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THE FEASIBILITY OF USING SOME WHEY PROTEINS CONCENTRATE PREPARATIONS IN MANUFACTURE OF INFANT FORMULA

[54]

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ABSTRACT

Different types of whey proteins concentrate (WPC) were prepared in Brewaster Dairy pilot plant at Ohio State Univ. USA and used for formulating an infant formula and compared with Alacen 841 (Commercial WPC) as control to select the best WPC preparations which succeed in manufacture of infant formula. Infant formula was prepared with different types of WPC (Alacen 841, UF 5x, UF4x, DF 8x and DF 4x). The ratio of WPC: sodium caseinate was 40: 60. Salt, sucrose, lecithin and vegetable oil were added then homogenized and sterilized after sealing at 121°C for 6 minute, cooled in an ice bath and stored at room temperature. Samples were analyzed chemically and organoleptically when fresh and after storage at room temperature for three months. Emulsion volume index (EVI), viscosity, protein solubility, sedimentation and particle size were determined before and after sterilization. Results showed that EVI, viscosity, sedimentation and particle size increased after sterilization than before where as protein solubility take an opposite trend. Emulsion volume index, viscosity, sedimentation and particle sizes were increased after storage at room temperature for three months than fresh. Whereas proteins solubility deceased after storage than fresh. Statistical analysis showed high significant difference ($\alpha 0.05$) for emulsion volume index, protein solubility, viscosity, particle size and sedimentation.

Key words: Whey protein concentrates, Infant formula, Ultrafiltration, Difiltration

INTRODUCTION

Concentrated infants formula are complex systems and are formulated to resemble the human milk. Since the composition of bovine and human milk are different, whey proteins are utilized in infant's formula to adjust the casein/whey proteins ratio and provide their functionality (e.g. emulsion). The ratio of cow's milk is 80 casein/20 whey proteins, while that of human milk range being 30-40/70-60. **Leman and Kinsella (1989)** stated that whey proteins generally show lower emulsion capacity than caseinates, whereas emulsions prepared with whey

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proteins form more stable emulsions than caseinate. Higher proportion of whey protein was suggested to form more stable emulsion with a ratio of 60% whey proteins concentrate to 40% casein **Fligner *et al* (1990)**. Sterilization of infant formula is necessary in order to meet the requirements of safety and long shelf life. The addition of whey proteins can cause some heat stability problems in the forms of proteins agglomeration, gelation and sedimentation. The emulsion instability is creaming, which can be a major problem in infant formula **Miles, (1982)**. When creaming has occurred, products may not be accepted by consumers. The purpose of this study is to prepare different types of WPC and utilize it in preparing infant formula to select the best WPC preparation which succeed in formulating an infant formula.

MATERIAL AND METHODS

Freeze dried difiltrated whey proteins concentrate (WPC df4xFD) was prepared in the Brewaster Dairy pilot plant at Ohio State Univ. USA. Lecithin was provided by KERRY Ingredients.

Sucrose and vegetable oil were purchased from the market, cheese starter was provided from Dri-Vac Lactic culture, number 44 Chr. Hansens Laboratory, Milwaukee, WI. Cheddar cheese whey was prepared from cow's milk using lactic acid culture and rennet as coagulant.

Preparation of whey proteins concentrate

WPC was prepared by using procedure of **Hwang, (1994)** with some modifications. Whey was ultrafiltrated around 45°C with an Uf unit equipped with Romicon. UF unit was operated at inlet pressure of 27 psi and an outlet pressure at 12 psi. Fat was separated from whey at (55°C), followed by pasteurization at 72°C for 15 sec. Before the UF running the pH of the whey was adjust to 6.0 by adding citric acid. Whey was concentrated to 5, 4, fold by ultrafiltration and to 8, 4 fold by difilteration. The UF running was carried out at 55°C to 5 fold. The obtained retentate was ultrafiltrated to 4 fold and adjusted to pH 6.0 with citric acid. Followed by difiltration 8 fold and difiltration to 4 fold. The final products of retentates were freez dried in freez-drying Unit (The Virtis Company, Inc., Gahhdine, NY).

Infant Formula preparation

* Distilled water (73.8 gm)

Total protein (4.1 gm)

(40:60 (WPC: sodium caseinate)

Salt	(0.1 gm)
Sucrose	(14.4 gm)
Lecithin	(0.4 gm)
Vegetable oil	(7.2 gm)
Total	= 100 gm

Except for oil, the above ingredients were hydrated for at least one hour. The dispersion and oil were prewarmed to 60°C before mixing. The mixture was stirred by a magnetic stirrer and homogenized by an Electric pump 4- stage valve homogenizer (HillerØd, Denmark) with 60°C water running through a tube that wrapped around the valve. After homogenization, 5 ml sample was placed in a serum bottle and sealed with rubber stop-

per and aluminum cap. The samples were sterilized at 121°C for 6 minutes .After autoclaving of samples, the bottles were cooled in an ice bath and stored at room temperature. Samples were analyzed before and after sterilization as well as after storage at room temperature for three months for protein solubility, viscosity, particle size and sedimentation. Emulsion volume index (EVI) was determined on fresh samples and after storage for3 months.

Methods of Analysis

pH was determined by Oakion pH a corn series. Protein content was determined by the **AOAC, (1980)** microkjeldahl method. A tecator ZOZO Digestor and a Tecator Kjeletec auto sampler system 1035 analyzer (perstorp analytical, Inc., Silver Spring, MI) were used.

For viscosity determination a Brook field viscometer LVDVII (Brook field Engineering laboratories, Inc., Stoughton, MA) with a/uL adapter was used. 16 ml of samples were placed in the UL container and viscosity was determined at 25°C at 4 rpm for 2 minutes.

Protein solubility was determined by the method of **Morr, *et al* (1985)** with some modifications. 1%, 3% and 5% of different WPC solution were prepared by hydrating WPC for 1 hour and centrifuged (20.000 xg) for 30 minutes by using a sorvall RC-Estates refrigerated supper speed centrifuge (Dupont Instruments, Hoffman Estates, IL). The supernant was filtered and protein content was measured by kjeldhal procedure. Protein solubility was computed from the protein content of the supernatant, as percentage of the total protein content.

Calcium content was determined according to **Perkin Elmer Corp (1973)** by using perkin-Elmer HGA 2001 controller using a hallo cathode lamp (Ca^{2+} / Mg^{2+}/Zn^{2+}) at a wave length of 422.7 nm (air-acetylene flame). Concentration of 0, 1.25, 2.5, 3.75, 5 and 6.25 mg Ca^{+}/ml solutions were used to make a standard curve.

Particle size distribution was measured as described by **Bunville, (1984)** by Malvern Instrument Ltd., (Worcester shire, WR 141 AT, UK). The particle size distribution of WPC infant formula was determined as follows: after samples preparation, the pump arm was lowered into a beaker filled with approximately 500 ml of distilled water. The stirrer speed was controlled by the pump speed that was set at 1600. Then few drops of the sample were dropped into the beaker until the measured beam obscuration showed 20% and 30%.

Sedimentation was determined by using centrifugal method described by **Mcdermott, *et al* (1981).**

Emulsion volume index (EVI) was determined according to **Mcdermott, *et al* (1981)**, as follows; one ml of the sample was placed in a porcelain plate and mixed with 10 µl of oil Red "0" and 10µL methylene blue. Microhematocrit capillary tubes were filled with the stained sample and sealed with red stained sealant. Those capillary tubes were centrifuged at 13, 500Xg for 30 min. After centrifugation the total length of the sample and length of the emulsion layer were determined. The EVI was calculated by the following equation:

$$EVI = \frac{\textit{(Emulsion length)}}{\textit{(Total length)}} X\ 100$$

Statistical analysis was according **Snedecor and Cochran (1968).**

RESULTS AND DISCUSSION

Table (1), illustrates the effect of using different preparations of WPC and sterilization at 121°C for 6 minute one emulsion volume index (EVI) of infant formula. It is clear that EVI increased after sterilization than before in all treatments. The WPC preparation by difiltration to 8 fold (DF8x) had the highest EVI than all treatments whereas WPC (DF4x) had the lowest EVI than all treatments. This may be due to the method of preparation. **Fligner *et al* (1991)** showed that sterilization of infant formula results in aggregate of protein which will increase turbidity and possibly falsely predict a stable emulsion and the high value of EVI is desirable. During the formation of emulsion, protein molecules, diffuse to the interface and spread and unfold to form a continuous cohesive film. Low molecular weight emulsifier aids in emulsion formation however, proteins lead to more stable emulsion. Sterilization of infant formula is necessary in order to meet the requirements of safety and long shelf life. The greater stability could also be attributed to increase viscosity. This result is agreeable to the result which in Table, (2) WPC (DF 8x) had a highest viscosity than all treatments. Table, (2) shows the effect of different preparation of WPC on protein solubility %. It is clear that protein solubility decreased after sterilization than before sterilization in all treatments. These results agree with those of **Hassan (2002a).** WPC (DF4x) had the lowest protein solubility than all treatments whereas Alacen (841) had the highest protein solubility than all treatments. WPC (DF8x) showed intermediate values. Sterilization often causes denaturation of whey proteins. Whey proteins begins denaturation by heating above 65°C followed by aggregation and precipitation. It is known that α-la is the most heat resistant whey protein and β-Lg which is the most heat-labile whey protein. Though the denaturation temperature of α-La is 65°C, its denaturation is strongly reversible **Shu-Wei-Pal (1995).** Generally, heating at high temperature results in worse solubility and denaturation and aggregation of proteins. So heat denaturation cause proteins to unfold and increase viscosity and may even result in gelation.

Table, (2) show the effect of different preparations of of WPC on viscosity before and after sterilization at 121°C for 6 minute. It could be notice that WPC prepared with difiltration to 8 fold WPC (DF 8x) had highest viscosity begin 1.94 mp than all treatments followed by WPC (DF4x) being 1.89 mp whereas, Alacen 841, commercial WPC had lowest viscosity being 0.89 mp. After sterilization the viscosity increased than before in all treatments. These results are in agreement with **Kinsella, (1984)** who reported that aggregation and precipitation of protein after thermal processing resulted in an increase of viscosity. Also, these results are in agreement with **Hassan, (2002b)** who found that viscosity of beverages prepared with different whey protein concentrates increased after sterilization than before. The increase in viscosity could enhance greater stability **Fligner *et al* (1990).**

Table, (2) show the effect of different prepared WPC and sterilization at 121°C for 6 minute on particle size. Particle size of all different prepared WPC increased

Table 1. Effect of different prepared whey protein concentrates (WPC) and sterilization at 121°C for 6 minute on emulsion volume index (EVI) mm model (infant formula)

WPC*	Before sterilization	After sterilization
Alacen 841	0.18	0.23
UF5x	0.22	0.25
UF 4x	0.21	0.26
DF 8x	0.23	0.27
DF 4x	0.17	0.26

* Alacen 841 : Commercial WPC
UF 5x : Ultrafiltrated to five fold.
UF 4x : Ultrafiltrated to four fold.
DF 8x : Difilterated to eight fold.
DF 4x : Difilterated to four fold.

Table 2. Effect of different prepared whey protein concentrates (WPC) and sterilization at 121°C for 6 minute on protein solubility (P)%, viscosity (V) mP, particle size (PS) μm and sedimentation (S) mm. of a model infant formula

WPC*	Before sterilization				After sterilization			
	P %	V (mp)	PS (μm)	S (mm)	P %	V (mp)	PS (μm)	S (mm)
Alacen 841	89.21	0.89	0.42	0.075	84.10	1.40	0.46	0.140
UF5x	77.32	1.00	0.50	0.062	73.20	2.86	0.56	0.125
UF 4x	71.24	1.23	0.48	0.090	70.86	2.34	0.53	0.180
DF 8x	74.62	1.94	0.65	0.085	68.30	3.54	0.70	0.190
DF 4x	73.56	1.89	0.72	0.091	70.24	3.00	0.75	0.195

* see Table (1)

after sterilization at 121°C for 6 minute than before sterilization. Alacen 841 sample showed the lowest particle size (μm) followed by WPC treatments (UF4x) and WPC (UF5x) whereas WPC (DF8x) was intermediat. These results are in agreement with **Hassan, (2002a),** who found that particle size of WPC in beverages increased after sterilization than before.

Table, (2) indicates the effect of using different prepared WPC on sedimentation. It is clear that UF WPC to 5 folds (UF5x) had low sedimentation whereas WPC difiltrated to 8 folds had intermediate sedimentation. On the other hand WPC difilterated to four folds had the highest sedimentation. Also sedimentation was increased after sterilization than before in all treatments. These results agree with **Hassan, (2002b)** who found that sedimentation of beverages prepared with different WPC increased after sterilization than before. This may be due to the intensive effect of heating on whey proteins denaturation. **Table, (3)** summarize the organoleptic properties of infant formula prepared with different WPC. Infant formula prepared with WPC difiltration to eight folds had gained the highest scores for appearance, taste and flavour followed by WPC difiltration to four folds and Alacen 841 (commercial WPC). On the other hand WPC ultrafiltrated to five folds had the lowest score for appearance, taste and flavour. **Table, (4)** show the effect of storage at room temperature on emulsion value index (EVI). It is clear that EVI increased after storage at room temperature for 3 months than before storage in all treatments. The infant formula prepared with WPC (DF8x) had the highest EVI than all treatments whereas Alacen 841 had the lowest EVI. These results agree with the results of **Table, (2).** Where increasing of viscosity led to increase EVI.

Table, (5) shows the effect of storage on protein solubility. It is clear that protein solubility decreased after storage at room temperature for 3 months. **Table, (5)** illustrates the effect of storage at room temperature on viscosity (mp). It could be notice that viscosity increased after storage than before in all treatments this may be due to the effect of storage. **Table, (5)** summarized the effect of storage on particle size (PS) μm. PS which increased after storage than before **(Table, 2)** in all treatments, summarized the effect of storage on particle size (PS) (μm) which increased after storage than before in all treatments. Regarding sedimentation it increased after storage than before in all treatments. **Table, (6)** indicate the effect of storage at room temperature on organoleptic properties of infant formula containing different WPC treatments. It was clear that appearance, taste and flavour decreased after storage than before in all treatments.

CONCLUSION

Finally it could be conduced that, whey proteins concentrate (WPC), (DF 8x) was the best treatment that enhance the functionality of WPC and is successful in preparing infant formula.

Table 3. Organoleptic properties of infant formula treatments prepared with different prepared whey protein concentrates (WPC)

WPC*	Appearance (10)	Taste (60)	Flavour (30)	Total (100)
Alacen 841	8	53	26	87
UF5x	6	22	24	80
UF4x	7	51	23	81
DF 8x	9	55	27	91
DF 4x	8	54	25	87

* See Table (1)

Table 4. Effect of storage at room temperature of infant formula with different prepared whey protein concentrates (WPC) on emulsion volume index (EVI) mm

WPC*	Fresh	After 3 months
Alacen 841	0.23	1.75
UF5x	0.25	1.90
UF 4x	0.26	1.59
DF 8x	0.27	2.13
DF 4x	0.26	1.98

* See Table (1)

Table 5. Effect of storage at room temperature on protein solubility (P)%, viscosity (V)mp, particle size (PS) μm and sedimentation (S) (mm.) of a model infant formula

WPC*	Three months			
	P %	V (mp)	PS (μm)	S (mm)
Alacen 841	80.32	3.60	1.86	0.321
UF5x	69.35	3.90	1.90	0.300
UF 4x	66.35	3.50	1.98	0.426
DF 8x	65.22	4.92	2.20	0.567
DF 4x	67.12	5.21	2.55	0.721

* See Table (1)

Table 6. Effect of storage at room temperature on organoleptic properties of a model infant formula

WPC*	Three months			
	Appearance (10)	Taste (60)	Flavour (30)	Total (100)
Alacen 841	7	51	24	82
UF5x	5	50	21	76
UF 4x	6	48	20	72
DF 8x	8	53	25	86
DF 4x	7	50	22	79

* See Table (1)

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امكانية استخدام بعض مستحضرات مركزات بروتين الشرش فى صناعة وجبات الأطفال الرضع

[٥٤]

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تم تحضير أنواع مختلفة من مركزات بروتين الشرش فى وحدة الانتاج نصف الصناعى بجامعة أوهايو فى الولايات المتحدة الامريكية و استخدمت فى تحضير وجبات للأطفال الرضع ومقارنتها بوجبة محضرة باستخدام مركز بروتين شرش تجارية يسمى Alacen 841 . وذلك لاختيار أفضل مركز بروتين شرش ينجح فى تصنيع وجبات للأطفال. حيث تم تحضير وجبات الأطفال باستخدام انواع مختلفة من مركزات بروتين الشرش المحضرة وتشمل مركز بروتين شرش تجارى(Alacen841) ، مركز بروتين شرش محضرة باستخدام الترشيح الفائق بمعامل تركيز ٥ (UF5x) ومركز بروتين شرش محضر باستخدام الترشيح الفائق لتركيز ٤ (UF4x) ومركز بروتين شرش محضر باستخدام اعادة الترشيح لتركيز ٨ (DF8x) ومركز بروتين شرش تم اعادة الترشيح لمعامل تركيز ٤ (DF4x) وتم إضافة كازينات صوديوم بحيث تكون النسبة مابين مركز بروتين الشرش: كازينات الصوديوم نسبة ٤٠ : ٦٠ ثم إضافة ملح، سكروز،اليسيثين وزيت ثم تم عمل تجنيس والتعقيم على ١٢١° م مدة ٦ دقائق ثم

التبريد فى حمام ثلجى والتخزين على درجة حرارة الغرفة ولقد تم عمل تحليل حسى وكيماوى للعينات وهى طازجة وبعد التخزين على درجة حرارة الغرفة مدة ثلاثة شهور . ولقد اشارت النتائج إلى حدوث زيادة فى معامل حجم الاستحلاب (EVI) واللزوجة ومعدل الترسيب وحجم الجزيئات بعد التعقيم عن قبل التعقيم بينما قابليةالبروتين للذوبان اتخذت الاتجاه العكسى. وايضا حدث زيادة فى معامل حجم الاستحلاب واللزوجة ومعدل الترسيب وحجم الجزيئات بعد التخزين على درجة حرارة الغرفة مدة ثلاثة شهور عن العينات الطازجة بينما حدث انخفاض فى قابلية البروتين للذوبان بعد التخزين عن العينات الطازجة. ولقد اشارت نتائج التحليل الإحصائي إلى وجود فروق معنوية عالية عند مستوى (α 0.05) لكلا من معامل حجم الاستحلاب واللزوجة وقابلية البروتين للذوبان وحجم الجزيئات ومعدل الترسيب.

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