Functional Properties of a Novel Processed Oat Milk Product

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Abstract

This project aims to characterize the unique emulsion and foaming properties exhibited by a novel oat milk homogenate whilst investigating its nutritional value. This oat milk is produced under the novel 'whole ingredient nutrient extraction' (WINX) system utilizing enzymatic and high-pressure processes (HPP) to produce a stable oat milk emulsion. The release and exposure of β-glucan soluble fibres are hypothesized to be the functional compound within this WINX oat milk, but this is yet to be substantiated. Physical experiments have been conducted based on protein concentration by homogenizing varying oat milk samples. This is done to understand the effects the enzymatic and high-pressure production steps have on the formation of the stable oat milk emulsion and foam. These physical experiments have been concluded to be non-reproducible with this current experimental method as results varied too greatly between samples of the same oat milks further supporting the idea β-glucans are functionally stabilizing the oat milk emulsion. Current experimental focuses are now on determining the bioavailability of B-glucans through in vitro digestions followed by β-glucan assays. This will help substantiate both the physical composition and the nutritional relevance of this oat milk as this soluble fibre has glucose-regulating and cholesterol-reducing effects.

1. Introduction

1.1 Problem Statement

There is a demand and market for nutritional, sustainable, and novel oat milks that can adequately supplement the rising demand for plant-based milk alternatives. Whole Green Foods (Whole.) is a Perth-based biotechnology company that has developed proprietary food processing technology that presents a novel means to produce food extracts through high-pressure processing (HPP), while limiting waste. This processor has been named 'Whole Ingredient Nutrient Extraction' or WINX. WINX processed novel oat product is to be marketed as a chemical and additive-free emulsified oat milk that possesses the creamier texture of current oat milks on the market. This will allow lower cost production of a higher consumer and environmentally friendly product catering to many dietary preferences.

Although oats have been observed to contain proteins of dietary nutraceutical value, this is only relevant to oats that have been hydrolysed (Rafique et al., 2020). Rolled oat flour provided by Avena Mills to Whole. have not been through any chemical processes to hydrolyse the grain.

However, soluble dietary fibres, such as β-glucans, affect physiological responses in the gastrointestinal track and are highly abundant in most milled oats (Rafique et al., 2020)(Henrion et al., 2019). Whole.'s novel oat concentrate has yet to be analysed for such functional nutritional components nor has the stability of this oat products' structure been experimentally characterised. It is hypothesised that the stability of the WINX oat milk foam and emulsion are a result of specific aspects of the WINX process. The outcome of this experiment is to attribute these processing steps to the stability of WINX emulsions by making samples omitting different processing steps including the starch enzymatic processing and high-pressure processing hoping to gain insight into the structure of this oat milk emulsion. Thus, the aim of this experiment is to determine the nutritional value and structural properties of WINX-produced novel oat product and in achieving this, the foundation for developing a patent for this oat product can be provided.

1.2 Background Information

Alternative diets currently have focused on soya, almond and rice-based sources which have their own sustainability and nutritional concerns such as the use of genetically modified crops, high waste production or insufficient nutritional profiles (Jeske et al., 2019).This creates a focus on developing plant-based substitutes that address both the nutritional and sustainability concerns presented in the current market. With the production of plant-based milks, important steps are taken to maintain the stability of milk concentrates regardless of the base plant ingredient. This is typically done through additives such as hydrocolloids and emulsifiers, including oils or gum extracts. Thus, novel methods and technologies are needed to protect plant-based milk stability without any of these additives (Aydar et al., 2020). There are a few technologies that achieve this including high-pressure processing or homogenization (Aydar et al., 2020)

Seed storage proteins may not be the only contributing emulsifier in oat milks. In a study investigating oat gels, it was observed that significantly more stable and smooth gels were produced under HPP (Fan et al., 2019). They used two different oats with variable molecular weights and used them in varying concentrations under varying pressures to see how these factors affect the formation of oat gels. Optimal conditions did not depolymerise β-glucans preserving the core molecular structure and MW. This allowed for ideal Hydrogen bonding and electrostatic attractions to occur between β-glucan molecules which are observed to be the main gel-forming interactions under high pressures (Fan et al., 2019).

2. Process

2.1 Emulsion Stability and Activity

The stability of emulsion of each oat milk sample was experimentally determined using a ULTRA-TURRAX T 25 basic homogenizer. 5mL 0.1% sodium dodecyl sulphate solution was used to stablise each emulsion sample before measuring absorbance. For each sample, 400mLs was homogenized at 19000rpm for 1 minute. 50μL samples were pipetted from the bottom of the sample beaker 0-minutes and 10 minutes after homogenizing. Each sample falcon tube was thoroughly vortexed before pipetting 100μL in triplicate into a 96-well plate.

Using the previously determined protein concentrations, the following calculations were used to determine the emulsion activity index (EAI), equation 1.1, and the emulsion stability index (ESI), equation 1.2, of each oat milk sample (Konak et al. 2014). For the EAI to be calculated,

the protein concentration of each sample was determined through a Coomassie blue Bradford assay.

$$EAI = \frac{2 * 2.303 * A_0}{0.25 * protein(g)} \quad \textbf{1.1}$$

Emulsion activity Index (EAI) has units of area of interface stabilised per unit weight of protein and is a function of oil volume fraction, protein concentration and type of equipment used to produce the emulsion (Pearce et al., 1978). A_0 is the absorbance read at 0 minutes after homogenizing. Δt is the absorbance after 10 minutes. ΔA is the change in turbidity and is the difference between A0 and Δt.

$$ESI = A_0 \frac{\Delta t}{\Delta A} \quad \textbf{1.2}$$

Emulsion stability index (ESI) is the change of turbidity within the defined time interval and has units of time defined by the input and in this case, seconds. A_0 is the absorbance read at 0 minutes after homogenizing. Δt is the 10-minute absorbance. ΔA is the difference between 0- and 10-minute absorbances.

2.2 In-vitro Digestion

An in-vitro digestion of oat milk was established based on a standardized static in-vitro method (Minekus et al. 2013). This experiment paired with a mixed-linkage β-glucan assay experiment is aimed to determine the bioavailability of β-glucans within the sample both free and within the human gastrointestinal system, thus, inferring its abundance within the milk and its nutritional contribution to the WINX oat milk emulsion. Preparation of the in vitro digestion includes making basic electrolytic stock solutions of correct concentrations to then make up three separate digestive electrolytic stock solutions to best simulate the contents of human salivary, gastric and intestinal fluid. Each simulated digestive fluid would then be made by adding the oat milk food sample to digestive electrolytic stock solutions along with appropriate enzymes. The WINX oat milk food sample is added to the salivary digestive stock, including the electrolytes and relevant enzymes, to be in a ratio of 50:50. The digestive product from one step will become the food input for the subsequent step until intestinal digestion is complete. Final digestive samples will then be aliquoted and snap-frozen in liquid nitrogen.

2.3 Bile Salt Assay

Before bile salts can be used during intestinal digestion, the concentration must first be determined through a total bile acids assay kit (#80268) provided by Crystal Chem in order to produce the correct concentration of bile solution. The principle of this assay is based on the enzymatic conversion of bile acids into 3-keto steroids and NADH by 3-α hydroxy-steroid dehydrogenase. These products react with a dye named nitrotetrazolium blue which is detected, and its absorbance is measured at 540nm. This reading is directly proportional to the concentration of the bile acids concentration.

2.4 Total β-glucan Assay (mixed-linkages)

The total β-glucan assay (mixed-linkages) test kit (K-BGLU) provided by Megazyme will be used to determine the bioavailability of β-glucan within the WINX oat milk undigested and digested sample. The principle of this assay is that β-glucan samples are suspended in a buffer solution of pH 6.5 where it is then incubated with lichenase and filtered. The filtrate is then

aliquoted and then hydrolysed to completion with β-glucosidase. The D-glucose product is assayed using a glucose oxidase/peroxidase reagent. The absorbance measured at 510nm can then be used to calculate the amount of β-glucan (g/100mL). This is done by first calculating the factor for conversion from absorbance to µg of glucose (µg D-glucose / abs. of 100 µg of D-glucose). Then after factoring in volume correction, adjustment, conversion to grams and the factor to convert free D-glucose to anhydro-D-glucose occurring in β-glucans, the amount of β-glucans (g/100mL) is determined.

3. Results and Discussion

Experimental work to date has covered the physical emulsion experiments, while the in vitro digest is still being investigated. The first emulsion experiment testing the stability of the four samples, WINX enzymatic processed oat milk, commercial oat milk, negative enzyme milk and oat milk slurry, showed varying results as seen in Table 1. Regarding the first set of data, only the WINX and commercial oat milk saw a decrease in absorbance over 10 minutes. When compared to the second data set, the opposite is observed, WINX and commercial oat milk are the only samples to increase in absorbance over the 10-minute period. This resulted in inconsistent emulsification stability and activity index calculations. This was enough to question the reliability of the experimental data and conditions of the experiment in analysing WINX oat milk.

Next, a variance test for this emulsion experiment was designed to determine if this experiment was able to produce consistent results. The Vitasoy unsweetened oat milk was emulsified over 8 tests from 4 separate batches with varying results once again, as seen in Table 1. None of the EAI or ESI calculations appeared to support a coherent conclusion. To confidently conclude on the reproducibility of this experiment, an ANOVA statistical test was performed on the mean from separate batches. The ANOVA test, Table 2, analysed the effects the independent variable, time after homogenizing, had on the absorbance levels. The resulting F-statistic was calculated to be lower than the F-critical value meaning the null hypothesis cannot be rejected. This is also the case with the calculated P-value being higher than 0.05, failing to reject the null hypothesis meaning there is insufficient evidence to claim the stability of commercial oat milk can be measured by the current experimental procedures. This could be a result of low sample size of 8 trials, or some unforeseen variance introduced by the nature of the experiment and the oat milk used.

Test 1

	Abs. 500nm	mL	g	m^2/g	min
sample	average	Volume	Protein	EAI	ESI
oat 0	0.92067	0.1	0.92839	**15.7477**	**1.35017**
oat 10	0.5474	0.1			
enz 0	0.641	0.1	0.51194	**6.04591**	**1.07251**
enz 10	1.5932	0.1			
slu 0	0.68323	0.1	0.41804	**5.26218**	**3.39251**
slu 10	0.85553	0.1			
com 0	0.26073	0.1	0.50345	**2.41846**	**3.76981**
com 10	0.24387	0.1			

Test 2

	Abs. 500nm	mL	g	m^2/g	min
sample	average	Volume	Protein	EAI	ESI
oat 0	0.34353	0.1	0.92839	**5.87602**	**1.30745**
oat 10	0.46597	0.1			
enz 0	1.23323	0.1	0.51194	**11.6319**	**1.74224**
enz 10	0.7221	0.1			
slu 0	0.65073	0.1	0.41804	**5.01187**	**7.61985**
slu 10	0.59953	0.1			
com 0	0.17033	0.1	0.50345	**1.57995**	**1.21398**
com 10	0.19813	0.1			

Table 1 Results from two separate emulsion stability and activity experiments. Samples followed by the number 0 are those sampled immediately after homogenizing. Samples followed by the number 10 are those sampled 10 minutes after homogenizing. “oat” is the WINX with enzymatic process oat milk. “enz” is WINX processed oat milk without enzyme processing. “slu” is a slurry of oat flour (20% m/v) mixed with water. “com” is a commercial oat milk brand (Vitasoy unsweetened oat milk).

A

Batch No.	Minute	Abs (500nm)
1	0	0.0442
1	0	0.1877
2	0	0.11483333
2	0	0.2824
3	0	0.03746667
3	0	0.1235
4	0	0.0629
4	0	0.1235
1	10	0.0797
1	10	0.1832
2	10	0.061
2	10	0.17003333
3	10	0.0438
3	10	0.12853333
4	10	0.0438
4	10	0.12853333

B

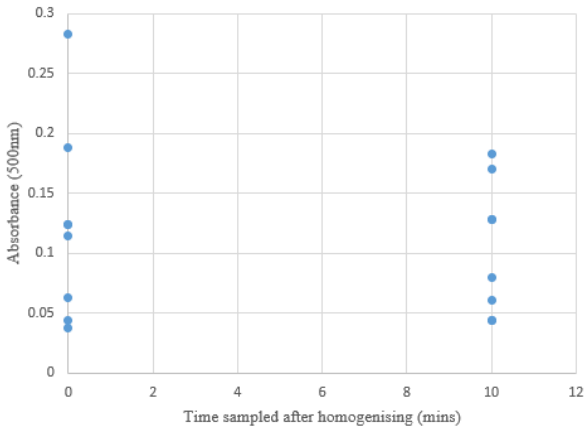

Figure 1 Emulsion experimental absorbances (500nm) from eight separate experiments from four batches of commercial oat milk (Vitasoy unsweetened oat milk). **A.** Tabled results of absorbance values gained from homogenizing commercial oat milk and letting sit for 10 minutes. **B.** Graphical representation of results presented in A.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0 minutes	8	0.9765	0.122063	0.006685
10 minutes	8	0.8386	0.104825	0.003074

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001189	1	0.001189	0.243583	0.629283	4.60011
Within Groups	0.068311	14	0.004879			
Total	0.069499	15				

Table 2 Analysis of variance (ANOVA) statistical test calculations conducted between 0- and 10-minute series of results across 8 experiments. 'SS' is the sum of squares due to the source. 'df' is the degrees of freedom in the source. 'MS' is the mean sum of squares due to the source. 'F' is the F-statistic. ($P < 0.05$) was used to determine statistical significance.

In the paper originally describing the emulsion stability index (Pearce et al., 1978), it is emphasized that different emulsions may break down through different processes and there are dependent factors affecting this such as the concentration of oils. Where an emulsion that is stable and oil undergoes no separation, then the emulsion stability will depend solely on the distribution of particles in terms of number or surface area. Commercial oat milk, such as the one chosen for this experiment, contains gum emulsifiers and stabilising oils drastically improving the stability of the oat milk emulsion. Both the ESI and EAI are based on emulsification by the properties of proteins, particularly whey and yeast proteins (Pearce et al., 1978). It may be the case that commercial oat milk emulsions are more of a product of oils and gum emulsifiers rather than proteins. Moreover, WINX-produced oat milks which do not contain these additives are likely emulsified by alternative mechanisms such as the presence of soluble β-glucan fibres (Fan et al., 2019) which would not be accounted for by the current emulsion indexes. Though these results did not provide insights into which processing step contributes to the stability of WINX oat milk emulsions, they did give deeper insights into the likely emulsion mechanisms involved.

4. Conclusions and Future Work

Current emulsion experimental data suggest the presence of structurally functional β-glucan contributing to the emulsification of WINX-produced oat milk. Emulsion stability data is not reproducible through the current experimental design with the current oat milk samples. This is likely due to the abundance of oil stabilisers and gum emulsifiers within commercial oat milks and the non-protein-specific mechanisms of WINX oat milk emulsions. A longer time period and more frequent sampling over more experimental replicates would be needed to provide more thorough results which cannot be realistically conducted within the timeframe of this project. Current focuses are now shifted towards conducting an in-vitro digestion of WINX oat milk in order to determine the presence and bioavailability of β-glucans, which is hypothesized to be the main contributing factor to WINX milks stable emulsion formation. Due to the established health benefits of β-glucans, analysing the possible bioavailability of this fibre within WINX oat milk could substantiate positive nutraceutical properties.

Future endeavours in this line of food science are to measure and analyse the stability of foam produced by WINX milk and possibly link this to the presence of β-glucans pushing its potential to be used as a barista milk alternative. Establishing a new emulsion experimental design focused on contributions by fibre could possibly make analyzing WINX oat milk substantially easier while also providing more evidence on the structural function of β-glucan in oat milks.

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6. References

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