Production of Functional Whey Protein Concentrate by Monitoring the Process of Ultrafilteration

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ABSTRACT : This investigation was undertaken in order to elicit the relationship between the extent of ultrafiltration processing of whey and its effect on composition and yield of resultant whey protein concentrate (WPC). Cheddar cheese whey was fractionated through ultrafiltration to an extent of 70, 80, 90, 95, 97.5% and 97.5% volume reduction followed by I stage and II stage diafiltration. After each level of ultrafiltration, the composition of WPC was monitored. Similarly, the initial whey was adjusted to 3.0, 6.2 and 7.0 pH levels and ultrafiltration was carried out to elicit the effect of pH of ultrafiltration on the composition. Further, initial whey was adjusted to different levels of whey protein content ranging from 0.5 to 1.0 per cent and subjected to ultrafiltration to different levels. The various range of retentate obtained were further condensed and spray dried in order to assess the yield of WPC per unit volume of whey used and the quantity of whey required to produce unit weight of product. With the progress of ultrafiltration, there was a progressive increase in protein content and decrease in lactose and ash content. The regression study led to good relationships with R^2 values of more than 0.95 between the extents of permeate removed and the resultant changes in composition of each of the constituents. Whey processed at pH 3.0 had significantly a very low ash content and high protein content as compared to processing at 6.2 and 7.0. The yield of WPC per unit volume of whey varied significantly with the initial protein content. Higher initial protein content led to higher yield of all ranges of WPC and the quantity of whey required per unit weight of spray dried WPC significantly reduced. Regression equations establishing the relationship between initial protein content of whey and the yield of various types of WPC have been derived with very high R^2 values of 0.99. This study revealed that, the yield and composition of whey can be monitored strictly by controlling the processing parameters and WPC can be produced depending on the food formulation requirement. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 433-438)***

Key Words : Whey, Ultrafiltration, Diafiltration, Processing Parameters, Whey Protein Concentrate, Yield and Composition

INTRODUCTION

Worldwide, whey processing represents a challenge to food technologist to find interesting ways for its utilization. The most successful way to redeem whey solids is considered to be the production of WPC by the application of ultrafiltration (UF) system (Cheryan and Kuo, 1984; Marshall and Harper, 1988). Nowadays, whey proteins are widely used as a food ingredient, because they are highly nutritious and possess useful functional properties. Whey proteins are being considered as versatile constituent of whey due to their excellent nutritional and functional properties (Huffman, 1996; Jayaprakasha and Brueckner, 1999). UF is the unique tool to recover these proteins and presently, it is possible to recover proteins from whey in their native form (Morr and Foegeding, 1990). The flux during ultrafiltration and the composition of resultant WPC are the prime factors for the commercial exploitation of this technology for sustainable and economic operation (Kessler et al., 1982). A relatively higher flux was observed at pH 3.0 and 7.0 as compared to native pH of whey and other range of pH attempted, while processing whey by ultrafiltration along with the significant change in the composition of WPC (Hiddink et al., 1981; Jayaprakasha et al., 1994, 1996). Now this technology has become well established to conserve whey solids in the form of spray dried whey protein concentrate and has been commercially made available. The terminology WPC is being used for the dried whey having more than 25% protein and as such there is a wide variation in composition of resultant WPC, ranging from 25 to 90% protein (Renner and Abd El-Salam, 1991). Owing to their excellent nutritional and functional properties of WPC, they find varieties of application in various food formulations. However, the end use of WPC depends on its protein content and the composition of other constituents (Huffman, 1996; Jayaprakasha and Brueckner, 1999). The functionality of WPC in a food system depends not only on its protein content but also on the proportion of other constituents (Melachouris, 1984). Each food application needs specific level protein possessing WPC and accordingly the process has to be carefully monitored, otherwise the basic purpose of these ingredients application in food formulation will be defeated (Lee and Hong, 2003). Hence, it is utmost important to monitor the composition of the resultant WPC to meet the specific end application (de Wit, 1984; Jayaprakasha et al., 1995). It is also equally important to assess the yield of WPC per unit volume of whey as there is wide variation in the composition of whey. The whey processor is facing problems in this context as

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the demand for WPC depends on its functionality and end use. A great care need to be bestowed to carefully process whey to spray dried WPC to make its application more economical. As such information with respect of this is limited.

Therefore, this investigation was undertaken with a view to monitor the compositional changes during ultrafiltration to tailor the WPC depending on the end use. This also helps to establish the relationship between the initial composition and the final yield of a range of WPC to be obtained per unit volume of whey. Thus the data generated will be very useful to the industry to explore this technique for sustainable commercial application.

MATERIAL AND METHODS

Processing of whey through ultrafiltration

Fresh cheddar cheese whey was clarified and subjected to heat treatment in a vat pasteurizer to 70°C/5 min. followed by cooling to 50°C. Exactly 250 liters of heat treated whey was taken for each experiment in the balance tank of the ultrafiltration unit and ultrafiltration was carried out at 50°C and at a pressure of 100 PSI and 25 PSI inlet and outlet pressures, respectively by using Spiral wound type UF plant possessing polysulphone membrane of 25,000 Daltons and effective area 1.8 m^2. Whey was processed through UF separately to different levels of volume reduction viz., 70, 80, 90, 95, 97.5 and 97.5% followed by I and II diafiltration. After removing the required level of permeate, the process was stopped and the retentate was further subjected to vacuum condensing followed by spray drying or directly spray drying to obtain dried WPC. The spray dried WPC obtained by ultrafiltering to different degree of volume reduction was subjected to chemical analysis to evaluate the extent of retention of various constituents. The data obtained was subjected to regression analysis to establish the relationship of extent of ultrafiltration and the composition of WPC.

Volume reduction : Volume reduction was determined by using the following formula.

$$VR = \frac{V}{VO} \times 100$$

Where,
VR = the percentage volume reduction,
VP = the volume of permeate removed (ml) and
VO = original volume of the whey

Diafiltration : 250 L of fresh pretreated cheddar cheese whey was ultrafiltered to a level of 97.5% volume reduction with the above processing conditions. When 97.5% volume reduction was attained, distilled water (50°C) was added to the retentate at 1:1 proportion. Filtration was carried out till all the added water was removed in the form of permeate. Similarly a second diafiltration was carried out.

Condensing : The retentate obtained through ultrafiltration was further condensed to 35 per cent total solids in a single stage raising film vacuum evaporator (Anhydro Copenhagen, Denmark 35 kg water evaporation/ hour) at a temperature of 54-56°C and a vacuum of 630-635 mm. The condensed retentate was further used for spray drying.

Spray drying : The condensed UF retentate was further spray dried in a spray drier (Anhydro Copenhagen, Denmark of 7 kg water vapor capacity per hour) at an inlet and outlet temperature of 180°C and 80°C, respectively so as to have moisture content of less than five per cent in the dried WPC. The resultant WPC was used in the experiments.

Extent of retention : At regular interval of volume reduction the retentate was further processed in to spray dried WPC and subjected to various compositional attributes. The yield of each component was calculated by the following formula.

$$Y = C_1/f\,C_0$$

Where,
Y is the yield of component,
C_0 is the initial concentration of the given component,
C_1 is the final concentration of the given component and f is the concentration factor

Effect of pH of whey on composition of WPC

Clarified whey was adjusted to 3 levels of pH, viz. 3.0, 6.2 and 7.0, either by the addition of 10 per cent sodium hydroxide or by 10 per cent hydrochloric acid and subjected to a heat treatment of 70°C/5 min. followed by cooling to 50°C. Ultrafiltration was carried out as indicated above at 50°C to the above said levels of volume reduction by removing the known volume of permeate followed by diafiltration. The resultant retentate was further processed by vacuum condensing and spray drying to obtain spray dried WPC. The spray dried WPC was subjected to analysis for various components to assess the effect of pH of processing on the composition of WPC.

Effect of initial protein content of whey on yield of WPC

The protein content of initial cheddar cheese whey was estimated and further the protein content was standardized to 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 per cent by using previously prepared spray dried high protein (80%) WPC and ultrafiltration was carried out to required degree of volume reduction so as to have protein content of 35, 45, 60, 70 and 80 per cent on dry matter basis in the UF retentate. The retentate thus obtained was subjected to vacuum condensing and spray drying to obtain spray dried WPC. The quantity

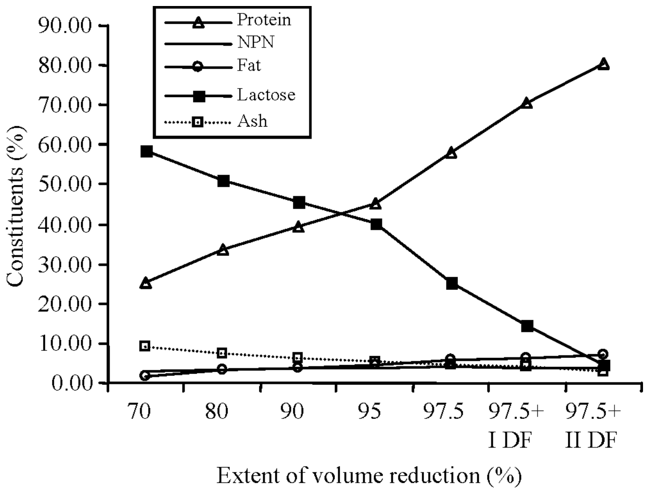

Figure 1. The effect of extent of ultrafiltration and diafiltration (DF) on composition of spray dried WPC. I DF: I diafiltration and II DF: II diafiltration.

of spray dried WPC was measured at each instance and yield was assessed. Similarly, based on these data the quantity of whey required to prepare a unit weight of spray dried WPC was computed to establish the relationship of initial protein content and yield of WPC. The data obtained were subjected to regression analysis to develop equations for predicting the yield of WPC based on the initial protein content.

Compositional analysis

Total solids, protein, fat, and ash contents in whey and spray dried WPC were determined by the methods described in AOAC (1980). The lactose content of various samples was estimated by phenol-sulphuric acid method as per the procedure recommended by Lawrence (1963). Non-protein nitrogen content of the samples was estimated by precipitating the sample using 17% trichloroacetic acid (Rowland, 1938) and filtering. The nitrogen content of the filtrate was analyzed by kjeldahl method (AOAC, 1980).

Statistical analysis

The data were analyzed statistically for test of significance by ANOVA and regression analysis by the application of SAS package with the help computers installed at the University of Agricultural Sciences, Bangalore, India.

RESULTS AND DISCUSSION

Extent of ultrafiltration and diafiltration on changes in composition of various constituents

The effect of the extent of removal of permeates on the composition of various constituents of spray dried WPC is depicted in Figure 1. The regression equations establishing the relationship of unit volume of permeate removed and addition of water on various constituents of WPC is also presented in Table 1.

Table 1. Regression equations establishing the relationship of influence of extent of ultrafiltration and diafiltration on various constituents of WPC

Constituents	Intercept	Extent of ultrafiltration	Diafiltration	R^2
Total protein	-53.05	1.088 (9.6744)**	14.22 (9.385)**	0.95
NPN	-0.015	0.041 (30.9186)**	-0.111 (6.2266)**	0.98
Fat	-6.693	0.1209 (15.023)**	1.1441 (10.4076)**	0.97
Lactose	132.356	-1.0184 (8.8999)**	-15.1188 (9.6742)**	0.95
Ash	20.554	-0.1622 (38.5032)**	-0.9124 (15.8557)**	0.99

Results are mean values of 3 trials.
Figures in the parenthesis are the calculated 't' values.

The protein content of spray dried WPC was 25.50, 33.50, 39.40, 45.30, 58.05, 70.40 and 80.60 when whey was subjected to 70, 80, 90, 95 and 97.5% volume reduction and 97.5% volume reduction followed by I and II stage diafiltration, respectively. In contrast to protein, the lactose content of retentate decreased proportionately with the increasing volume reduction. The lactose content at these levels of volume reductions was 58.20, 51.20, 45.50, 40.30, 25.10, 14.10 and 4.60%, respectively. The extent of decrease in ash content was less compared to the decrease in lactose content. The ash content of WPC was 9.20, 7.45, 6.05, 5.20, 4.55, 4.05 and 2.80%, respectively.

From the results it is very pertinent to indicate that, to obtain 25% proteins in WPC, the extent of permeate removal should be at least 70% of the original volume. According to US Federal standards to designate as WPC, it should possess a minimum of 25% protein (Renner and Abd-El Salam, 1991). As could be seen from the figure, as the extent of permeate removal increased, the protein content in the retentate proportionately increased and the lactose, NPN and ash content decreased significantly at all levels of volume reduction. From the results it is evident that as the ultrafiltration progresses, the protein content increases as UF membrane (Polysulphone) is capable to retain protein molecule. As the extent of permeate removal increased, lactose content, which is a predominant component decreased with the proportionate increase in protein content. Though there was decrease in total ash content of the retentate with the progress of ultrafiltration, the changes in ash content did not follow the similar pattern as those of lactose and protein, as some of the salts are not permeable through membrane. However, as the process progressed the degree of permeation of salts decreased, as some of the minerals are still in bound form as they are associated with β-lactaglobulin which cannot permeate

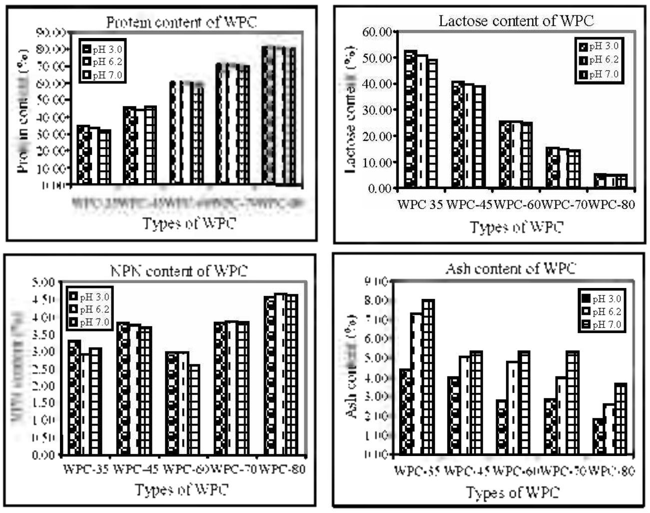

Figure 2. Effect of pH of ultrafiltration of cheese whey on the composition of WPC.

through membrane (Patocka and Jelen, 1987). Lower lactose and ash contents in higher protein content WPC products have been reported by several earlier workers (Hugunin, 1987; Marshall and Harper, 1988). The residual fat content increased uniformly with the increase in the extent of permeate removal as the constituent fat being impermeable also gets concentrated during ultrafiltration (Morr, 1979; Daufin et al., 1992).

The results of the experiment indicated that to obtain spray dried WPC equivalent to skim milk powder composition, UF of whey has to be carried out to an extent 80% volume reduction, which corresponds nearly to a protein content of skim milk powder. These results suggest that whey can be processed in such way that it could be used as a substitute for skim milk powder. Similar observations were reported by some of earlier workers (Renner and Abd El-Salam, 1991; Jayaprakasha et al., 1995). To obtain spray dried WPC of 35, 45 and 60% protein content UF has to be carried out to an extent of 80, 90 and 97.5% volume reduction. However, for higher protein content, it was necessary to follow diafiltration after 97.5% volume reduction. By I stage diafiltration, the protein content could be increased to a level of 70% and by II stage to a level of 80%. The regression relationship (Table 1) established that for every unit increase in volume reduction, the protein content in the final product increases by 1.088 and the lactose and ash content decrease by 1.0184 and 0.1622 units, respectively. Diafiltration results in increase in protein content by 14.22 units with a very high regression coefficient (r^2) of 0.95. Similarly, the decrease in lactose content for diafiltartion was 15.118 with r^2 values of 0.95. The equations also fitted very well with respect to ash content of various types of WPC with r^2 values of 0.99.

The increase in protein content and decrease in lactose and ash content during diafiltration could be attributed to the addition of water to the UF retentate, which aids in reducing the viscosity and, thereby more of lactose, minerals and low molecular weight compounds pass along with the permeate (Derham and Chanton, 1986; Vuillemand et.al., 1989). As the diafiltration aids in dilution of retentate, more and more of salts and lactose permeate through the membrane resulting in exponential increase in protein content. The diafiltration is of great significance for obtaining high protein spray dried WPC products.

Effect of pH of whey on the composition of WPC

Fresh whey was adjusted to 3 ranges of pH, namely 3.0, 6.2 (native pH) and 7.0 prior to ultrafiltration and processing was carried out by ultrafiltration. The composition of spray dried WPC obtained by ultrafiltration to various levels of volume reduction as affected by pH of whey is presented in Figure 2. It is apparent from the results that the pH of whey has a significant ($p \leq 0.05$) effect on the composition of WPC. As could be seen from the figure as the pH is increased from 3.0 to 7.0, the ash content of the retentate increased significantly irrespective of the types of WPC. The yield of protein in WPC-60 varied from 60.40 to 59.55. The variation in protein yield when processed at different levels of pH could be attributed to variation in permeation behavior of other constituents especially mineral matter rather than permeation behavior of true proteins (Hiddink et al., 1981; Tratnik and Krsev, 1991; Jayaprakash et al., 1995). Little variation in the NPN content of the retentate was observed with the change in pH of whey. The lactose content of WPC-35 varied from 52.40 to 49.10% and for WPC-80 from 5.05 to 4.70.

Wide variation in the ash content was observed when whey was ultrafiltered at different pH values. It is evident from Figure 2 that as the pH of UF increased from 3.0 to 7.0, the ash content of retentate increased dramatically. The lowest ash content was observed at pH 3.0 and the highest at pH 7.0. The ash content of WPC at pH 7.0 was nearly double than that of the retentate at pH 3.0 in all most all the types of spray dried WPC. For example the ash content of WPC-35 when ultrafiltered at pH 3.0 was only 4.4% whereas it was 8.0% when ultrafiltered at pH 7.0. Similarly it was 1.85% and 3.65% for WPC-80, respectively when ultrafiltered at 3.0 and 7.0 pH. It was clear from the results that the ash content of the retentate could be monitored by adjusting the pH of whey during ultrafiltration. The lowest ash content of pH 3.0 could be ascribed to the greater permeation of some minerals, especially calcium and phosphorus. These minerals which are associated with the protein could be dislodged by decreasing the pH and such soluble minerals especially calcium and phosphorus easily permeate through the UF membrane leading to lower ash content of the retentate (Hayes et al., 1974; Patocka and Jelen, 1987; Jayaprakasha et al., 1996). These results are of

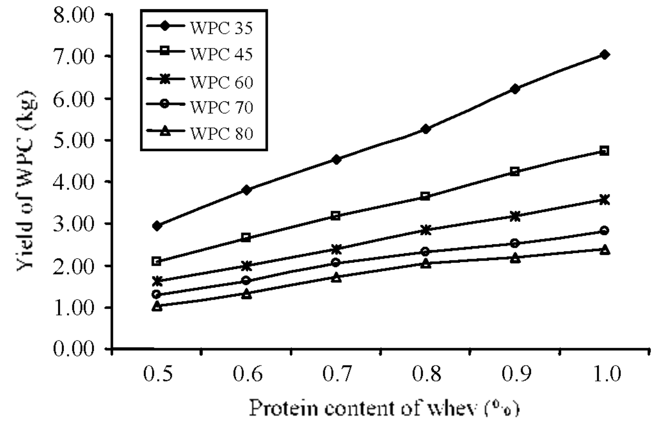

Figure 3. Relationship between protein content of whey and yeld of spray dried whey protein concentrate for 250 L of whey.

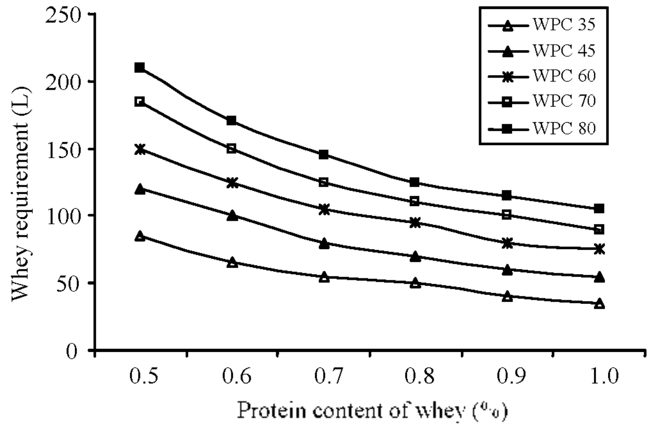

Figure 4. Quantity of whey required for one kg spray dried WPC production as a function of intial protein content of whey.

Table 2. Regression equations establishing relationship between initial protein content of whey and yield of spray dried whey protein concentrate

Type of WPC	Constant	Yield of spray dried WPC	R^2
WPC 35	-1.188	8.188 (16.49)*	0.99
WPC 45	-0.367	4.96 (39.11)*	0.99
WPC 60	-0.055	3.469 (23.15)*	0.99
WPC 70	-0.027	2.826 (30.104)*	0.99
WPC 80	0.045	2.3714 (24.08)*	0.99

Results are mean values of 3 trials.
* Figures in the parenthesis are the calculated 't' values.

great significance when preparing WPC intended for hospital and infant food formulations where in lesser ash content is preferred.

Yield of whey protein concentrate

The yield of spray dried WPC as affected by the initial protein content of whey could be seen in Figure 3. As represented in the figure it is evident that for low protein products (WPC-35), the yield of WPC per unit volume of whey was significantly higher as compared to higher protein product. From 250 kg of whey it was possible to obtain 4.54, 3.17, 2.38, 2.05 and 1.72 kg of spray dried WPC of 35, 45, 60, 70 and 80 per cent protein products, respectively for the whey having initial protein content of 0.7%. Where as these yields increased to a level of 7.04, 4.74, 3.58, 2.82 and 2.38, respectively when the protein content of whey was increased to level of 1.0%, indicating that the initial protein content is of significant factor for the yield of final spray dried WPC. The higher yield of lower protein products (WPC 35 and WPC-45) as compared to higher protein products (WPC-60, WPC-70 and WPC-80) could be attributed to higher retention of lactose and ash as the extent of removal of permeate and in turn the removal of lactose was lesser with respect to low protein products (Marshall and Harper, 1988; Renner and Abd El-salam; 1991; Jayaprakasha et al., 1995). As ultrafiltration progresses more and more of lactose and minerals get permeated and thus the protein levels increased but the yield per unit volume of whey decreased. The relationship between the initial protein content of whey and the expected yield of spray dried WPC is represented in Table 2. The regression equations clearly demonstrate that the yield of various types of WPC could be predicted if the initial protein content of whey is known. For every unit increase in protein content, there will be 8.188 units increase in yield in case of spray dried WPC-35, but it will be only 2.3714 units incase spray dried WPC-80, with a R^2 values of 0.99. In case of higher protein WPC as we remove more and more of lactose and ash with the progress of ultrafiltration and diafiltration, one would expect lesser yield of product per unit volume.

Computation of whey requirement for production unit weight of spray dried WPC

The whey requirement for production of unit weight of spray dried WPC at different initial levels of protein content is represented in Figure 4. As could be seen from the figure, as the level of protein increased in the initial whey, the volume of whey required producing unit weight of spray dried WPC decreased significantly. To produce 1 kg of spray dried WPC 80, WPC-70, WPC 60, WPC-45 and WPC 35, the whey requirement was 170, 150, 125, 105 and 65 liters at 0.6% of initial protein content, whereas it was only 125, 110, 95, 70 and 50 liters respectively at 0.80 per cent protein level. Similarly, the whey requirement at other levels of protein is also presented in the figure. It is apparent from the studies that, the whey consumption to produce spray dried WPC to higher protein content is significantly higher. For example to produce 1 kg of spray dried WPC having 80% protein, the whey required (0.7% protein) was 145 L as against only 80 and 55 liters for the dried WPC possessing 45% and 35% protein. The content of true protein is also very important for the yield as the retention of non-protein nitrogen is very low. The lower the protein content, higher is the whey consumption and vice versa. It is therefore very important to know the content of NPN in relation to the content of total protein when whey consumption for production of WPC has to be calculated. From the regression equations one could compute the yield that could be obtained per unit volume of whey processed. The data generated helps the whey processor to compute the yield of any type spray dried WPC.

CONCLUSIONS

The foregoing study revealed that the composition of spray dried WPC is widely variable depending on the extent of ultrafiltration and diafiltration carried out. To designate as WPC, at least 70% volume reduction of whey is needed. By ultrafiltering whey to about 80% volume reduction, the retentate that could possess composition of skim milk could be obtained. For higher protein content of more than 60% protein, ultrafiltration to an extent of 97.50% followed by diafiltration is required. By varying the pH of whey, the composition of WPC can be altered to a great extent especially with respect to ash content. Ultrafiltering at pH 3.0, the ash content of WPC can reduced to a minimum level. The study demonstrated that the composition of WPC can be altered by monitoring ultrafiltration process and imparting appropriate treatment. The changes in composition of spray dried WPC and yield per unit volume of whey processed could be predicted by the regression equations provided. The composition of spray dried WPC can be tailor made by monitoring the ultrafiltration process depending on the end use applications.

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