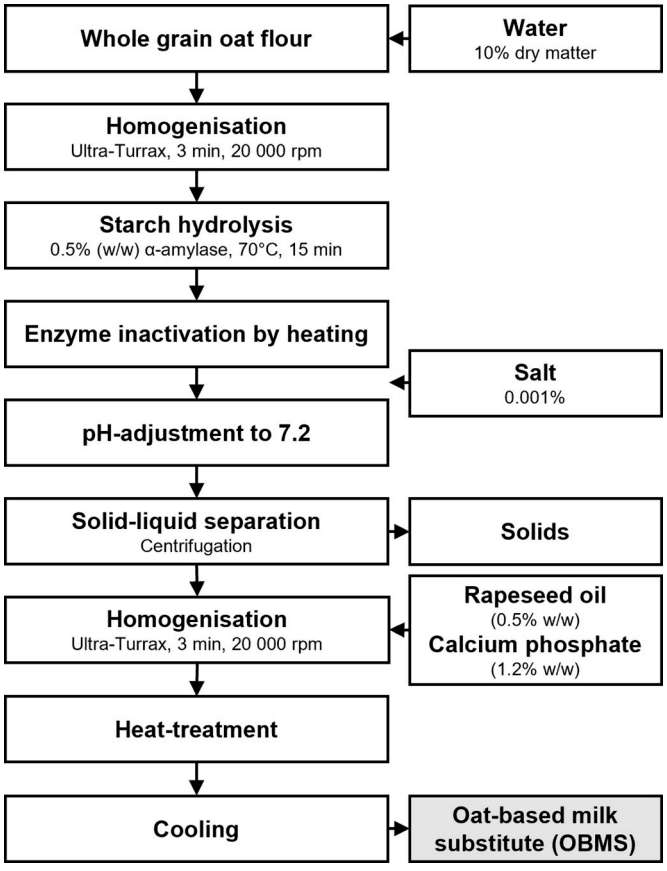

Fig. 1. Processing scheme describing the preparation of the oat-based milk substitute (OBMS).

2.3. Compositional characterisation of the oat-based milk substitutes

2.3.1. Mass yield

Mass yield of the OBMS was calculated from the solid-liquid separation step as follows (Equation (1)):

$$\text{Mass yield of OBMS } (\%) = \frac{\text{weighed liquid after solid-liquid separation step (g)}}{\text{whole supsension centrifuged (g)}} \times 100\% \quad (1)$$

2.3.2. Dry matter content and dry matter yield

Dry matter content of final OBMS was determined from thawed samples by drying the samples together with Supelco Silicon dioxide sea sand (Sigma-Aldrich, Saint Louis, MI, United States) overnight in an oven at 105 °C. The dry substance yield was calculated according to Equation (2). The amount of salt, calcium phosphate and oil added after the solid-liquid separation step was subtracted from the solids amount present in the OBMS in order to calculate the actual yield of oat mass from the flour to the OBMS.

$$\text{Dry substance yield in OBMS } (\%) = \frac{\text{dry substance in OBMS (g)} - \text{salt (g)} - \text{calcium phosphate (g)} - \text{oil (g)}}{\text{whole suspension centrifuged (g)} \times \frac{\text{solid content of suspension (\%)}}{100}} \times 100\% \quad (2)$$

2.3.3. Protein content and protein yield

The protein analysis was performed for the OBMS samples with Kjeldahl method based on nitrogen content (N × 6.25) using Kjeltec 2300 autoanalyser (Foss Tecator Digestion System, Kjeltec2300 analyser unit, Höganäs, Sweden). The protein yield to the OBMS was calculated according to Equation (3).

$$\text{Protein yield } (\%) = \frac{\text{liquid from solid} - \text{liquid separation (g)} \times \frac{\text{protein content of OBMS (\%)}}{100}}{\frac{\text{protein content of oat groat (\% db)}}{100} \times \text{whole suspension centrifuged (g)} \times \frac{\text{solid content of suspension (\%)}}{100}} \times 100\% \quad (3)$$

2.3.4. Free and total reducing sugars

The amount of free reducing sugars was measured from the aliquot samples (see section 2.2) taken directly after the solid-liquid separation step. The liquid samples were centrifuged again (9632×*g*, 10 min, 20 °C), frozen, thawed and re-centrifuged (20854×*g*, 10 min, 23 °C) and the amount of free reducing sugars was quantified using a 3,5-dinitrosalicylic acid (DNS) method with glucose as a standard. Additionally, aliquot samples underwent acid hydrolysis (1 M H_2SO_4, 1.5 h, 100 °C) after the first centrifugation and the acid-hydrolysed samples were re-centrifuged (10 min, 23 °C, 20854×*g*) and the DNS-analysis was again performed to determine the amount of total reducing sugars (i.e., free sugars and oligosaccharides) present in OBMS. DNS analysis was performed twice for each of the duplicate samples (n = 4).

2.4. Physical properties of the oat-based milk substitutes

2.4.1. Dispersion stability

Dispersion stability of OBMS samples was measured with the optical scanner TurbiScan® LAB Expert (Formulaction SA, France) by determining the sedimentation at time points 0 h, 24 h, 1 week and 2 weeks at 22 °C. Between the measurements the samples were stored at 8 °C. The results were analysed with Turbisoft-software (Turbisoft, version 2.1.0.52, Formulaction, Toulouse, France) and the parameter used to evaluate the stability was the global TSI (Turbiscan Stability Index), which is a dimensionless parameter representing the destabilisation phenomena in the sample measured utilising static multiple light scattering. TSI analysis was performed in duplicate for duplicate OBMS samples (n = 4). Additionally, photographs of the sample vessels were taken at the same time-points for visual observation of the sample sedimentation in time.

2.4.2. Colour

Colour analysis of thawed OBMS samples was carried out using Minolta Chroma Meter CR-200 (Minolta Camera Co., Ltd., Osaka, Japan). The recorded parameters were lightness (L*), red-green (a*) and blue-yellow (b*) intensities. 15 ml of OBMS sample was placed into a Petri dish and the colour was measured three times, namely from middle, left and right of the Petri dish, from each duplicate sample. The measuring head was positioned upside down so that the sample was placed on top of the measuring head and a white paper was placed on top of the sample so that the surface was similar for each sample.

2.4.3. Particle size

Particle size of OBMS was measured immediately after sample preparation by laser diffraction with a Mastersizer 3000 Hydro (Malvern Analytical, Worcestershire, UK) using a refractive index of 1.339 (Mäkinen et al., 2015). Ten replicate analysis results from each of the duplicate samples was utilised in calculation of the D50, i.e. median particle size value.

2.4.4. Viscosity

Viscosity analysis of OBMS samples was carried out at 6 °C using an ARES-G2 rheometer (TA Instruments, New Castle, DE, United States) and DIN concentric cylinder (Peltier Steel, 992324) after overnight storage of the samples at 6 °C. Viscosity (Pa*s) was recorded as a function of twenty shear rates ranging between 0.1 and 100 1/s.

2.5. Statistical analysis

Principal component analysis (PCA) of the averaged indicator data was performed using RStudio 2023 06 1 with R version 4 3 1 and the FactoMineR library. Prior to analysis, data was mean-centered and autoscaled. The PCA aimed to uncover underlying patterns and relationships within the dataset by extracting orthogonal principal components that captured maximum variance. The relationships between oat flour and OBMS properties were also estimated based on Pearson correlation coefficients using SPSS-software (IBM SPSS Statistics, version 26, IBM, New York, NY, USA).

3. Results and discussion

3.1. Differences in oat-based milk substitute properties

Yield of the total mass of the original dispersion to OBMS varied

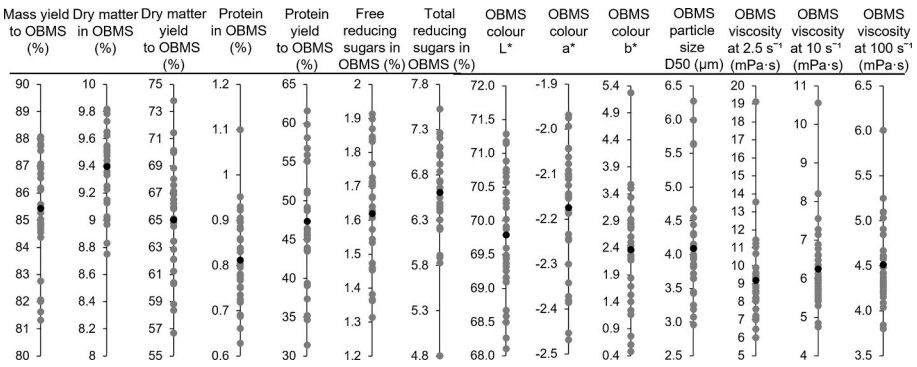

Fig. 2. Composition, yields and physical properties of oat-based milk substitutes (OBMS). Black dots represent the average values, whereas grey dots show the distribution of the 30 individual samples. D50: median particle size.

between 81.3 and 88.1% (average 85.4%) for different oat samples and variation was also observed in dry matter contents of OBMS (8.8–9.8%; average 9.4%) and dry matter yields to OBMS (56.7–73.8%; average 65.0%; Fig. 2; S1). Protein contents showed also considerable differences between the samples and the maximum value (1.1%) was almost double as high as the lowest value (0.6%) while most of the values ranged between 0.7 and 0.9% (average 0.8%). In addition to protein content, protein yield to OBMS varied considerably and ranged from 31.4 to 61.6% (average 47.4%). Amounts of free and total reducing sugars were 1.3–1.9% (average 1.6%) and 4.8–7.5% (average 6.6%), respectively.

Bonke et al. (2020) showed that a somewhat similar process for preparing the oat drink from a 10% oat-base including a pre-heating, enzymatic hydrolysis using alpha-amylase, glucoamylase and beta-amylases, inactivation by heating and solid-liquid separation by sieving allowed to recover 59% of the original dispersion mass, 22% of dry matter and 38% of protein. Interestingly, mass and dry matter yields were considerably lower than in the current study despite their use of broader spectrum of enzymes. However, in the current study, a much higher alpha-amylase dosage (0.5% w/w) was used when compared with the dosage by Bonke et al. (2020) (0.0125 v%). Protein recovery in their study was at a similar level compared to the samples with the lowest recovery in the current work. Higher protein recovery values obtained in average in this work may result from the higher pH of the initial drink in this work (7.2) compared with that in Bonke et al. (2020, pH 6.2) as well as from the added salt in this work since oat protein solubility is known to increase at higher pH values (Nivala, Mäkinen, Kruus, Nordlund, & Ercili-Cura, 2017) and higher salt concentrations (Loponen, Sontag-Strohm, Venäläinen, & Salovaara, 2007). Likewise, lower mass yields of 53.9–78.9% have been reported also by Deswal et al. (2014) who, on the other hand, reported higher dry matter contents of OBMS (24–30%). However, they studied the impact of initial slurry concentration (25–35% w/w) on the solid content of the OBMS and noticed dry matter content to increase with increasing initial slurry concentration. Thus, it is reasonable that the OBMS products obtained in this work from slurries with lower initial concentration compared with those in Deswal et al. (2014) had also lower dry matter contents.

Low protein content is generally considered as one of the nutritionally limiting factors in plant-based milk substitutes and it was also evident in this work that the protein content did not reach values typically reported for cow's milk (3.3–3.8%; Sethi et al., 2016; Vanga & Raghavan, 2018). However, when compared to the OBMS samples of the current study, a commercial OBMS from Oatly (0.7 g/100g; Jeske et al. (2017)) and OBMS samples previously reported in the literature (0.8–1%; Mäkinen et al., 2015; Martínez-Padilla et al., 2020; Ravindran & RadhaiSri, 2020) have similar protein levels. The low protein content of plant-based milk substitutes manufactured in a process containing a solid-liquid separation step results partly from the insolubility of plant proteins, and especially oat proteins are known to exhibit low solubility in slightly acidic and neutral conditions (Nivala et al., 2017). However, it must be noted that the solubility of proteins is usually determined after more intensive solid-liquid separation step (i.e., by applying higher centrifugation forces) and thus the protein yield in OBMS cannot be unambiguously referred to as protein solubility. For example, Mäkinen et al. (2015) showed that the protein present in a commercial OBMS containing 0.8% protein was nearly totally insoluble at pH range 3–8 when centrifugation was carried out at 5000×*g*. Contrary to many other research, Demir et al., 2021 obtained from a 16.7% oat dispersion an OBMS with a 10.3% dry matter content and a considerably higher protein content of 4.6% at pH 7.3, but they did not report the size of the cheesecloth used in the solid-liquid separation step.

Free sugars were determined to evaluate the efficiency of starch hydrolysis by alpha-amylase. The amount of total sugar content was, on the other hand, determined to detect how much the OBMS samples differed in regard to the amounts of free sugars and soluble di-, tri- and oligosaccharides that are analysed as free sugars after acid hydrolysis. The amounts of free and total reducing sugars were 1.1–1.9% and 4.8–7.5%, respectively. Jeske et al. (2017) reported sum of reducing monosaccharide glucose and disaccharide maltose to be 3.4% in a commercial OBMS. The result is higher than reported in this work presumably due to application of also other than only alpha-amylase enzyme resulting in formation of e.g. higher amount maltose and less dextrins (Triantafyllou, 2014).

Regarding physical properties of OBMS, a considerable variation was seen for example in the yellowness (b*) values (0.5–5.3; average 2.4) while redness (a*; −2.5–2.0; average −2.2) and lightness (L*; 68.1–71.3; average 69.8) values varied less (Fig. 2). Similar value for L* was reported previously by Demir et al. (2021; 68.6) for an experimental OBMS while an OBMS prepared without an enzymatic hydrolysis step by Yao, He, Cai, Bekhit, and Xu (2022) exhibited higher L* value of 87.5. In the current work, a* and b* values were much lower than those determined by Demir et al. (2021; −0.9 and 11.3, respectively) whereas a somewhat similar a* value was reported by Yao et al. (2022; −1.8). Some of the variation may derive from the fact that no oil was added in the OBMS products by Demir et al. (2021) and Yao et al. (2022). Furthermore, type and quality of oil added may also play a role in the colour of the final OBMS. For comparison, L* values for pasteurised cow's milk have been determined to vary from 81.7 to 86.2, b* values from 4.1 to 7.8 and a* values from −4.8 to −1.7 depending on the fat content of the milk (Kneifel, Ulberth, & Schaffer, 1992). This shows, that OBMS exhibit considerably lower lightness when compared with cow's milk, which can be regarded as a challenge in terms of consumer acceptance. For particle size, Jeske et al. (2017) reported values d3,2 of 1.7 μm and d4,3 of 3.8 μm, both of which are in the same order of magnitude with the current median values ranging from 3.0 to 6.3 μm (average 4.1 μm). Viscosity values also varied a lot between the samples; however viscosity of 6.8 mPa·s measured at 10 1/s for a commercial OBMS by Jeske et al. (2017) was well in line with the current results

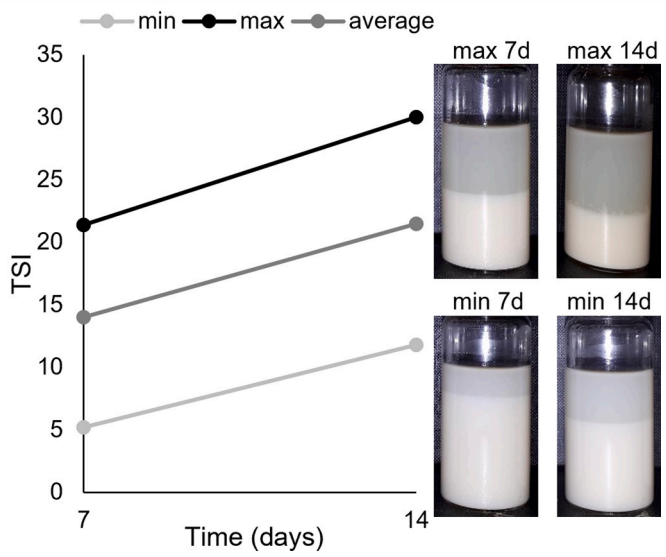

Fig. 3. Visual appearance and Turbiscan Stability Index (TSI) values for the most (min TSI) and least (max TSI) stable oat-based milk substitute samples after 7 and 14 days of storage as well as the average TSI values for all the 30 samples.

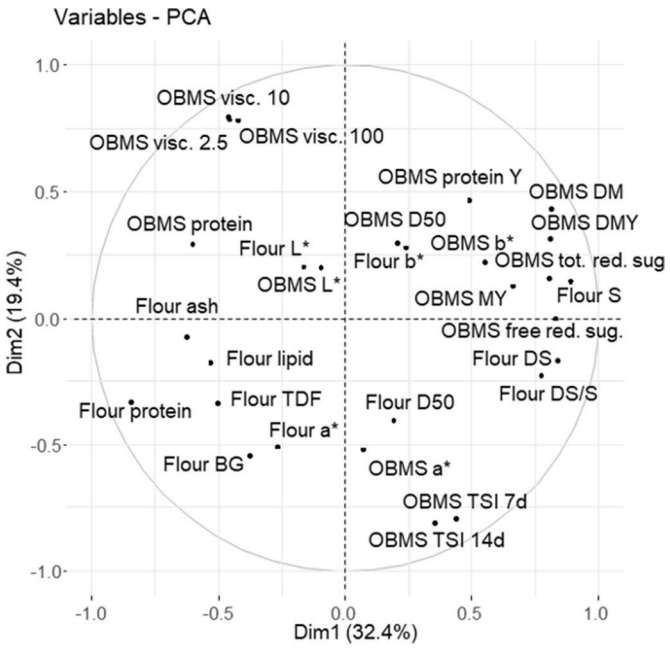

Fig. 4. Principal component analysis (PCA) correlation loadings of the oat-based milk substitute (OBMS) properties and chemical composition of the oat flours. Flour a*, b* and L* are flour colour values a*, b* and L*; flour ash, BG, S, DS, DS/S, protein, lipid, TDF and D50 are flour ash content, β-glucan content, starch content, damaged starch content, ratio between damaged starch and starch contents, protein content, lipid content, total dietary fibre content and median particle size, respectively. OBMS MY, DM, DMY, protein, protein Y, free red. sug., tot. red. sug., TSI 7d, TSI 14d, L*, a*, b*, D50, visc. 2.5, visc. 10, visc. 100 are oat-based milk substitute mass yield, dry matter content, dry matter yield, protein content, protein yield, free reducing sugar content, total reducing sugar content, Turbiscan Stability Index at 7 and 14 d, colour values L*, a* and b* and viscosity values measured at shear rates 2.5 s^{-1}, 10 s^{-1} and 100 s^{-1}.

showing values of 6.0–19.1 mPa·s (average 9.2 mPa·s), 4.8–10.6 mPa·s (average 6.3 mPa·s) and 3.8–6.0 mPa·s (average 4.5 mPa·s) at 2.5, 10 and 100 1/s, respectively. Stabilities of the OBMS samples against sedimentation varied considerably between the most and least stable sample and the sample that exhibited the lowest TSI value after 7 d of storage was also the most stable sample after 14 d of storage (Fig. 3; S2).

3.2. Impact of oat flour composition on composition of oat-based milk substitute

According to the visual projection of the correlation loadings in principal component analysis (PCA), systematic variation was detected between multiple OBMS and oat flour properties (Fig. 4). Firstly, contents of flour starch, flour damaged starch and ratio between damaged starch and starch in the flour grouped with OBMS properties dry matter content, dry matter yield, mass yield, protein yield and free and total reducing sugar contents while flour protein content was located on the opposite edge of the correlation loadings. On the contrary, protein content of OBMS was grouped with flour ash content. Indeed, more thorough evaluation of the relationships between the different properties by Pearson correlation coefficients confirmed these findings as contents of many oat components in the flour showed significant correlation with the composition of the OBMS (Table 1). Flour starch content correlated positively with mass yield of the OBMS ($r = 0.51$, $p < 0.01$), dry matter content of the OBMS ($r = 0.76$, $p < 0.01$), dry matter yield to the OBMS ($r = 0.77$, $p < 0.01$), and amounts of both free ($r = 0.68$, $p < 0.01$) and total ($r = 0.67$, $p < 0.01$) reducing sugar contents of the OBMS. In addition, an interesting positive correlation ($r = 0.69$, $p < 0.01$) was noticed between the starch content of the flour and protein yield to OBMS, whereas the flour starch content correlated negatively with the protein content of OBMS ($r = -0.41$, $p < 0.05$). Close to similar correlations were also observed between the content of damaged starch in the flour and OBMS composition as well as between the share of damaged starch in the flour of the total starch content of the flour (DS/S) and OBMS composition. On the contrary, protein content of the flour showed significant negative correlation with all other compositional attributes and yields of OBMS except protein content of OBMS with which the content in the flour correlated positively ($r = 0.39$, $p < 0.05$). Negative correlations were also observed between the total dietary fibre content of the flour and dry matter content of OBMS ($r = -0.46$, $p < 0.05$), dry matter yield to OBMS ($r = -0.51$, $p < 0.01$) and amount of total reducing sugars in OBMS ($r = -0.56$, $p < 0.01$). However, regarding the flour β-glucan content, significant correlations were only observed with dry matter content of OBMS ($r = -0.44$, $p < 0.05$), protein content of OBMS ($r = 0.43$, $p < 0.05$) and amount of total reducing sugars in OBMS ($r = -0.41$, $p < 0.05$). Median particle size of the flour did not show significant correlation with any of the compositional attributes of OBMS.

The overall oat groat structure and composition supposedly affected the final composition of OBMS. For example, the positive correlation between the flour starch content and the mass yield to OBMS, dry matter content of OBMS and dry matter yield to OBMS was expected since starch forms the largest share of individual components in oat grain (Jokinen et al., 2021; Lapveteläinen et al., 2001), and thus, variation in starch content directly affects the yield of starch-derived components in the OBMS after alpha-amylase treatment. The positive correlation indicates that more OBMS can be obtained from a high-starch oat samples with sufficient enzyme dosage (Deswal et al., 2014). Similarly to starch content, damaged starch content of the flour correlated positively with these OBMS attributes which was expected since damaged starch is more prone to hydrolysis by alpha-amylase (Barrera, Tadini, León, & Ribotta, 2016). Previously, it has been shown that thousand seed weight correlates positively with starch content and negatively with protein content of oat groats (Jokinen et al., 2021) showing that larger groats contain more starch and less protein. This may explain the negative correlation between protein content of OBMS and starch content of the flour since less protein can be solubilised from the low-protein flours to OBMS. On the contrary, positive correlation between starch content of the flour and protein yield to OBMS presumably results from the fact that the share of bran is smaller in the flour with lower protein content (Youngs, 1972)

Table 1
Correlations between oat flour composition and composition and yields of oat-based milk substitutes (OBMS) calculated as Pearson correlation coefficients (n = 30).

	Oat-based milk substitute composition and yields to oat-based milk substitute						
	Mass yield to OBMS	Dry matter content of OBMS	Dry matter yield to OBMS	Protein content of OBMS	Protein yield to OBMS	Amount of free reducing sugars in OBMS	Amount of total reducing sugars in OBMS
Flour starch (S)	.51**	.76**	.77**	−.41*	.69**	.68**	.67**
Flour damaged starch (DS)	.44*	.58**	.58**	−.45*	.40*	.59**	.60**
Ratio of DS/S in flour	.39*	.51**	.50**	−.43*	–	.53**	.55**
Flour protein	−.55**	−.84**	−.78**	.39*	−.77**	−.64**	−.63**
Flour lipid	–	−.42*	−.45*	–	–	−.42*	−.50**
Flour ash	−.55**	−.48**	−.47**	.66**	–	−.60**	−.39*
Flour total dietary fibre	–	−.46*	−.51**	–	–	–	−.56**
Flour β-glucan	–	−.44*	–	.43*	–	–	−.41*

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

and thus less physically entrapped proteins are removed with the bran during the solid-liquid separation step.

Due to the known negative correlation between starch content and total dietary fibre content of oat flour (Jokinen et al., 2021) it was evident that total dietary fibre content correlated negatively with dry matter content of OBMS, dry matter yield to OBMS and amount of total reducing sugars in OBMS. Furthermore, the high amount of dietary fibre in the flour may indicate presence of higher share of large bran-derived oat groat particles (Youngs, 1972) which are presumably removed during the solid-liquid separation step. In addition to dietary fibre, β-glucan content of oat flour correlated also negatively with dry matter yield of OBMS and amount of total reducing sugars in OBMS. As β-glucan is enriched in the outer grain layers of oat (Doehlert & Moore, 1997), β-glucan content of the flour was expected to correlate negatively with the dry matter yield to OBMS since larger share of bran in the groat reduces the share of the endosperm from which most of the solubilised components derive. This was indeed further supported by the observation that flour β-glucan content correlated negatively with the total reducing sugar content of OBMS which suggests that high β-glucan content of the flour did not increase the amount of soluble β-glucan present in the flour and further its solubilisation to OBMS but β-glucan was rather removed as a part of the large particles in the solid-liquid separation. On the contrary, protein content of OBMS was the only parameter correlating significantly positively with the flour β-glucan content and this may be associated with the positive correlation between flour protein and flour β-glucan contents (Jokinen et al., 2021). However, also negative correlations between flour protein and β-glucan contents have been earlier reported (Lapveteläinen et al., 2001). Ash content of the flour showed similar correlations with flour protein content which further supports the impact of the relative share of the bran part of the oat, which is known to be enriched in both protein (Youngs, 1972) and ash (Peterson, Senturia, Youngs, & Schrader, 1975), on properties of OBMS.

3.3. Impact of oat flour properties on physical properties of oat-based milk substitute

PCA correlation loadings showed that each colour value of OBMS and flour were grouped to some extent with each other (Fig. 4). Correlations confirmed that colour values of the oat flour showed various significant correlations with the colour values of the OBMS (Table 2). The b* value of the OBMS correlated positively with the b* value of the colour of the flour revealing yellowness of both flour and OBMS ($r = 0.52$, $p < 0.01$). Additionally, a* value, which is related to redness, in OBMS and flour correlated positively with each other ($r = 0.66$, $p < 0.01$) while no significant correlation was found between lightness-related L* values of OBMS and the flours.

PCA showed also that starch content of the flour was grouped with b* value of OBMS. Indeed, in regard to flour composition, starch content of the flour correlated positively with the b* value of the colour of the OBMS ($r = 0.51$, $p < 0.01$) while protein content of the flour correlated negatively with the colour value b* of the OBMS ($r = -0.54$, $p < 0.01$) which indicates that OBMS prepared from flours with higher starch contents were more yellow in colour compared with those richer in protein. In regard to consumer acceptance, colour of the plant-based milk substitutes play an important role due to the fact that unwanted visual appearance may reduce the use of these food products and light-coloured products similar to cow's milk are sought after (McClements et al., 2019).

Regarding the impact of flour composition on the physical OBMS properties, correlations verified the findings of PCA since the content of damaged starch in the flour correlated negatively with viscosity of the OBMS regardless of the shear rates. Furthermore, the amount of damaged starch in flour showed positive correlations with TSI values, i.e. higher amount of damaged starch made the OBMS less stable. Damaged starch is known to be more prone to degradation by enzymes when compared to native starch and thus it may be expected that shorter

Table 2
Correlations between oat flour composition and physical properties of oat-based milk substitutes (OBMS) calculated as Pearson correlation coefficients (n = 30).

	Physical properties of oat-based milk substitute								
	OBMS colour L*	OBMS colour a*	OBMS colour b*	OBMS TSI 7d	OBMS TSI 14d	OBMS particle size D50[a]	OBMS viscosity at 2.5 1/s	OBMS viscosity at 10 1/s	OBMS viscosity at 100 1/s
Flour starch (S)	–	–	.51**	–	–	–	–	–	–
Flour damaged starch (DS)	–	–	.37*	.45*	.42*	–	−.54**	−.49**	−.40*
Ratio of DS/S in flour	–	–	–	.46*	.43*	–	−.56**	−.51**	−.42*
Flour protein	–	–	−.54**	–	–	–	–	–	–
Flour β-glucan	–	.47**	–	–	.43*	−.40*	–	–	–
Flour colour L*	–	–	−.46*	–	–	–	–	–	–
Flour colour a*	–	.66**	−.41*	–	–	–	–	–	–
Flour colour b*	–	−.43*	.52**	–	–	–	–	–	–

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table 3
Correlations between compositional and physical attributes of oat-based milk substitutes (OBMS) calculated as Pearson correlation coefficients (n = 30).

	Composition, yields and physical properties of oat-based milk substitute														
	Dry matter content	Dry matter yield	Protein content	Protein yield	Amount of free reducing sugars	Amount of total reducing sugars	TSI 7d	TSI 14d	Colour L*	Colour a*	Colour b*	Particle size D50[a]	Viscosity at 2.5 1/s	Viscosity at 10 1/s	Viscosity at 100 1/s
Mass yield	.72**	.83**	−.43*	–	.65**	.53**	–	–	–	.40*	–	–	–	–	–
Dry matter content		.92**	–	.71**	.67**	.74**	–	–	–	–	.58**	–	–	–	–
Dry matter yield			–	.67**	.74**	.66**	–	–	–	–	.43*	–	–	–	–
Protein content				–	−.55**	−.43*	−.55**	−.37*	–	–	–	–	.40*	.49**	.57**
Protein yield					–	–	–	–	–	–	.55**	–	–	–	–
Amount of free reducing sugars						.66**	.40*	–	–	–	.38*	–	–	−.41*	−.45*
Amount of total reducing sugars							–	–	–	–	.53**	–	–	–	–
OBMS TSI 7d								.93**	–	.41*	–	–	−.79**	−.81**	−.79**
OBMS TSI 14d									–	.45*	–	–	−.76**	−.75**	−.70**
OBMS colour L*										–	–	–	–	–	–
OBMS colour a*											−.48**	–	–	–	–
OBMS colour b*												–	–	–	–
OBMS particle size D50													–	–	–
OBMS viscosity at 2.5 1/s														.98**	.88**
OBMS viscosity at 10 1/s															.95**

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).
[a] Median particle size.

dextrins are formed from the damaged starch. Shorter dextrins are known to exhibit lower viscosity when compared with longer dextrins (Sun, Zhao, Zeng, Li, & Li, 2010) and this decrease in viscosity might have been detected also in the current negative correlation between the content of damaged starch in the flour and viscosity of OBMS.

3.4. Relationship between composition and physical properties of oat-based milk substitute

As also shown by PCA, mass yield to the OBMS correlated positively with dry matter content of the OBMS ($r = 0.72$, $p < 0.01$), dry matter yield to the OBMS ($r = 0.83$, $p < 0.01$) and amounts of free ($r = 0.65$, $p < 0.01$) and total ($r = 0.53$, $p < 0.01$) reducing sugars in the OBMS (Table 3). Likewise, dry matter content of OBMS showed positive correlation with dry matter yield to OBMS ($r = 0.92$, $p < 0.01$), protein yield to OBMS ($r = 0.71$, $p < 0.01$) and amounts of free ($r = 0.67$, $p < 0.01$) and total ($r = 0.74$, $p < 0.01$) reducing sugars in OBMS. Furthermore, also dry matter yield to OBMS correlated positively with protein yield and amounts of free and total reducing sugars in OBMS. Negative correlations were observed between mass yield and protein content of OBMS ($r = -0.43$, $p < 0.05$) and between protein content and amounts of both free ($r = -0.55$, $p < 0.01$) and total ($r = -0.43$, $p < 0.05$) reducing sugars in OBMS. Finally, amounts of free and total reducing sugars in OBMS correlated positively with each other ($r = 0.66$, $p < 0.01$).

As earlier discussed, many of the observed correlations most potentially result from the oat groat structure and composition. The groats with higher amount of starch allow formation of higher amounts of soluble components transferring to the liquid phase after starch hydrolysis and centrifugation, which explains the positive correlations between the dry matter- and sugar-related attributes. The negative correlation between protein content of the OBMS and mass yield and sugar constituents presumably derives also from the groat structure since less protein is transferred to the liquid phase from those raw materials that initially contain more hydrolysable starch. Additionally, the positive correlation between free and total reducing sugars in OBMS, in combination with the positive correlations found between flour starch content and free and total reducing sugars in OBMS, proposes that starch is hydrolysed to both longer dextrins and free sugars.

PCA revealed also connections between the different physical OBMS properties (Fig. 4). Especially viscosities measured at different shear rates were grouped together, and also TSI-values measured at different time-points were grouped with each other. Additionally, those stability-related properties, i.e. viscosities and TSI-values, were grouped on the opposite edges of the correlation loadings. Pearson correlation coefficients verified these findings. Dispersion stabilities analysed as TSI-values showed at both time points negative correlations with viscosity values measured at shear rate of 2.5 1/s (i.e. the higher the viscosity, the more stable the sample; Table 3). This negative correlation was expected since the lower the viscosity, the less stabilising effect the continuous phase of the system has against gravitational separation, including sedimentation and creaming, and particle aggregation (McClements et al., 2019). Likewise, expectedly, a strong positive correlation was observed between TSI-values after 7 and 14 days of storage ($r = 0.93$, $p < 0.01$), which indicates that the stability of the OBMS after a shorter storage time predicts well the stability also after longer time. Protein content of OBMS correlated negatively with dispersion instabilities of OBMS at both 7 and 14 days ($r = -0.55$, $p < 0.01$ and $r = -0.37$, $p < 0.05$, respectively). In addition to the negative correlation between dispersion instability values and protein content of OBMS, the protein content of OBMS correlated positively with the viscosity suggesting that higher amount of protein in OBMS may stabilise the system through viscosity increase.

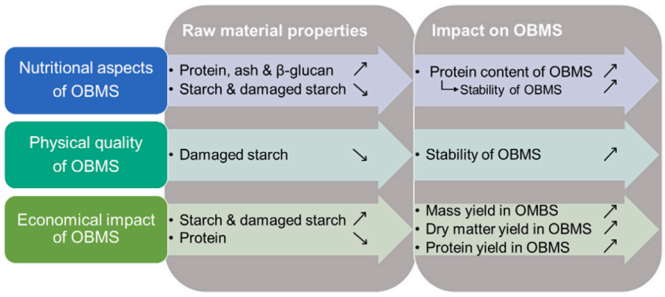

Fig. 5. Practical recommendations for selection of raw materials with most suitable properties aiming at production of oat-based milk substitutes (OBMS) with high nutritional properties, physical quality or economic feasibility.

4. Conclusions

Understanding the applicability of different oat raw materials in liquid food systems, such as dairy substitutes, has a considerable impact in terms of consumer acceptance, economic feasibility and resource efficiency. This study revealed multiple correlations that can be exploited when targeting production of OBMS with high nutritional and physical quality or economic feasibility (Fig. 5). In regard to nutritional aspects, raw materials with high contents of protein, ash and β-glucan and low contents of starch and damaged starch are associated with high protein content in OBMS, which in turn improves the stability of OBMS. Low content of damaged starch in the flour also improves stability of OBMS, which is an important quality characteristic of OBMS. High contents of starch and damaged starch as well as low protein content of the flour improve the economic feasibility via increases in mass, dry matter and protein yields of OBMS. In addition, low flour lipid, ash and total dietary fibre contents improve mass yield of OBMS.

Considering these recommendations, it can be concluded that different raw material properties should be valued depending on the targeted properties of the final OBMS while further process optimisation may presumably improve overall quality of the production process and final product. Given the fact that no single raw material property was found to result in good quality OBMS in terms of nutrition, physical quality and feasibility, the future research should focus on mitigation of the impact of compositional differences in the raw materials and modifying the desired properties via optimisation of the production process. This optimisation could include selection of the most suitable enzymes for each raw material, adjusting the unit operations and conditions in the production process depending on the raw material and modifying the level of added components. It should be, however, also noted that the correlations identified in this research were confirmed to apply for the OBMSs prepared only with the applied production process and differing correlations might be observed for the differently prepared OBMSs. To enhance and ensure the nutritional quality of OBMS, protein solubilisation and digestibility of the proteins should be considered in the future research. Regarding raw material utilisation, more research is needed to harness the full potential of nutritionally relevant protein and dietary fibre components, that currently remain vastly in the insoluble residual fraction which is removed during the solid-liquid separation step of OBMS manufacturing.

Funding

This research was funded by Business Finland (grant number 5703/31/2018) as a part of the project “Novel indicators and technologies for oat quality and applicability” (OatHow) cofunded by the University of Helsinki, the University of Turku, Luke Natural Resources Institute Finland and VTT Technical Research Centre of Finland Ltd.

CRediT author statement

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Declaration of competing interest

The authors declare that they have no conflict of interests.

Data availability

Data will be made available on request.

Acknowledgements

Riitta Pasanen, Riitta Alander, Tytti Salminen and Eero Mattila are acknowledged for their assistance in the experimental part of the work. Heikki Aisala is thanked for his contributions in the principal component analysis. Martina Lille is thanked for her help in the viscosity measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2023.109402.

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