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Recovery Of Fat From Buffalo Milk Whey Using Coagulation By Chitosan

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Abstract :*Whey derived from buffalo milk has a higher dry matter content compared to the one from bovine milk, presenting an interesting nutritional profile. This work aims at the recovery of fat from buffalo milk whey by coagulation with chitosan. Coagulation process was investigated in range of pH 5.8 to 7.2, temperature 10^oC to 20^oC and chitosan concentration 150 mg. L^{-1} to 350 mg.L^{-1}, using Rotational Central Composite Factorial Design. Basing on turbidity removal and on economy of the process, the optimum coagulation conditions was determined as chitosan concentration of 250mg. L^{-1}, temperature of 15^oC and pH of 6.5, in which up to 94.7% of turbidity was removed. The coagulated material was dried in a spray dryer at 150^oC, obtaining a powder with humidity of 2%, 52% of fat, 13% of lactose and 13% of protein. Results showed good performance of the proposed method for recovering fat from buffalo milk whey.*

Keywords *-buffalo milk,, chitosan, coagulation, fat, whey*

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I INTRODUCTION

Dairy industries are responsible for manufacturing many milk derivatives, including cheese. In cheese manufacturing, whey is obtained as a residual fraction of milk after coagulation and separation of casein. It is necessary to reduce the environmental impact when this residual whey stream is disposed, once it presents a high organic content, being a potential polluter. On the other hand, it presents a high nutritional and functional value and the efforts to process whey have been increased. Researches on concentrating and producing whey powder of high commercial value which can be used in the Food, Cosmetic and Pharmaceutical Industry have been developed. It can improve technological properties such as homogeneity and foam control, important for those industries [1, 2].

There are several studies carried out with bovine milk whey, but the same is not true for buffalo whey, which has a high fat and nutritional content with higher caloric value comparing to bovine milk [3, 4, 5]. Buffalo whey has, on average, 9.30 to 10.4% total solids, 4.07 to 5.84% lactose, 1.20 to 1.51% fat, and 0.8 to 1.19 of protein. Besides, the consumption of dairy products from buffalo milk, specially cheese, has increased worldwide [6].

An alternative that can provide a better use of whey is obtaining a concentrated through coagulation process. The use of chitosan as a coagulant has already been reported in some studies with bovine serum, however there are no studies with buffalo serum, being necessary to establish the adequate process conditions of this raw material which is an unexplored and rich source of lipids [7, 8].

Chitosan is a biodegradable polymer, non-toxic, with a high molecular weight derived from chitin present in highly polluting waste from shrimp and crab processing, which justifies its use on whey coagulation [9, 10]. Several toxicological studies about chitosan have shown it to be nontoxic. Therefore, the use of chitosan should be health safe [11].

Whey fat is present in the form of globules, consisting of a nucleus, composed mainly of triglycerides, protected by a lipoprotein membrane. The composition and structure of the membrane are influenced by several factors, but, in general, it is constituted by about 25% of proteins, mainly glycoproteins, and 70% of lipids. During milk processing in products such as cheese, polar lipids and fat globule membrane proteins are preferably distributed in the aqueous phase. In milk whey, 35% of total milk phospholipids are found [12]. Hwang &Damadoran [11] made use of chitosan to remove lipids from bovine cheese serum and achieved satisfactory results, showing that it consists in a simple, economical and effective process route. In their study, turbidity was the parameter that represented the efficiency of fat removal. Other authores also employed successfully chitosan in recovery of beta-lactoglobulin [13].

Milk fat has an interesting economic value and contributes to supply a characteristic flavor and improve the texture in the foods. The total cholesterol of the buffalo milk is lower when compared to cow's milk (275 mg and 330mg per 100 g) [14, 15].

This work aims to recovering the fat from whey derived from buffalo milk by the use of chitosan as coagulant agent, establishing proper conditions of process. All results were analyzed based on turbidity removal from the original whey, considering that, in the established conditions, chitosan is more selective for fat, being the main substance removed such as reported by Hwang &Damadoran [11].

II MATERIAL AND METHODS

II.1 Whey Samples and Chitosan

Buffalo milk whey samples were supplied by Bom Destino Dairy Factory, located at the state of Mina Gerais (Brazil), from the buffalo muzzarella cheese manufactory plant. Samples were filtered through cotton to remove solids residues resulting from the cheese production process.

Chitosan used as a coagulant was obtained from *Sigma-Aldrich*, in powder, mean molecular weight and degree of deacetylation equal to or greater than 75%. Chitosan solutions were obtained from the dilution of stock solutions prepared previously for each set of tests. For the preparation of the stock solutions, chitosan was dissolved in acetic acid 1% v/v under magnetic stirring for 2h.

II.2 - Whey Compositional Determination

The whey samples were characterized according to the procedures presented by IAL [16] and by the APHA [17], which are: pH, by electrometric determination with pHmeter (*Phtek, PHS-3B*); acidity in lactic *acid*, by titration of the solution with sodium hydroxide 0,1 M; turbidity, through spectrophotometer reading (*CELM, E-225D*); chemical oxygen demand *(COD)* using colorimetric method of closed reflux, readings in spectrophotometer (*CELM, E-225D*); total dry extract, by gravimetric method, determining the dried solid mass after 24h in a convective oven at 105 ° C; lactose, by titration with Fehling's solution; protein using determination of nitrogen by the *Kjeldahl* method; lipids (fat), through the extraction of petroleum ether, in *Soxhlet* extractor; and ash content, measuring the remaining mass of the solid incinerated at 550 °C after 2 hours. All analyzes were performed in triplicates, to determine the experimental error.

II.3 - Coagulation Process

Chemical coagulation was carried out using the Jar-test (*MILAN, model JT-203*), with 6 jars with stirring regulation.

Experiments were conducted by adding 285 mL of whey sample to the jars, under agitation, 15 mL of the chitosan solution, stirring the material at 120 rpm for 2 minutes (rapid step), and then for 5 minutes at 10 rpm (slow stage). After that, the material was left for 2 hours at rest to float. The coagulated was separated by vacuum filtering.

The influence of three parameters was analyzed: concentration of chitosan, from 150 mg L^{-1} to 350 mg L^{-1}, temperature from 10 ° C to 20 ° C and pH in the range of 5.8 to 7.2.

A rotational central compound design (RCCD) was used in order to evaluate the influence of the three parameters chitosan concentration, initial temperature and pH on the process efficiency, which was measured by the percentage of turbidity removal. Full factorial design 2^3, randomized and in one block, was performed with six axial points and six replicates at the central point. TABLE 1 presents levels used for each parameter, in codified and real values.

TABLE1:Levels of parameters investigated in coagulation experiments - DCCR

Codified level	Chitosan Concentration mg/L	Temperature	pH
-1.68179	81.8	6.6	5.3
-1	150.0	10.0	5.8
0	250.0	15.0	6.5
1	350.0	20.0	7.2
1.68179	418.2	23.4	7.7

Coagulation experiments were conducted as designed, in random order and performed at the Food Engineering Laboratory at the Chemical Engineering Department (DEQ), Universidade Federal de Minas Gerais (UFMG).

Turbidity removal was selected as the parameter for evaluating the process efficiency. Since the main component of the concentrated is fat, it was considered that turbidity removal can express this efficiency. The result can be calculated by the equation (1):

$$\text{Turbidity removal } (\%) = [(NTUi - NTUf) \div NTUi] \times 100 \quad (1)$$

where: NTU_i = whey initial turbidity (NTU - *Nephelometric Turbity Unit)* NTU_f = supernatant turbidity, after coagulation and filtering processes (NTU*)*

Results were submitted to a statistical treatment using *Minitab®*, in order to verify the influence of each one of the factors analyzed in the answer variable (turbidity removal).

II.4 Drying

Coagulated was dried in spray dryer (*Mini Spray Dryer, model MSDi 1.0, LM Equipment and instruments*), obtaining a powder concentrate, at drying air flow rate of 0.60 m³ min, maximum operating temperature of 190°C, and maximum pump flow of 17 ml min^{-1} with nominal drying capacity of 1.0 L h^{-1}.

II.5 Compositional Determination of the Filtrate and of the Concentrate

The composition of the filtrate obtained from separation after coagulation in the best process condition was determined by the same parameters used for the whey samples, which are: pH, acid in lactic acid, ash content, COD, dry extract, lipids (fat), lactose, protein and turbidity. The procedures used were the same described in item 2.2 (whey composition determination).

Concentrate obtained from the drying of coagulate was analyzed in terms of humidity, fat, lactose, protein and ash contents. Humidity were determinate by gravimetric method, in which 5 g of the sample is dried in a convective oven, at 105°C, for 24 h and the final mass is evaluated [18].

The other concentrate properties were obtained following the same described in item II.2. All procedures were performed in triplicate, in order to determine the experimental error.

III RESULTS AND DISCUSSION

III.1 Whey Characterization

The results of the means and standard deviations obtained in the characterization of the buffalo whey sample are shown in TABLE 2.

TABLE 2: Mean values and standard deviation of the results obtained for buffalo whey samples

Parameter	*Values for buffalo whey*
pH	6.00 ± 0.05
acidity in lactic acid (ºD)	10.98 ± 0.35
Turbidity (NTU)	2230 ± 69
COD (μg O_2 L^{-1})	76.596 ± 5.687
Total dry extract (%)	7.9 ± 0.2
Lactose (%)	4.78 ± 0.35
Protein (%)	0.89 ± 0.08
Lipids (%)	0.7 ± 0.2
Ash (%)	0.63 ± 0.08

Lira et al. [6] reported for buffalo milk a pH of 6.31 and lactic acidity of 10ºD, values very similar to those found in this study. Vogelaar&Pawlowsky [8] registered a lower value of 878 NTU for bovine whey. This difference may be due to differences in the cheese manufacturing process, besides the different origin of them.

Chemical oxygen demand (COD) indicates that buffalo whey has an environment polluting potential and is close to that reported by Vogelaar&Pawlowsky [8], which was 70.956 μg of oxygen. mL^{-1} The total dry extract indicates the total amount of solids present in relation to the analyzed sample. Nishanthi, Vasiljevic, &Chandrapala [2] found values from 5.5 to 21.9% for whey from bovine milk, range in which the one here determined is situated. On the other hand, Lima et al. [19] reported a greater value of 14.39 to 15.63% for buffalo whey, variance which may be due to the difference in the buffalo races.

Lima et al. [19] determined lactose values from 4.6 to 4.9%, which agree with this work. For protein, these authors related a value of 0.91%, very similar to this work. Nishanthi, Vasiljevic, &Chandrapala [2] found 0.24% to 1.24% of protein for whey from bovine milk.The value for lipids reported by Lira et al. [6] is 1.20% and by Lima et al.[19] is 1.51%, greater than that found here. The differences found can be probably attributed to different cheese production methods. For ash, Lima et al. [19] determined 0.45%, which is of the same order than that found here.Buffalo whey is a product very susceptible to variations in its chemical composition due to several factors such as animal, climate, and manufacturing method, among others, which explains the small differences in composition values when compared to other works.

III.2 - Coagulation Results

The results for coagulation tests are shown in TABLE 3. As it can be observed, high indices of turbidity removal were achieved, which demonstrated the efficiency of the coagulation using chitosan.

TABLE 3: Coagulation experiments: turbidity removal

Experiment	*Chitosan concentration mg L^{-1}*	*Temperature (°C)*	*pH*	*Turbidity removal (%)*
1	350.0	10.0	5.8	90.1
2	150.0	10.0	5.8	87.6
3	350.0	20.0	5.8	90.2
4	150.0	20.0	5.8	94.0
5	250.0	15.0	6.5	88.7
6	250.0	15.0	6.5	94.7
7	418.0	15.0	6.5	96.6
8	250.0	15.0	5.3	85.3
9	150.0	10.0	7.2	87.8
10	250.0	15.00	6.50	92.6
11	81.8	15.00	6.0	81.9
12	250.0	15.0	6.5	86.7
13	250.0	15.0	6.5	87.0
14	250.0	23.4	6.5	91.8
15	350.0	20.0	5.80	89.3
16	350.0	10.0	7.2	93.3
17	250.0	6.6	6.5	93.9
18	150.0	20.0	5.80	91.7
19	250.0	15.00	6.50	90.0
20	250.0	15.00	7.68	89.4

ANOVA (Analysis of variance) performed showed that, at 90% confidence interval, there is a significant dependence of the turbidity removal with the chitosan concentration (p-value <0.1). This tendency can be observed in Fig. 1.

The higher value achieved is 96.6% of turbidity removal, at 418 mg/L of chitosan, 15°C and pH of 6.5. However, considering the process economy, using chitosan concentration of 250mg/L is recommended, resulting in a 94.7% turbidity removal, which is very satisfactory. The small difference in removal does not justify an approximately 80% increase in chitosan concentration, which would directly reflect in the process costs.

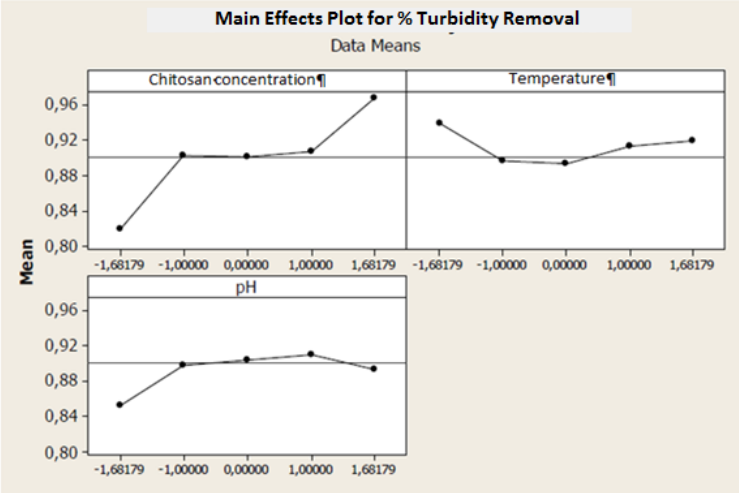

Figure 1:effect of the process variation on turbidity removal.

III.3 - Characterization of Filtrate and of the Concentrate

TABLE 4 shows the results of the chemical determination for the filtered and for the dry concentrate. It is also presented again the values for buffalo whey samples, in order to facilitate the comparison.

TABLE 4 : Chemical determination - Mean values and standard deviation

Parameter	*Whey*	*Filtered*	*Dry concentrate*
pH	6.00 ± 0.05	5.73 ± 0.03	-
acidity in lactic acid (ºD)	10.98 ± 0.35	10.31 ± 0.47	-
Turbidity (NTU)	2230 ± 69	96 ± 4	-
COD (µg O_2 L^{-1})	76.596 ± 5.687	65.596 ± 6.698	-
Total dry extract (%)	7.9 ± 0.2	6.62 ± 0.2	-
Lactose (%)	4.78 ± 0.35	4.41 ± 0.43	25.90 ± 0.33
Protein (%)	0.89 ± 0.08	0.78 ± 0.05	13 ± 0.2
Lipids (%)	0.7 ± 0.2	-	52 ± 0.3

Results of turbidity removal and the fat content of the coagulate indicate that chitosan was efficient for coagulation of lipids (fat), resulting in a concentrate of 52% of fat, proving that there was drag of fat to the solid material during the coagulation process. This good performance can be accredited to the fact of the milk fat is in the form of small globules suspended in water. As chitosan has a high density of positive charge, it attracts and binds to the lipids whose molecules are negative in nature, being this coagulant agent very efficient for this system [12].

This lipid concentrate becomes a value-added product once the recovered lipids are mostly phospholipids (lecithin) [20]. These are considered essential nutrients for the proper functioning of the human body and can be used as an ingredient for the Food Industry. The pharmacological use of lecithin includes the treatment of hypercholesterolemia, neurological disorders, and liver diseases [21, 22].

According to the results, there was a decrease in COD, but the final value for the filtered indicates that is necessary additional processes that minimize this parameter.

There was a slight reduction in lactose and protein contents, which confirm the requirement of other process that remove these components, especially lactose, which is very responsible for greater values of COD.

Humidity of 2% observed for the dry coagulate is adequate for powder product, where low values prevent from deterioration.

Considering the amount of fat contained in the original sample and the one detected in the concentrate, it is possible verify that the process yield is about 71%, which means that for each liter of buffalo whey, it can be recovered about 5 g of fat.

Although chitosan was not removed from the coagulate and, therefore, from the dry concentrate, it is considered that, according to literature, it is safe to be used even for food purposes [11]. Besides, the amount of chitosan is presented in a small concentration of the dried material (around 5%, considering that no chitosan was held in the supernatant).

IV CONCLUSION

Coagulation of buffalo whey using chitosan showed to be very efficient, removing turbidity satisfactorily and recovering a considering amount of fat from the original solution. Using chitosan concentration of 250mg L^{-1}, pH 6.5 and temperature of 15°C, coagulation allowed a turbidity removal of the 96%, resulting in a coagulate that, after drying, contains of 52% of lipids. The yield based on fat recovery is around 71% in mass. This fat rich product may be used in food, cosmetics and pharmaceuticals products. The COD result for the filtrate after coagulation indicates that complementary methods should be used to treat the filtered in order to achieve attend the environmental legislation for effluent disposal.

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