Functional Properties of a High Protein Beverage Stabilized with Oat-β-Glucan

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Abstract: This study evaluated the effect of oat flour and milk protein on the functional properties and sensory acceptability of shelf stable high protein dairy beverages containing at least 0.75 g of oat-β-glucan per serving size. Formulations adjusted to levels of 1.50% to 2.30% oat flour and 2.50% to 4.00% milk protein isolate (MPI) were thermal processed in a rotary retort. The finished product exhibited good suspension stability (>80%). The increase of oat and MPI contents lead to nectar-like beverages (51 to 100 mPas). However, oat flour was the component showing the highest effect on the viscosity coefficient values of the beverages. Sensory evaluation indicated that formulations with less than 1.9% oat flour and 2.5% MPI (thin liquid, <50 mPas) were the most accepted. Mouthfeel (perceived thickness), sweetness and aftertaste had the most influence on overall liking of the beverages.

Keywords: functional beverage, high protein, oat-β-glucan, surface response methodology, suspension stability

Practical Application: Overall, this study comprises the development of a functional food product. Supplementation of beverages with fiber from oats is an innovative approach to stabilize high protein beverages. Ready to drink protein beverage formulations use gums to stabilize the product and provide a desirable mouthfeel. The levels of oat-β-glucan used in the beverage increased the thickness and meet the requirement of the FDA approved health claim for reduction of the cardiovascular disease risk (21 CFR 101.81).

Introduction

In the United States, functional food market sales reached $43.9 billion in 2012, and functional beverages were 59% of the market (Sloan, 2012). Currently, some trends are focused on the use of specialty nutritional ingredients, claims to prevent or delay chronic diseases and use of food ingredient alternatives (Sloan, 2014). Beverages are the most active product in the functional food market and represent an ideal matrix for the addition of micronutrients and bioactive compound (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014).

Oats are recognized among cereals for their high protein content, which is composed of 70% to 80% globulins (Robert, Nozzolillo, Cudjoe, & Altosaar, 1983). Its soluble dietary fiber compounds, β-glucan, composes about 75% of the oat endosperm cell walls. The form of (1→3)-β-D (30%) and (1→4)-β-D (70%) mixed linkage glucan is found in oats (Ahmad, Anjum, Zahoor, Nawaz, & Dilshad, 2012; Wood, 1991). The intake of β-glucan is beneficial in lowering coronary heart disease risk. Incorporation of β-glucan in the diet (3 g β-glucan/day) supports cardiovascular heart disease risk reduction related with reduction in cholesterol levels. At least 0.75 g of oat β-glucan is required per Reference Amount Customarily Consumed (RACC) to be claimed as having these health benefits (21 CFR 101.81) (FDA, 2013a). β-Glucan consumption has been linked with diabetes prevention (lowers postprandial glucose and insulin responses), a decrease in the risk of cancer and improving the immune functions. The high viscosity promoted by β-glucan seems to be related with serum cholesterol, insulin, and glucose lowering effects (Daou & Zhang, 2012; Jing & Hu, 2012). Additionally, fiber-containing beverages reduce hunger and promote satiety (Pentikäinen et al., 2014; Rebello et al., 2014). Other studies have also reported that oat fiber can improve intestinal regularity (Stephen, Dahl, Johns, & Englyst, 1997).

Moreover, protein fractions of milk provide high nutritional value as a source of essential amino acids. Casein provides all the essential amino acids but cysteine and whey has high contents of leucine, isoleucine, and valine (Hall, Millward, Long, & Morgan, 2003). Overall, a favorable effect on metabolic control has been related to the ingestion of dairy products (McGregor & Poppitt, 2013). Based on the FDA (21 CFR 101.54), the term "high" can be used in food containing 20% or more of the Reference Daily Intake (RDI) per RACC (FDA, 2013; Mellema, de Groot, & Golding, 2009).

Incorporating functional ingredients in foods might imply numerous challenges for improvement of stability and sensorial properties such as flavor and mouthfeel. Formulations with gums and hydrocolloids are commonly used for protein and emulsion stabilization properties, as well as the mouthfeel and good suspension these additives produced in beverages. Hydrocolloids and gums are also a source of dietary soluble fiber (Beristain et al., 2006; Mellema et al., 2009). The high molecular weight of oat β-glucan influences viscosity and it can be suitable as a thickening agent in beverages. The aim of this study is to (1) assess the effect of oat flour and milk protein on the physicochemical and sensory properties of a ready to drink high protein dairy beverage enriched with β-glucan, (2) meet the "cardiovascular disease" health claim for β-glucan content (21 CFR 101.81) and a "high protein" nutrient content labeling claim (21 CFR 101.54).

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Table 1–Formulation for the high protein dairy beverages with oat-β-glucan.

Ingredients	Percentage (% w/w)
Skim milk	75.00
Water	9.07–11.37
Cream	5.00
Sucrose	3.50
Milk protein isolate	2.50–4.00[a]
Oat flour	1.50–2.30[a]
Oat-β-glucan isolate	0.70
Mono- di- glycerides	0.20
Dipotassium phosphate	0.10
Salt	0.10
Carrageenan	0.03

[a]Low and high levels of ingredient based on a central composite design (CCD).

Materials and Methods

Beverage formulation and processing

The experimental formulations did not include reference samples without oat-β-glucan or without oat flour because the objective of the study was to investigate the effect of oat-β-glucan addition, which would meet the FDA health claim in the finished product, and not to replace a specific stabilizer. Therefore, in order to determine the concentration levels of oat-β-glucan, preliminary laboratory-scale trials (data not shown) were conducted with formulations containing different levels of oat flour, including formulations with oat flour only and others without it. Furthermore, the formulation beverages of the preliminary work were also sensory evaluated for their physical attributes. Products with oat flour only resulted with a pudding-like consistency and products with oat-β-glucan isolate only, did not have any effect on the texture attributes. Consequently, the preliminary work findings led to the use of the central composite experimental design for two factors at five levels as described below. The formulation for beverage production is described in Table 1. Dry ingredients (oat flour, β-glucan isolate, MPI, carrageenan, and sucrose) and liquid ingredients (skim milk, cream, mono-di-glycerides, and water) were mixed separately, then all ingredients were mixed while heating to 80 $\pm$ 5 °C for 10 min in a steam kettle, and the resulting solution was homogenized at 30 MPa pressure and 60 °C in a 2-stage Lab 100 M-G homogenizer (Lubeck-Schlutut, Germany). Beverages (approximately 355 mL) were hand-filled into 300 $\times$ 407 (14 fl-oz, #300) metal cans. An F_0 value of 10 min was used as target (Richardson, 2004). Calculation of the thermal process was carried out by the General Method for Process Calculation (Bigelow & Esty, 1920), assuming a z value of 10 °C. Standard D value for *Clostridium botulinum* is $D_{121.1°C} = 0.2$ min ($12D = 2.4$ min) (Lund, Karel, Fennema, & Lund, 1975). Beverages were stored at room temperature for at least 24 hr but no more than 1 week before analysis.

Physicochemical properties

The β-glucan content for beverages, and ingredients was determined enzymatically by the AOAC method 995.16 using a Megazyme β-glucan (mixed linkage) assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Total protein content was determined by Kjeldahl nitrogen analysis according to the AOAC official method 991.20 (AOAC, 1995). Total solids and fat content were measured using a CEM SMART Trac II Analyzer according to the AOAC Official Method 2005.06 (CEM Corporation, Matthews, N.C., U.S.A.) (Cartwright, McManus, Leffler, & Moser, 2005; AOAC, 2000). Suspension stability (%SS) was measured as the ratio of total solids (TS) in the top (upper one-third) portion to the bottom (lower one-third) portion of 12 mL of beverage in a glass tube (Priepke, Wei, Nelson, & Steinberg, 1980). All samples were centrifuged at 3000 $\times$ g for 20 min to accelerate settling. Apparent viscosity was determined by using a controlled-strain modular compact rheometer (Anton Paar MCR 302 Rheometer, Ashland, VA, U.S.A.) equipped with a Peltier temperature controller. The measurements were performed using a plate-plate geometry (plate diameter 40 mm, gap size 0.5 mm) with 800 μL of each sample placed on the plate of the rheometer. Apparent viscosities were evaluated as function of temperature range (4 to 25 °C) at a shear rate of 50 s^{-1}.

Sensory evaluation

Sensory evaluations were conducted to assess the acceptability of the high protein dairy beverage formulated with oat-β-glucan. Acceptability tests on the basis of a 9-point hedonic scale and evaluation in a 5-point Just-About Right (JAR) scale were conducted on overall liking, mouthfeel (thickness), flavor, sweetness and aftertaste. About 1 oz of each refrigerated (at approximately 10 °C) sample was served in plastic cups and presented in a monadic sequential order to panelists ($n = 70$). Subjects were average consumers age 18 to 65 years old from different backgrounds and social economic status subscribed to the database of The Ohio State Univ.'s Consumer Sensory Testing. Penalty analysis or "mean drop" was calculated as described by Lawless and Heymann (2010).

Statistical analysis

The experiments were based on a central composite design (CCD) for two factors at five levels. Therefore, 13 prototype formulations, including five replicates of the center point were carried out based on the CCD. The independent variables studied were: Oat flour content ($x_1 = 1.50\%$ to 2.30% w/w) and MPI content ($x_2 = 2.50\%$ to 4.00% w/w). MINITAB 17 statistical software package (Minitab Inc., PA, U.S.A.) was used for the experimental design and SPSS Software was used for data analysis. Prediction equation was generated using the second order polynomials function as follows:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{11}x_2^2$$

where y is the dependent variable, b the regression coefficients and x the independent variables.

Additionally, physicochemical measurements and sensory acceptance data was expressed as mean $\pm$ standard deviation and as mean $\pm$ standard error of the mean, respectively. All data was statistically analyzed with SPSS software (IBM, SPSS Incorporation, Chicago, IL, U.S.A., version 23.0) using a one-way ANOVA with a subsequent least significant difference (LSD) test, applied for multiple sample comparison, to test for any significant differences ($P < 0.05$) in the mean values. All physicochemical measurements were done in triplicate.

Results and Discussion

Physicochemical properties

The β-glucan content of the high protein dairy beverage formulations is shown in Table 2. Based on the contents found in this study (0.43% to 0.52 % of β-glucan), a beverage with a serving

Table 2–Values of β-glucan, total solids and suspension stability for the oat-β-glucan high protein beverages.

Formulation*	MPI (%)	Oat flour (%)	β-Glucan (%)	Total protein (%)	Total solids (%)	Suspension stability (%)
1	2.72	1.62	0.47 ± 0.02^{a}	5.16 ± 0.41^{a}	18.19 ± 0.04^{a}	$90.7 \pm 5.3^{a,b}$
2	2.72	2.18	0.51 ± 0.02^{b}	$5.59 \pm 0.1^{a,b}$	19.12 ± 0.26^{b}	$93.5 \pm 1.2^{a,b}$
3	3.78	1.62	0.50 ± 0.01^{b}	6.41 ± 0.07^{c}	18.34 ± 0.10^{c}	$91.7 \pm 2.1^{a,b}$
4	3.78	2.18	0.52 ± 0.01^{b}	6.44 ± 0.11^{c}	20.22 ± 0.10^{d}	$94.5 \pm 2.5^{a,b}$
5	3.25	1.50	0.43 ± 0.01^{c}	5.81 ± 0.09^{d}	17.82 ± 0.08^{e}	87.4 ± 4.9^{a}
6	3.25	2.30	0.52 ± 0.01^{b}	6.04 ± 0.04^{e}	19.73 ± 0.17^{f}	97.2 ± 0.5^{b}
7	2.50	1.90	0.52 ± 0.01^{b}	5.07 ± 0.33^{a}	18.99 ± 0.19^{b}	$90.8 \pm 0.8^{a,b}$
8	4.00	1.90	0.50 ± 0.01^{b}	6.47 ± 0.09^{c}	20.66 ± 0.09^{g}	$96.9 \pm 2.8^{a,b}$
9 to 13 (C)	3.25	1.90	0.51 ± 0.01^{b}	$5.9 \pm 0.02^{d,e}$	$19.37 \pm 0.24^{b,d,f}$	$94.5 \pm 1.1^{a,b}$

[a,b,c,d,e,f,g]Superscripts indicate significant differences ($P \leq 0.05$) among columns. MPI = Milk Protein Isolate. 1 to 13 = formulations based on central composite design (CCD). 9 to 13 (C) = average of replicated batches for the central point.

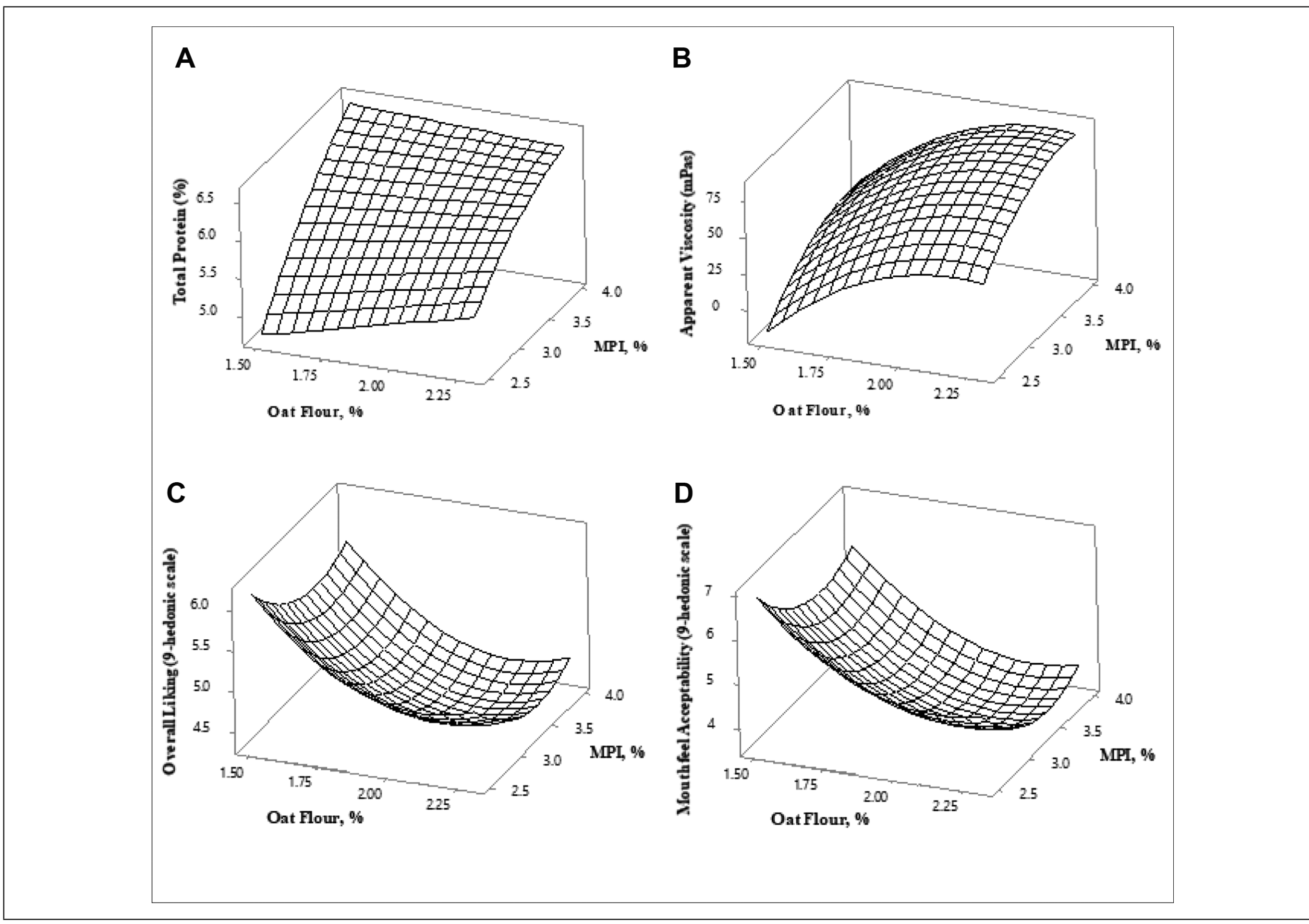

Figure 1–Surfaces responses for protein content (A), apparent viscosity at 10 °C (B), overall liking (C) and mouthfeel acceptability (D) as a function of varied levels of oat flour and MPI.

size of 8 fl-oz (240 mL) will provide 1.0 to 1.2 g of the 3 g of β-glucan, recommended for daily ingestion. This corresponds to more than the minimum amount required by the health claim 21 CFR 101.81. (0.75 g β-glucan per RACC). In comparison to other dairy beverages containing β-glucan, similar contents were found. For instance, 0.5% β-glucan in an orange-whey juice (Temelli, Bansema, & Stobbe, 2004) and 0.62% of β-glucan in milk (Bangari, 2011) corresponded to the highest contents that did not negatively affect the texture properties of those drinks. Moreover, beverages were formulated to contain more than 10 g protein per serving size (>20% of DRI). Total protein content varied from 5.07% to 6.47%. As shown in Figure 1 and Table 3, the most significant changes in protein content were influenced by MPI concentration ($P = 0.000$). Oat flour had a less significant, but positive effect ($P = 0.004$). The response equation for protein content showed no significant lack of fit ($P > 0.05$) with a R^2 of 0.9838; which implies that the model was accurate. Based on the protein levels found in this study, the beverages contain 12 to 14 g per 8 fl-oz serving size; thus, all formulations correspond to high protein beverages.

The prototypes displayed total solids contents of 17.8% to 20.6% (Table 2). The increase of oat flour and MPI leads to an increase in total solids. The formulation with 4% of MPI showed a higher total solids percentage value (20.7%) than the prototype made

Table 3–Regression coefficients, R^2, adjusted R^2, *P*-value (response) and lack of fit (*P*-value and *F*-value) for the final reduced models equations for protein, apparent viscosity at 10 °C, overall liking and mouthfeel acceptability.

Parameters	Protein content (y_1)	Apparent viscosity at 10 °C (y_2)	Overall liking[f] (y_3)	Mouthfeel acceptability[f] (y_4)
b_o	−5.33	−839	26.63	42.08
b_1 (Oat)[a]	2.509	533	−13.34	−21.19
b_2 (MPI)[a]	4.319	176.1	−4.63	−9.05
b_{11} (Oat∗Oat)[b]	–	−121.3	3.181	4.95
b_{22} (MPI∗MPI)[b]	–0.3215	−23.0	0.676	1.293
b_{12} (Oat∗MPI)[c]	−0.666	–	–	–
R^2[d]	0.9838	0.9345	0.8773	0.9005
R^2 (adj)[e]	0.9757	0.9017	0.8160	0.8507
Response (*p*-value)	0.000	0.000	0.001	0.000
Response (*F*-value)	121.54	28.53	14.30	18.09
Lack of fit (*p*-value)	0.804	0.641	0.583	0.328
Lack of fit (*F*-value)	0.40	0.68	0.80	1.61

$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{11}x_2^2$.
[a]b_1, b_2 the estimated regression coefficient for the main linear effects.
[b]b_{11}, b_{22} the estimated regression coefficient for the quadratic effects.
[c]b_{12}, the estimated regression coefficient for the interaction effects.
[d]R^2 is the correlation coefficient.
[e]R^2 (adj) is the adjusted correlation coefficient.
[f]Sensory acceptance in a 9-hedonic scale.

with higher content of oat flour (2.3%). Although, there was an increase in the total solids with the increase of oat flour, there was no difference between samples containing 1.9% and 2.3% oat flour. No significant differences were found in terms of color (L* 86.1 ± 2.1, a* 3.7 ± 0.7, and b* 17.9 ± 1.1) and a pH of 6.73 ± 0.03 (data not shown). Visually, there were no clear difference observed among beverages. As presented in Table 2, all beverages showed stabilities higher than 80%. Since Priepke et al. (1980) suggest that drinks with suspension stabilities higher than 70% can be classified as stable, all prototypes can be considered as stable. The highest stability value of 97.2 ± 0.7% was achieved with the highest amount of oat flour (2.30%) while the lowest stability at 87.4 ± 5.9% corresponded to the formulation with the lowest amount of oat flour (1.50%). A viscous/thicker texture developed by oat flour avoids the sedimentation of particles. This thickening effect of β-glucan, oat flour and carrageenan provide a stable matrix for milk proteins with no visual signs of separation. In a similar matrix composed of milk and 0.30% to 0.62% β-glucan, Bangari (2011) observed that the addition of carrageenan favored the stability of the system; after 21 days, samples without added carrageenan presented syneresis.

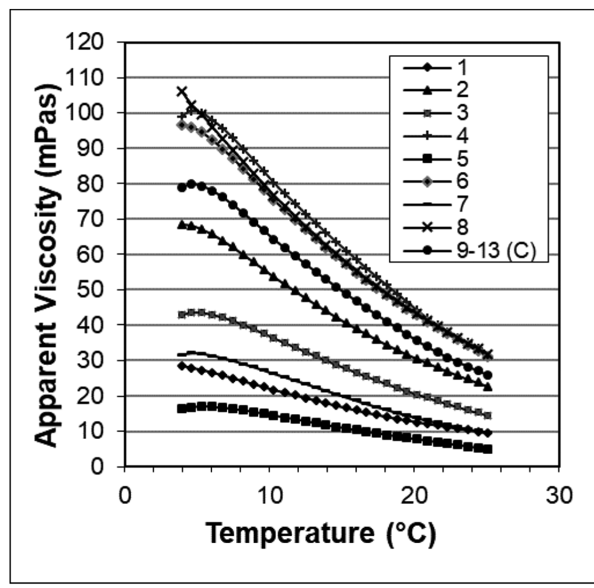

Figure 2–Apparent viscosity measured over temperature (4 to 25 °C) for high protein dairy beverages with oat-β-glucan at a shear rate of 50 s^{-1}. 1 o 13 = formulations based on central composite design (CCD). 9 – 13 (C) = average of replicated batches for the central point formulation (1.90% oat flour, 3.25% MPI).

The data in Figure 2 illustrates the apparent viscosity for the high protein dairy beverages at 4 to 25 °C. As expected, there was a relevant decrease in apparent viscosity with the increase of temperature. Apparent viscosities of values >60 mPas and 17 to 43 mPas at approximately 4 °C were reduced to <40 mPas and 5 to 15 mPas at 25 °C, respectively. According to the National Dysphagia Diet (NDD), viscosity values lower than 50 mPas correspond to thin liquids (NDD, 2002). Thus, formulations with contents <1.90% oat flour and <2.5% MPI are considered thin beverages at both refrigeration and room temperature. On the other hand, formulations with contents >1.90% oat flour and >2.5% MPI are nectar-like liquids (>50 mPas). As shown in Figure 1 and Table 3, both oat and milk proteins increase the viscosity of the beverages. Oat content had a positive and slightly more significant effect ($P < 0.001$) than MPI ($P < 0.003$) on solids and viscosity at 10 °C. At constant β-glucan content, the milk protein, and oat flour, which provide protein and starch, have potential influence on the apparent viscosity. Low increments of oat flour have more influence on viscosity because of the starch content. This interpretation might be supported by the work of Kim and White (2012). These authors evaluated the interaction of components in oat suspension (slurries) by enzymatic treatment. Their findings showed that starch had the highest impact on viscosity. After the addition of amylase, they found an 89% decrease in viscosity. The protein from oats was reported to have minor influence on this property (Kim & White, 2012).

Sensory evaluation

Regression coefficients of the overall liking and mouthfeel acceptability responses (Table 3), show that main effects for the independent variable (Oat and MPI) had a minor effect on the overall acceptability, while the quadratic effects of the independent variables (Oat∗Oat; MPI∗MPI) had a significant positive effect on the beverages' acceptability. From the response surface curves

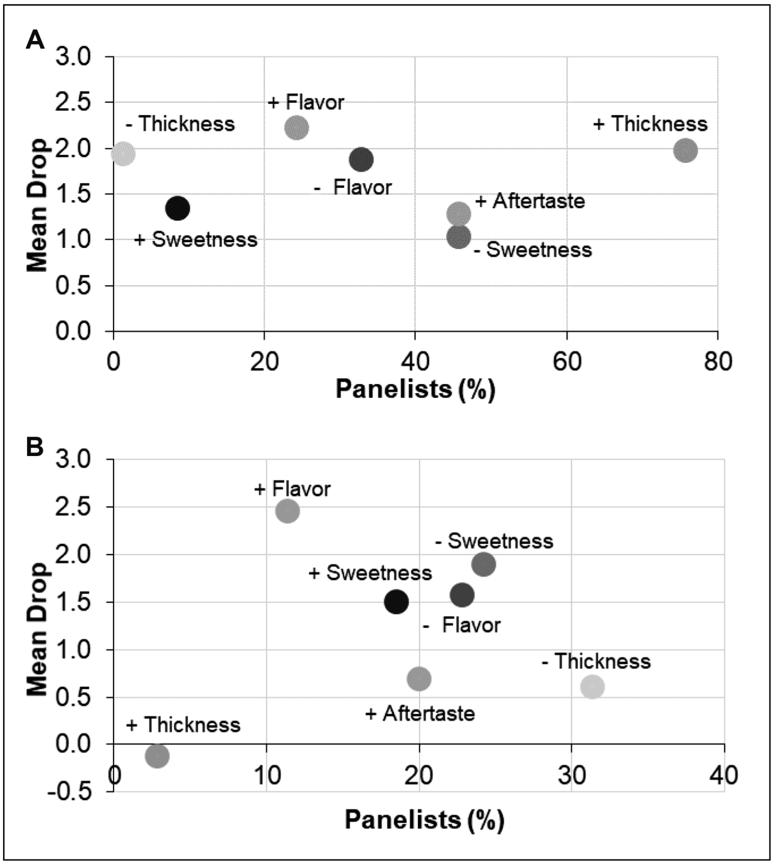

Figure 3–Penalty analysis plot of nonacceptable formulation with contents of 2.30% oat flour and 3.25% MPI (A). Acceptable formulation with contents of 1.50% oat flour and 3.25% MPI (B). Overall liking >5 in a 9-point hedonic scale was considered as acceptable.

(Figure 1), it is clear that a decrease in the levels of oat flour and MPI is related with an increase in overall liking and acceptability for thickness. Specifically, levels of <1.7% of oat flour and <3.3% MPI resulted in a sensory acceptance >5 for all attributes. As in the case of mouthfeel (thickness) acceptance, this attribute showed slightly higher hedonic scores than the overall liking at the same concentrations of oat and MPI. The R^2 values were 0.877 and 0.900 for overall liking and mouthfeel acceptance, respectively. The more significant coefficient was for oat flour content (Oat), which presented a $P = 0.001$ overall liking, and $P = 0.000$ for the mouthfeel acceptance models.

The penalty analyses for an acceptable (overall liking >5) and a nonacceptable (overall liking <5) formulation are presented in Figure 3. Attributes with a large mean drop and a high percentage of panelists (upper right corner of the plots) imply a negative effect on the overall liking and are subject to reformulation. Beverages with low overall acceptability presented similar characteristics. Mouthfeel (thickness) had the most effect on acceptability among other attributes. Too much thickness (+) received the highest penalty with mean drops of approximately 2.0 for more than 60% of the panelists in lesser accepted formulations, which contained >1.9% oat flour and >3.25% of MPI (Figure 3A). On the contrary, too much thickness (+) did not account for any penalty in the acceptable prototype (Figure 3B). For a low percentage of panelists, a decrease in overall liking was caused by the low thickness of this sample (30%, mean drop = 1). Therefore, there might be a strong correlation between thickness acceptability and apparent viscosity measured at 10 °C. Viscosities >40 mPas were not acceptable (hedonic score <5).

Mouthfeel might be influenced by different attributes that can act as drivers of texture acceptability. It had been reported that perceived thickness and sliminess were strongly correlated with instrumental viscosity in beverages with contents of 0.5% to 1% β-glucan (Lyly et al., 2003). However, not all the attributes related with mouthfeel can be correlated with an instrumental viscosity. According to de Wijk and Prinz (2005), other textural attributes like creaminess, smoothness or stickiness were not correlated with apparent viscosity values at 50 s^{-1}, which is the shear rate for swallowing. Moreover, total solids correlate negatively with the mouthfeel acceptability; formulations with more solids showed higher viscosity, thus, lower acceptance. These findings suggest that the increase in viscosity is one of the main concerns when including a large amount of oat and protein in the beverage. High viscous beverages are difficult to swallow, which might result in a poor acceptability for the product. Even though the level of sugar added was the same (3.5% sucrose), the percentage of panelists that penalized the beverages because of a lack of sweetness increased markedly for the nonacceptable formulation (Figure 3C). The mean drops for this attribute ranged from 0.6 to 2.3 for less than 47% of the panelists, compared to less than 25% of acceptable formulations. As previously mentioned, these beverages differ in their viscosity level, which might have had a slight influence on the perception of sweetness.

The above observations are supported by evidence of increased hydrocolloid content contributing to decreased perceived taste in beverages (Matta, Chambers, Garcia, & Helverson, 2006, Pangborn, Gibbs, & Tassan, 1978, Stone & Oliver, 1966). However, the JAR scores for sweetness indicated that >50% of panelists considered the sweetness level as adequate in most of the beverages (data not shown). A slight increase in the sucrose content will lead to higher overall liking scores. It is well known there is a strong influence of sweetness on food acceptability; there is a predisposition to prefer sweet tastes, which varies among individuals (Birch, 1999; Drewnowski, Mennella, Johnson, & Bellisle, 2012; Kim, Prescott, & Kim, 2014). Another notable penalty was due to perception of an aftertaste. Panelists (>40%) considered a strong aftertaste a negative attribute with mean drops of 1.1 to 2.2, which might be related to either protein or oat taste.

Conclusion

It was demonstrated that it was possible to formulate an acceptable functional dairy beverage that meets the cardiovascular disease risk reduction (21 CFR 101.81) and high protein content (21 CFR 101.54) claims by the addition of oat flour, oat-β-glucan and milk protein. All formulations showed high suspension stability while the thickening effect of oat flour helped to prevent particle sedimentation. It can be concluded that the main attributes influencing the acceptability of the beverages were mouthfeel (thickness), sweetness and aftertaste. The decrease in oat flour and MPI content was found to increase the overall sensory acceptability. Therefore, this improvement was thought to be caused by the decrease in perceived thickness.

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Author Contributions

E. Vasquez-Orejarena designed the study, collected test data, interpreted the results and drafted the manuscript. C. Simons advised on designing the sensory study. J. Litchfield provided expertise on Beta-Glucan, reviewed the study design and interpreted the results. V. Alvarez designed the study, interpreted the results and drafted the manuscript.

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