Full Length Research Paper

Effect of whey protein enrichment on selected engineering and sensory properties of Pasteurised yoghurt

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The effect of whey protein concentrate enrichment on the electrical and themophysical characteristics of pasteurized yoghurt was studied. Six samples consisting of control (unpasteurized yoghurt without whey) and yoghurt pasteurized at 50°C for 12 min and enriched with 0, 3, 6, 9 and 12% whey protein concentrate (WPC) were used. Results showed that electrical conductivity (G) increased with both temperature and whey protein concentrate. At 15 to 30°C and constant whey concentration of 3%, G ranged from 0.643 to 0.677 $\mu\Omega^{-1}$.cm⁻¹. Similarly, at 12%, G ranged from 0.643 to 0.725 $\mu\Omega^{-1}$.cm⁻¹ corresponding to the same temperature range. Specific heat of samples increased significantly (p < 0.05) with increase in WPC from 5.812 to 10.823 Jkg⁻¹K⁻¹ corresponding to 0 to 12% WPC. At constant temperature of 25°C, density (ρ) increased from 1645 to 1655 kgm⁻³ corresponding to 3 to 12% WPC. Statistically, there was no significant difference (p ≥ 0.05) in density among samples. Rheological characteristics of samples indicated that the consistency index and apparent viscosity decreased generally with temperature but increased with whey concentration. Both apparent viscosity and consistency index decreased with shear rate indicating shear thinning. The flow behavior index did not show any defined trend with changes in temperature but was generally less than one (n<1) indicating that the samples are pseudoplastic. The activation energies of samples decreased with temperature and increased with shear rate.

Key words: Protein enrichment, yoghurt, protein concentrate,

INTRODUCTION

Yoghurt is defined as the acidified coagulated cow's milk product made from raw milk or different parts of it, that is, (i) skimmed milk;(ii) concentrated milk;(iii) reconstituted dried milks; (iv) whey, etc, in which after pasteurisation, lactic acid has been produced within the product by bacterial cultures consisting of Lactobacillus bulgaricus with or without Streptococcus thermophilus (Early, 1992).

Yoghurt may be subject to heat treatment (pasteurization) after fermentation (Schmidt, 1992; Lucey et al., 1997; Egbere, 2008). Pasteurised yoghurt is yoghurt which has been heat treated to reduce the culture bacteria. However, pasteurised yoghurt is known for its low viscosity and low protein content (Alakali et al., 2007; 2007; Alakali et al., 2008).

Yoghurt products have achieved considerable economic importance worldwide owing to their high nutritional image. This positive image could be expanded further by adding nutraceutical ingredients such as native whey protein to the yoghurt. Whey protein, a by-product of cheese manufacture, offers several benefits to yoghurt formulations. Addition of 2% whey protein isolates (WPI) instead of 2% skim milk powder (SMP) has been reported to improve consistency of set-style yoghurt (Guggisberg et al., 2004). Whey also enhances the viscosity and protein content of the yoghurt products. Patocka et al. (2004, 2006) also reported textural effect of soluble whey protein isolate (WPI) added to stirred yoghurt after fermentation.

Three factors are reported to have a major contribution on yoghurt structure: the heat treatment of the milk base (casein/whey protein ratio), the starter culture, and

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technological influences such as pressure valves and flow properties (Sodini et al., 2004). The flow property does not only affect quality, it is important in plant and process design. Heat transfer and pasteurisation may be incorrectly evaluated if the actual flow properties are not assessed (Rao and Anantheswaran, 1982). Viscosity is an important flow property which is affected by temperature and shear rate amongst others (Rha, 1975; Lewis, 1987). It is important therefore, to assess the flow properties as an aid to rheological characterization and establish the temperature dependence on these properties.

Thermal processing of food products, ranging from mild to relatively severe treatments, is a common operation in the food industry, where physical modifications affecting the structure and stability of the final product are achieved, mainly to preserve its quality and prevent spoilage (Corrieu et al., 1985; Rao, 1986). The knowledge of engineering properties, such as density, specific heat and electrical conductivity, as well as the flow characteristics are important in thermal processing and proper design of industrial plants, definition of level of product quality and control of manufacturing processes.

The broad objective of this research is to study the effect of native whey protein addition on the quality of pasteurised yoghurt. The specific objectives are to produce pasteurized yoghurt enriched with whey protein concentrate and to determine the electrical and thermophysical properties of the samples.

MATERIALS AND METHODS

Isolation of whey protein concentrates

About 800 g of dried powdered full cream milk were dissolved in 2.5 L of distilled water containing 4 g citric acid. The citric acid was to facilitate the separation of the milk proteins and whey from the casein. The resultant solution was stored at 29 to 32°C for 30 min. to enable the insoluble material to sediment. Then the resultant solution was centrifuged using a Harrier 15/80, MSE-SANYN model centrifuge at the speed of 4500 rpm for 20 min. The supernatant whey was decanted and frozen at -5°C, after which it was freeze-dried at the temperature of 45°C for 15 to 24 h using a freeze driver to obtain the whey protein isolate concentrate in a freeze dried form.

Production of yoghurt

Yoghurt was made from reconstituted low-heat non fat (Skimmed) dry milk. This milk contains 50% (w/w) lactose. 36% (w/w) protein and 0.67% (w/w) fat. In each experiment, 10 L of reconstituted whole milk with total solids content of 12 and 7% (w/w) were prepared. The homogenised milk was heated to 95°C for 5 min. and cooled to 45°C, the milk was inoculated with 3% yogourmet freezedried yoghurt starter culture (a mixture of Streptococcus and thermophilus. Lactobacillus bulgaricus Lactobacillus acidophilus) and incubated at 43 to 45°C until the yoghurt coagulum was formed and the desired pH value (pH 4.4 to 4.6) was reached. This was achieved within 4 to 5 h. Then the yoghurt coagulum was broken manually by means of a perforated stirrer, by stirring the coagulum twenty five minutes in slow helicoidal movements. This was done in order to ensure that the yoghurt coagulum was

completely broken and that whey incorporation was promoted. 500 ml of unpasteurized yoghurt without whey were taken out of the whole bulk, the yoghurt was then thermised at 50°C for 12 min, after which 500 ml each mixed with 0% (control) 3, 6, 9 and 12% of native whey protein powder were prepared. The products were stored at 10 to 20°C for 24 h.

Physicochemical analyses

Moisture content determination

2 g of samples was weighed into already weighed, cleaned moisture dish with lid and oven-dried at 105°C to a constant weight. The dried sample was cooled in a dessicator for about forty-five minutes and weighed. Duplicate determination was made for each sample (AOAC, 1984).

Crude protein

Protein content of the sample was determined as described by AOAC (2006). Duplicate determination was made for each sample.

Fat determination

The fat content of samples was determined according to AOAC (2006). Duplicate determination was made for each sample.

Ash content determination

Ash content was determined as described by Pearson (1981). 2 g of sample were weighed into clean, dry ash crucibles and incinerated. They were then transferred into a muffle furnace at 530°C for 5 h. The ash were then cooled in a dessicator and weighed. The percentage ash was calculated as weight of ash over weight of sample times one hundred.

Determination of crude fibre

Crude fibre was determined as described by AOAC (2006). Duplicate determination was made for each sample.

Carbohydrate determination

The carbohydrate content of the sample was determined by difference as described by AOAC (2006). Duplicate determination was made for each sample.

Total solids determination

The total solids were determined by the gravimetric method as described by Pearson (1981). Duplicate determination was made for each sample.

Sensory evaluation

Six yoghurt samples made up of unpasteurized yoghurt without whey yoghurt, 0, 3, 6%, 9 and 12% pasteurized yoghurt with whey were evaluated by semi trained panel of 10 judges, comprising of students of the Department of Food Science and Technology, University of Agriculture Makurdi, Benue State, Nigeria. The attributes evaluated include colour, appearance, consistency, mouthfeel, flavour and general acceptability. A seven point hedonic scale was used where 7 was liked extremely and 1 disliked extremely.

Determination of electrical conductivity

Electrical conductivity of the yoghurt samples was determined using an electrical conductivity meter, model MC 126 (Mettler, Toledo, and MC 126-2 m). Electrical measurements were carried out with yoghurt samples previously prepared as outlined, each replicated. The probe was inserted into the yoghurt sample solution. The tip was immersed to or beyond the vent holes and the probe agitated vertically. Triplicate readings were taken for each temperature.

Determination of specific heat

The specific heat capacity of the yoghurt products were determined using electrical method of specific heat determination. A heating coil of resistance R Ohms was immersed in 63 to 66 g of yoghurt in a stainless steel colorimeter. A current of I amperes, measured by an ammeter, flows through the heat coil across which there was a potential drop of V volts, measured by a voltmeter. The power developed by the current in the resistance is given by Equation (1):

P= IV watts (Joules/s) (1)

If the current flows through the resistance for a total time of t seconds, then the total work done by the current is:

W = IVt joules (2)

Using Ohm's law,

V = IR (3)

Substituting Equation (2) into (3)

 $W = I^2 Rt$ joules (Joule's Law) (4)

Now this work W is transformed into heat which warms up the yoghurt, the inner calorimeter plus stirrer and the heating coil.

An electrical element was inserted in a stainless steel calorimeter containing the test fluid (yoghurt) and the temperature rise was measured by the application of known voltage and current for a fixed time. Heat losses were eliminated by cooling the yoghurt to below room temperature and applying electrical energy until the temperature reached a value above room temperature such that the temperature differences above and below room temperature are the same (Lewis, 1990).

In this case:

$$VIt = M_C C_C \Delta T + M_1 C_1 \Delta T$$
 (5)

where V (V) is the voltage, I(A) is the current, t (s) is the time, ΔT (°C) is the temperature difference, M₁ (kg) and M_c (kg) are the mass of yoghurt and calorimeter, respectively and C₁ and C_C (Jkg⁻¹K⁻¹) are the specific heat of the yoghurt and calorimeter respectively.

Heat lost by the coil = heat gained by calorimeter + yoghurt sample.

Methods and precaution for specific heat of foods have been described in more detail by Mohsenin (1980) and Ohlsson (1983).

Density measurement

The density of the yoghurt samples were measure using the

specific gravity method as described by Smith (1980).

Rheological characterization

The methods used to assess the temperature effect on the rheological properties of yoghurt are essentially similar to those reported previously by Afonso and Maia (1999). The experiments were performed in a Brookfield rotational viscometer Model LV8 Visocmeter UK Ltd London). Samples of unpasteurized yoghurt without whey and pasteurised yoghurt were collected and the temperature effect on the rheological properties of yoghurt was studied by means of apparent viscosity measurement at 15, 20, 25 and 30°C.Temperature was maintained by immersing the sample holder in warm water bath at the appropriate temperature. The viscosity readings were taken at three minutes interval for 5 shear rates or rotational speeds of 3, 6, 12, 30, 60 rpm, with three replications.

Apparent viscosity was determined based on (Bourne, 1982) as in Equation (6):

$$\mu_a = k \gamma^{n-1} \tag{6}$$

where μ_a = apparent viscosity, k = consistency index (NS/m²), γ = rotational shear rate (rpm or s⁻¹) and n = power law index.

Activation energy E_a was by Equation (7):

$$\mu_a = \mu_o \exp\left(-\frac{Ea}{RT}\right)$$
(7)

where μ_o = apparent viscosity at a reference temperature, E_a = activation energy of flow (kJmol⁻¹), R = 8.314 J.K⁻¹.mol⁻¹, T =absolute temperature (K)

Statistical analysis

Data were obtained in triplicate analysed using the analysis of variance (ANOVA) as described by Iwe (2002).

RESULTS AND DISCUSSION

Chemical properties of whey protein isolate

The proximate composition of whey protein concentrate (WPC) is shown in Table 1. Result showed that the whey protein was high in crude fibre, protein, total solid but low in carbohydrate, moisture, fat and ash contents compared to values reported in literature for whey protein isolates (Guggisberg et al., 2007). However, carbohydrate and crude fibre values are not in available literature for comparison. The ash content values were observed to be the same with those reported by Guggisberg et al. (2007) for WPI. The differences observed in the composition of WPC and literature values could be because the amount of lactose and salts is much higher in WPC than WPI. It could also be attributed to the source of milk protein as reported by Muir (1992). According to the author, the major determinant of milk composition include breed of cows, diet, age and stage of lactation, among other factors.

Concentration (g kg⁻¹) Component Fat 5 52 Moisture Protein 782 Total solid 722 Crude fibre 888 Ash 35 Carbohydrate 37

Table 1. Proximate composition of whey protein concentrate (WPC).

Effect of whey protein enrichment on specific heat and density

Table 2 shows values of specific heat (C_p) and density (ρ) of samples. The results showed that C_p increased generally with increased whey concentration ranging from 5.438 to 10.823 JkgK⁻¹, corresponding to 0 to12% WPC. This implied that as the whey concentration increased, more heat was required to raise the temperature of the sample by one degree Kelvin (Moshenin, 1980). Increase in C_{p} due to the addition of WPC increased the heat requirement of samples. Thus the sample with 12% WPC required more heat to raise its temperature than the sample with 3%WPC. Statistically there were significant differences (P<0.05) among samples as whev concentration increase from 0 to 12%.

The density dependence of yoghurt samples on whey concentration was also investigated at the constant temperature (25°C) under study (Table 2). A weak dependence of density on whey concentration was observed since statistically there was no significant difference (P>0.05) among samples. The density values of the samples ranged from 1633 to 1655 kgm⁻³. Afonso et al. (2003) reported 1042 to 1071 kgm⁻³ for stirred yoghurt enriched with whey protein isolate.

Effects of whey protein on sensory of yoghurt samples

The result of sensory qualities of the samples is shown in Table 3. The table shows that yoghurt sample with 12% whey concentration had the highest rating by panelists in terms of appearance (colour), consistency (mouthfeel), flavour and general (overall) acceptability. This agreed with works published by Patocka et al.(2004) stirred yoghurt fortified with protein isolates and Guggisberg et al.(2007) for set yoghurt produced with addition of native whey protein.

Effect of whey protein enrichment on electrical conductivity

The result presented in Table 4 shows that electrical conductivity (G) increased with both temperature and

concentration (WPC). For 3% WPC whey and temperatures of 15 and 30°C, G increased from 0.615 to 0.677 mmhocm⁻¹. Similarly, for 12% WPC, G increased from 0.725 to 0.759 $\mu\Omega^{\text{-1}}\text{.cm}^{\text{-1}}$ in the same temperature range. As temperature increased, the density and consistency of samples decreased (Tables 2 and 5) favouring electrical conductivity. Electrical conductivity for unpasteurized yoghurt without whey (UYWW) and pasteurised yoghurt without whey (0%PYW) were not significantly different (p > 0.05), indicating that pasteurisation did not influence electrical conductivity of samples. However, the results showed that electrical conductivity increased as whey concentration was increased from 3 to 12% and values of 9 to 12%PYW were statistically different (p < 0.05) with those of UYWW and 0%PYW. Increased WPC probably favoured ionic transfer and increased G of samples (Lewis, 1990).

Effect of whey protein enrichment on rheological characteristics

The relationship between apparent viscosity and shear rate is typically presented in Figure 1. Apparent viscosity of yoghurt samples decreased generally with increase in shear rates at all temperatures studied as reported by Moshenin (1980) and Alakali et al. (2003). Several other researchers reported inverse relationship between viscosity and shear rate for pseudoplastic fluids. Notable are Sopade and Kassum (1992) for liquid and semi liquid foods; Awonori (1993) for sausage made from broiler chicken and guinea fowl. The researchers suggested that the inverse relationship between viscosity and shear rate might be due to increase in alignment of fluid molecules in the flow direction.

Apparent viscosity of the yoghurt did not change significantly with increase in whey protein concentrates. The works of Patocka et al. (2004) on textural effect of soluble whey protein isolate of stirred yoghurt; Patocka et al. (2006) on rheological behvaiour of dairy products as affected whey protein isolate; and Guggisberg et al. (2007) on heat transfer and food products showed that addition of soluble whey protein isolates (WPI) to stirred yoghurt after fermentation at different concentrations acted as a nutraceutical rather than as a technological

Sample	Density $ ho$ (kgm ⁻³)	Specific heat (Cp), Jkg ⁻¹ K ⁻¹
UYWW	1633 ^ª	6.285 [°]
0% PYW	1638 ^a	5.438c
3% PYW	1643 ^a	8.412 ^b
6% PYW	1645 ^a	8.540 ^b
9% PYW	1651 ^a	10.519 ^a
12% PYW	1655 ^a	10.823 ^a
t-test (LSD)	224.03	0.6115

Table 2. Density and specific heat (Cp) of yoghurt samples.

*Means with the same superscript in the same column are not significantly different at p<0.05. UYWW – Unpasteurized yoghurt without whey. PYW – Pasteurised yoghurt with whey.

	Sensory attribute					
Product/ Sample	Colour/ Appearance Consistency/ Mouthfeel Flavor General acceptabi					
UYWW	5.9 ^a	4.4 ^d	5.8 ^a	5.3 ^b		
0% PYW	5.5 ^ª	4.0 ^d	5.5 ^a	5.0 ^b		
3% PYW	4.5 ^b	4.0 ^d	4.6 ^b	4.5 ^{bc}		
6% PYW	4.9 ^a	4.8 ^c	5.1 ^a	4.8 ^{bc}		
9% PYW	5.0 ^a	5.7 ^b	4.3 ^b	5.0 ^b		
12% PYW	5.7 ^a	6.9 ^a	5.7 ^a	6.1 ^a		
t-test (LSD)	1.06	0.6	1.23	0.72		

Table 3. Sensory values for yoghurt products.

* Results represented as mean value. *Means with same superscripts are not UYWW- Unpasteurized yoghurt without whey yoghurt without whey. Significantly different at ($P \ge 0.05$) PYW – Pasteurised yoghurt with whey.

Table 4. Electrical conductivity (G) of yoghurt	samples at vari	ous temperatures.
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	G (mmhocm ⁻¹)					
Sample temperature (°C)	UYWW	0% PYW	3%PYW	6%PYW	9%PYW	12%PYW
15	0.615±0.200	0.615±0.100	0.643±0.300	0.680±0.080	0.712±0.100	0.725±0.025
20	0.609±0.200	0.619±0.100	0.657±0.300	0.689±0.080	0.714±0.100	0.740±0.030
25	0.626±0.200	0.623±0.027	0.677±0.300	0.723±0.020	0.740±0.100	0.747±0.100
30	0.6320.200	0.632±0.029	0.677±0.300	0.723±0.020	0.740±0.100	0.759±0.100
LSD: K Vs %Whey conc.	0.1081					
LSD K Vs Temp.	0.1324					

Key: UYWW - Unpasteurized yoghurt without whey yoghurt without whey.PYW - Pasteurised yoghurt with whey.

Sample	Temperature (°C)	Consistency index (K)(NSm ⁻²)	Flow behaviour index (n)	R ²
	15	5.256	1.96E-04	0.902
	20	5.185	1.77 E-04	0.874
	25	5.126	1.84 E-04	0.880
	30	4.357	4.78 E-04	0.896
01000	15	5.081	2.29 E-04	0.910
	20	4.928	2.28 E-04	0.887
	25	4.878	2.50 E-04	0.881
	30	3.608	1.12 E-04	0.893
0% PYV	15	5.096	2.26 E-04	0.910
	20	4.949	2.38 E-04	0.881
	25	4.926	2.40 E-04	0.881
	30	4.521	3.52 E-04	0.872
3% PYV	15	5.093	2.26 E-04	0.910
	20	4.944	2.38 E-04	0.881
	25	4.942	2.29 E-04	0.882
	30	4.484	3.65 E-04	0.876
0%P1VV	15	5.087	2.28 E-04	0.910
	20	4.941	2.38 E-04	0.879
	25	4.939	2.29 E-04	0.881
	30	4.555	3.27 E-04	0.856
9% 110	15	5.084	2.28 E-04	0.909
	20	4.918	2.46 E-04	0.878
400/ D)////	25	4.898	2.45 E-04	0.880
12%PYW	30	4.51	3.43 E-04	0.856

Table 5. Consistency and flow behaviour indices of yoghurt at 15 to 30°C.

UYWW = Unpasteurized yoghurt without whey.PYW = Pasteurised yoghurt with whey.

The log-log relationship between apparent viscosity and Shear rate of yoghurt samples was plotted. The regression analysis (Table 5) shows that the power law model is appropriate ($\mathbb{R}^2 \ge 0.916$) in describing the rheological behaviour of yoghurt samples. Table 5 shows that the flow behaviour index did not show any defined trend with changes in temperature but was less than one (n<1) indicating that the samples are pseudoplastics.

Table 5 shows the effect of temperature on consistency index of yoghurt samples. Reference to the table shows that as the temperature increased, consistency index decreased. This agreed with Lewis (1990) who reported that the consistency and flow behaviour indices of dilute and concentrated protein in solution depend upon pH, ionic strength and temperature. In the case of pasteurized yoghurt fortified with protein concentrate, as the temperature increased, the consistency index decreased. Alakali et al. (2003) and Sopade and Kassum (1992) also reported similar trends for canarium oil, kunu zaki and kunu gyuda.

The predictive equations for UYWW and PYW (0 to 12%) based on Equation (6) are presented in Table 7. Equation (8) is the mean for pasteurized yoghurt enriched with 3 to 12% whey protein concentrate.

 $\tau = 4.868\gamma 0.000262$ (8)

Equation 8 can be used to predict average rheological behaviour of yoghurt samples enriched with whey protein concentrate within the limit of the concentrations.

The temperature dependence of consistency index was investigated using the Arrhenius relationship (Equation 7). The activation energy (E_a) at various shear rates (3 to 60 rpm) were obtained from the log plots of apparent viscosity versus inverse of absolute temperature and the values ranged from 0.288 to 0.908 kJ/mol and the regression coefficient (R^2) ranged from 0.675 to 0.953 (Table 6). High R^2 values imply that temperature effect



Figure 1. Typical graph of apparent viscosity vs. Shear rate at various temperatures for UYWW.

-	Activation energy Ea (kJ/mol)				
Product	Shear rate (rpm)				
	3	6	12	30	60
UYWW	0.661	0.576	0.738	0.749	0.908
R ²	0.719	0.708	0.871	0.927	0.867
PYW	0.661	0.288	0.735	0.638	0.988
R^2	0.717	0.673	0.857	0.982	0.724
3% PYW	0.658	0.870	0.788	0.645	0.968
R^2	0.729	0.953	0.675	0.770	0.833
6% PYW	0.659	0.834	0.790	0.621	0.970
R ²	0.727	0.863	0.686	0.913	0.815
9% PYW	0.660	0.935	0.7655	0.631	0.970
R ²	0.724	0.704	0.779	0.852	0.793
12% PYW	0.659	0.896	0.740	0.630	0.972
R ²	0.782	0.930	0.812	0.859	0.750

Table 6. Temperature sensitivity of consistency index at 15 to 30°C.

UYWW = Unpasteurized yoghurt without whey, PYW = pasteurised yoghurt with whey, R^2 = coefficient of determination.

Table 7. Predictive power	law model	l for yoghurt	samples.
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Product	Gradient	Equation (mean value)	R ²
UYWW	0.000259	$\tau = 4.981 \ \gamma \ 0.000205$	0.888
0% PYW	0.000205	$\tau = 4.624 \ \gamma \ 0.000205$	0.893
3% PYW	0.000264	$\tau = 4.873 \ \gamma \ 0.000264$	0.886
6% PYW	0.000265	$\tau = 4.866 \ \gamma \ 0.000265$	0.887
9% PYW	0.000256	$\tau = 4.881 \ \gamma \ 0.000256$	0.882
12% PYW	0.000266	$\tau = 4.853 \ \gamma \ 0.000266$	0.881

UYWW = Unpasteurized yoghurt without whey yoghurt without whey, PYW = Pasteurised yoghurt with whey, $R^2 = coefficient$ of determination.

can be explained by Arrhenius type relationship. E_a showed increase with the shear rate and slight decrease with whey concentration. This indicates that as whey concentration increased viscosity becomes less sensitive to temperature and more energy was required.

Conclusion

The results of this study showed that the electrical conductivity (G) increased with increased whey concentration and temperature, respectively. The specific heat and apparent viscosity of yoghurt increased with increased whey concentration. The density of yoghurt samples showed weak dependency whey concentration. Apparent viscosity decreased with increased shear rate indicative of shear-thinning. The flow rate index did not show major change with temperature and its value was generally below one, indicative of pseudoplastic behaviour. Furthermore, the activation energies were higher at higher shear rate and lower at higher whey concentration.

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