Research Article

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Physicochemical and nutritional properties of high protein emulsion-type lupin-based model milk alternatives: effect of protein source and homogenization pressure

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Abstract

BACKGROUND: Plant-based milk alternatives are becoming more popular. However, many are low in nutrients, particularly protein. More attention is being given to plant protein isolates / concentrates as potential ingredients in high-protein milk alternative formulations.

RESULTS: The effect of lupin protein source on the physicochemical, functional, and nutritional characteristics of model milk alternatives was investigated. Milk alternatives were produced with either blue lupin or white lupin protein isolate, formulated to contain similar levels of protein and fat as low-fat cow's milk. Nutritional composition and predicted glycemic properties were measured. The effect of homogenization pressure on the physicochemical properties and storage stability was also assessed, with cow's milk and soy milk alternative analyzed for comparison. Both blue and white lupin milk alternatives were high in protein, low in fermentable oligo-, di- and monosaccharides, and polyols (FODMAPs), and had a low predicted glycemic index. White lupin milk alternatives had smaller particle size as well as greater stability, with less creaming compared to blue lupin milk alternatives, although the former showed slightly higher sediment layers. Increasing homogenization pressure from 180 to 780 bar resulted in smaller particle size, lower separation rate, and greater foamability for both blue and white lupin milk alternatives. White lupin milk alternative homogenized at 780 bar was found to be the most stable product, with a similar separation rate to cow's milk.

CONCLUSIONS: These results indicate that protein source and processing can influence functional properties significantly along with product stability, and this is an important consideration when formulating high-protein milk alternatives.

INTRODUCTION

Recent years have seen increased demand for plant-based food and beverage products that provide alternatives to traditional animal-based products.[1] One reason for this change is greater environmental awareness. It is now recognized that animal-based foods, such as dairy and meat, are often associated with far higher environmental impact than plant-based foods, requiring higher energy input and resulting in higher greenhouse gas emissions.[2] Aside from environmental concerns, consumers opt for plant-based alternatives to meat, dairy or eggs for ethical, health and lifestyle choice reasons. Plant-based milk alternatives have been on the market for many years, and in the USA they represented the largest category of plant-based products by sales, at $2 billion in 2019.[1] However, a potential disadvantage of replacing cow's milk with plant-based alternatives is the inferior nutritional value of many of the latter. With the notable exception of soy-based products, most commercially available milk alternatives (MA) are lower in nutrients compared with cow's milk. Many are much lower in protein, and some are high in sugar, with a higher glycemic index than cow's milk.[3,4] Interestingly, sales of almond MA are now more than six times higher than soy MA in the USA,[1] although the protein content of almond MA tends to be considerably lower than soy MA.[3,4] In general, the nutritional composition of plant-based MAs depends on the starting material, as well as the manufacturing process. Traditionally, plant-based MAs are produced by extracting plant material in water, either from whole seeds (with wet milling) or flour, to yield a product resembling milk in terms of taste and appearance. Heat treatment and homogenization technologies can be applied, and various components such as sweeteners, flavorings, stabilizers, and micronutrients may be added.[5,6] With this approach, the overall nutritional composition resembles that of the input seed material, minus any separated solids, such as insoluble fibers. Extracted

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components include carbohydrates, protein, and fat, which is typically in the form of oil bodies.[7,8] Where starchy grains are used, for example oat or rice MA, enzymatic liquefaction of starch may be necessary.[5] As many of the grains and nuts used to produce plant-based MAs are low in protein, it can be difficult to reach a similar composition to milk or soy MA in the final product.

Interest has been building in the use of pulses for production of nutritious plant-based beverages, due to their health benefits as well as functional properties.[9,10] Pulses tend to be relatively rich in protein, and there is interest in producing milk alternatives directly from milled seeds, using a similar method to the production of soy MA. A recent study focused on the production of milk alternatives in this manner from lupin and chickpea.[11] However, for many pulses, the majority of the seed is composed of carbohydrates,[12] which could limit the achievable protein content of the milk alternative. Furthermore, pulses can be limited by their sensory attributes in this type of application.[8] Protein isolates and concentrates are now being recognized for their potential as ingredients in plant-based MAs, as their high protein and low carbohydrate content opens up the possibility of higher protein formulations. In combination with vegetable oils and other ingredients, they can be used to formulate products with a similar nutritional profile to cow's milk and soy MA. Recent studies have focused on emulsion-type milk alternatives produced with lupin protein isolate,[13,14] and lentil protein isolate.[15] Furthermore, commercial high-protein MAs formulated with pea protein isolate are now available.[16]

In this study, the nutritional composition, physicochemical / functional properties, physical stability and *in vitro* glycemic properties of lupin-based model milk alternatives were assessed. Protein and fat content of the milk alternatives were modeled on that of low-fat cow's milk. Lupin protein isolates were chosen due to their excellent functional properties including relatively high-protein solubility.[17] Protein isolates from two different species of lupin, blue lupin (*L. angustifolius*) and white lupin (*L. albus*) were used in order to compare the influence of protein source on the product properties. The effect of homogenization pressure on certain properties was also assessed, with two different pressures employed. Homogenization pressure is a key consideration in the design of milk alternatives, as it can have a significant influence on protein solubilization, particle / droplet size, and consequently product physical stability.[15] Commercial cow's milk and soy MA were also used as reference products.

MATERIALS AND METHODS

Materials and chemicals

All chemicals were purchased from Sigma-Aldrich (St Louis, MO, USA). Blue lupin protein isolate and white lupin protein isolate were provided by ProLupin GmbH and the Fraunhofer Institute for Process Engineering and Packaging (Freising, Germany), respectively. Both lupin protein isolates were the focus of a previous study, where the manufacturing process has been outlined.[17] Cow's milk (low fat) and soy MA were purchased at a local supermarket; both were pasteurized products in the chilled section. Coconut oil and sucrose were also purchased at a local supermarket.

Production of lupin-based milk alternatives

Lupin-based milk alternatives (LMAs) were produced according to the method described by Jacobs *et al.*[13] with modifications from Jeske *et al.*[15] Blue lupin protein isolate (872.8 g.kg^{-1} protein and 9.2 g.kg^{-1} fat), and white lupin protein isolate (908.6 g.kg^{-1} protein and 10.7 g.kg^{-1} fat) were used as the protein source. The target composition for LMAs was modeled on the protein and fat content of low-fat cow's milk (35 g.kg^{-1} protein and 15 g.kg^{-1} fat), with 24 g.kg^{-1} sugar. Accordingly, the formulation for a 500 g batch of blue lupin milk alternative (BLMA) was 460.63 g water, 7.32 g coconut oil, 20.05 g protein isolate, and 12 g sucrose, and the formulation for white lupin milk alternative (WLMA) was 461.46 g water, 7.29 g coconut oil, 19.26 g protein isolate, and 12 g sucrose. The protein isolate and sucrose were dispersed in the water using a magnetic stirrer and pH was adjusted to 7 using 2 mol L^{-1} NaOH. Dispersions were then heated to 50 °C in a water bath and held for 1 h at this temperature. The dispersions were then stirred using a magnetic stirrer and simultaneously sheared at 4600 rpm for 10 min using a Ultraturrax T18 high shear mixer (Janke & Kunkel IKA Labortechnik, Germany). Pre-heated coconut oil was then added, followed by a further 10 min of shearing with the same settings. The pre-emulsions were homogenized with a two-stage high-pressure homogenizer (APV-2000, SPX FLOW Inc., Charlotte, NC, USA) at 180 bar (150 bar and 30 bar), or 780 bar (650 bar and 130 bar). To ensure microbial stability, samples were pasteurized at 85 °C for 2 min in a stirring water bath (Lochner mashing device LP electronic, Berching, Germany). Samples were refrigerated (4 °C) overnight and measured on the following day. Additionally, samples were stored for 21 days at 4 °C to assess changes in particle size and viscosity during storage, supplemented with sodium azide (0.02%) to prevent microbial spoilage.

Compositional analysis

For LMAs, compositional analysis was carried out only on the samples homogenized at 180 bar, as the higher homogenization pressure was not expected to influence the composition. The total nitrogen content was analyzed according to the Kjeldahl method using the general nitrogen-to-protein conversion factor of 6.25 for plant-based samples, and 6.38 for cow's milk samples.[18,19] Fat content was measured using the Soxhlet method, using Celite® R566 as an adsorbent, and petroleum ether as the solvent.[3] Amino acid composition was determined on freeze-dried samples by Chelab S.r.l. using ion chromatography with post-column ninhydrin derivatization (fluorescence detection; UV detection for tryptophan) after adequate extraction and protein hydrolysis (separate hydrolysis procedures for the determination of tryptophan, sulfur-containing amino acids and remaining amino acids).

FODMAP analysis

For LMAs, fermentable oligo-, di- and monosaccharides, and polyols (FODMAP) analysis was carried out only on the samples homogenized at 180 bar. The quantification of mono-, di-, galactooligosaccharides, and polyols was conducted using high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), performed on a Dionex™ ICS-5000+ system (Sunnyvale, CA, USA), as described by Ispiryan *et al.*[20] All carbohydrates were quantified using authentic reference standards.[20] Samples were filtered through 0.2 µm syringe filters.

Protein solubility

Protein solubility of the LMAs and commercial products was analyzed to give an indication of stability. Protein contents of whole samples and supernatants (centrifuged at 3000×*g* for 10 min) were determined using the Kjeldahl method. Protein solubility

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was expressed as protein content of the supernatant as a percentage of the protein content of the non-centrifuged sample.

Particle size analysis

Particle size distribution (PSD) was measured using a static laser light diffraction unit (Mastersizer 3000, Malvern Panalytical Ltd, Malvern, UK), covering a size range of 0.01–3000 μm. Lupin-based milk alternative samples were analyzed on day 1 and day 21. Samples were shaken vigorously by hand prior to dilution to ensure homogeneity of sampling. The particle refractive index was set at 1.45, the absorption used was 0.1, and the dispersant refractive index was 1.33. Samples were introduced into the dispersing unit using ultrapure water as dispersant until a laser obscuration of ~12% was achieved. Samples were diluted 1:10 with ultrapure water before analysis, and LMA samples were diluted 1:10 in 1% sodium dodecyl sulfate (SDS) to disrupt flocs and assess the potential effects of flocculation and coalescence on particle size.[21]

Accelerated physical stability analysis

Stability was measured using an analytical centrifuge (LUMiSizer®, LUM GmbH, Berlin, Germany). The samples were centrifuged at 1000×*g* for 90 min at 15 °C (equivalent to approximately 2 months under gravity). During the analysis, the sample was illuminated with near infra-red light, and transmitted light was detected by sensors across the entire sample length. The results are shown as transmission profiles over the sample length and measurement time, with 30 s intervals between each profile. The separation rate was also calculated using the Lumisizer software. The percentage integrated light transmission (across the entire sample length) increased over time. The percentage integral transmission is plotted against time and the slope of the line fit to this curve is referred to as the separation rate.

Rheological behavior

The rheological behavior of the products was characterized using a controlled stress rheometer (MCR301, Anton Paar GmbH, Austria) equipped with a concentric cylinder measuring system (C-CC27-T200/SS, Anton Paar GmbH). The shear stress was measured as a function of shear rate ranging from 0.5 to 200 s^{-1}. The measurements were carried out at 20 °C and the power law model was fitted from 10–200 s^{-1} to determine the flow behavior index (n). LMA samples were analyzed on day 1 and day 21. Apparent viscosity measured at 40.1 s^{-1} is referred to as viscosity.

Foaming properties

Samples were frothed using an Ultraturrax T18 (Ika-Labortechnik, Janke and Kunkel GmbH, Staufen) at 8000 rpm for 1 min in a graduated cylinder. The heights of the foam and unfoamed sample were measured after 3 min (as a clear interface was visible) and several timepoints up to 60 min. Foaming capacity was taken as percentage sample expansion at 3 min, while foam stability was taken as sample expansion at 30 min as a percentage of sample expansion at 3 min. Sample expansion was calculated using the following equation:

$$\text{Sample expansion}\,(\%) = [\text{Foam volume}/\text{Initial sample volume}]\cdot 100$$

Color analysis

The color values were measured using the CIE *L*a*b** color system. The instrument used was a colorimeter (CR-400, Konica Minolta, Osaka, Japan). Color of samples was characterized according to whiteness index (WI).[22]

$$WI = 100 - \sqrt{\left(100 - L^{*}\right)^{2} + a^{*2} + b^{*2}}$$

Confocal laser scanning microscopy

The microstructural analysis of LMAs and commercial samples was performed using a confocal laser scanning microscope (CLSM) (Olympus FV1000, incorporating an IX81 inverted microscope, Germany), according to Jeske *et al.*[15] A saturated solution of Nile blue was used to label both protein and lipid; 0.5 mL of Nile blue was added to 1 mL of sample.

Predicted glycemic index and glycemic load

In vitro determination of the predicted glycemic index (GI) was evaluated according to Magaletta and DiCataldo[23] on the LMAs homogenized at 180 bar as well as commercial samples. A sample volume equivalent to 0.5 g of available carbohydrates was digested by a multi-enzyme preparation. The digestate was analyzed for glucose, fructose, lactose, and galactose using high-performance liquid chromatography with a HPLC Agilent 1260 Infinity (Agilent Technologies, Santa Clara CA, USA) equipped with a refractive index detector (RID) and a Sugar-Pak I 10 μm, 6.5 × 300 mm column (Waters, Milford MA, USA), with 50 mg/L Ca-EDTA as mobile phase and a flow rate of 0.5 mL min^{-1} at 80 °C. Quantification was achieved by external standards in a calibration range of 0.1 to 10 g $.L^{-1}$. These results, together with the results from the protein and fat content of the original samples, were used as inputs for the following calculation:

$$\begin{aligned}\text{GI} = {} & 26.264529 - 1.048186\ \text{protein}\ (\%) - 0.248138\ \text{fat}\ (\%) \\ & + 621.7824\ \text{glucose}\ (\%) - 52.7993\ \text{fructose}\ (\%) \\ & + 233.67679\ \text{lactose}\ (\%) - 61.21071\end{aligned}$$

Glycemic load (GL) was calculated as described by Atkinson *et al.*[24] and the portion size was set to 250 mL:

$$\text{GL} = (\text{GI}\cdot\text{available carbohydrate}\,(\text{g})\,\text{per portion})/100$$

Statistical data analysis

The results shown are the mean values and standard deviation for analyses of three batches of LMAs, or in the case of commercial products, three packages, with the exception of the amino acid analysis. Amino acid analysis was carried out by an external analytical laboratory and validated uncertainty values are shown. Means were compared using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test ($P < 0.05$) using IBM SPSS version 26 (Armonk, NY, USA).

RESULTS AND DISCUSSION

Composition

Protein and fat are important nutritional and functional components in milk. Due to the low nutritional value of many plant-based MAs, particularly low protein content, there is increasing interest in developing higher protein MA formulations. At the same time, plant proteins generally have less favorable amino acid profiles compared to milk protein and other animal proteins.[25] Protein and fat content for LMAs, cow's milk and soy MA

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Table 1. Nutritional composition of lupin-based milk alternatives and commercial products

	Protein (%)	Fat (%)
BLMA	3.54 ± 0.04^{c}	1.43 ± 0.05^{a}
WLMA	3.44 ± 0.04^{b}	1.37 ± 0.06^{a}
Cow's milk	3.83 ± 0.03^{d}	1.28 ± 0.08^{a}
Soy MA	3.20 ± 0.01^{a}	1.69 ± 0.10^{b}

Means ± standard deviations are shown. Values within a column that share the same letter are not significantly different ($P < 0.05$).

are shown in Table 1. The results differ slightly from the target protein and fat contents for BL and WL milk alternatives. For cow's milk, the measured protein content was slightly higher than displayed in the nutritional information on the package, whereas for soy MA the measured protein content was lower (3.5% protein was displayed on the package for both cow's milk and soy MA), although all values were within the discrepancy range set by the European Commission.[26] All products also met the EU legal requirements necessary to display a 'high protein' claim.[27] Previous studies have used a similar process to produce milk alternatives with lupin protein isolate, either intended as a beverage or as a base for yogurt production; however, a lower protein content was used in both cases (2% protein isolate).[13,14] Aside from increased protein content, this method allows flexibility in terms of fat content and composition, where potentially low or full fat products could be developed, using various types of vegetable oils to give a desired fatty acid profile. Either neutral or flavor-contributing oils can also be chosen. Products such as these LMAs could help bridge the nutritional gap between cow's milk and plant-based MAs. This is particularly important for certain consumers, such as children, who may be at risk of nutrient deficiencies when replacing cow's milk with commercial plant-based products.[28]

The amino acid composition for LMAs, cow's milk and soy MA is shown in Table 2. These results are generally in line with previously reported values for lupin,[29,30] cow's milk,[31] and soybeans.[32] The profiles for BL, WL, and soy MA are quite similar, while cow's milk has some notable differences, including higher tryptophan, methionine, proline, and lysine content, as well as lower aspartic acid, glycine, and arginine levels, compared to the lupin and soy samples. The indispensable and conditionally indispensable amino acid contents of LBAs, cow's milk and soy MA are shown in Fig. 1, as a percentage of the World Health Organization requirements for adults.[33] Both LMAs provide most of these amino acids above the requirements. However, there are several amino acids that fall below the requirement, including sulfur-containing amino acids (methionine and cysteine) and valine. Tryptophan is above the requirement for BLMA, but below the requirement for WLMA. For both BLMA and WLMA, lysine almost reaches the requirement. Only sulfur-containing amino acids fall below the requirement for soy MA, while cow's milk contains all the amino acids above the requirement. The limiting amino acids are sulfur-containing amino acids for BLMA and soy MA, and tryptophan for WLMA. The differences in the amino acid profile reflect the biological functions of the respective proteins. From a nutritional perspective, milk proteins provide the amino acids needed for growth by the neonate,[34] whereas legume seed proteins are mainly storage proteins, which provide free amino acids upon germination, along with ammonia and carbon skeletons.[35]

FODMAP analysis

Fermentable oligo-, di- and monosaccharides, and polyols (FODMAPs) are a family of carbohydrates that are poorly digestible, and therefore are fermented in the gut, which can result in increased fluid and gas production along with discomfort.[36] Consequently, a low FODMAP diet may be recommended for individuals with irritable bowel syndrome (IBS).[37] Sucrose, which does not fall into this category, was also analyzed, as it was the sugar used in the LMA and soy MA formulations. Polyols, glucose/galactose, fructose, and verbascose were not present in any of the samples. Only sucrose was present for the LMAs. The measured sucrose contents of BLMA and WLMA were 2.33 ± 0.034 and 2.28 ± 0.012 g/100 mL, respectively. Sucrose (2.43 ± 0.107 g/100 mL) and raffinose/stachyose (0.189 ± 0.007 g/100 mL) were present in soy MA. Raffinose and stachyose together contribute to a large proportion of the carbohydrate content of legume seeds such as soybeans and lupins, with 1.2–8.3% of dry matter in soybeans, and up to ~10% of dry matter in lupins.[32,38] The effective removal of these components during processing of lupin seeds into protein isolates allows for very low FODMAP content in the isolates, and consequently in the resulting milk alternatives.[17] In cow's milk, only lactose was present (4.55 ± 0.074 g/100 mL). Lactose is poorly digested by many adults, as lactase synthesis decreases with age.[39] FODMAP levels of <0.3 g (galactooligosaccharides) and <1 g (lactose) per portion in foods a considered suitable for inclusion in low FODMAP diets.[40] For a portion size of 250 mL, soy MA and cow's milk would contain 0.47 g and 11.4 g of FODMAPs per serving, respectively, meaning they would be unsuitable for a low FODMAP diet under this definition, particularly in the case of cow's milk. On the other hand, both LMAs could be considered suitable for inclusion.

Protein solubility

As protein represents the largest proportion of the solids in this formulation, protein solubility is a critical consideration, especially due to the poor solubility of many commercial plant protein isolates.[41] Many plant-based milk alternatives are prone to sedimentation of poorly soluble components during storage,[3] which is undesirable, especially if they are nutritionally important components, which could remain unconsumed if the package is not shaken sufficiently. Protein solubility, along with emulsifying properties, should therefore be considered carefully when selecting protein ingredients.

Protein solubility for LMAs as well as commercial reference products is show in Fig. 2. The blue lupin milk alternative 780 bar (BL-780) had the highest protein solubility at 99.7%, followed by cow's milk with 98.2%. The white lupin milk alternative 180 bar (WL-180) had the lowest protein solubility at 80.3%. Slightly higher protein solubility was apparent for blue lupin compared to white lupin in this milk alternative formulation. The slight difference in solubility between these two lupin protein sources may be expected, and is in line with previous analysis on these protein isolates in dispersions, where slightly higher solubility was observed for blue lupin compared to white lupin protein isolate dispersed in water at pH 7.[17] Additionally, for both blue and white lupin, homogenization at 780 bar compared to 180 bar resulted in slightly higher solubility, possibly due to further reduction of particle size with the higher pressure treatment.

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Table 2. Amino acid composition of lupin-based milk alternatives and commercial reference products. Results are expressed as g/100 g of total amino acids, ± uncertainty values

	BLMA	WLMA	Cow's milk	Soy MA
Tryptophan	0.63 ± 0.07	0.40 ± 0.04	2.15 ± 0.22	0.70 ± 0.08
Cysteine	1.21 ± 0.15	1.10 ± 0.13	0.93 ± 0.11	1.18 ± 0.14
Methionine	0.36 ± 0.04	0.46 ± 0.06	2.08 ± 0.25	0.83 ± 0.10
Aspartic acid	10.76 ± 1.50	11.14 ± 1.55	7.70 ± 1.07	11.62 ± 1.62
Threonine	3.38 ± 0.47	3.49 ± 0.49	4.10 ± 0.57	3.97 ± 0.55
Serine	5.99 ± 0.84	5.90 ± 0.82	5.65 ± 0.79	5.85 ± 0.81
Glutamic acid	24.02 ± 3.34	23.40 ± 3.26	20.22 ± 2.82	19.21 ± 2.67
Proline	4.41 ± 0.61	3.87 ± 0.54	10.33 ± 1.44	5.68 ± 0.79
Glycine	4.89 ± 0.68	4.40 ± 0.61	2.02 ± 0.28	4.70 ± 0.65
Alanine	3.43 ± 0.48	3.21 ± 0.45	3.21 ± 0.45	4.28 ± 0.60
Valine	2.83 ± 0.39	3.00 ± 0.42	5.37 ± 0.75	4.25 ± 0.59
Isoleucine	3.34 ± 0.46	4.15 ± 0.58	4.02 ± 0.56	3.64 ± 0.51
Leucine	7.95 ± 1.11	8.68 ± 1.21	9.86 ± 1.37	8.08 ± 1.12
Tyrosine	3.50 ± 0.49	5.24 ± 0.73	4.87 ± 0.68	3.73 ± 0.52
Phenylalanine	4.07 ± 0.57	4.21 ± 0.59	4.68 ± 0.65	5.21 ± 0.73
Lysine	4.27 ± 0.59	4.37 ± 0.61	7.67 ± 1.07	6.41 ± 0.89
Histidine	2.56 ± 0.36	2.12 ± 0.30	2.25 ± 0.31	2.56 ± 0.36
Arginine	12.39 ± 1.73	10.86 ± 1.51	2.88 ± 0.40	8.10 ± 1.13

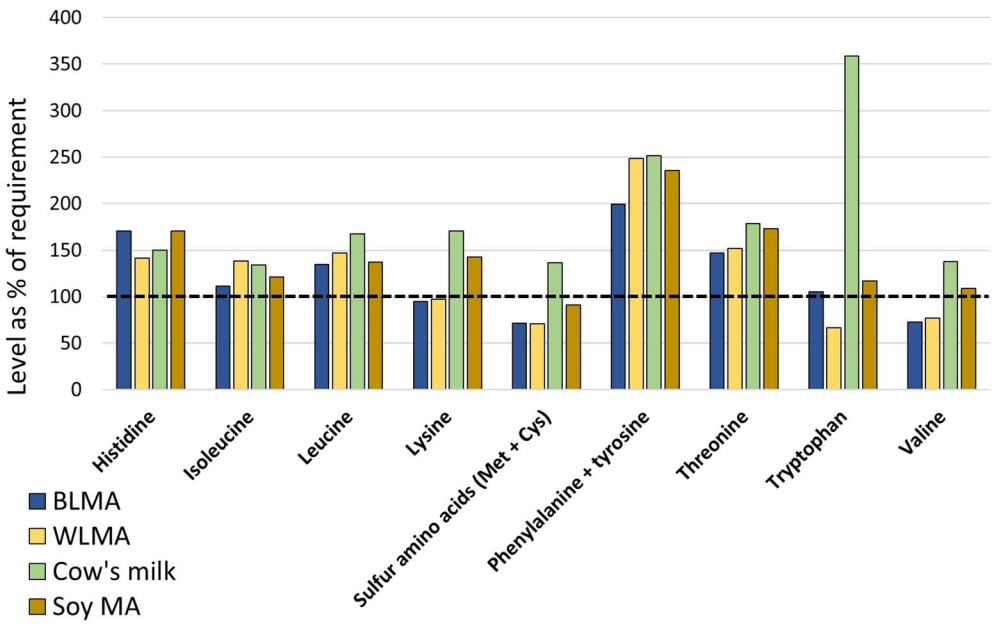

Figure 1. Indispensable / conditionally indispensable amino acid levels for lupin-based milk alternatives and commercial reference products as percentages of the World Health Organization requirements for adults (mg of each amino acid required per g of protein).[33] The amount of each amino acid as a percentage of total amino acids was calculated, and shown here as a percentage of the recommended level.

Particle size distribution

Due to the high protein content it can be expected that the lupin-based samples could contain both protein-coated fat droplets, as well as dispersed protein particles. In emulsions, droplet size / size distribution is important as it is one of the factors that determine the rate of creaming according to Stokes law.[42] Similarly, the size of insoluble protein particles present will affect the rate of sedimentation. Volume-weighted particle size distributions for LMAs, cow's milk and soy MA are shown in Fig. 3, for LMAs at day 1 and after 21 days' storage, with and without sodium dodecyl sulfate (SDS). All samples showed a similar size range, although the distribution varied slightly depending on the sample. For LMAs, homogenization at 780 bar resulted in a smaller particle size compared to homogenization at 180 bar. For BLMA, volume weighted mean particle size ($D_{4,3}$) was 1.26 μm for 180 bar, compared to 0.6 μm for 780 bar; for WLMA, $D_{4,3}$ was found to be 0.69 μm for 180 bar and 0.29 μm for 780 bar. Generally, the WLMAs had smaller $D_{4,3}$ than BLMAs, although the distribution curves are relatively similar. This could be due to the slightly higher presence of very large particles in the BLMAs. For all

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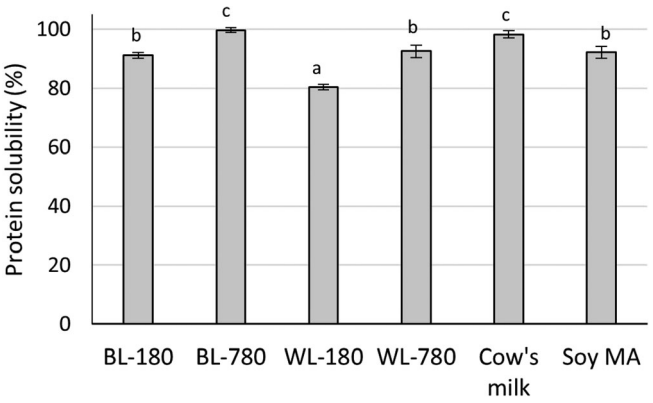

Figure 2. Protein solubility of lupin-based milk alternatives and commercial reference products. BL-180 and BL-780 are blue lupin-based milk alternative homogenized at 180 and 780 bar, respectively. WL-180 and WL-780 are white lupin-based milk alternative homogenized at 180 and 780 bar, respectively. Error bars show standard deviation. Values that share the same letter are not significantly different.

samples, addition of SDS resulted in a slight shift of the distribution to smaller particle size, suggesting aggregation or bridging flocculation of fat droplets in samples without SDS.[21] The addition of SDS had a greater effect on size distribution of WLMAs compared to BLMAs, suggesting a greater degree of noncovalent aggregation/flocculation in WLMAs. Heat treatment of protein stabilized emulsions can result in unfolding of proteins, exposing reactive groups such as hydrophobic side chains and sulfhydryl groups, which can cause flocculation and coalescence of fat droplets.[21] There was no major difference in the size distribution of samples after 21 days storage compared to samples from day 1, both with and without SDS. This indicates a stable emulsion was formed, without any noticeable coalescence of fat droplets during storage.

Rheological properties

Viscosity is an important property for beverages as it influences mouthfeel, and it may be desirable to mimic the mouthfeel of cow's milk when formulating plant-based milk alternatives. Apparent viscosity at 40.1 s^{-1} is shown in Table 3. All lupin-based samples had a relatively similar viscosity compared to cow's milk, with WLMAs showing slightly lower values than BLMAs. No significant differences related to homogenization pressure were observed. By contrast, soy MA had a considerably higher viscosity, which could be attributed to its higher fat content, as well as the addition of gellan gum in the formulation. Previous work has shown that most types of plant-based MAs tend to have higher viscosity than cow's milk.[3] This could be in part due to the type of plant material used; however, many products also contain hydrocolloids as stabilizers which could increase viscosity.[3] With the exception of BL-180 day 1, all LMA samples as well as cow's milk had a flow behavior index slightly higher than 1, indicating slightly dilatant behavior. On the other hand, Soy MA had a flow behavior index of 0.832, indicating slightly pseudoplastic behavior. No significant differences were observed for any of the samples between day 1 and day 21, with the exception of BL-180, where the flow index was slightly higher for day 21 compared to day 1.

Foaming properties

Foaming properties are important for milk-type beverages, and foam formation may be desirable when they are used as an ingredient, e.g., in cappuccinos, or desserts.[43,44] Conversely, excessive foaming could be undesirable during processing and packaging. Foaming capacity and foam stability are shown in Table 3, while the dissipation of foam over time is depicted in Fig. 4. WL-780 showed the greatest foaming capacity at 229%, whereas BL-180 showed the lowest at 54.4%. Soy MA had the highest foam stability after 30 min, with a value of 86.8%, while for cow's milk there was no foam remaining after 30 min. Even though the foaming capacity varied considerably, all the LMAs appeared to have a similar pattern of dissipation (Fig. 4). For cow's milk the volume of foam declined more rapidly, while for soy MA the foam dissipated more slowly compared to LMAs. The higher viscosity of soy MA may have contributed to its higher stability. In general, WLMAs showed higher foamability than BLMAs. Higher homogenization pressure also resulted in significantly higher foaming capacity, as well as higher foam stability. The smaller fat droplet size resulting from higher homogenization pressure could possibly account for this. A study on whole cow's milk showed that raw milk had lower foamability compared to homogenized milk (pasteurized or UHT), depending on the temperature. This was attributed to smaller fat droplets, thus reducing the spread of fat on disruption, which could, in turn, destabilize foam.[43] The study by Ho *et al.*[44] also showed that reduction of fat globule size led to increased foamability in cow's milk. Previous work has shown that when dispersed in water, blue lupin and white lupin protein isolate had similar foaming properties,[17] suggesting that the differences seen here may be influenced by differences in emulsifying ability of blue lupin and white lupin proteins.

Color

Whiteness index, shown in Table 3, is a useful tool for examining the color of milk alternatives, as it is desirable to replicate the characteristic white color of cow's milk, to make products more attractive to consumers. Cow's milk had the highest whiteness index, followed by WLMAs, followed by BLMAs, while soy MA had the lowest whiteness index. There was no significant difference in the whiteness index between samples treated at 180 bar or 780 bar. The color of milk alternatives primarily depends on the natural components of the plant material used in their formulation. Increasing fat content can also increase whiteness, and heat treatments may also influence color.[15] Ideally, milk alternatives should possess similar characteristics to cow's milk, including appearance,[7] although the color and whiteness index of popular commercial milk alternatives can vary widely.[3]

Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) is a useful technique for examining differences in microstructure of complex food and beverage products, as it allows labeling of different components. The CLSM images of LMAs and commercial products are shown in Fig. 5. A clear difference is apparent between samples homogenized at 780 bar compared to 180 bar, with larger protein particles visible at the lower homogenization pressure. Additionally, at 180 bar, BLMA seems to have larger protein particles compared to WLMA. This is in line with the particle size analysis results, and suggests possible aggregation of blue lupin proteins to a greater extent than the white lupin proteins. For the plant-based samples, the images are dominated by protein, whereas for cow's milk the fat is more prominent. This may be due to protein particles in lupin and soy-based samples, which are likely larger and more visible compared to the casein micelles found in cow's milk.

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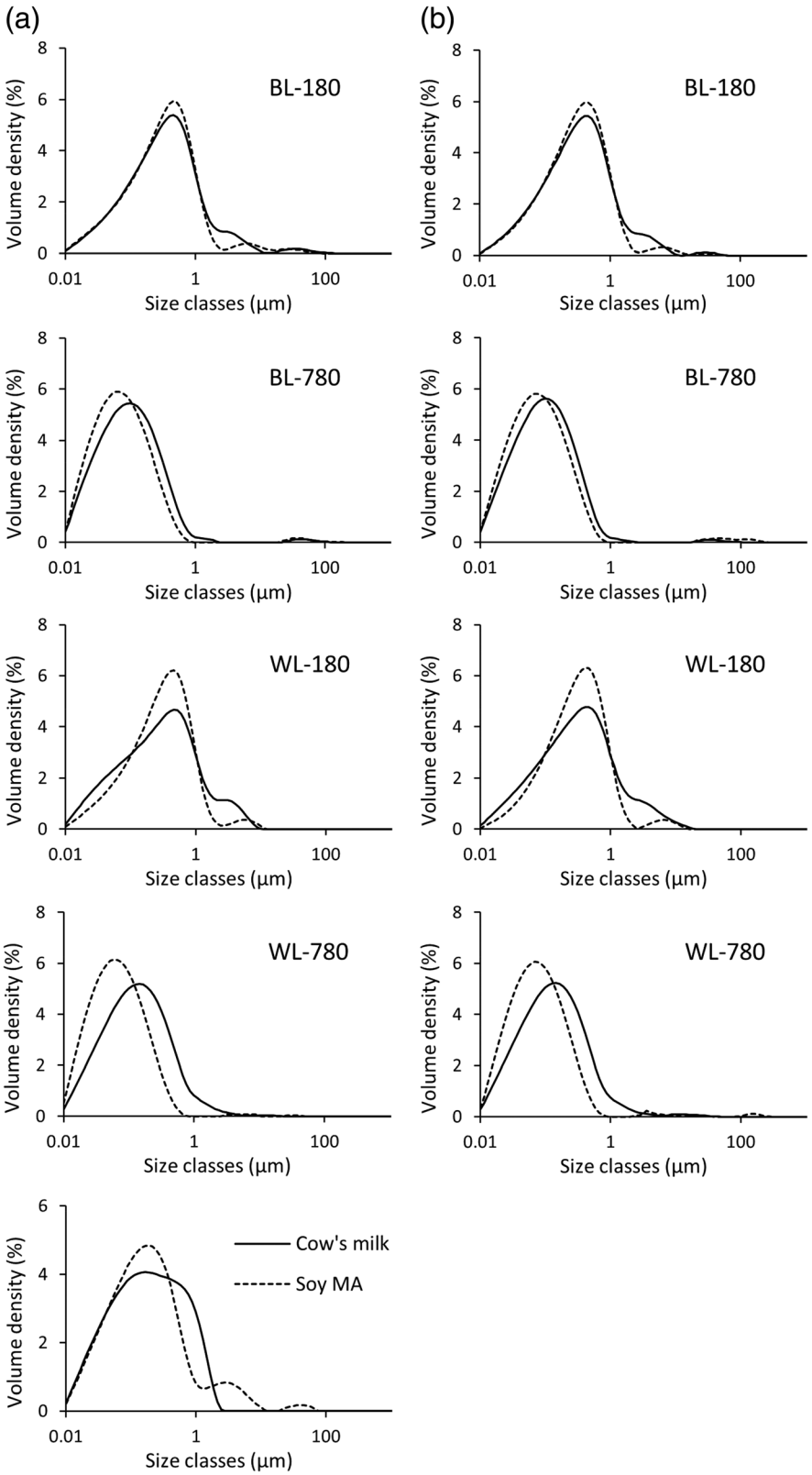

Figure 3. Volume-weighted particle size distribution of LMAs and commercial products. Results from day 1 and day 21 are shown in columns (a) and (b), respectively. For LMAs, continuous lines represent dilution in water, whereas dashed lines represent dilution in SDS solution.

The particle size results (Fig. 3) also show that distribution curves for lupin and soy-based MAs generally extend to a larger size range compared to cow's milk.

Accelerated physical stability analysis

Plant-based MAs are typically prone to gravitational separation during storage, in the form of creaming, or sedimentation,

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Table 3. Viscosity, flow behavior index (n), whiteness index, and foaming properties of LMAs and commercial reference products

	Viscosity (mPas)		Flow behavior index (–)		Whiteness index (–)	Foaming capacity (%)	Foam stability (%)
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 1	Day 1
BL-180	1.80 ± 0.09^{c}	1.65 ± 0.02^{c}	0.974 ± 0.20^{b}	1.07 ± 0.01^{c}	82.5 ± 0.25^{b}	54.4 ± 7.58^{a}	3.67 ± 3.26^{a}
BL-780	1.64 ± 0.02^{c}	$1.61 \pm 0.08^{b,c}$	1.04 ± 0.03^{c}	1.07 ± 0.03^{c}	82.1 ± 0.37^{b}	193 ± 4.38^{d}	58.8 ± 4.64^{b}
WL-180	$1.41 \pm 0.02^{a,b}$	1.36 ± 0.03^{a}	1.09 ± 0.02^{c}	1.09 ± 0.03^{c}	86.1 ± 0.30^{c}	141 ± 11.90^{c}	53.9 ± 3.05^{b}
WL-780	1.38 ± 0.02^{a}	1.39 ± 0.01^{a}	1.08 ± 0.02^{c}	1.07 ± 0.02^{c}	86.4 ± 0.64^{c}	229 ± 7.58^{e}	59.5 ± 4.83^{b}
Cow's milk	1.65 ± 0.06^{c}	N/A	1.07 ± 0.01^{c}	N/A	89.1 ± 0.19^{d}	126 ± 22.4^{c}	0 ± 0^{a}
Soy MA	4.90 ± 0.19^{d}	N/A	0.832 ± 0.02^{a}	N/A	80.6 ± 0.47^{a}	86.9 ± 5.96^{b}	86.8 ± 0.94^{c}

Means ± standard deviations are shown. Values within a column (including both day 1 and day 21 for viscosity and flow behavior index), which share the same letter, are not significantly different ($P < 0.05$). N/A = not applicable.

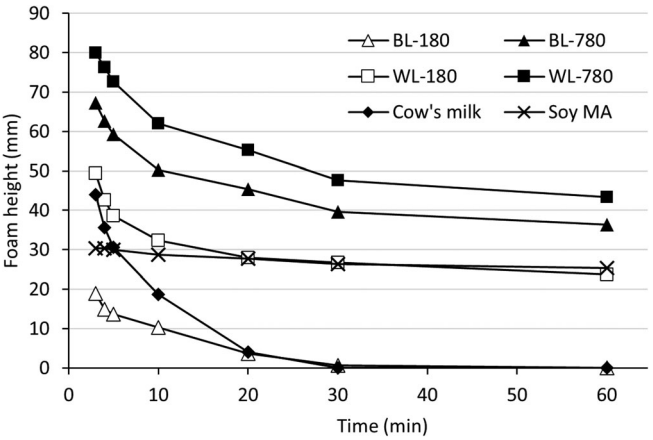

Figure 4. Foam height as a function of time for LMAs and commercial products.

depending on the density of the components.[8] Accelerated stability analysis using analytical centrifugation is a useful rapid means of predicting gravitational separation behavior. The Lumisizer transmission profiles for LMAs and commercial samples are shown in Fig. 6. The centrifugation experienced by the samples is equivalent to approximately 2 months of gravitational separation. Clear differences can be seen between all samples. Overall, it is apparent that the 780 bar homogenization treatment resulted in lower separation for LMAs, which can be explained by the smaller particle size with 780 bar homogenization.[7,42] The WLMAs showed lower separation compared to BLMAs, which might also be explained by the slight differences in particle size distribution between BLMAs and WLMAs. The transmission profiles of BL-180 and WL-180 show a relatively similar shape, whereas more difference is seen between BL-780 and WL-780. In WL 780, an initial increase in transmission from the top of the sample indicates sedimentation. For BL-780 this is also apparent to some extent. However, creaming behavior, visible as increased transmission coming from the bottom of the sample,[45] is more pronounced in BLMAs. The WLMAs also show higher sediment layers than BLMAs, indicated by a larger distance from the sample bottom before transmission increases. For each lupin type, higher homogenization pressure also resulted in a slightly lower sediment layer.[45] The profile for cow's milk shows creaming behavior and very little sedimentation, while the soy MA profile shows mixed behavior with both sedimentation and creaming, and the most clearance appearing in the center of the sample. The separation rate, i.e., overall rate of change of the integral transmission, is shown in Fig. 7. The lowest rate of separation was apparent for WL-780, whereas the highest was found for BL-180. By this metric, WLMAs showed greater stability compared to BLMAs, with the BLMAs also showing more susceptibility to creaming in the transmission profiles. There was no significant difference for separation rate between WLMAs and cow's milk. However, the higher sediment layer for WLMA samples indicates more sedimentation of insoluble protein compared to BLMAs, which is also reflected in the slightly lower protein solubility of WLMAs (Fig. 2).

These results indicate better emulsifying ability for white lupin compared to blue lupin, as demonstrated by slower creaming. It has been demonstrated previously that proteins from different legume sources showed differences in emulsion stability.[46] With regard to the protein isolates used here, previous characterization showed differences that could affect emulsifying properties. Blue and white lupin displayed major differences in electrophoretic protein profile, with blue lupin overall showing smaller protein sizes. Blue lupin was found to have higher surface hydrophobicity compared to white lupin.[17] Overall, the lupin-based milk alternatives could be considered to have good physical stability, especially as some plant-based beverages have shown very rapid separation.[3]

In vitro predicted glycemic properties

The glycemic index is defined as the post-prandial glycemic (blood glucose) response elicited after ingestion of a food portion containing a specified amount of available carbohydrate, as a percentage of the glycemic response of a reference carbohydrate.[47] The predicted glycemic properties of LMAs and commercial samples are shown in Table 4. Cow's milk had the lowest glycemic index (GI), followed by the LMAs, which had similar GI values, and soy MA had the highest GI. The slightly higher GI of soy MA may be explained by the presence of starch, resulting in glucose on digestion, as soybeans were used as the main input material. Glycemic load (GL), which is also dependent on the amount of carbohydrate per serving, was highest for cow's milk, which has a higher overall carbohydrate content. The LMAs, as well as cow's milk, can be considered low-GI foods based on these values as the GI is ≤55. Soy MA is slightly higher and could be classified as a medium GI food.[24] The GI values reported here for cow's milk and soy MA are similar to those reported by Jeske *et al.*[3] for whole milk and various soy MAs using the same *in vitro* method.

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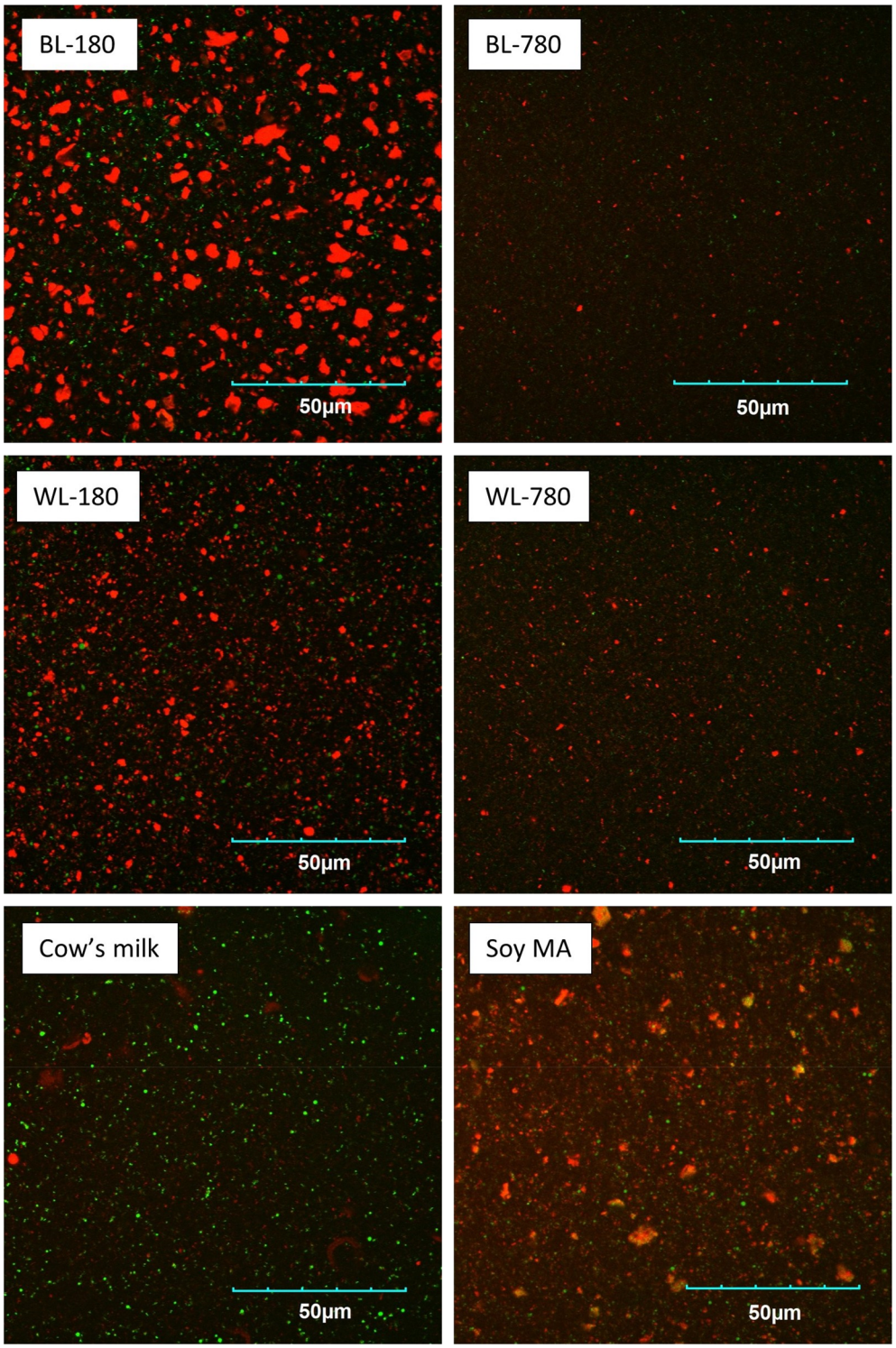

Figure 5. Confocal laser scanning microscopy (CLSM) of LMAs and commercial products stained with Nile blue. Protein is visible as red, whereas fat appears as green.

However, *in vivo* GI measurements for similar products are slightly lower, with 39 ± 3 for whole milk, 37 ± 4 for skim milk, and 34 ± 4 for soy MA.[48] Overall, the LMAs compare favorably to some categories of plant-based MAs, such as rice MAs, which have been shown to have very high *in vitro* GI.[3] It is also possible to formulate unsweetened versions if very low GL is desired.

CONCLUSIONS

Both blue lupin and white lupin protein isolate could be used to produce milk alternatives with good stability and somewhat similar physical and functional characteristics compared to cow's milk. However, white lupin MAs showed greater emulsion stability with separation rates comparable to cow's milk, while blue lupin

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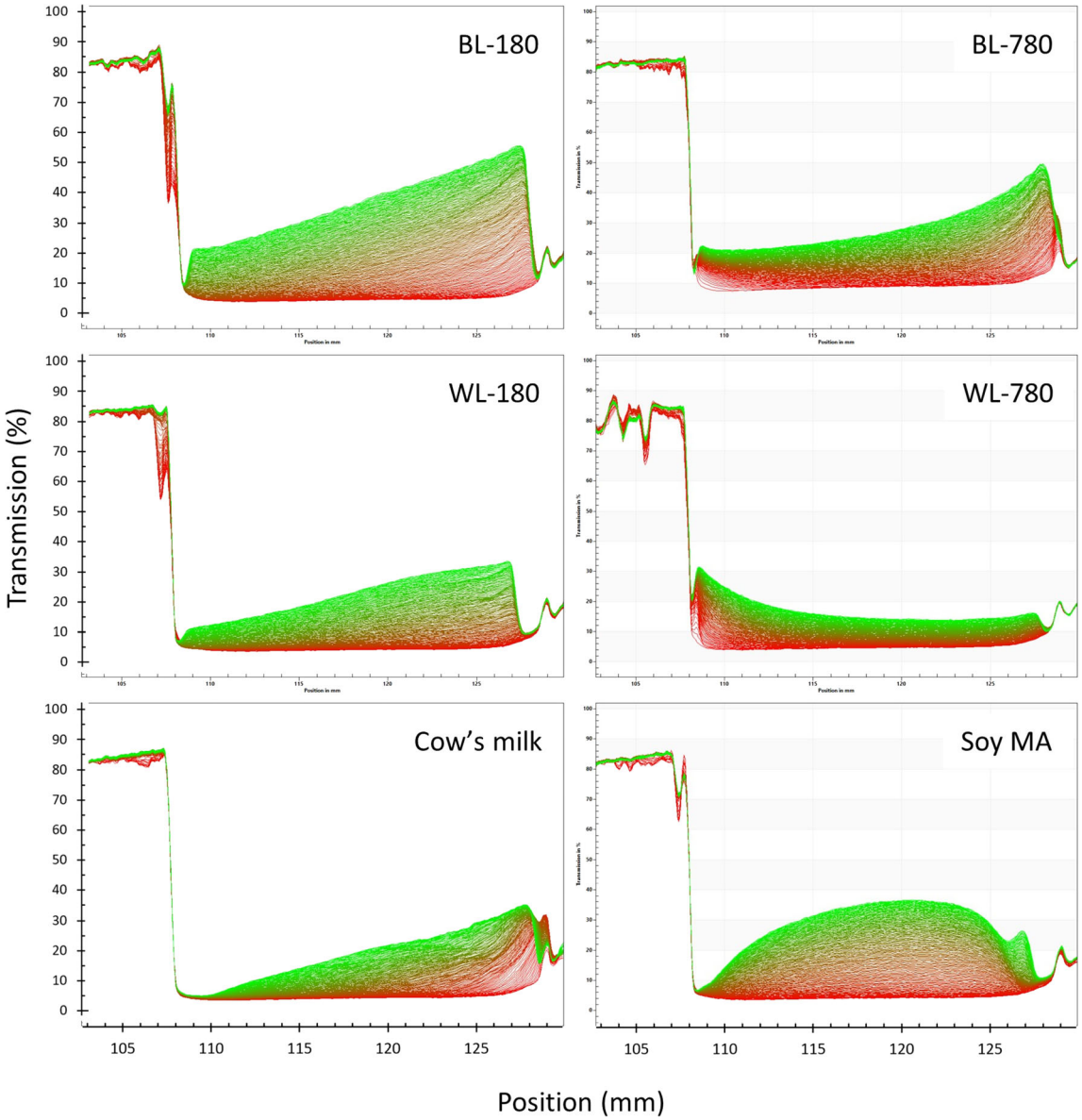

Figure 6. Representative Lumisizer graphs showing transmission of near infra-red (NIR) light as a function of position. The top of the sample begins at the left side of the graph. The first transmission profile is shown in red, while the last is shown in green.

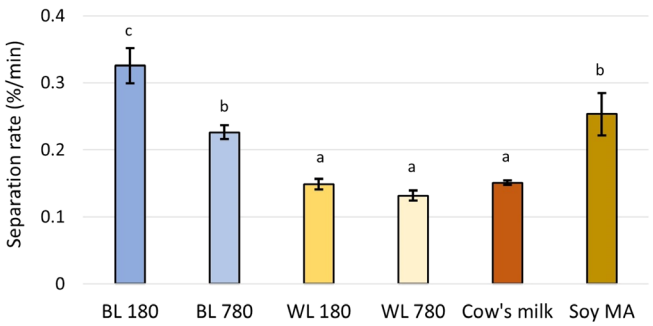

Figure 7. Separation rate in percentage/min for lupin-based milk alternatives and commercial products. Separation rate represents the slope of the change in integral transmission over time. Error bars show standard deviation. Values that share the same letter are not significantly different.

Table 4. *In vitro* predicted glycemic properties for lupin-based milk alternatives and commercial products

	Glycemic index (–)	Glycemic load (–)
BLMA	51.15 ± 0.23^{b}	2.98 ± 0.04^{a}
WLMA	53.71 ± 2.16^{b}	3.06 ± 0.11^{a}
Cow's milk	44.66 ± 0.73^{a}	5.08 ± 0.16^{c}
Soy MA	57.73 ± 0.13^{c}	3.76 ± 0.15^{b}

Means ± standard deviations are shown. Values within the same column that share the same letter are not significantly different ($P < 0.05$).

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MAs were less stable and showed more creaming. On the other hand, higher sediment layers were apparent for WLMAs. For both BLMAs and WLMAs, increasing homogenization pressure from 180 to 780 bar resulted in smaller particle size and greater stability. The WLMA homogenized at 780 bar was the most stable product. Lupin MAs could also be classed as high-protein, low-glycemic index and low FODMAP products. With good functionality, lupin protein isolates, and in particular white lupin, show promise as a source of protein for milk alternatives with higher nutritional value than many of the plant-based beverages currently available. Along with processing technique, protein source was shown to be an important consideration, as considerable differences were apparent between blue and white lupin. Future studies focusing on sensory qualities, micronutrient fortification, and improvement of amino acid profile would be useful.

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